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398.	Sensory systems—visual psychophysics and behavior II	Slide				thAM	
175.	Somatic and visceral afferents I	Poster		tuAM			
293.	Somatic and visceral afferents II	Poster			wAM		
365.	Somatic and visceral afferents III	Poster			wPM		

Session Number	Session Title	Type	Day & Time Mon.	Tue.	Wed.	Thu.	Fri.
528.	Somatic and visceral afferents IV	Slide					fAM
103.	Somatic and visceral afferents: capsaicin	Poster	mPM				
100.	Somatosensory cortex and thalamocortical relationships I	Poster	mPM				
101.	Somatosensory cortex and thalamocortical relationships II	Poster	mPM				
443.	Somatosensory cortex and thalamocortical relationships III	Poster				thAM	
500.	Somatosensory cortex and thalamocortical relationships IV	Poster				thPM	
235.	Spinal cord: anatomy and physiology	Poster		tuPM			
174.	Spinal cord: neurotransmitters	Poster		tuAM			
99.	Subcortical somatosensory pathways	Poster	mPM				
	Theme G: Motor Systems and Sensorimotor Integration						
104.	Basal ganglia and thalamus I	Poster	mPM				
105.	Basal ganglia and thalamus II	Poster	mPM				
176.	Basal ganglia and thalamus III	Poster		tuAM			
179.	Basal ganglia and thalamus IV	Poster		tuAM			
180.	Basal ganglia and thalamus V	Poster		tuAM			
394.	Basal ganglia and thalamus VI	Slide				thAM	
505.	Basal ganglia and thalamus VII	Poster				thPM	
506.	Basal ganglia and thalamus VIII	Poster				thPM	
301.	Circuitry and pattern generation I	Poster			wAM		
446.	Circuitry and pattern generation II	Poster				thAM	
465.	Circuitry and pattern generation III	Slide				thPM	
50.	Control of posture and movement I	Poster	mAM				
69.	Control of posture and movement II	Slide	mPM				
368.	Control of posture and movement: animal locomotion	Poster			wPM		
445.	Control of posture and movement: arm and hand	Poster				thAM	
544.	Control of posture and movement: clinical studies	Poster					fAM
545.	Control of posture and movement: humans	Poster					fAM
369.	Control of posture and movement: learning and development	Poster			wPM		
106.	Cortex I	Poster	mPM				
107.	Cortex II	Poster	mPM				
178.	Cortex III	Poster		tuAM			
466.	Cortex IV	Slide				thPM	
503.	Cortex V	Poster				thPM	
504.	Invertebrate motor function	Poster				thPM	
268.	Motor systems and sensorimotor integration: cerebellum I	Slide			wAM		
370.	Motor systems and sensorimotor integration: cerebellum II	Poster			wPM		
371.	Motor systems and sensorimotor integration: cerebellum III	Poster			wPM		
372.	Motor systems and sensorimotor integration: oculomotor system I	Poster			wPM		
373.	Motor systems and sensorimotor integration: oculomotor system II	Poster			wPM		
444.	Motor systems and sensorimotor integration: oculomotor system III	Poster				thAM	
304.	Motor systems and sensorimotor integration: vestibular system I	Poster			wAM		
305.	Motor systems and sensorimotor integration: vestibular system II	Poster			wAM		
401.	Motor systems and sensorimotor integration: vestibular system III	Slide				thAM	
52.	Muscle: general	Poster	mAM				
177.	Muscle: human studies	Poster		tuAM			
51.	Muscle: molecular studies	Poster	mAM				
367.	Reflex function: animal studies	Poster			wPM		
366.	Reflex function: human	Poster			wPM		
4.	**Regulation of Pattern Generating Networks**	**Symp.**	**mAM**				
48.	Spinal cord and brainstem	Poster	mAM				
303.	Spinal cord and brainstem: anatomy	Poster			wAM		
49.	Spinal cord and brainstem: motoneurons	Poster	mAM				
302.	Spinal cord and brainstem: pharmacological studies	Poster			wAM		
	Theme H: Other Systems of the CNS						
447.	Association cortex and thalamocortical relationships	Poster				thAM	
129.	Brain metabolism and blood flow: central influences	Slide		tuAM			
239.	Brain metabolism and blood flow: endogenous factors	Poster		tuPM			
307.	Brain metabolism and blood flow: exogenous factors	Poster			wAM		
16.	Brain metabolism and blood flow: methods	Slide	mAM				
507.	Brainstem systems	Poster				thPM	
55.	Comparative neuroanatomy: fish and amphibia	Poster	mAM				

Session Number	Session Title	Type	Day & Time Mon.	Tue.	Wed.	Thu.	Fri.
108.	Comparative neuroanatomy: reptiles, birds, mammals	Poster	mPM				
546.	Hippocampus and amygdala: behavior	Poster					fAM
53.	Hippocampus and amygdala: neuroanatomy	Poster	mAM				
181.	Hippocampus and amygdala: neurocytology	Poster		tuAM			
306.	Hippocampus and amygdala: neurophysiology I	Poster			wAM		
448.	Hippocampus and amygdala: neurophysiology II	Poster				thAM	
240.	Hypothalamus	Poster		tuPM			
54.	Limbic system I	Poster	mAM				

Theme I: Neural Basis of Behavior

248.	Biological rhythms and sleep I	Poster		tuPM			
270.	Biological rhythms and sleep II	Slide			wAM		
317.	Biological rhythms and sleep III	Poster			wAM		
514.	Biological rhythms and sleep IV	Poster				thPM	
550.	Biological rhythms and sleep V	Poster					fAM
451.	Drugs of abuse	Poster				thAM	
57.	Drugs of abuse: alcohol I	Poster	mAM				
112.	Drugs of abuse: alcohol II	Poster	mPM				
183.	Drugs of abuse: alcohol III	Poster		tuAM			
198.	Drugs of abuse: alcohol IV	Slide		tuPM			
312.	Drugs of abuse: alcohol V	Poster			wAM		
243.	Drugs of abuse: amphetamine	Poster		tuPM			
136.	Drugs of abuse: amphetamine and cocaine	Slide		tuAM			
450.	Drugs of abuse: cannabinoids, nicotine and PCP	Poster				thAM	
11.	Drugs of abuse: cocaine	Slide	mAM				
242.	Drugs of abuse: cocaine and others	Poster		tuPM			
310.	Drugs of abuse: cocaine, behavior	Poster			wAM		
309.	Drugs of abuse: cocaine, cellular	Poster			wAM		
110.	Drugs of abuse: cocaine, dopamine	Poster	mPM				
111.	Drugs of abuse: cocaine, serotonin	Poster	mPM				
382.	Drugs of abuse: opioids	Poster			wPM		
204.	Hormonal control of behavior I	Slide		tuPM			
308.	Hormonal control of behavior II	Poster			wAM		
315.	Hormonal control of behavior III	Poster			wAM		
380.	Hormonal control of behavior IV	Poster			wPM		
18.	Human behavioral neurobiology	Slide	mAM				
241.	Human behavioral neurobiology: event related potentials, attention, audition	Poster		tuPM			
509.	Human behavioral neurobiology: history, memory, imaging, other	Poster				thPM	
513.	Ingestive behavior: body weight and eating	Poster				thPM	
376.	Ingestive behavior: monoamines	Poster			wPM		
131.	Ingestive behavior: monoamines and nutrients	Slide		tuAM			
318.	Ingestive behavior: peptides I	Poster			wAM		
406.	Ingestive behavior: peptides II	Slide				thAM	
510.	Ingestive behavior: salt, water and aversion	Poster				thPM	
381.	Interhemispheric relations	Poster			wPM		
14.	Invertebrate learning and behavior I	Slide	mAM				
246.	Invertebrate learning and behavior II	Poster		tuPM			
263.	Invertebrate learning and behavior III	Slide			wAM		
249.	Learning and memory: anatomy I	Poster		tuPM			
250.	Learning and memory: anatomy II	Poster		tuPM			
115.	Learning and memory: conditioning	Poster	mPM				
127.	Learning and memory: human lesion studies	Slide		tuAM			
258.	Learning and memory: non-human primate lesion studies	Slide			wAM		
549.	Learning and memory—pharmacology	Poster					fAM
58.	Learning and memory—pharmacology: acetylcholine I	Poster	mAM				
374.	Learning and memory—pharmacology: acetylcholine II	Poster			wPM		
316.	Learning and memory—pharmacology: excitatory amino acids	Poster			wAM		
508.	Learning and memory—pharmacology: monoamines	Poster				thPM	
113.	Learning and memory: physiology I	Poster	mPM				
185.	Learning and memory: physiology II	Poster		tuAM			
205.	Learning and memory: physiology III	Slide		tuPM			
314.	Learning and memory: physiology IV	Poster			wAM		

Session Number	Session Title	Type	Day & Time Mon.	Tue.	Wed.	Thu.	Fri.
378.	Learning and memory: physiology V	Poster			wPM		
511.	Learning and memory: spatial	Poster				thPM	
56.	Monoamines and behavior I	Poster	mAM				
75.	Monoamines and behavior II	Slide	mPM				
184.	Monoamines and behavior III	Poster		tuAM			
311.	Monoamines and behavior IV	Poster			wAM		
247.	Motivation and emotion	Poster		tuPM			
245.	Motivation and self-stimulation	Poster		tuPM			
449.	Neuroethology: avian song	Poster				thAM	
512.	Neuroethology: avian song and other	Poster				thPM	
548.	Neuroethology: fish	Poster					fAM
313.	Neuroethology: invertebrates	Poster			wAM		
379.	Neuroethology: mammals, reptiles, amphibians	Poster			wPM		
114.	Neuropeptides and behavior I	Poster	mPM				
186.	Neuropeptides and behavior II	Poster		tuAM			
375.	Neuropeptides and behavior III	Poster			wPM		
547.	Psychotherapeutic drugs	Poster					fAM
377.	Psychotherapeutic drugs: antidepressants	Poster			wPM		
109.	Psychotherapeutic drugs: antipsychotics I	Poster	mPM				
244.	Psychotherapeutic drugs: antipsychotics II	Poster		tuPM			
66.	Stress, hormones and the autonomic nervous system I	Slide	mPM				
116.	Stress, hormones and the autonomic nervous system II	Poster	mPM				
182.	Stress, hormones and the autonomic nervous system III	Poster		tuAM			
124.	**The Olivocerebellar System: Its Possible Role in Learning**	**Symp.**		**tuAM**			

Theme J: Disorders of the Nervous System

12.	Alzheimer's disease: amyloid I	Slide	mAM				
190.	Alzheimer's disease: amyloid II	Poster		tuAM			
322.	Alzheimer's disease: amyloid III	Poster			wAM		
120.	Alzheimer's disease: biochemistry and clinical studies	Poster	mPM				
67.	Alzheimer's disease: cognitive and clinical studies	Slide	mPM				
387.	Alzheimer's disease: cytoskeleton	Poster			wPM		
555.	Alzheimer's disease: models	Poster					fAM
519.	Alzheimer's disease: molecular studies	Poster				thPM	
199.	Alzheimer's disease: neuropathology I	Slide		tuPM			
389.	Alzheimer's disease: neuropathology II	Poster			wPM		
517.	Alzheimer's disease: neuropathology III	Poster				thPM	
252.	Alzheimer's disease: pharmacology	Poster		tuPM			
516.	Clinical CNS neurophysiology	Poster				thPM	
457.	Degenerative disease—other: basal ganglia	Poster				thAM	
554.	Degenerative disease—other: MS, ALS and others	Poster					fAM
337.	Degenerative disease—Parkinson's	Slide			wPM		
453.	Degenerative disease—Parkinson's: humans and treatment	Poster				thAM	
553.	Degenerative disease—Parkinson's: MPTP monkeys and rodents	Poster					fAM
323.	Disorders of the nervous system: developmental models	Poster			wAM		
320.	Epilepsy: animal genetic models	Poster			wAM		
551.	Epilepsy: animal models	Poster					fAM
321.	Epilepsy: anti-convulsant drugs	Poster			wAM		
15.	Epilepsy: basic mechanisms I	Slide	mAM				
388.	Epilepsy: basic mechanisms II	Poster			wPM		
188.	Epilepsy: human studies	Poster		tuAM			
138.	Epilepsy: human studies and animal models	Slide		tuAM			
452.	Epilepsy: kindling I	Poster				thAM	
518.	Epilepsy: kindling II	Poster				thPM	
119.	Epilepsy: status epilepticus	Poster	mPM				
189.	Genetic models of nervous disorders I	Poster		tuAM			
383.	Genetic models of nervous disorders II	Poster			wPM		
469.	Genetic models of nervous disorders III	Slide				thPM	
253.	Infectious diseases	Poster		tuPM			
13.	Ischemia I	Slide	mAM				
117.	Ischemia II	Poster	mPM				
118.	Ischemia III	Poster	mPM				
384.	Ischemia IV	Poster			wPM		

Session Number	Session Title	Type	Day & Time Mon.	Tue.	Wed.	Thu.	Fri.
385.	Ischemia V	Poster			wPM		
386.	Ischemia VI	Poster			wPM		
527.	Ischemia VII	Slide					fAM
59.	Mental illness: depression, suicide, other	Poster	mAM				
556.	Mental illness: schizophrenia	Poster					fAM
60.	Neuromuscular diseases	Poster	mAM				
454.	Neurotoxicity: amino acids	Poster				thAM	
187.	Neurotoxicity: metals	Poster		tuAM			
515.	Neurotoxicity: MPTP	Poster				thPM	
455.	Neurotoxicity: other I	Poster				thAM	
251.	Neurotoxicity: PNS and retina	Poster		tuPM			
256.	**Recapitulation of Developmental Mechanisms in Neurodegenerative Disorders**	**Symp.**			**wAM**		
206.	Trauma	Slide		tuPM			
319.	Trauma: brain injury	Poster			wAM		
552.	Trauma: spinal cord, NMDA and other	Poster					fAM

Other:

325.	**NINDS: Forty Years of Progress**	**Symp.**			**wPM**		

3

SYMPOSIUM: MOLECULAR MECHANISMS OF NEURONAL GUIDANCE M.E. Hatten, Columbia Univ. Col. Phys. & Surg.(chairperson); L.F. Reichardt, U.C.S.F.; B. Ranscht, La Jolla Cancer Res. Fndn.; T.M. Jessell, Columbia Univ. Col. Phys. Surg.; and C.S. Goodman, U.C. Berkeley

In celebration of the 100th anniversary of Cajal's naming of the "growth cone", this symposium will focus on the molecular mechanisms of neuronal migration and growth cone navigation. Molecular cloning of cell adhesion receptor systems will be used to examine the relationships between cell adhesion molecules, cadherins and integrins, and both *in vivo* and *in vitro* approaches will be used to analyze the function and expression of these ligands. Receptor systems that guide neuronal migration will be analyzed with an *in vitro* model system for cerebellar granule neurons, focusing on the neuron-glia ligand astrotactin and its role in orchestrating the movement of the leading process, a growth cone like structure, along the glial fiber. The role of neuronal receptors that regulate axonal growth and growth cone motility will be discussed, focussing on *in vitro*, functional studies with integrins, cadherins and cell adhesion molecules of the immunoglobulin superfamily. Cell adhesion molecules in nerve guidance will be examined by analyzing the molecular interactions of growing axons with cell adhesion ligands marked by specific intermediate targets during nerve growth. Cell surface and diffusible molecules that guide developing axons will be examined by analysis of the spatial and temporal regulation of neural members of the immunoglobulin superfamily, and the role of chemotrophic molecules in axon guidance in the developing spinal cord will be discussed. Finally, the molecular genetics of cell adhesion and cell recognition in Drosophila will be examined by a genetic and molecular genetic analysis of the structure and function of neural cell adhesion molecules expressed by growth cones in Drosophila.

4

SYMPOSIUM. REGULATION OF PATTERN GENERATING NETWORKS. K.G.Pearson, Univ. of Alberta (Chairperson); E.E.Marder, Bradeis Univ.; P.Wallen, Karolinska Institute; A.Roberts, Bristol Univ.; J.L.Feldman, U.C.L.A.

Many rhythmic pattern generating networks have been shown to be modified strongly by neuromodulators and by central and peripheral neural signals. This symposium will review recent progress in establishing the cellular and synaptic events associated with these regulatory processes. E.E.Marder will describe how modifications of synaptic efficacy and intrinsic membrane properties can reconfigure pattern generating networks in lobsters and crabs. This can result in the same neural elements participating in several different neural networks. The modulatory control of the network for locomotion in the lamprey by serotonergic fibres and by sensory feedback from intraspinal stretch receptors will be reviewed by P.Wallen. The use of computer simulations in the analysis of this system will be discussed. Likewise, A.Roberts will present data from modelling studies on the network for swimming in the frog tadpole aimed at explaining how the frequency of oscillation in this network is regulated by central neural signals. K.G.Pearson will review data from the locomotor systems of mammals, birds and insects demonstrating that phasic proprioceptive feedback is responsible for establishing the timing of some features of the normal motor pattern. Finally, J.L.Feldman will describe how the neural control mechanisms for respiration in neonatal and adult mammals can be changed either by modulation of the pattern generating network or by transformations of the properties of elements in this network.

CELLULAR AND MOLECULAR BIOLOGY OF CATECHOLAMINE RECEPTORS

5.1

D1 RECEPTOR ACTIVATION INDUCES C-FOS EXPRESSION IN STRIATO-NIGRAL NEURONS FOLLOWING DENERVATION. G.S. Robertson, S.R. Vincent and H.C. Fibiger. Div. Neurol. Sci., Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C., Canada. V6T 2W5

D1, but not D2, receptor agonists activate the proto-oncogene, c-fos, in the striatum (ST) ipsilateral to a 6-hydroxydopamine (6-OHDA) lesion of the nigrostriatal pathway. The ST sends major outputs to the globus pallidus (GP) and the substantia nigra pars reticulata (SNR). The present study examined the nature of striatal neurons containing D1 receptor induced c-fos expression using the fluorescent retrograde tracer Fluoro-Gold (FG). FG was injected into the SNR (0.1-0.5μl) or GP (iontophoretic) ipsilateral to a 6-OHDA-lesion of the medial forebrain bundle. Five days later, c-fos expression was induced by injection of the selective D1 receptor agonist SKF 38393 (4 mg/kg) which produced circling (12-14 turns/min) that was directed away from the lesioned side. Two hr following SKF 38393 injection, rats were anesthetized and perfused with 4% PFA. Sections 20μM thick were incubated with sheep anti-c-fos antibody for 48 hr. After incubation for 1 hr with Texas Red-labelled rabbit anti-sheep antibody sections were were observed using a Leitz fluorescence microscope either under ultraviolet light to examine FG, or green light to look at Texas Red fluorescence. C-fos-positive nuclei were frequently located in striatal cell bodies retrogradely labelled with FG from the SNR but seldom in cell bodies labelled from the GP. These results are in agreement with autoradiographic receptor binding studies which suggest that D1 receptors are located predominantly on striato-nigral rather than on striato-pallidal neurons.

5.2

CHARACTERIZATION AND REGULATION OF DA_1 DOPAMINE RECEPTORS IN OPOSSUM KIDNEY (OK) CELLS. M. D. Bates*, M. G. Caron, and J. R. Raymond*. Depts. of Cell Biology and Medicine, Duke University Medical Center, Durham, NC 27710.

Peripheral dopamine receptors have been classified on the basis of synaptic location and pharmacology into two categories, DA_1 and DA_2, which are similar to but distinct from central D_1 and D_2 dopamine receptors. Here we report the presence of DA_1 dopamine receptors in opossum kidney (OK) cells, a cell line having characteristics of renal proximal tubules. The specific activity of receptors in OK cell membranes, as defined by [^{125}I]SCH 23982 binding, is ~100 fmol/mg. Competition with various agonists and antagonists gives a pharmacological profile appropriate for a DA_1 receptor. The receptor is functionally coupled to the stimulation of adenylyl cyclase. Dopamine stimulates OK cell membrane adenylyl cyclase activity ~4-fold, with an EC_{50} of ~3 μM; this stimulation is blocked specifically by SCH 23390. Treatment of OK cells with dopamine results in desensitization of dopaminergic adenylyl cyclase stimulation in a time- and dose-dependent manner. With greatest desensitization, adenylyl cyclase stimulation is decreased to ~20% of control. Treatment of cells with the membrane permeable cAMP analog 8-bromo-cAMP also diminishes adenylyl cyclase stimulation by dopamine, suggesting that heterologous regulation of DA_1 receptors may occur. Thus, OK cells provide a model system for the study of the peripheral actions of dopamine at DA_1 receptors and the regulation of these receptors.

5.3

DIFFERENTIAL LOCALIZATION OF EXPRESSION OF THE GENES ENCODING THE D_1 AND D_2 DOPAMINE RECEPTORS IN THE RAT BRAIN. R. T. Fremeau Jr., G. Duncan, G. Breese, J. A. Gingrich*, P. Falardeau, M. G. Caron, & A. Dearry. Depts. of Neurobiol. & Cell Biol., Duke Univ. Med. Ctr., Durham, NC 27710 and BSRC, University of North Carolina, Chapel Hill, NC 27599

D_1 and D_2 dopamine receptors functionally interact to control behavior in both synergistic and opposing manners in different brain regions. To provide a framework for studying the molecular basis of D_1/D_2 receptor interactions, we examined the expression of the genes encoding the D_1 and D_2 receptors in the rat brain by *in situ* hybridization using ^{35}S-riboprobes. The 0.45kb EcoR1/Cla1 fragment of the rat D_1 cDNA (Dearry *et. al.*, this meeting) and the 1.5kb Sac1/BamH1 fragment of the rat D_2 receptor cDNA (Bunzow *et. al.*, Nature 336: 783, 1988) were subcloned into pBluescript®. High levels of D_1 mRNA were detected in striatum, nucleus accumbens, and olfactory tubercle. No D_1 mRNA was detected in substantia nigra or amygdala. These results confirm that substantia nigra D_1 receptors are on the terminals of the striatonigral pathway neurons. In contrast, hybridization of serial sections with a D_2-specific probe revealed the expected high level of D_2 mRNA in substantia nigra zona compacta, striatum, nucleus accumbens, and olfactory tubercle. Emulsion autoradiograms indicate that D_1 and D_2 receptors are expressed by distinct populations of neurons in the olfactory tubercle. Thus, D_1/D_2 interactions are more complicated than can be accounted for by co-expression of both receptors in the same neuron.

5.4

FUNCTIONAL ACTIVITY AND mRNA LOCALIZATION OF A CLONED D1 DOPAMINE RECEPTOR. EVIDENCE FOR MULTIPLE SUBTYPES. P. Falardeau, M. D. Bates*, J. A. Gingrich*, A. Dearry, R.T.Fremeau Jr., and M.G. Caron. Dept of Cell Biology. Duke Univ. Med. Ctr., Durham, N.C.

We have recently isolated a genomic clone for a human D1 dopamine receptor. In mouse L(tk-) cells stably transfected with the D1 receptor, a 5-fold stimulation of adenylyl cyclase (AC) was elicited by dopamine. The selective D1 agonist SKF38393 also stimulated the enzyme activity, whereas the selective D2 agonist quinpirole was ineffective. The stimulation by dopamine was blocked by the D1 antagonist SCH23390 but not by the D2 antagonist raclopride. Interestingly, dopamine failed to stimulate phosphatidylinositol (PI) metabolism in COS-7 cells transiently transfected with the D1 dopamine receptor . The specificity of coupling of this D1 receptor to AC but not to PI metabolism is in contrast to the recent reports documenting the presence of a D1 dopamine receptor coupled to PI metabolism in striatum and kidney. By northern blot and PCR analysis of mRNA, this receptor appears to be expressed in high abundance in caudate and at lower levels in frontal cortex and hippocampus. Much lower levels in cerebellum and ventral tegmental area were found. No message was detected in kidney, heart and liver. These results agree with the expected distribution for D1 receptor message in the CNS, but suggest that the D1 dopaminergic stimulation of AC and PI metabolism in kidney must be mediated by one or more distinct D1 receptor subtypes.

These results coupled with the presence of multiple hybridizing bands on genomic Southern blot analysis at low stringency indicate the possible existence of additional D1 dopamine receptor subtypes.

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

5.5

MOLECULAR CLONING AND EXPRESSION OF THE GENE FOR A HUMAN D1 DOPAMINE RECEPTOR. A. Dearry, J. Gingrich, P. Falardeau, R. Fremeau, M. Bates, W. Leung, T. Yang-Feng, and M. Caron. Duke Univ. Medical Center, Durham, N.C., & Yale Univ. School of Medicine, New Haven, Ct.

The diverse physiological actions of dopamine are mediated by its interaction with two basic types of G protein-coupled receptor: D1 and D2, which respectively stimulate and inhibit the enzyme adenylyl cyclase. We have isolated and characterized the gene encoding a human D1 dopamine receptor (D1 R). This gene is intronless within its coding region in contrast to that encoding the D2 dopamine receptor (D2 R). The D1 R gene encodes a protein of 446 amino acids having a predicted molecular weight of 49,300 daltons and a transmembrane topology similar to that of other G protein-coupled receptors. Within the putative transmembrane domains, the human D1 R has a sequence identity of approximately 40% with the D2 R, α2-, β1-, and β2-adrenergic receptors, and 5HT1A receptor. The most homologous regions shared by the D1 R and D2 R are found in transmembranes II and III. The D1 R possesses two sites for N-linked glycosylation and several potential sites for regulatory phosphorylation. Expression of the cloned gene into host cells established specific ligand binding characteristic of a D1 R. PCR analysis of tissue RNA revealed that D1 R message is most abundant in caudate. The gene for the D1 R localizes to the long arm of human chromosome 5. Cloning of the gene for a D1 R will facilitate investigation of the molecular processes involved in activation and regulation of this receptor.

5.6

Comparison of Expression and Genomic Organization of the D2 Receptor Locus in Inbred and Outbred Strains of Rats. R.R. Luedtke, R.P. Artymyshyn*, B.R. Monks* and P. B. Molinoff. *Department of Pharmacology, University. of Pennsylvania, Philadelphia, PA 19104*

Radioligand binding and Southern blot hybridization techniques were used to look for phenotypic and genotypic differences at the rat D2 receptor locus. Three outbred strains (Sprague Dawley, Wistar and Long Evans) and five inbred strains (Brown Norway, Buffalo, DA, Fisher and Lewis) were used. Radioligand binding studies were performed using ^{125}I-IBZM, a D2-selective antagonist. Comparison of the affinities (K_d = 0.3 nM) and densities (450-580 fmol/mg protein) of binding sites for ^{125}I-IBZM in the striatum of each rat strain did not reveal statistically significant differences. A comparison was made of Southern hybridization blots using genomic DNA from each strain. Blots were hybridized with a DNA probe that codes for the $D2_L$ receptor isoform. Restriction fragment lengths were conserved between strains for DNA digested with *Bam* HI, *Eco* RI, *Hind* III, *Pst* I and *Taq* I. A restriction fragment length polymorphism (RFLP) was identified when DNA was digested with *Xba* I. The genetic polymorphism was mapped to within 2 kb of the exon coding for the third intracellular loop of the D2 receptor. This RFLP differentiated Sprague Dawley and Brown Norway rats from Wistar, Long Evans, Buffalo, DA, Lewis and Fisher rats. The RFLP for the rat D2 receptor locus provides a genetic marker that can be used to test whether strain differences in behavior and/or in the response to pharmacologic intervention are determined by genetic elements linked to the D2 receptor locus. (U.S.P.H.S grant NS18591)

5.7

A LARGE RECEPTOR RESERVE (RR) EXISTS FOR DOPAMINERGIC REGULATION OF PROLACTIN (PRL) SECRETION IN VIVO AND IN ANTERIOR PITUITARY (AP) CELLS IN PRIMARY CULTURE. E. Meller, T. Puza*, J.C. Miller, A.J. Friedhoff and J.S. Schweitzer*. Dept. of Psychiatry, NYU Medical Center, New York, NY 10016

Dopamine (DA) D2 receptors on lactotrophs of the AP mediate inhibition of PRL secretion. These receptors are functionally similar to D2 autoreceptors since serum PRL levels are reduced by autoreceptor-selective agonists such as 3-PPP. We previously showed that autoreceptor-selectivity can be explained by the presence of a large RR at D2 auto- but not postsynaptic receptors. We therefore determined if AP D2 receptors also have a large RR for agonists.

Male rats were pretreated with vehicle or N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) and dose-response (DR) curves for N-propylnorapomorphine (NPA)-induced reduction of serum PRL levels in GBL-treated animals were obtained. In control rats the ED_{50} for NPA was 0.12 ug/kg. The DR curve for NPA was shifted more than 5-fold to the right after EEDQ (1x6 mg/kg) (ED_{50}=0.64 ug/kg), but the maximal response was not reduced. EEDQ (2x6 mg/kg) further shifted the DR curve to the right (ED_{50}=1.04 ug/kg) but the maximal response was still not reduced, indicating the presence of a large RR. A large RR for NPA was also demonstrated in AP cells in primary culture. Interestingly, however, there was no RR for NPA inhibition of forskolin-stimulated extracellular cAMP accumulation, suggesting that D2 receptor coupling to other transduction pathways (e.g. ion channels) may be more related to the observed RR.

5.8

BETA ADRENOCEPTOR-INDUCED EXPRESSION OF EARLY RESPONSE GENES IN THE RAT CEREBRAL CORTEX. G. Bing, E.A. Stone, J.C. Miller, A.J. Friedhoff, D. Filer*. Millhauser Laboratories, Dept. Psychiatry, New York Univ. Sch. Med., New York, NY 10016.

Previous studies have shown that activation of beta adrenoceptors in brain cells both in vitro and in vivo produces expression of various early response genes. In the present study we have replicated this finding for the c-fos gene in vivo and have shown that it also occurs in vivo for the TIS-1 gene. Rats were subjected either to stress (handling plus i.p. saline injection or brief restraint) or to yohimbine injection (5 mg/kg) to increase brain norepinephrine release and were killed 1 hr later. Treatments were given with or without prior i.p. administration of propranolol (10 mg/kg). c-Fos mRNA in the cerebral cortex was assayed by Northern blot. The results indicated that both stress and yohimbine administration were effective in eliciting increased c-fos mRNA. Yohimbine was also found to increase TIS-1 mRNA. All responses were attenuated by propranolol. The results indicate therefore that pharmacological and physiological activation of beta adrenergic receptors in the brain in vivo causes expression of various early response genes. Studies on the involvement of beta receptor subtypes in these responses and their cellular localization are currently in progress. Supported in part by grants AFOSR 89-0208, MH45265 and MH08618.

5.9

LOCALIZATION OF α_2 ADRENERGIC RECEPTOR SUBTYPES IN BRAIN. [1]D.B. Bylund, M.E. Alburges, M. Hunt, L.L. Longlet and J.K. Wamsley. Neuropsychiatric Res. Institute, 120 Eighth Street South, Fargo, ND 58103; [1]Department of Pharmacology, Univ. of Nebraska Medical Center, 42nd and Dewey Avenue, Omaha, NE 68198.

Molecular biological studies have confirmed the existence of multiple α_2 adrenergic receptor subtypes originally identified on the basis of their pharmacology. Tritiated rauwolscine ([^{3}H]RAU) has been shown to have a similar affinity for α_{2A} and α_{2B} subtypes whereas, oxymetazoline (OXY) preferentially binds to the α_{2A} subtype, and ARC-239 (ARC) preferentially labels α_{2B}. By inhibiting [^{3}H]RAU binding to rat brain, the IC_{50} value concentrations of these agents were determined and used to inhibit [^{3}H]RAU binding from slices in autoradiographic experiments. Many brain areas indicated a similar reduction in [^{3}H]RAU binding with each of the two agents, however, there were a few regions which showed a predominance of one site over the other. Lamina IV of the cerebral cortex was inhibited with OXY but not ARC. These receptors are therefore α_{2A}. The substantia nigra pars reticulata, on the other hand, was inhibited by ARC but not OXY, and consequently does not show the presence of α_{2A} receptors. These studies indicate the existence of α_{21} receptor subtypes in individual regions of the brain and pinpoint areas for future research. (This research was supported by grant GM 40784.)

5.10

A NEW ALPHA-1 ADRENERGIC RECEPTOR: CLONING AND CHARACTERIZATION. J.W. Lomasney*, D. Schwinn*, S. Cotecchia*, M. Brownstein+, D. Anthony, M.G. Caron and R.J. Lefkowitz*. HHMI, Duke Univ. Med. Ctr., Durham NC 27710 and +NIMH Bethesda, MD 20892.

The adrenergic receptors are members of the G-protein coupled receptor superfamily, and as such have a putative topology which includes 7 highly conserved stretches of 20-25 hydrophobic aa each thought to span the membrane. Recent pharmacological and functional studies have suggested heterogeneity of α_1AR. Using a fragment of the hamster α_{1B} cDNA as a probe, we have isolated two different clones from a rat cerebral cortex cDNA and a rat genomic library. The first clone is the rat homologue of the hamster α_{1B} receptor. The deduced aa sequence of the second clone α_{1L} while demonstrating features characteristic of G-protein coupled receptors, is most similar to the α_1AR, having 88% amino acid identity in the seven putative transmembrane domains. COS-7 cells transfected with α_{1L} DNA bound the selective α_1AR antagonist ^{125}I-HEAT specifically. In addition, norepinephrine was able to stimulate incorporation of ^{32}P into phosphatidylinositol in these cells. Northern blot analysis of rat tissues shows the α_{1B} mRNA distribution to be liver≥heart>cerebral cortex>kidney>hippocampus while that of the α_{1L} as vas deferens>hippocampus≥cerebral cortex>heart>kidney. The tissue localization of the α_{1L} mRNA suggests that this receptor may be equivalent to the pharmacologically defined α_{1A} subtype.

5.11

COUPLING OF ALPHA$_2$-ADRENERGIC RECEPTOR (α_2-AR) SUBTYPES TO DIFFERENT G-PROTEINS. E Duzic*, S Downing*, I Coupry* and SM Lanier*. (Spon. K. Sweadner) Card Unit, Mass Gen Hosp, Harvard Med School, Boston, MA 02114.

α_2-AR subtypes are expressed in a tissue specific manner and differ in their ligand recognition properties and perhaps their mechanism of signal transduction. The transduction pathway utilized by the receptor subtypes likely depends on the particular G-protein or effector molecule in any given cell. To address this issue we have stably transfected NIH3T3 cells with genes encoding α_2-AR subtypes(RG10, RG20) and determined 1) the ability of the receptor to exist in a high agonist affinity state and 2) receptor coupling to adenylyl cyclase. RG10 and RG20 differ by 20 fold in their affinity for the α_2-AR antagonist ^{3}H-rauwolscine(RG10 K_d=0.6nM) and also in the potency order of competing ligands (RG10 rau> phent> pra> oxy; RG20 phent > rau> oxy> pra). In cells expressing RG20 (200-3000 fmol/mg) but not in cells expressing RG10 (80-1000 fmol/mg) receptor activation inhibited basal and forskolin-stimulated adenylyl cyclase activity by 40%. For RG10, agonist competition curves were monophasic, of low affinity(K_i=1.2 uM) and Gpp(NH)p insensitive. In contrast, agonist competition curves with RG20 were biphasic, could be resolved into high (K_i=10 nM) and low (K_i=1uM) agonist affinity states and were Gpp(NH)p sensitive. These data suggest that NIH3T3 cells express G-proteins capable of interacting with RG20 but not RG10. The major PT sensitive G-protein in NIH-3T3 cells is apparently $G_{i\alpha 2}$ suggesting that RG20 but not RG10 can "recognize" this $G_{i\alpha}$ isoform.

POTASSIUM CHANNELS I

6.1

CHARACTERIZATION OF TWO VOLTAGE-SENSITIVE POTASSIUM CHANNEL SUBTYPES IN MAMMALIAN SKELETAL MUSCLE. J. S. Trimmer, L. K. Kaczmarek, and W. S. Agnew. Depts. of Cellular and Molecular Physiology and Pharmacology, Yale School of Medicine, New Haven, CT 06510

We have undertaken molecular cloning studies that have established the existence of multiple potassium channel transcripts in rat skeletal muscle. We have isolated cDNAs from a denervated rat skeletal muscle library corresponding to the rat brain DRK1 (Frech et al, Nature 340:642) and Kv1 (Swanson et al, Neuron, in press) potassium channels. We have used these cDNAs to study the regulation of expression of the corresponding transcripts in skeletal muscle, and to produce fusion proteins for the production of subtype-specific antisera. We find that the expression of DRK1 in skeletal muscle is constitutve throughout postnatal development, yet increases dramatically upon denervation of adult muscle. In contrast, Kv1 transcripts change both during development and after denervation. Antibodies raised to fusion proteins containing DRK1 and Kv1 fragments react with both brain and skeletal muscle membrane preparations. The DRK1 antibodies react with a Mr=130 kD band on Western blots of brain and muscle membranes, indicating that the 99 kD core peptide predicted from the deduced amino acid sequence has been modified post-translationally. The Kv1 antibodies react with a band of Mr= 84 kD (compare to 66 kDpredicted core peptide). These results indicate that the differential regulation of these two potassium channels in skeletal muscle may underlie the changes in excitability in this tissue during development and after denervation.

6.2

DESCRIPTION OF A NEW CLASS OF POTASSIUM CHANNEL GENES. Vega-Saenz de Miera, E., Sen, K., Serodio, P., McCormack, T. and Rudy, B. Depts. of Physiology & Biophysics. and Biochemistry. NYU Med. Ctr. NY, NY 10016.

Several K^+ channel genes cloned from mammals show large homology to the Shaker gene in *Drosophila*. These genes form three groups based on sequence homology and thus evolutionary relatedness. The members of the first group (Class I genes) are more closely related to the *Drosophila* Shaker gene, Class II to the *Drosophila* Shab gene and Class III to Shaw. Several members of Class I genes have been cloned in *Aplysia*, mouse, rat and human. We now report that Class III genes are also a large gene family. One member (RKShIIIA) was cloned from a rat brain library by this laboratory (PNAS, in press), and another from NG108 cells (NGk2) by Yokoyama et al. (FEBS. Lett 1989). Southern analysis of rat genomic DNA using probes derived from RKShIIIA indicates the presence of multiple genes of this class. Based on the sequence of RKShIIIA, primers were synthesized for polymerase chain reaction (PCR) to obtain partial sequences of other members of this class. Three genes different from RKShIIIA and NGK2 were found. The PCR fragments as well as sequences from the RKShIIIA gene were used as probes to obtain further sequences of Class III genes from rat and human. (Supported by NHI grant GM26976 to B. Rudy).

6.3

MOLECULAR ANALYSIS OF POTASSIUM CHANNELS IN IDENTIFIED CELLS OF *APLYSIA*. Paul Pfaffinger*, Y. Furukawa*, T. Kubo*, B. Zhao*, & E.R. Kandel. Ctr. Neurobiol. & Behav., Columbia Univ., & HHMI, NY, NY 10032.

The molecular diversity of K$^+$ channels is important for aspects of neuronal modulation and learning-related plasticity. We are attempting to determine how specific cloned K$^+$ channels contribute to the functioning and modulation of identified neurons in *Aplysia*. As a first step we have cloned the *Aplysia* homologues of 3 *Drosophila* K$^+$ channels -- *Shaker*, *Shab* and *Shaw* -- and have identified other clones that may encode new K$^+$ channels. We next compared the A type K$^+$ currents in identified *Aplysia* neurons to the *Aplysia Shaker* currents expressed in *Xenopus* oocytes. In *Aplysia* two different 4AP-sensitive A currents are expressed respectively in LUQ cells (L2 to L6) and in RUQ cells (R3 to R8). The LUQ cell A current ($I_{A,fast}$) inactivates about five times more rapidly than the RUQ cell A current ($I_{A,slow}$). Also, the inactivation rate of $I_{A,fast}$ is voltage-dependent whereas that of $I_{A,slow}$ is not. Finally, forskolin depresses $I_{A,fast}$ with no change in kinetics, but it both depresses $I_{A,slow}$ and changes inactivation to a double exponential. In *Xenopus* oocytes the *Aplysia Shaker* clone AK01a directs the synthesis of a 4AP-sensitive A type K$^+$ channel similar to $I_{A,slow}$. It has a voltage-independent rate of inactivation. In response to forskolin the peak current decreases and inactivation becomes double exponential. However, some properties of AK01a in *Xenopus* oocytes differ: gating is shifted to more positive voltages and the inactivation rate is faster. To determine whether these differences reflect the properties of expression in oocytes, or are functionally important in nerve cells, we are now trying to express AK01a in *Aplysia* neurons by RNA microinjection. Combined with parallel studies on other K$^+$ channel clones, these experiments should provide insight into the roles various cloned K$^+$ channels normally play in the nervous system.

6.4

HYBRID K^+ CHANNELS ARE NOT FORMED BETWEEN FOUR SUBFAMILIES OF K^+ CHANNEL GENES. Manuel Covarrubias*, Aguan Wei, David McKinnon and Lawrence Salkoff. Dept. of Anatomy and Neurobiol., Washington Univ. Sch. of Med., St. Louis, MO 63110.

K^+ channel diversity results from a highly conserved extended gene family. In *Drosophila* four separate genes (*Shaker*, *Shal*, *Shab* and *Shaw*), code for homologous proteins which form distinct K^+ channels when expressed in *Xenopus* oocytes. Each of the four *Drosophila* genes is representative of a separate gene subfamily which is conserved in mammals (Wei, et al., *Science*, in press). Hybrid assembly of different gene products is a possible mechanism for generating K^+ channel functional diversity. This has been demonstrated for genes within the *Shaker* subfamily (Christie, *et al.*, *Neuron*, 4:405, 1990). We investigated whether hybrids were formed between the products of different subfamilies. cRNAs from *Shaker*, *Shal*, *Shab* and *Shaw* were coinjected in pairwise combinations in *Xenopus* oocytes, then assayed by two-electrode voltage-clamp. Currents from coinjected oocytes were analyzed for novel properties. The expressed currents could be separated by kinetics of inactivation, steady-state inactivation and pharmacology into components that closely resemble the properties of currents expressed by singly injected oocytes. In all cases, no novel properties were observed and currents could be modeled as a linear sum of currents from singly injected oocytes. This was in contrast to a novel component of inactivation observed by coexpression of two members of the *Shaker* subfamily, *ShakerH37* and BK2 (a rat homolog). Thus, hybrid assembly between gene products of K^+ channel subfamilies appears not to be a mechanism of diversity. Supported by NIH 1 RO1 NS24785-01, and research grants from the MDA and Monsanto-Searle.

6.5

DECREASED CONDUCTANCE OF THE A CHANNEL BY MUTATION OF A CONSERVED CORE REGION IN SHAKER.
Andrea J. Yool and Thomas L. Schwarz. Department of Molecular & Cellular Physiology, Stanford Univ. Med. Center, Stanford CA 94305-5426.

The high homology of a sequence designated H5 (amino acids 428 to 455 in ShakerB)* in all reported sequences of K channels, but not Ca or Na channels, suggests an important role in K channel-specific function. The H5 region is of intermediate hydrophobicity, with many thr, ser and gly residues. Homomeric channels with single amino acid substitutions in H5 were expressed in *Xenopus* oocytes and screened for function by 2-electrode voltage clamp. More than 50% of the mutants gave no detectable currents. Mutations that generated A currents were primarily conservative, including asp to glu, phe to tyr, thr to ser, and gly to ala. Three of these mutants when analyzed by patch clamp showed 20-50% decreases in single channel amplitude and slope conductance. No shifts in reversal potential or voltage-dependent activation have been detected as yet. Channel kinetics were affected in one case, with an increase in mean open time and slowed inactivation. The low conductance mutations may reflect alteration of the pore structure, or stabilization of a subconductance state. Ongoing studies will test our hypothesis that the H5 region of Shaker is involved in K+ ion permeation. (Supported by NIH R01 GM42376 to TLS, and NIH R01-15963 to L. Jan.)

*Schwarz et al., 1988. *Nature* 331: 137-142.

6.6

A SYNTHETIC PEPTIDE WITH *SHAKER B* SEQUENCE RESTORES INACTIVATION IN MUTANT CHANNELS THAT DO NOT INACTIVATE
T. Hoshi, W. N. Zagotta* and R. W. Aldrich, Dept. of Molecular and Cellular Physiology, Stanford University, Stanford, CA 94305.

We have found that deletions or point mutations near the amino terminal drastically slow inactivation of *ShB* potassium channels expressed in *Xenopus* oocytes. The lack of voltage dependence of inactivation rates, the ability of internal proteolytic agents to modify inactivation, and the effects of mutations in cytoplasmic domains that alter inactivation suggest a mechanism of inactivation similar to the ball and chain model originally proposed by Armstrong and Bezanilla for voltage-gated Na channels. To test this model further, we have examined the ability of the putative "ball" region to cause inactivation when it is not covalently attached to the rest of the channel protein. We applied a synthetic peptide with the amino acid sequence of the first 20 amino acids of *ShB* to the cytoplasmic surface of noninactivating mutant channels in inside-out patches. The peptide restored inactivation in a concentration-dependent manner with concentrations of 50 to 100 μM mimicking wild type inactivation at macroscopic and single-channel levels. Like wild type inactivation, peptide-induced inactivation has very little if any voltage dependence. Recovery from peptide-induced inactivation is slower than wild type inactivation and requires more negative voltages. Trypsin-treated peptide was ineffective as was externally applied peptide. Arginine or lysine (50 μM) did not block the channels. Additional peptides with sequences derived from the first twenty amino acids of noninactivating mutants, including one with only a single amino acid change, did not restore inactivation at comparable concentrations. These results further support the idea that inactivation occurs by a cytoplasmic domain of the protein occluding the pore. They suggest that the structural features of the amino terminal region important for inactivation exist even when the domain is not covalently linked to the rest of the channel protein. Supported by NS 23294 and the American Heart Association.

6.7

FAST AND SLOW INACTIVATION OF *SHAKER* POTASSIUM CHANNELS
W. N. Zagotta*, T. Hoshi and R. W. Aldrich, Dept. of Molecular and Cellular Physiology, Stanford University, Stanford, CA 94305.

Deletions or point mutations near the amino terminal drastically slow inactivation of *ShB* potassium channels expressed in *Xenopus* oocytes. All of these mutant channels, including large deletion mutations, exhibit a slow rate of inactivation, with macroscopic time constants on the order of one to two seconds . This residual slow inactivation occurs at a characteristic rate in the amino terminal mutations and in wild type channels treated with internal trypsin. The similarity of inactivation rates among amino-terminal deletion mutations suggests a slow inactivation process separate from the fast inactivation occurring when the amino terminal is intact. Both macroscopic and single-channel experiments suggest that wild-type *ShB* channels exhibit both fast and slow inactivation processes. We have found that the rate of the slow inactivation is independent of voltage and coupled to activation, similar to fast inactivation. The slow inactivation process can be influenced by altering the carboxyl terminus. Alternative splicing of the *Shaker* gene provides one source for variation. The *ShA* variant is identical to *ShB* except in the carboxyl terminal region where the sequences diverge near the last proposed transmembrane segment. *ShA* channels with mutations in their amino terminus that disrupt fast inactivation exhibit a significantly faster slow inactivation process than *ShB* channels with the same mutations. Large deletion mutations in the putative intracellular carboxyl terminus of *ShB* produced little or no alteration in the rate of slow inactivation. These results suggest two different inactivation processes; one controlled by the amino terminal cytoplasmic domain and one controlled by the carboxyl terminal domain. According to this scheme, wild type *ShB* channels inactivate primarily by means of the amino terminal domain whereas wild type *ShA* channels inactivate by means of both amino and carboxyl terminal domains. Supported by NS 23294 and the American Heart Association.

6.8

Mutations in the Leucine-Heptad Repeat of Shaker Potassium Channels Alter Voltage-Dependent Gating.
K. McCormack*[1], J.W. Lin[2], T. McCormack*[1], M.K. Mathew*[1], M.Tanouye[1] & B.Rudy[2]. [1]Division of Biology, CalTech, Pasadena, CA 91125 ?, [2]Department of Physiology, New York University, New York, NY 10016.

The *Shaker* (*Sh*) gene encodes several protein products which form voltage-dependent K+ channels through multimeric association. In order to investigate the mechanism(s) of gating in *Sh* , we substituted amino acid residues within the leucine-heptad repeat region (which is adjacent to the S4 domain).We expressed these constructs in *Xenopus* oocytes from which we recorded with two electrode voltage clamp . Substitution of valines for the first two leucines in the heptad (V1 and V2) shifts the voltage-dependence up to 100 mV in the depolarizing direction while substitution of a nearby hydrophobic residue in S4 has essentially no effect .The V1 and V2 mutations also change the slopes of the conductance-voltage and steady-state inactivation curves. Similar substitution for the other three leucines (V3, V4 and V5) shifts the voltage-dependence in the opposite direction by as much as 25 mV with little or no change in slope. Our data is consistent with the ideas that the heptad-repeat region is important in determining the position of the "voltage-sensing" charges or that it transduces the conformational changes produced by charge movement into opening and closing of the pore, or both. Because all of the leucines do not appear to play equivalent roles as might be expected for a leucine zipper, or a "bent-zipper" where the leucines interact through intra-subunit contacts, it is difficult to understand this region in terms of structural models. We are further investigating the extent and properties of this region through random mutagenesis and single channel analysis.

6.9

STRUCTURE-FUNCTION ANALYSIS OF *DRK1*: DELETIONS AT N- AND C-TERMINI AND POINT MUTATIONS IN TRANSMEMBRANE SEGMENTS.
A.M.J. VanDongen, G.C. Frech*, J.A. Drewe, R.H. Joho and A.M. Brown. Department of Molecular Physiology and Biophysics, Baylor College of Medicine, One Baylor Plaza, Houston, Texas 77030.

Voltage-dependent ion channels are thought to consist of a repeated structural motif of six transmembrane segments (S1-S6), flanked by cytoplasmic domains. S4 appears to form the voltage sensor, while the functions of the other transmembrane segments or the cytoplasmic domains are not known. We have investigated the functional importance of the N- and C-terminus, for a delayed rectifier K^+ channel from rat brain, *drk1* (Frech *et al.*, Nature 340:642). Deletions in these cytoplasmic domains altered activation, deactivation and inactivation. Deletion mutants ΔN16 (missing 16 amino acids from the N-terminus) and ΔC318 (missing 318 aa's from the C-terminus) had only minor effects on kinetics: activation kinetics and steady state activation curves were shifted 5 mV in the hyperpolarizing direction. For two N-terminal deletion mutants (ΔN101 and ΔN139) activation threshold was shifted 30 mV in depolarizing direction, kinetics were slowed dramatically and inactivation was removed. A double mutant (ΔN139+ΔC318), which had most of both termini removed, restored both activation and inactivation kinetics. Therefore, a core region, which includes the six transmembrane segments, is sufficient for the formation of channels with the kinetics of a delayed rectifier. One of the functional roles of the N- and C-terminus could be to modulate activation and inactivation.

Point mutations were made in transmembrane segments S4 and S5. The S4 segment of *drk1* contains a threonine at position 311. *Shaker* and *rck* channels have a lysine at the corresponding position. A T311K mutation shifted the steady state activation curve 25 mV in the hyperpolarizing direction without changing the slope or kinetics. The S5 segment is flanked by two aspartates (positions 326 and 349), which are strictly conserved amongst all K^+ channels. Four point mutations were made (E326D, E326Q, E349D and E349Q) that conserved either charge or bulkiness. The effect of these mutations is being studied at the single channel level.

6.10

REGULATED GENE EXPRESSION OF A DELAYED RECTIFIER K^+ CHANNEL IN RAT ASTROCYTOMA C6 CELLS.
S-Y Wang, Y. Liu,* N. Castle* and G.K. Wang. Dept. of Biology, SUNY at Albany, Albany, NY 12203 and Dept. of Anesthesia, Harvard Med. Sch., Boston, MA 02115

Freshly dissociated astrocytes are known to express both voltage-dependent Na^+ and delayed rectifier K^+ channels. To check whether rat astocytoma C6 cells also contain similar type of channels we measured the ionic currents in these cells under patch clamp conditions. Only the delayed rectifier K^+ current could be readily detected in most of cells whereas the Na^+ current was absent. The K^+ current was blocked by TEA with IC_{50} = ~0.5 mM and was abolished by 100 nM Toxin I dendrotoxin. Through cDNA cloning and sequencing we found that a delayed rectifier K^+ channel gene with the coding sequence identical to that of RCK1 gene was expressed. The level of K^+ channel gene transcript in C6 cells is comparable to that in rat brain. Addition of 0.5 mM dibutyryl cAMP and 0.5 mM theophylline reduced the level of gene transcript by more than 70% within 24 hrs, which then remained constant up to 4 days. This result demonstrates that cAMP can down regulate the expression of RCK1 gene in vitro. Whether or not a similar phenomenon occurs in vivo remains to be seen.

6.11

MORPHOLOGICAL DIFFERENTIATION AND K CHANNEL EXPRESSION IN A NEUROBLASTOMA CELL LINE. T. Begenisich and M. Weisenhaus*. Dept. of Physiology, Univ. of Rochester Sch. of Med. and Dent., Rochester, NY 14642.

Untreated cells from the neuroblastoma line, N_2AB-1, are relatively homogeneous in their morphology: about 90% of the cells are round; the rest express neurites longer than the cell diameter. After exposure to retinoic acid, about 75% of the cells express extensive neurites and the rest remain round. In a previous study we showed that the round, untreated cells did not express a delayed rectifier K channel but did after morphological differentiation with DMSO/low serum. Our new study was designed to determine if K channel expression was obligatorily linked to morphological differentiation. We used standard, whole cell patch clamp techniques. We found that untreated N_2AB-1 cells, even those with extensive neurites did not express K channels. Cells treated with retinoic acid always expressed K channels including those with a round morphology. These results show that, in this cell line, expression of the delayed rectifier K channel is not obligatorily linked to morphological differentiation.

6.12

M-LIKE POTASSIUM CHANNELS IN PATCHES FROM CULTURED RAT SYMPATHETIC NEURONS C.E.Stansfeld, S.J.Marsh* & D.A. Brown, Dept. Pharmacology, University College, London, WC1E 6BT, U.K.

Cultured rat superior cervical sympathetic neurons possess a prominent time- and voltage-dependent K current, the M-current (Marrion, N.V., Smart, T.G., & Brown D.A. : Neurosci. Lett., 77, 55). Using isolated outside-out membrane patches we have identified single channels which might be responsible for the M-current in these cells. We used asymmetric K (2.5/150 mM) with 0.5 Ca / 5 Mg in the external solution and 1 mM EGTA internally. Sustained activity of single channels with maximum conductance around 9 pS but multiple substates (predominantly 3 - 7pS) were recorded following small depolarizations from -50 mV. maximum P_{open} was about 0.7 near 0 mV. Channels were insensitive to 1 - 5 mM TEA and 100 uM dTC (which blocks Ca-activated K currents) but were blocked by 1 mM Ba (which blocks M-current but not delayed rectifier or A current). Averaged currents from >80 depolarizing steps yielded slow activation/deactivation relaxations, fitted by single exponentials with mean tau_{on} 147 msec (n=7) between -30 and -10 mV. These kinetics match those of macroscopic M-currents in these cells.

Supported by the U.K. Medical Research Council and Wellcome Trust.

SENSORY SYSTEMS—VISUAL CORTEX: MOTION PATHWAYS

7.1

PROJECTION PATTERNS OF MEYNERT CELLS IN PRIMATE VISUAL CORTEX IDENTIFIED BY IMMUNOREACTIVITY FOR NEUROFILAMENTS. B.A. McGuire & R.M. Siegel, The Rockefeller University, New York, NY 10021 & IBM Research Center, Yorktown Heights, NY 10598.

Meynert cells are the largest pyramidal cells in primary visual cortex of primates. They are sparsely arranged in a thin region of upper layer 6. We have previously shown that Meynert cells in *Macaca mulatta* are stained with an antibody specific to neurofilaments (McGuire & Naegele, '88). The present study was undertaken to examine the projection patterns of the Meynert cells. In *Macaca fascicularis* several small injections of retrograde label (red beads or true blue) were made in area 18 near the V1/V2 border. Patches of retrogradely-labeled cells were found in area 17 in both the superficial layers and layer 4B, confirming earlier reports (e.g. Livingstone & Hubel, '87), but no labeled layer 6 cells were found. Thus we found no evidence for a Meynert cell projection to V1.

Area MT is known to receive a prominent input from layers 4B and 6 of striate cortex, and many of the layer 6 cells are thought to be Meynert cells (Tigges et al., '81). Therefore we retrogradely labeled area 17 in squirrel monkeys by making several small injections of red beads in area MT. In two hemispheres, there was strong retrograde labeling in area 17 that consisted of densely packed layer 4B cells in register with a sparser group of layer 6 cells, confirming Tigges et al., '81. Using the neurofilament antibody SMI-32, it was seen that almost all (96%) of the MT-projecting cells in layer 6 were double-labeled with immunofluorescence. These double-labeled cells were large (26 μm avg. diam. $\pm$4.6 μm). Scattered throughout the 3.5 x 1.5 mm region of labeled Meynert cells were other large neurons (24 μm avg. diam. $\pm$4.2 μm) that were neurofilament-positive but not retrogradely labeled. About thirty percent of the double-labeled cells were solely neurofilament labeled. The lack of retrograde label in these neurofilament-positive Meynert cells may be due to the small size of the injection sites or might reflect projections to targets other than area MT. Thus it appears that Meynert cells can be identified by neurofilament staining, and they have a dominant projection to MT and no apparent projection to V2.

McGuire & Naegele (1988) Neurosci. Soc. Abst. 14:601.
Tigges et al. (1981) J. Comp. Neurol. 202: 539-560.
Livingstone & Hubel (1987) J. Neurosci. 7: 3371-3377.

7.2

FUNCTIONAL DEVELOPMENT OF THE CORTICAL VISUAL PATHWAY FOR MOTION ANALYSIS IN RHESUS MONKEYS. C. Distler, L.G. Ungerleider, J. Bachevalier and M. Mishkin. Lab. of Neuropsychology, NIMH, Bethesda, MD 20892.

In primates, the cortical system for motion analysis consists of visual inputs from areas V1, V2, and V3 to MT, outputs of MT to VIP, MST, and FST, and subsequent outputs of MST and FST to the anterior superior temporal sulcus (ASTS). Functional development of this system was studied by means of the 2-deoxyglucose method in 1 adult and 8 infant monkeys (age range 2 days - 6 months) that had received transection of the corpus callosum, anterior commissure, and one optic tract. Local cerebral glucose utilization (LCGU) was measured in visually stimulated awake animals. In animals of all ages, comparison between the intact and visually deafferented hemispheres revealed a progressive decline in hemispheric differences in LCGU along the motion analysis pathway, from a high in V1 to a low in ASTS. Hemispheric differences, however, varied with age. Thus, for each cortical area, the relative difference between the intact and deafferented hemispheres was smallest in the younger animals and reached the differences seen in the adult only at 3 months of age. These data suggest that the motion analysis system is not fully developed at birth, but may mature slightly earlier than the occipitotemporal system for object recognition, which reaches adultlike levels at 4 months of age (Macko et al., Soc. Neurosci. Abstr., 9:375, 1983).

7.3

IMPAIRED SPEED DISCRIMINATION RESTRICTED TO THE HEMIFIELD CONTRALATERAL TO AN OCCIPITO-PARIETAL SURGICAL RESECTION. G.T.Plant*, K.D.Laxer*, N.M.Barbaro, K. Nakayama, Smith-Kettlewell Eye Research Institute, 2232 Webster, SF, CA 94115 and Northern California Comprehensive Epilepsy Center, U.C.S.F., CA 94143-0138.

We have studied a patient with partial epilepsy due to a left occipito-parietal cortical developmental anomaly. A variety of types of visual aura were experienced including a sensation of motion in the right (contralateral) visual field. Drifting sine-wave gratings (spatial frequency 0.5 c/deg) were used to measure visual function before and after resection of occipito-parietal cortex as treatment for intractable epilepsy. The stimuli were located at an eccentricity of 10° and performance compared in the left and right upper quadrants of the visual field. A 2AFC paradigm was employed to measure contrast thresholds for three tasks: grating detection; discrimination of direction of drift; and discrimination between two gratings drifting at 8Hz and at 10Hz respectively (speed discrimination). Pre-operatively there was no difference in performance at these tasks between the two hemifields. Post-operatively there was a ten-fold rise in contrast threshold for speed discrimination in the right hemifield only, the remaining parameters were unaffected by the lesion. (G.T.P. is a Travelling Fellow of the Medical Research Council of the United Kingdom).

7.4

PERCEPTUAL EFFECTS OF CORTICAL MICROSTIMULATION ARE SPATIALLY AND TEMPORALLY RESTRICTED. C. Daniel Salzman*, Chieko M. Murasugi* and William T. Newsome, Dept. of Neurobiology, Stanford University School of Medicine.

We have recently shown that microstimulation of a cluster of MT neurons with similar preferred directions can influence a monkey's perceptual judgements of motion direction (ARVO Abst., 1990). In those experiments, rhesus monkeys discriminated the direction of motion in a dynamic random dot display placed directly over the receptive field of the stimulated neurons. Microstimulation (10 μA, 200 hz, biphasic) occurred on half of the trials, coinciding in time with the onset and offset of the visual motion display. In 29 of 62 experiments, microstimulation significantly biased the monkeys' perceptual decisions toward the preferred direction of the stimulated neurons. We now report the effects of microstimulation when it is dissociated from the visual display in space or in time.

Temporal offset experiments were identical to the previous experiments in all ways except that microstimulation occurred during the intertrial interval rather than during the visual display interval. In all 20 of these experiments, microstimulation had little or no effect on the monkey's psychophysical performance. In the spatial offset experiments, we applied microstimulation during the visual display interval, but at a site in MT remote from the topographic location of the display aperture. We also included control trials in which the visual stimulus overlapped the receptive field of the stimulated neurons. In 15 experiments, microstimulation significantly biased the monkey's choices in the overlapped condition but had little or no effect in the dissociated condition. These results indicate that the effect of microstimulation on perceptual judgements of motion direction is localized spatially within MT and temporally in relation to the occurence of the visual display.

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7.5

THE INTEGRATION OF DIRECTION INFORMATION IN AREA MT OF MACAQUE. Robert J. Snowden, Stefan Treue, Richard A. Andersen, Department of Brain and Cognitive Sciences, MIT, Cambridge, MA, 02139.

We have shown the response of MT neurons in awake behaving monkeys to a random dot pattern moving in its preferred direction is reduced by the addition of dots moving in the opposite (antipreferred) direction (Snowden et al. 1989). We have extended this study to examine the suppressive effect for a range of directions and for motion discontinuities.

The direction tuning of individual MT cells was assessed by drifting dot patterns in 8 directions. This was repeated save that a constant number of dots were always superimposed drifting in the preferred direction. It was found that directions other than the antipreferred also reduced the response of the cell below that to the preferred alone. The size of this suppressive effect increased with angular difference in directions up to around 90 deg. Directions between 90 - 180 deg generally give similar suppressive effects. When compared to the direction tuning alone it is apparent that a stimulus which by itself causes strong excitation, can cause a suppression of the response to the preferred stimulus. Thus a given stimulus can increase or decrease a cell's firing rate depending upon the context in which it is presented.

We also repeated the direction tuning in the presence of dots moving in the antipreferred direction. Under these conditions we found that the effect of the antipreferred dots was to reduce the response by a constant factor, rather than a constant number of spikes. The suppressive effect can therefore be thought of as divisive rather than subtractive inhibition.

A final set of experiments examined whether the suppressive effect was exclusive to 'transparent' stimuli. The dots were divided into stripes moving in opposite directions. It was found that the suppressive effects remain (though slightly reduced) even if the stimulus was composed of only two sections. Thus the effects described are not exclusive to transparent motions but occur for two spatially segregated motions occur within the cell's receptive field. These results therefore have important implications for processing edges formed by motion discontinuities.

Supported by NIH grant EYO7492

7.6

SURROUND PROPERTIES OF MT NEURONS SHOW LAMINAR ORGANIZATION. L. Lagae*, S. Raiguel, D-K. Xiao* and G.A. Orban, Laboratorium voor Neuro- en Psychofysiologie, K.U.Leuven, B-3000 Leuven, Belgium.

We examined the spatial extent and organization of the classical receptive field and its surround in area MT of macaque monkeys. To obtain an area-response curve, we used a series of moving textured patterns of increasing radii centered upon the receptive field. Stimuli were computer-generated white dots on a black background presented at a refresh rate of 100 Hz. For most cells, the response increased linearly for increasing stimulus area up to the border of the receptive field. In 40% of the cells, located predominantly in layer 4, no surround could be detected. In the remaining 60% of the cells, the response gradually decreased to about 35% of maximum as the stimuli exceeded the receptive field, indicating an inhibitory surround. The maximum extent of this surround was taken as the point at which this decrease began to level off. For cells in layer 4, the average area of the surround was only half that of cells in other layers.

Our study shows that the excitatory receptive field of an MT cell and its surround are spatially separated. The laminar differences of surround properties support a hierarchical scheme of receptive field organization in MT. Moreover, these differences may be related to our previous finding that only surrounds of cells outside layer 4 modulate direction selectivity of a moving bar (Lagae *et al.*, *Brain Res.*, 496:361-367, 1989).

7.7

A MEAN FIELD MODEL OF OPTIC FLOW ESTIMATION BY MT NEURONS

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We consider the problem of obtaining reliable estimates of the optic flow field and its spatial and temporal variation, and develop a dynamical model of neural elements and circuits in the primate motion pathway. Neurons in the middle temporal visual area (MT) are direction and speed selective and many also have direction and speed-dependent antagonism between the RF center and surround. Consequently, such neurons should respond well to spatially restricted translation or to appropriately curving, accelerating, or discontinuous flows. In our model the circuitry in MT is arranged to provide mean field approximations of the optical flow field — maximal neighbourhoods over which the local field is well-approximated as uniform, parallel translation. The basic intuition behind the scheme is this: for a neuron in which the RF center and surround share speed and direction tuning, successful suppression of the center by the surround means that the flow field can be approximated as uniform over a larger area than the RF center of the neuron in question. On the other hand if the neuron is firing strongly, the flow field is approximately constant in the center and either spatially limited or with large spatial variation (rapidly changing speed or direction) in the surround. Therefore the heart of the model is an ensemble of RFs of different size with inhibitory connections running from large to small RF cells. This is repeated at each position and for each of a set of direction and speed classes. Actively estimating the flow field variation with area around each point determines the permissible area for interpolation. The principal neural interactions in the network occur locally within each direction and speed class. One consequence is that unlike most computational schemes proposed to date multi-valued flow fields are not excluded, hence permitting representation of multiple, transparent surfaces. Simulations of the model's behaviour with steady and time-varying optic flow fields will be presented.

7.8

A COARSE-TO-FINE MULTIRESOLUTION NETWORK MODEL OF MOTION COMPUTATION IN PRIMATE AREA MT. H. T. Wang*, B. P. Mathur* and C. Koch, Rockwell International Science Center, Thousand Oaks, CA 91360 and CNS Program, 216-76 Caltech, Pasadena, CA 91125.

All current models of motion processing in the mammalian visual system operate at a single spatial scale. However, receptive field size of direction selective cells in V1 and MT at a constant eccentricity vary by at least one order of magnitude. We developed an adaptive coarse-to-fine motion algorithm in the context of computer vision (Battiti, Koch & Amaldi, 1990), which derives a significantly better estimate of the optical flow fields than single scale algorithms. We here adapt this multiscale strategy to our previous model of the primate's motion system (Wang, Mathur & Koch, 1989).

Our model consists of two stages: 1) the local velocity estimates (component selectivity) are computed at multiple spatial scales in the input layers of V1; 2) these estimates of local motion are then combined, using smoothness, to yield the 2-D motion fields (pattern selectivity). We compute the local error, arising primarily due to spatial and temporal aliasing, associated with the motion field at different spatial scales. If this error is below a certain threshold at any spatial scale, the algorithm has converged to the correct solution. Our model neurons show behavior reminiscent of the non-classical type I surround cells in MT (Allman, Miezin & McGuinness, 1985) and provides a novel explanation for the spatial frequency effect in motion capture (Ramachandran & Anstis, 1985). Our multiscale algorithm converges significantly faster than our previous single scale model.

7.9

ORGANIZATION OF OPTIC FLOW SENSITIVE RECEPTIVE FIELDS IN CORTICAL AREA MST. C. J. Duffy and R. H. Wurtz, Lab. of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

We have classified MSTd neurons as: single component, responding to planar, circular or radial stimuli; double component, responding to planar & circular or planar & radial stimuli; triple component, responding to planar, circular & radial stimuli. We determined whether the circular and radial responses could be understood from the planar responses of sub-fields within the receptive field. In 50 neurons we presented segment stimuli (33° X 33°) consisting of one-ninth of a large-field (100° X 100°) stimulus. Segment studies showed that circular and radial responses could be the product of partial activation of planar directional sub-fields. These findings were consistent with the hypothesis that the convergence of planar direction selective inputs creates circular and radial selectivity.

In 160 neurons we presented smaller versions of the full pattern of large-field stimuli. Reducing the size of such patterns and changing the position of these small-field stimuli did not eliminate circular or radial direction selectivity. In addition, small-field stimuli at different positions in the receptive field did not reveal areas of differing planar directionality. These findings suggest that the convergence of planar direction selective inputs does not fully account for circular and radial selectivity.

Triple component neurons yielded responses which were most consistent with the view that circular and radial selectivity are derived from the convergence of planar direction selectivities. Single component neurons yielded responses which were more consistent with the view that circular and radial selectivity represent neuronal extraction of a fundamental geometric feature of optic flow fields. These differences suggest that there might be a hierarchy of planar resolvability extending from triple component to single component neurons.

7.10

STIMULUS SELECTIVITY OF NEURONS IN MACAQUE MST. M. Graziano, R. Andersen, and R. Snowden, Dept. of Brain and Cognitive Sciences, MIT, Cambridge, MA, 02139.

We recorded from single neurons in the medial superior temporal visual area (MST) of the macaque, where previous investigators reported cells selective for stimuli such as a rotating field of dots, or an "expansion" made when a dot field is magnified. We used a novel stimulus which avoided artifacts, such as increase in dot size, decrease in dot density, and decrease in luminance, which appear in expansion, or local shape cues which appear in both expansion and rotation. In our stimuli each dot moved with a constant velocity for 200ms, before being relocated and given a new velocity appropriate to its new location. The effect is an overall motion, such as rotation, or expansion, or contraction, but without confounding artifacts.

We confirmed that MST cells are often selective for expansion or contraction, clockwise or counter-clockwise rotation, or pure translational motion; and that some cells respond to more than one class of motion. Some cells which were selective only for rotation responded regardless of the direction of rotation.

To test if expansion/contraction and rotation selectivities are special, or are just a few of many motion pattern selectivities in MST, we devised a method of comparing expansion, contraction, rotations, and spiraling stimuli, on one scale. Each stimulus can be represented as a velocity vector in a coordinate frame with radial speed on one axis and angular speed on the other. We plotted directional tuning curves in this coordinate system. In preliminary tests, most cells preferred expansion, contraction, or rotation, over any of the spiraling stimuli.

7.11

TRANSLATIONAL INVARIANCE AND ATTENTIONAL MODULATION OF MST CELLS. R. Andersen, M. Graziano, and R. Snowden. Dept. of Brain and Cognitive Sciences, MIT., Cambridge, MA, 02139.

Area MST neurons are often selective for expansion, contraction, or for clockwise or counter-clockwise rotation. We tested if these selectivities persist regardless of the location of the stimulus in the receptive field. MST receptive fields are usually at least 40 degrees in diameter. We tested with 10-degree diameter stimuli at five overlapping locations in the receptive field, arranged in a clover-leaf pattern. In every case cells retained the same stimulus selectivity at all five retinal locations, even though, in the area of overlap, the local direction of motion was reversed depending on stimulus location. Thus MST responses to these motion patterns are translation invariant. The cells also showed a degree of size invariance, responding well to 10 and 20 degree diameter stimuli, although they tended to respond better to the larger stimulus.

Attentional modulation was examined using a reaction time task, in which the animal was required to release a lever in response either to a change in the motion of the stimulus, or to a slight dimming of the fixation point. The two types of trials were randomly interleaved and the color of the fixation point indicated which detection was required. Many MST cells altered their response when the animal attended to the motion stimulus, showing a facilitated response to the preferred direction of motion, and often a reduction to the non-preferred direction. This result suggests that in some cases the tuning of area MST neurons is sharpened by attention.

7.12

Vestibular input to visual-tracking neurons in area FST of awake rhesus monkeys. P. Thier* and R.G. Erickson, Dept. Neurology, Univ. of Tübingen, 74 Tübingen, W. Germ. (Spon: European Neuroscience Association.)

During visual tracking with the head stationary, the activity of visual tracking (VT) cells within areas MST and FST of the superior temporal sulcus (STS) reflects integration of directionally selective visual responses and directionally selective non-visual responses. While it has been proposed that the non-visual signal may represent an eye movement efference copy, we have found that the pursuit-related discharge of such neurons is the same during "head-tracking" (with the eye stationary in the orbit) and "eye-tracking" (with the head stationary). This observation indicates that non-visual input to VT cells includes both eye- and head-movement components and may be equated to a gaze signal or to motion of the target in extrapersonal space rather than to eye movement alone. A preliminary sample of 20 VT neurons observed during alternating sets of both "eye-" and "head-tracking" have been analysed. The majority had the same preferred directions for visual tracking and visual pattern movement and all were recorded from a small region (referred to as FST or MST1 by different labs) on the lower anterior bank of the STS in 2 adult rhesus monkeys. Eye-tracking was studied using step-ramp and circular motion of a target rear-projected onto a tangent screen. Head-tracking involved suppression of the horizontal or vertical vestibulo-ocular reflex (VOR) by fixation of an LED rotated in synchrony with the animal in an otherwise dark room. The most striking result was that the activity of all 20 neurons was driven to a similar extent by both head- and eye-tracking, and that the same preferred direction of gaze movement was preserved in each case. The dual nature of the non-visual tracking response was verified by the lack of visual responsiveness to brief periods of target blinks or retinal stabilization during "eye-tracking", and by the consistent observation of vestibular modulation during rotation in complete darkness (VOR alone). The residual modulation by vestibular input amounted, on average, to 50% of the response observed during head-tracking. In summary, our results show that a representation of gaze or target motion in space is available in a small, well-defined visual area within the STS. *Support: DFG SFB 307.*

PEPTIDES—RECEPTORS

8.1

MOLECULAR CLONING AND FUNCTIONAL CHARACTERIZATION OF A cDNA ENCODING THE SWISS 3T3 BOMBESIN/GRP RECEPTOR. E. Giladi, T. Segerson*, P. Brehm, R.H. Goodman and E.R. Spindel. Division of Neuroscience, Oregon Regional Primate Res. Center, Beaverton, OR 97006 and Vollum Institute for Advanced Biomedical Research, Portland, OR 97201.

Bombesin and its mammalian homolog, gastrin-releasing peptide (GRP) function as growth factors, neurotransmitters and paracrine regulators of gastrointestinal function. To better characterize the physiologic roles of bombesin-like peptides, our laboratories have cloned the receptor for bombesin that is expressed in murine Swiss 3T3 fibroblasts. A cDNA library was prepared from Swiss 3T3 RNA in the vector λZapII. RNA transcribed from pools of 50,000 cDNA clones was expressed in *Xenopus* oocytes and monitored by two-electrode voltage clamp and by monitoring changes in intracellular calcium with aequorin. By this process, an initial positive pool of 50,000 clones was narrowed to a single clone by successive rounds of sib selection. Oocytes injected with RNA transcribed from this clone showed a log-linear response to bombesin from 10^{-10} to 10^{-6} M bombesin. The ED50 was 3 X 10^{-9} M and the response to 3 X 10^{-9} M bombesin could be completely blocked by 3 x 10^{-8} M of the specific bombesin antagonist [D-Phe^6]bombesin(6-13)propylamide (*Biochem.* **29**:616-622, 1990). DNA sequence analysis revealed a 1.8 kb cDNA encoding a 384 amino acid protein. The protein contained 7 hydrophobic domains and 5 N-linked glycosylation sites. Data base analysis showed highest homology to the tachykinin receptors with next highest homology to the β2-adrenergic receptor. RNA blot analysis showed a major hybridizing band 9.5 kb in size. Consistent with the known distribution of the bombesin receptor, this band was present in RNA from Swiss 3T3 cells but absent from NIH 3T3 cells.

8.2

FUNCTIONAL EXPRESSION OF NATIVE AND MUTATED MURINE BOMBESIN/GASTRIN-RELEASING PEPTIDE RECEPTOR cDNA IN MAMMALIAN AND AMPHIBIAN CELLS. TP Segerson*, S Montgomery*, PJS Stork*, K. Walton, E Giladi, RH Goodman*, ER Spindel. *Vollum Institute for Advanced Biomedical Research, Portland OR 97201 & Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.*

Bombesin and its mammalian homologue gastrin-releasing peptide (GRP) are mitogenic in fibroblast and some neuroendocrine cell lines. Our laboratories have cloned a cDNA for the bombesin/GRP receptor (B/GRP-R) by expression of a Swiss 3T3 cell library in *Xenopus* oocytes. To study the growth effects of bombesin peptides, we have introduced the B/GRP-R into heterologous mammalian cells. B/GRP-R cDNA cloned into the expression vector pCDM8 was transfected into COS 7 cells by calcium phosphate precipitation. Transfected cells were incubated with 0.4 μCi ^{125}I-bombesin (90 pM) for 2h and were washed and fixed with 1% glutaraldehyde. Four day exposure autoradiographs of dried cell monolayers showed increasing numbers of strongly labeled cells with increasing amounts of B/GRP-R plasmid transfected, blocked by 5 nM cold bombesin. An eight amino acid epitope for a monoclonal antibody (FLAG™, Immunex) was introduced into the N-terminal portion of the B/GRP-R in pCDM8. Transcripts of this mutant receptor injected into *Xenopus* oocytes produced bombesin-dependent inward currents of 1-2 μA (V_{hold}=-60 mV). Introduction of the mutated B/GRP-R into COS 7 cells resulted in ^{125}I-bombesin labeling equivalent to the native murine receptor. Antibody to this epitope precipitated a protein of appropriate size from *in vitro* translated mutant receptor transcript. In conclusion, we have 1) expressed a cloned B/GRP-R in heterologous cells and demonstrated binding of labeled bombesin to these cells; and 2) introduced an epitope into this receptor without loss of activity in oocytes or binding in mammalian cells. We are isolating stably-transfected fibroblast cell lines with the native and mutated receptor to study the role of receptor structure and receptor-protein interactions in bombesin-dependent mitogenesis.

8.3

CLONING PEPTIDE RECEPTORS BY EXPRESSION IN XENOPUS OOCYTES. H. Meiri, G. Omri, R.D. Blitzer, E.M. Landau, C. Cyr*, E. Scheindling*, J. Schlessinger* and R.M. Kris. Dept. Psych., Mt. Sinai Med. Ctr. and Bronx VA Med. Ctr., Bronx NY 10468, Technion Faculty of Medicine, Haifa, Israel, and Dept. Pharmacol., NYU Med Sch., New York, NY 10016.

Peptide receptors that couple to the phosphoinositide pathway in oocytes stimulate a Ca^{2+} dependent Cl^- current. We have used this current to clone a number of peptide receptors. A cDNA library was constructed from rat whole brain RNA in lambdaZAP. Oocytes injected with RNA transcribed from this library displayed Cl^- currents in the presence of NE, 5HT, CCK, neurotensin, substance K, bombesin and bradykinin. Fractionation of this library by sib selection produced small DNA pools which contained the clones for the receptors for bradykinin, bombesin and other peptides. Isolation and sequencing of the receptor clones for bombesin, bradykinin, and other neuropeptide receptors should be possible using this approach. Supported by the Irma T. Herschl Found. and the VA Merit Program.

8.4

CLONING, EXPRESSION AND BIOCHEMICAL ANALYSIS OF HUMAN SUBSTANCE - K RECEPTOR IN NIH 3T3 CELLS. R.M. Kris and C. Cyr. Department of Pharmacology, 550 First Avenue, N.Y.U. Medical Center, New York, NY 10016.

The human substance-K receptor was cloned from a human small intestine cDNA library. The receptor consists of 398 amino acids, including seven putative transmembrane regions. The human substance-K receptor was expressed in mouse 3T3 fibroblasts and Scatchard analysis indicates 97,000 receptors per cell with a single affinity of 12 nM Kd. Cross-linking experiments utilizing ^{125}I-substance-K demonstrate an apparent molecular weight of 45 kilodaltons, consistent with little or no N-linked-glycosylation. Early signal transduction events were measured following the binding of substance-K to its receptor. There is a rapid increase in the production of total inositol phosphates and the release of Ca^{++} from internal stores. Growth of the cells transfected with the human substance-K receptor is stimulated by the addition of substance-K to the medium. Therefore, the human substance-K receptor can function as a growth factor receptor when expressed in mouse 3T3 fibroblasts. Stimulation of alternative pathways stimulated by substance-K will also be discussed.

8.5

SOLUBILIZATION OF RAT BRAIN BOMBESIN/GRP RECEPTORS IN A HIGH AFFINITY LIGAND BINDING CONFORMATION. T.W. Moody, J. Staley,* L. Naldini,* D. Cirillo,* P. Comoglio* and R.M. Kris,* Dept. Biochem. & Mol. Biol., George Washington Univ. Med. Sch. Washington, D.C. 20037, and Dept. Biomed. Sciences and Oncology, Univ. Torino Med. Sch., Torino, Italy, and Dept. Pharm. NYU Sch. Med., New York, N.Y. 10016.

Bombesin/gastrin releasing peptide (BN/GRP) may function as a modulatory agent in the CNS. BN/GRP-like peptides and receptors are localized to certain regions of the rat brain such as the paraventricular nucleus of the hypothalamus where BN is a potent satiety and grooming agent (Chronwall et al., Brain Res. 338:97 (1985)) and Zarbin et al., J. Neurosci. 5:429 (1985)). While the gene for GRP has been cloned, little is known about the molecular properties of the BN/GRP receptor. Here the BN/GRP was solubilized from rat brain membranes using CHAPS/cholesterol hemisuccinate (CHS) and its binding properties determined. The soluble receptor bound (^{125}I-Tyr^4)BN with high affinity (Kd = 2 nM) to a single class of sites using a filtration assay. GRP and C-terminal fragments such as GRP$^{14-27}$ and GRP$^{18-27}$ inhibited specific (^{125}I-Tyr^4)BN binding activity with IC_{50} values of 5, 1 and 2 nM whereas N-terminal fragments such as GRP$^{1-16}$ were inactive. Also, BN/GRP receptor antagonists such as (D-Phe^6)BN$^{6-13}$propylamide and (Psi^{13,14}, Leu^{14})BN had IC_{50} values of 5 and 50 nM. Using gel filtration techniques the main peak of ^{125}I-(Tyr^4)BN binding activity had a Mr of 200 Kdaltons using Sephacryl S-300. The purification of the solubilized receptor using affinity chromatography techniques will be discussed. Because the membrane bound and extracted receptor have almost identicle binding properties, the BN/GRP receptor may be solubilized in its native conformation. Supported by NSF grant BNS 88-15133.

8.6

THE RAT SUBSTANCE P RECEPTOR: MOLECULAR CHARACTERIZATION OF ITS GENE AND EXPRESSION OF ITS mRNA. A.D. Hershey, L. Polenzani*, R. Miledi and J.E. Krause, Washington University School of Medicine, St. Louis, MO 63110 and University of California, Irvine, CA 92717.

We have recently reported the cloning and expression of a cDNA encoding a rat Substance P receptor (SPR) and a preliminary analysis of mRNA distribution (Science **247**, 958-962, 1990). Ligand binding analysis of this expressed SPR in COS-7 cells with a variety of tachykinins demonstrates that SP is the preferred natural agonist for this receptor. In this study, we have examined functional SP-mediated responses, examined patterns of mRNA expression and determined the structural organization of the SPR gene. When mRNA from this cloned SPR is expressed in *Xenopus* oocytes, SP application to the oocyte activates an oscillatory inward chloride current which rapidly desensitizes. Examination of mRNA levels encoding the SPR using sensitive nuclease protection assay procedures has revealed that SPR is expressed throughout the CNS and in many peripheral tissues, representing 0.0001 to 0.0016% of total RNA. Using coding region probes, the highest abundance detected is in the urinary bladder, striatum, spinal cord, submandibular gland and sublingual gland, with the majority of the signal represented by a single protected species. These localization results agree with *in vitro* autoradiographic studies for the distribution of SP binding sites. These results suggest that this cloned SPR mediates the primary actions of SP on its target neurons and tissues throughout the rat. The SPR gene was isolated and extensively characterized, and the transcriptional start site has been defined by nuclease protections assays. The gene consists of five exons containing 958, 195, 151, 197, and 2068 bp and extends over 45 kb. The locations of the exon splice sites are adjacent to the membrane spanning domains of the SPR. Overall, these studies define the molecular nature of a rat SPR and its gene. This work provides a basis for the further exploration of the structure and function of the SPR, and of the mechanism and regulation of SPR gene expression.

8.7

NK_2 RECEPTOR MEDIATED INTRACELLULAR CALCIUM MOBILIZATION IN TRANSFECTED MURINE FIBROBLASTS A.K. Henderson, T.L. Smith, J. Lai, H.I. Yamamura, and W.R. Roeske. Department of Pharmacology, The University of Arizona Health Sciences Center, Tucson, AZ 85724

We previously demonstrated in vitro expression of a bovine stomach cDNA encoding for an NK_2 receptor into the murine B82 fibroblast cell line. The transfected cells exhibited specific [^{125}I]NKA binding. Agonist induced phosphatidylinositol hydrolysis displayed a rank order of potency of NKA>SP>>senktide. NKA also mediated an increase in intracellular Ca^{+2} concentration ($[Ca^{+2}]_i$) in a dose dependent manner. An EC_{50} value of 40 nM was fluorometerically measured with fluo-3. Basal $[Ca^{+2}]_i$ was 57 ± 18 nM and maximal accumulation of $[Ca^{+2}]_i$ at 1 μM NKA was 45 ± 18 nM over basal levels. In vitro expression of NK_2 receptors provides a correlation of the structurally defined receptor with its ligand binding properties and its pharmacological and functional mechanisms.

8.8

HETEROLOGOUS EXPRESSION OF NEUROPEPTIDE Y (NPY) Y1-RECEPTORS IN *XENOPUS* OOCYTES. C. Wahlestedt, S. Nakanishi* and D.J. Reis, Div. of Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021, USA, and Inst. for Immunol., Kyoto Univ., Kyoto, JAPAN

The Y1-receptor is the major vascular NPY receptor and mediates many central NPY actions. Human neuroblastoma cells of the SK-N-MC line preferentially express the Y1-receptors (Springston, et al., *Soc. Neurosci. Abstr.*, 1990). We sought to determine whether this receptor can be expressed in *Xenopus* oocytes and to identify coupled signal transduction mechanisms. mRNA was prepared from SK-N-MC cells and microinjected into oocytes which were subsequently exposed to NPY. First, in voltage-clamped oocytes, NPY alone elicited negligible changes in chloride currents. However, oocytes in which SK-N-MC mRNA and *in vitro* transcribed mRNA for the cloned (phosphatidyl inositide (PI)-coupled) substance K (SK) receptor were coinjected, NPY potentiated threshold SK-evoked currents; native Y1-receptors also have the capacity to potentiate PI-coupled responses (Wahlestedt et al., *Am. J. Physiol.* 258:R736, 1990). Second, NPY elevated intracellular Ca^{++} concentrations in fluo-3 (a Ca^{++}-sensitive dye) injected cells. Third, NPY, applied to oocytes in which SK-N-MC mRNA and *in vitro* transcribed β_2-adrenoceptor mRNA were coinjected, attenuated by ≤60% the isoproterenol-elicited elevations in cAMP concentrations. We conclude: (a) NPY Y1-receptor mRNA from SK-N-MC cells can be expressed in *Xenopus* oocytes; (b) the expressed receptors initiate increased intracellular Ca^{++} concentration, inhibition of cAMP accumulation, and possibly facilitation of PI-associated chloride conductances. Such coupling may therefore represent signal transduction mechanisms characteristic of both the native and the heterologously expressed Y1-receptor and may make its expression cloning possible.

8.9

ENDOTHELIN RECEPTORS IN RAT BRAIN TISSUE. H.F. Cheng, S.M. Lee*, J.Y. Wang* and K.J. Chang. Institute of Biomedical Sciences, Academia Sinica and Department of Physiology and Biophysics, National Defence Medical Center, Taipei, Taiwan, R.O.C.

Endothelins (ETs), potent vasoconstrictors originally discovered from cultured porcine aortic endothelial cells, are neuropeptides in the brain. This study was to identify and characterize the receptors for ETs in rat brain by using radioligand binding technique. The iodinated ET bound specifically to receptors in rat brain membrane in a protein-, time- and dose-dependent manner. Scatchard analysis indicated a single class of high affinity binding sites on rat brain membrane. The dissociation rate of iodinated ET from ligand-receptor complex was very slow. Mg^{+2} increased iodinated ET binding at the optimal concentration of 5mM. On the other hand, GTPrS inhibited the specific binding of iodinated ET at mM range, suggesting endothelin receptors in rat brain might be coupled to a G-protein. The order of potency for ETs and sarafotoxin S6B to displace the specific binding of iodinated ET from brain membrane is ET-3 > ET-1 ≥ sarafotoxin S6B > ET-2. All these result suggest the presence of endothelin receptors on rat brain membrane which have the highest affinity for ET-3.

8.10

ENDOTHELIN RECEPTORS ON FETAL RAT DIENCEPHALIC ASTROGLIA. E.R. Levin, H.J.L. Frank*, A. Pedram*, Dept. of Med., Univ. of Calif., Irvine and Long Beach VAMC, Irvine, CA 92717.

Binding activity for endothelin has been found in the brain. To determine the cell type expressing receptors, binding characteristics and potential functions of these peptides in neural cells, we bound I^{125}-Endothelin-3 (ET-3) to glial (>90% GFAP positive) and neuron-predominant cultures (85% NSE positive) from fetal rat diencephalon. Affinity crosslinking of ^{125}I-ET-3 and in-situ autoradiography identified dense binding on astroglia with scattered rim binding on a small subpopulation of neurons. In binding studies, both type 1 and type 2 astroglia specifically bound 8-13% of ET-3 added, while neuron-predominant cultures showed 2-3% binding. Scatchard analysis of saturation binding in glia identified a single class of receptors with a Kd of 0.4±0.05nM (SEM) and a receptor density of 45 fmol/mg protein (54,000 receptors/cell). Affinity crosslinking of labeled ET-3 and SDS-PAGE identified a single predominant band of approximate Mr 52,000. ET-3 at 10^{-8}M significantly reduced 10^{-8}M atrial natriuretic peptide generation of cGMP, while having no effect on isoproterenol-induced cAMP or basal cAMP or cGMP production. ET-3 caused a significant, dose-related increase in H^3-thymidine incorporation in the presence of 1% serum. These studies establish that high affinity, low mol. wt. endothelin receptors exist mainly on astroglia in rat diencephalon and that this peptide might act as a proliferative factor for these cells.

8.11

LOCALIZATION OF ^{32}P-LABELED RELAXIN BINDING SITES IN RAT BRAIN. P.L. Osheroff* and H.S. Phillips#. Dept. of Protein Chemistry* and Developmental Biology#, Genentech, Inc., South San Francisco, CA. 94080.

Relaxin belongs to the insulin family of polypeptide hormones and is best known for its biological activities in the mammalian reproductive system, notably inhibition of uterine contraction and softening of the cervical canal. The biological actions of relaxin are thought to be mediated through specific receptors located in the target tissues. Using a biologically active ^{32}P-labeled human relaxin we have previously localized by *in vitro* autoradiography specific relaxin binding sites in rat uterine and cervical tissue sections. Using the same approach we describe here the localization of relaxin binding sites in the rat brain. Displaceable relaxin binding sites are found to be distributed in discrete anatomical pattern including the neocortex, the hippocampus, the hypothalamus, the thalamus, the amygdala, and the olfactory bulb. Of special interest is the specific binding of relaxin to two of the circumventricular organs (the subfornical organ and the organum vasculosum of the lamina terminalis) and the neurosecretory magnocellular hypothalamic nuclei (the paraventricular nuclei and the supraoptic nuclei). In view of recent physiological studies showing the effects of centrally administered relaxin in the inhibition of reflex milk ejection and haemotensive response, and that the subfornical organ might mediate the relaxin-induced actions, the present study provides biochemical evidence for the possible site(s) of action of relaxin in the CNS.

8.12

SPECIFIC BINDING SITES FOR PITUITARY ADENYLATE CYCLASE ACTIVATING POLYPEPTIDE (PACAP) IN RAT ASTROCYTES : INTERACTION WITH VASOACTVE INTESTINAL PEPTIDE (VIP). I.Tatsuno*, P.E. Gottschall*, and A. Arimura U.S.-Japan Biomed. Res. Labs., Tulane Univ. Hebert Ctr., Belle Chasse, LA 70037; Depts. of Medicine, Anatomy & Physiology, Tulane Univ. Sch. of Med., New Orleans, LA 70112.

PACAP stimulates adenylate cyclase activity not only in rat pituitary cell cultures but also in neuron and astrocyte cultures. In particular, the considerable stimulation in astrocytes suggested that these cells might be one of the major target cells for PACAP. The existence of specific high (Kd=10^{-9} M) and low (Kd=10^{-6} M) affinity binding sites for PACAP was demonstrated in rat astrocyte membrane preparations using radioreceptor assay (RRA) with [^{125}I]PACAP27. PACAP shows a large degree of homology with VIP, but it was approximately 1000 times more potent than VIP in stimulating adenylate cyclase and displacing [^{125}I]PACAP27 binding. At the same concentrations of radiolabeled peptides, the specific binding of [^{125}I]PACAP27 was approximately 50 times greater than that of [^{125}I]VIP. In RRA using [^{125}I]PACAP27, 10^{-6} M VIP reduced the binding maximal capacity (Bmax) of high affinity binding site for PACAP27 by 50%. However, the Bmax of low affinity binding site and the dissociation constant (Kd) for both high and low affinity binding sites were not altered. This indicates that only high concentration of VIP (10^{-7} M or larger) shares the high affinity binding site for PACAP which may be linked with adenylate cyclase. These data suggest that PACAP might have an important regulatory role in astrocytes and probably in CNS.

ACETYLCHOLINE—RECEPTORS

9.1

Identification of Critical Amino Acids Specifying G Protein Recognition using Muscarinic Acetylcholine Receptor Chimeras. James Lechleiter, David Ennulat *#, Kevin Duerson *, Ned David *#, David Clapham * and Ernest Peralta *#. Dept. Pharmacol., Mayo Clinic, Rochester, MN 55905, *#Dept. Biochem. and Mol. Biol., Harvard Univ., Cambridge MA 02138.

Muscarinic acetylcholine receptors (mAChRs) are a family of at least five distinct subtypes, designated m1 - m5, which fall into two functional classes: m1, m3, and m5 receptors strongly stimulate phosphoinositide (PI) hydrolysis through G proteins, while m2 and m4 receptor subtypes only weakly stimulate PI turnover and preferentially inhibit adenylyl cyclase activity through G proteins. We constructed a series of reciprocal hybrid receptors composed of domains derived from the m2 and m3 subtypes, representing the two functional classes of mAChRs, to identify specific amino acid sequences important for G protein recognition. SP6 receptor transcripts were injected into individual *Xenopus* oocytes (100 ng/egg) and ACh-induced Ca2+ activated chloride current (I-Cl) responses were measured two days later using two electrode voltage clamp measurements. Hybrid receptors were evaluated by their ability to stimulate I-Cl in comparison to m3 receptors, which produced large rapid inward currents, and to m2 receptors, which produced distinctly slower and reduced I-Cl responses. Based on seven-transmembrane topology, we found that the identity of the third cytoplasmic loop largely determined the type of observed response. A single contiguous domain of 9 amino acids from the third loop was sufficient to transfer this m3-like coupling of PI hydrolysis and intracellular calcium mobilization to the m2 receptor subtype. Site-directed mutagenesis of this region of the m3 receptor also revealed the importance of these sequences in G protein recognition. These results imply that an analogous domain may be responsible for specifying the G protein coupling properties of related neurotransmitter and hormone receptors, however, the location of this domain may vary between different classes of G protein-linked receptors. Funded by Minn. AHA fellowship to J.L. and Glaxo grant to D.C. and E.P.

9.2

PRODUCTION OF MUSCARINIC RECEPTOR ANTIBODIES AND REACTIVITY WITH m1-m5 SUBTYPE PROTEINS FROM BRAIN. A. I. Levey, T. M. Stormann, and M. R. Brann. Dept. Neurology, Johns Hopkins, Baltimore, MD. and Lab of Molecular Biology, NINDS, Bethesda, MD.

Molecular cloning studies have identified five distinct muscarinic receptor genes (m1-m5) and each mRNA has a unique localization in brain. However, it has not been possible to determine whether all five receptor mRNAs are translated since there are no ligands specific for the encoded receptor proteins. For this reason we prepared subtype-specific antibodies using recombinant DNA methods. The gene segments corresponding to the i3 loops of human m1-m5 (~150-200 amino acids unique to each subtype) were subcloned into the bacterial expression vectors pET and pGEX2T. High yields of fusion proteins were obtained (~30-50% of total culture protein) and two rabbits were immunized with each SDS-PAGE purified protein. Antibody specificity was verified with two assays. First, all antisera reacted specifically with the respective receptor fusion proteins on immunoblots. Second, cloned receptor subtypes independently expressed in CHO-K cells were labeled with 3[H]-QNB and 3[H]-PrBCM, solubilized, and tested for antibody immunoprecipitation. Each antisera bound to the appropriate receptor subtype without significant cross-reactivity to the other subtypes. The antibodies were also able to precipitate labeled receptor subtypes from rat brain in varying proportions depending on brain region. In conclusion, antisera specific for the five muscarinic receptor subtypes have been produced against fusion proteins and have been used to provide the first direct evidence for the proteins in brain.

9.3

EFFECTS OF THYMOPOIETIN (TPO) ON NICOTINIC ACETYLCHOLINE RECEPTOR (nAChR) AND NEURONAL NICOTINIC ALPHA-BUNGAROTOXIN BINDING SITE (nBgtS) EXPRESSION AND FUNCTION. R.J. Lukas, T. Audhya*, G. Goldstein* and L. Lucero*. Div. of Neurobiol. Barrow Neurol. Inst., Phoenix, AZ 85013 (RJL, LL) and Immunobiol. Research Inst., Annandale, NJ 08801.

TPO is a pleiotropic thymic hormone that influences T-cell maturation and immune system balance. It was initially isolated as a thymic factor that could interact with muscle cell nAChR and might play a role in myasthenia gravis. TPO blocks functional responses (IC50 = 30nM) and high affinity radiolabeled alpha-bungarotoxin (Bgt) or acetylcholine (ACh) binding (IC50 = 10nM) of muscle-type nAChR expressed by cells of the TE671 human clone or the BC3H-1 mouse muscle clone. Ganglia-type nAChR expressed by cells of the SH-SY5Y or IMR-32 human neuroblastoma clones are refractory to functional blockade by TPO at concentrations up to luM, but it blocks (IC50 = 80nM) high affinity binding of Bgt to nBgtS, which do not appear to function as ligand-gated ion channels, expressed by those cells. Chronic exposure to luM TPO induces a loss of muscle-type nAChR functional responses on TE671 or BC3H-1 cells. Chronic TPO treatment has no effect on expression of functional ganglia-type nAChR on SH-SY5Y cells. The data suggest that TPO or a related substance may be biologically active in both the immune and nervous systems. Some of TPO's functions may be attributable to its interaction with nBgtS or muscle-type nAChR.

9.4

THYMOPOIETIN, A THYMIC POLYPEPTIDE, INHIBITS FUNCTION AND NICOTINIC RECEPTOR BINDING AT THE NEUROMUSCULAR JUNCTION. M. Quik, B. Collier T. Audhya* and G. Goldstein. Dept. Pharmacol., McGill Univ., Montreal, Canada and Immunobiol. Res. Inst., Annandale, NJ, USA.

Thymopoietin (TPO) is a 49 amino acid polypeptide from thymus, linked to immune function and implicated in myasthenia gravis. The present results show that TPO completely blocked transmission at the rat phrenic nerve diaphragm at concentrations as low as 10^{-8} M. The TPO induced inhibition was dose and time dependent, with the polypeptide being only somewhat less potent than α-bungarotoxin (α-BGT). TPO did not inhibit muscle tension by an action on the muscle contractile mechanism per se. As well, TPO did not alter release of acetylcholine, suggesting it did not interact at a presynaptic level. On the other hand, TPO inhibited the binding of [^{125}I]α-BGT to diaphragm. In intact muscle tissue, the IC_{50} for inhibition of [^{125}I]α-BGT binding by TPO was 2.1 x 10^{-7} M, a value similar to the concentration of polypeptide required to inhibit phrenic nerve induced muscle contraction. In muscle membranes, the potency of the TPO in the [^{125}I]α-BGT binding assay was greatly increased (IC_{50} = 0.35 nM). The results suggest that TPO, a polypeptide linked to immune function, inhibits neuromuscular activity through an interaction at the nicotinic receptor.

9.5

STEROID MODULATION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTORS. D. Bertrand, S. Bertrand*, D. Rungger*, S. Valera* and M. Ballivet. Dpt of Physiology, CMU, 1 Rue Michel Servet, 1211 Geneva 4, Switzerland.

Nicotinic acetylcholine receptors (nAChRs) can be modulated by extracellular or intracellular substances. Application of substance P significantly increases the desensitization rate of neuronal nAChRs in rat sympathetic ganglion (Role, L.W., *Proc. Nat. Acad. Sci.*, 81:2924, 1984) and chromaffin cells (Clapham, D.E. and Neher, E., *J. Physiol.*, 347:255, 1984). More recently it was reported that ACh desensitization in chromaffin cell is also modulated by dexamethasone, a synthetic glucocorticoid. We examine here the action of dexamethasone and other steroids on reconstituted α4/nα1 neuronal nAChR expressed in Xenopus oocytes following nuclear injection of subunit cDNAs (Ballivet, M. et al., *Neuron*, 1:847, 1988). The effects of dexamethasone on the α4/nα1 channel were found to depend upon the batch of oocytes. In some batches 1 μM dexamethasone had no action while in other an immediate and significant increase of the ACh-induced current was observed in response to 1 μM agonist. In contrast, application of 10 μM progesterone always reduces immediately the amplitude of the ACh-induced current. Moreover, ACh-induced currents are abolished within four hours following a progesterone application sufficient to trigger oocyte maturation, whereas cytoplasmic injections of maturation promoting factor does not affect the response to ACh.

9.6

ACETYLCHOLINE RECEPTOR EXPRESSION IN DEVELOPING CHICK CILIARY GANGLION NEURONS. M.H.Jacob. Worc. Fnd. for Exp. Biol., Shrewsbury,MA 01545.

Innervation has been proposed to play a role in the induction of acetylcholine receptor (AChR) expression in neurons. I have used an anti-AChR mAb and immunolabeling at the ultrastructural level to compare the levels of AChRs in chick ciliary ganglion neurons before and after the establishment of innervation. Using immunolabeling with an anti-synaptic vesicle protein mAb, embryonic day 4.5 (St.24) was established as the earliest time at which synapses could be detected in the ganglion. In older neurons that are innervated, AChRs are present in both surface and internal pools. In embryonic day 4.5 neurons, AChRs are present in the rough endoplasmic reticulum, in the nuclear envelope, in the postsynaptic membrane and occasionally in small patches of extrasynaptic membrane. Before innervation, in embryonic day 4 (St 23) neurons, no AChRs can be detected on the surface and little to no AChRs appear to be present intracellularly. Preliminary results suggest that AChR levels are dramatically reduced in neurons developing in the absence of innervation. The results suggest that regulatory signals from presynaptic inputs cause a large increase in AChR expression in neurons.

9.7

Properties of embryonic and adult muscle acetylcholine receptors (AChRs) transiently expressed in COS cells. J.R. Forsayeth*, Y. Gu*, A. Franco, Jr.*, P. Gardner, J.B. Lansman and Z.W. Hall

We have used transient transfection in COS cells to compare the properties of mouse muscle nicotinic AChRs containing α, β, δ and either γ or ε subunits. Following transfection with cDNAs of either the γ- or ε-AChR, toxin-binding activity corresponding to fully assembled receptor was expressed on the surface of COS cells at a level comparable to that seen in cultured muscle cells. Toxin-binding to γ-, but not ε-, AChR was partially blocked by a myasthenic serum reported to be specific for the embryonic form of muscle AChR. The ε-AChR was also degraded more slowly in the membrane than the γ-AChR ($t_{1/2}$ ε: 19 h; $t_{1/2}$ γ: 11 h). Patch-clamp recordings showed that the γ-AChR had the characteristics of embryonic AChR (γ_C: 41pS; τ: 11 ms) and the ε-AChR had those of the adult AChR (γ_C: 61pS; τ: 1.0 ms). These results demonstrated that some but not all of the differences between AChRs at the adult endplates and those in the extrasynaptic membrane can be explained by the difference in subunit composition of γ- and ε- AChRs. Finally, transient expression of AChRs in COS cells is convenient, reproducible, yields high levels of surface AChR, and should be generally applicable to the study of other multi-subunit proteins. Supported by NIH grants (NS28062 and NS13521) to JRF and ZWH.

9.8

EVIDENCE THAT FUNCTIONAL NEURONAL NICOTINIC ACHRs HAVE PENTAMERIC STRUCTURES. E. Cooper. Dept. of Physiol. McGill Un. Montreal, Que. H3G 1Y6

Several lines of evidence suggest that nAChRs on neurons can be made up of only two separate subunits: an α (ligand-binding) subunit and a non-α (ß) subunit. However, their stoichiometric arrangement is not known. There are data suggesting that there are 2 α subunits in the functional receptor. Our experiments assess the number of non-α subunits. Experiments were done on outside-out patches from oocyte membranes 2-5 days after co-injecting cDNAs for chick α4 and non-α1 into the oocytes' nuclei. To determine the number of non-α subunits, we first mutated non-α1 at residue 260: the wild type lysine was changed to glutamic acid (referred to as nα1E260). This single residue change increased the conductance from 21pS for the wild type α4/nα1 receptor to 37.5pS for the mutant α4/nα1E260 receptor. Co-injection of nα1 and nα1E260 together with α4 resulted in patches containing 4 classes of receptors based on conductance: wild type(α4/nα1)-21pS; mutant type(α4/nα1-E260)-37.5pS; and 2 intermediate conductance receptors, one 26pS and the other 31pS. Our interpretation is that the 26pS channel is made up of 2 wild type nα1 subunits and 1 nα1E260, and the 31pS channnel is made up of 1 nα1 and 2 nα1E260 subunits. Therefore, we conclude that functional α4/nα1 receptors are pentameric structures containing 2 α subunits and 3 non-α subunits. (I am sincerely gratefully to Dr. M. Ballivet for providing the cDNAs.)

9.9

CLONING OF β5, A NICOTINIC RECEPTOR SUBUNIT. K. E. Isenberg. Department of Psychiatry, Washington Univ. Sch. of Med., St. Louis, MO 63110.

The neuronal nicotinic acetylcholine receptor is a multisubunit peripheral and central nervous system receptor. Several distinctive acetylcholine binding and nonacetylcholine binding or β subunits have been described in rat. I have cloned a new putative nonacetylcholine binding neuronal nicotinic acetylcholine receptor subunit. The clone was isolated from a rat superior cervical ganglion cDNA library (library provided by J. P. Merlie). The deduced amino acid sequence of the clone, designated β5, shows significant similarity with previously described neuronal receptor subunits and lesser but significant similarity with other ligand gated ion channel subunits. Analysis of the amino acid sequence suggests the presence of four putative transmembrane domains. The third and fourth transmembrane domains are separated by a large nonconserved cytoplasmic region. β5 lacks the side by side cysteine residues thought to be characteristic of acetylcholine binding subunits. These observations demonstrate the increasing complexity of the neuronal nicotinic acetylcholine receptor gene family.

9.10

MOLECULAR CLONING AND EXPRESSION OF HUMAN NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR SUBUNITS. Norman Nash, David Gdula, David Weiner, Mark Brann, and Tom Stormann. LMB/NINDS, Bldg., 36, Rm 3D-02, Bethesda, MD 20892 and Receptor Genetics, Inc..

Molecular cloning efforts have identifed seven rat cDNAs encoding subunits of neuronal nicotinic acetylcholine receptors (nAChRs). Coexpression of the α2, α3, or α4 subunits with the β2 or β4 subunits in frog oocytes gives rise to six functional nAChRs (Duvoisin et al, Neuron 1989). We have cloned the corresponding human cDNAs as a first step in developing model systems for screening drugs acting on human neuronal nAChR subtypes. Human retinal, cerebral cortical and hippocampal cDNA libraries were screened with oligonucleotide probes derived from the rat cDNA sequences. We have isolated cDNAs encoding the human α2, α3, α4, β2, and β4 subunits. Sequence analysis of these clones has demonstrated that the extracellular and transmembrane domains of the subunits are well conserved between rat and human, while the cytoplasmic domains exhibit much less conservation. The pharmacological profiles of the cloned receptor subtypes have been evaluated in transformed cells. We have found that κ-bungarotoxin binds to cells cotransfected with α3β2 but not to cells transfected with either of the single subunits. Subtype selective probes are being used to localize mRNA in human brain, via *in situ* hybridization.

9.11

EFFECT OF FORSKOLIN ON THE DEGRADATION RATE OF NICOTINIC ACETYLCHOLINE RECEPTORS. S.-L. Shyng and M.M. Salpeter. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

The degradation rate of nicotinic acetylcholine receptors (AChR) in the neuromuscular junction (nmj) of mammalian skeletal muscles depends on the state of innervation. Two populations of junctional AChRs have been described: junctional AChRs synthesized and inserted in innervated muscles (original AChR), whose degradation rate accelerates and decelerates in response to denervation and reinnervation, and junctional AChRs inserted after denervation (new AChR), whose degradation rate does not respond to reinnervation. The present study deals with the regulation of the original AChRs and aims at understanding how the degradation rate of these AChRs can be modulated after they are in the plasma membrane. We used mouse diaphragm muscle in which the degradation half-life ($t_{1/2}$) is 9 days when innervated and accelerates to 2.5 days within a few days after denervation. We found that when these muscles are placed in organ culture the accelerated degradation rate can be decelerated by the addition of forskolin, a compound known to activate adenylate cyclase. By 4 days in 20 μM forskolin the degradation rate decelerated from a $t_{1/2}$ of 2.3 days to one of 5.5 days. In 40 μM forskolin the switch occurred by 3 days and went to a $t_{1/2}$ of 8.5 days. The modulation effect of forskolin on the degradation of original AChRs is reversible. The decelerated degradation rate of original AChRs can be accelerated again by removing forskolin from the culture medium. Forskolin-1,9-dideoxy, a forskolin analog which does not increase the cAMP level, had no effect on the degradation of original AChRs. While the mechanism of post-insertion modification in AChR degradation rate by innervation is not yet understood, the present study suggests that this process might involve protein phosphorylation. Because of the delay in the action of forskolin, the effect of phosphorylation is probably indirect.

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CARDIOVASCULAR REGULATION I

10.1

SITE AND MECHANISM OF THE SYMPATHOLYTIC ACTION OF 8-OH DPAT. **R.B. McCall and M.E. Clement*. The Upjohn Co., Kalamazoo, MI 49001.**

Previous studies indicate that the 5-HT_{1A} agonist 8-OH DPAT acts centrally to inhibit sympathetic activity and lower arterial blood pressure. This study was designed to investigate the site and mechanism of the central sympatholytic action of 8-OH DPAT. The sympatholytic effect of 8-OH DPAT was not altered by mid-collicular transection or midline lesions of the pons and medulla. 8-OH DPAT inhibited the firing of medullospinal 5-HT neurons. However, the inhibition of 5-HT neuronal firing was not correlated with the inhibition of whole sympathetic nerve activity. Iontophoretic 8-OH DPAT failed to affect the firing of preganglionic neurons but blocked the excitatory effects of iontophoretic 5-HT. Intravenous 8-OH DPAT inhibited the firing of sympathoexcitatory (SE) neurons in the rostral ventrolateral medulla (RVLM). The inhibition of unit firing produced by 8-OH DPAT was exactly paralleled by the shutoff of inferior cardiac nerve activity. Iontophoretic 8-OH DPAT and 5-HT onto SE neurons in the RVLM failed to affect the firing rate of these neurons. These data suggest that 8-OH DPAT inhibits sympathetic activity in the lower brain stem by acting on central sympathetic neurons which lie antecedent to the RVLM SE neurons. Alternatively, 8-OH DPAT may act on distal dendrites of the RVLM SE neurons.

10.2

POSTNATAL MATURATION OF THE BAROREFLEX IN NEONATAL SWINE H.L. Cohen, P.M. Gootman, B.W. Hundley, G. Condemi* and L.P. Eberle*, Depts. Psychiatry and Physiology, SUNY, Health Science Center Brooklyn, Brooklyn, New York 11203.

The baroreflex was examined in 13 piglets from <1 to 33 days age, lightly anesthestized with Saffan, paralyzed and artificially ventilated (100% O_2). Simultaneous recordings of cervical sympathetic (CS) activity, aortic pressure (AoP) and EKG were made under control conditions and in response to bolus injections of Na nitroprusside (NP, 30 ug/kg, iv) and phenylephrine (PE, 20 ug/kg, iv). Data analyses included correlation, repeated measures t tests and power spectral techniques. Resting AoP increased significantly with postnatal age but a decreasing trend in resting heart rate (HR) was not significant. CS periodicities ranged from 1-50 Hz. The baroreflex was present at birth. NP reduced AoP 34.2% and significantly increased (28.6%) the magnitude of the CS power spectrum. The correlation between postnatal age and the NP-induced increase in HR just missed attaining statistical significance at $P<.059$. PE increased AoP 28.0% and significantly reduced the magnitude (-24.8%) of the CS power spectrum. The correlation between postnatal age and the PE-induced decrease in HR was signficant at $P < .05$. The results demonstrate that the baroreflex can be elicited in neonatal piglets at birth, but its varied components differ in their rate of postnatal maturation. (Supported by NIH Grant #HL - 20864 to PMG).

10.3

THE BED NUCLEUS OF THE TRANSTEGMENTAL TRACT: A MAJOR AUTONOMIC INTEGRATION CENTER OF THE MEDULLA OBLONGATA. D.J. Reis and D.A. Ruggiero Div. of Neurobiol., Cornell Univ. Med. Coll., NY, NY 10021.

A field of the lateral medullary reticular formation, bridging the nucleus tractus solitarii (NTS) and premotor autonomic regions of ventral lateral medulla (VLM) is important in autonomic regulation. We sought to establish the relationship between this field and the projection between NTS and VLM, the transtegmental tract (TT). The TT can be visualized throughout the medulla following: (a) injection of WGA-HRP into NTS or VLM; (b) immunostaining for PNMT, choline acetyltransferase, or enkephalin; (c) labeling, with specific radioligands for cholinergic, angiotensin II, or NPY receptors. Embedded in TT are clusters of neurons forming a bed nucleus of the transtegmental tract (BNTT). Transport studies demonstrate that BNTT neurons: (a) interconnect dorsoventrally, rostrocaudally and, contralaterally; (b) are innervated by and project to autonomic nuclei of brain e.g. parabrachial, lateral hypothalamic, and visceral cortex; (c) contribute to the cervical vagus. The BNTT has distinctive functional domains: e.g. the vasodepressor area of caudal VLM (CVL) which innervates sympathoexcitatory neurons of rostral VLM (RVL). The BNTT is a heretofore unrecognized nucleus of the medulla oblongata. It may serve as the principal interneuronal network integrating cardiovascular, respiratory and endocrine responses to stimulation of visceral afferents and centrally generated behaviors.

10.4

THE DISTRIBUTION OF GLUTAMATERGIC NEURONS IN CENTRAL AUTONOMIC PATHWAYS. D.A. Ruggiero, M. Anwar, M.P. Meeley, T. Kaneko & D.J. Reis. Div. Neurobiol., Cornell Univ. Med. Coll., NY, NY 10021.

L-glutamate (L-GLU) has been proposed as an excitatory neurotransmitter in a number of central autonomic pathways; however, the anatomical substrates for such glutamatergic neurotransmission have not been established. We, therefore, examined in adult Sprague-Dawley rats the immunocytochemical distribution within central autonomic networks of a polyclonal antibody to L-GLU (courtesy of Drs. P. Petrusz and A. Rustioni) and a monoclonal antibody against the GLU-biosynthetic enzyme phosphate-activated glutaminase (PAG). GLU-like immunoreactivity (GLU-LI) was detected in four principal areas relating to autonomic sensory-motor integration: (1) Sites in nucleus tractus solitarii (NTS) receiving primary visceral afferents of IXth and Xth cranial nerves (e.g. dorsal strip, subpostremal-medial parvocellular n.) contained cells and fibers exhibiting GLU-LI. In NTS PAG-labeled processes were abundant but neurons sparse. (2) Second order projection fields of NTS, the lateral parabrachial n. and locus ceruleus contained cells and processes with GLU-LI and PAG. (3) Brainstem nuclei projecting to spinal preganglionic (rostral ventrolateral, rostral ventromedial, raphe and A5 cell groups) or respiratory motor nuclei (retroambigual n. of n. CVL) contained neurons expressing GLU-LI and/or PAG. (4) Forebrain areas projecting to sensory and visceromotor nuclei of medulla and spinal cord. GLU-LI and PAG were concentrated in perikarya and processes in paraventricular, supraoptic, arcuate and paraventricular hypothalamic n., amygdala and visceral (insular-medial-prefrontal) cortex. These findings add support to pharmacological and physiological evidence that L-GLU may act as a neurotransmitter at multiple sites within autonomic centers of brain and spinal cord.

10.5

NEUROANATOMICAL SUBSTRATES FOR FUNCTIONAL DIFFERENTIATION OF THE ROSTRAL VENTRAL MEDULLA. S. Aicher, M. Anwar, E.P. Mtui, D.J. Reis & D.A. Ruggiero. Div. of Neurobiol., Cornell Univ. Med. Coll., NY, NY 10021.

The rostral ventral medulla contains two areas of importance in autonomic control: rostral ventrolateral reticular nucleus (RVL) and a poorly defined medial zone, the rostral ventral medial medulla (RVM). We sought to determine if these regions could be distinguished on the basis of their efferent projection fields. In anesthetized Sprague-Dawley rats (chloral hydrate, .5 g/kg i.p.), the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) was iontophoresed (6 μA, 15 min, 7 s on/off) into RVL or RVM. After 14 days, rats were reanesthetized, perfused and tissue was processed immunocytochemically for PHA-L. Although there was overlap of some projection fields, topographically distinct patterns of projections characterized efferents of RVL and RVM at all levels of the neuraxis. RVL and RVM project by lateral and medial transtegmental tracts, respectively. RVL targets include: dorsal and ventral nucleus tractus solitarius (NTS), n. retroambiguus, locus coeruleus, external medial and lateral parabrachial n., midline thalamic n. (paraventricular), midline hypothalamic n. (paraventricular, arcuate), medial preoptic n. and amygdala. RVM targets include: medial NTS, n. subcoeruleus, laterodorsal tegmental n., central gray, intralaminar thalamic n. (paracentral), dorsal hypothalamic n. and lateral preoptic n. In contrast to RVL, RVM deposits were distinguished by heavy transport to all spinal levels, including lumbosacral. Both sites project to the intermediolateral cell column of the cord. These distinct patterns of efferent projection fields may provide an anatomical basis for functional differentiation between lateral and medial sites in the rostral ventral medulla.

10.6

AUTONOMIC REFLEX ARCS FROM THE NUCLEUS TRACTUS SOLITARII TO THE SPINAL CORD. E.P. Mtui, D.A. Ruggiero, M. Anwar*, S. Aicher, R. Gomez* and D.J. Reis. Div. of Neurobiology, Cornell Univ. Med. Coll., NY, NY 10021.

Pathways subserving cardiopulmonary reflexes are believed to be indirect, involving projections from nucleus tractus solitarii (NTS) to reticulospinal neurons in ventrolateral medulla (VLM) that in turn innervate spinal respiratory and preganglionic motor nuclei. We sought to determine whether neurons of the NTS may directly innervate spinal autonomic neurons. Rats were anesthetized with chloral hydrate (0.5g/kg). Retrograde transport: WGA-HRP (2%) or rhodamine microbeads (50%) were microinjected into midcervical or upper thoracic cord. The ventral subnucleus of NTS (NTSv) from obex to spinomedullary junction contained: (a) a dense cluster of small horizontally-oriented neurons; (b) an array of larger diagonally-oriented neurons extending into adjacent n. reticularis dorsalis--a region within the bed nucleus of the transtegmental tract (BNTT) (Reis and Ruggiero, Neurosci. Abstr., 1990); Anterograde transport: Phaseolus lectin (PHA-L) was iontophoresed into loci of NTS containing retrogradely labeled neurons. PHA-L was transported to a diagonal sheet of axons and punctate varicosities radiating through the BNTT and VLM, descending through the lateral funiculus, terminating in laminae VII and X of the cervico-thoracic cord and innervating phrenic and intercostal motor nuclei and intermediolateral cell column. Neurons of NTS directly innervate spinal respiratory and sympathetic preganglionic neurons without synapsing in VLM. This solitaro-spinal pathway may regulate autonomic reflexes.

10.7

PROJECTIONS THROUGH THE FASTIGIAL NUCLEUS (FN) AND THE FASTIGIAL PRESSOR RESPONSE (FPR). W.T. Talman and S.C. Robertson*, Lab. of Neurobiology, VAMC & Univ. of Iowa, Iowa City, IA 52242

The FPR elicited by electrical stimulation of the FN likely results from stimulation of fibers of passage. We have sought to determine the potential origin of those fibers. In 7 rats the retrograde tracer fast blue, which is transported by fibers of passage, was injected unilaterally into the FN at sites where stimulation elicited the FPR. Dense fluorescent labeling was found bilaterally in the external cuneate, lateral reticular, medial vestibular (MVN), and caudal prepositus hypoglossi nuclei (NPH), and contralaterally in the inferior olivary nucleus. Lesser cellular labelling was present elsewhere. As the NPH had not previously been shown to modulate arterial pressure (AP), we sought to determine if electrical or chemical stimulation of the NPH or the adjacent MVN alterred AP or heart rate in 6 anesthetized rats. Both types of stimuli to the caudal, but not the rostral, pole of the NPH or to the MVN produced an increase of AP; bradycardia accompanied the former and tachycardia the latter. Both the NPH and MVN may participate in the FPR and in regulation of AP.

10.8

ROSTRAL VENTROLATERAL MEDULLA (RVLM) AND SYMPATHORESPIRATORY INTEGRATION IN RATS. P.G.Guyenet, R.L. Stornetta and R.A Darnall.*
Depts. of Pharmacology and Pediatrics, Univ. Virginia Sch. of Med., Charlottesville, VA 22908.

Phrenic nerve discharge- (PND-) triggered averages of the lumbar sympathetic nerve discharge (LSND) were examined in vagotomized, paralyzed, ventilated, halothan-anesthetized rats. The respiratory modulation of LSND (LSNDrm) was on average i) proportionnal to PND amplitude during CO2 inhalation and ii) attenuated by baroreceptor activation. Bilateral microinjections of kynurenate (2.25 nmoles/ side) into selected sites in the RVLM (N=8) produced central tachypnea, slight reductions in PND amplitude (33%), hypotension (15mmHg) and large reductions in LSNDrm (66%). Injections of bicuculline in the same area (0.22 nmoles/side, N=5) produced bradypnea, hypertension (27mmHg), reductions in PND amplitude (33%), loss of baroreflex and increases in LSNDrm (40%). Strychnine injections into RVLM (0.225 nmoles/side, N=5) were ineffective as were kynurenate or bicuculline injections into medullary raphe.

In conclusion, blockade of excitatory aminoacid or GABAa receptors in RVLM greatly modifies the coupling between central respiratory generator and sympathetic outflow. The results also indicate that baroreceptor- and CRG- related inputs to RVLM sympathetic premotoneurons are conveyed via largely independant routes. (Supported by NHLBI 28785 and 39841 to PGG).

10.9

CONTRASTING CARDIOVASCULAR EFFECTS OF MICROINJECTION OF ENDOTHELIN-1 AND -3 IN THE BRAINSTEM OF NORMOTENSIVE RATS. R. Mosqueda-Garcia, C. Beck, R.M. Robertson*, M. Appalsamy* and D. Robertson. Department of Pharmacology and Medicine, Vanderbilt University, Nashville, TN 37232.

At least three isoforms of the 21 amino acid peptide, endothelin, have been described. Endothelin-3 (ET-3) differs from endothelin-1 (ET-1) by six amino acids, exerts more profound initial depressor responses and exhibits less potent constrictor activity both in vivo and in vitro. It has been suggested that ET-3 may be a neural form of endothelin. The purpose of this study was to investigate whether ET-3 and ET-1 differ in their cardiovascular effects after microinjection in specific brainstem nuclei.

Male Sprague-Dawley rats were anesthetized with urethane, and blood pressure (BP) was monitored intraarterially. Intramedullary microinjection (60 nl) of ET-1 or ET-3 (0.5, 1, 2, 4, and 6 pmol) was made into the nucleus of the solitary tract (NTS), or into the area postrema (AP). Site confirmation was done by histological procedures.

Lower doses of ET-3 into the NTS increased BP and HR, an effect which peaked at 2 pmol (17 ± 3 mmHg, 14 ± 6 bpm, n=7). Maximal changes occurred within 45 seconds and recovered by 15 minutes. Higher doses of ET-3 modestly decreased BP and HR. In contrast, all the doses of ET-1 in the NTS evoked a hypotensive and bradycardic effect (0.5 pmol: -12 ± 4 mmHg, -48 ± 12 bpm, n=5). These effects were maximal between 5-8 minutes and recovered after 25 minutes. In the AP, ET-3 produced no change at the lowest dose but at higher doses, BP and HR fell in a dose-dependent manner. On the contrary, the lower doses of ET-1 in the AP decreased BP and HR, whereas higher doses produced no changes.

These studies indicate that ET isoforms exert distinct cardiovascular effects in the brainstem of anesthetized rats and suggest the presence in these medullary nuclei of more than one subtype of endothelin receptor.

10.10

AORTIC DEPRESSOR NERVE STIMULATION INFLUENCES AREA POSTREMA NEURONS IN THE RAT. S. Papas and A.V. Ferguson. Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The area postrema (AP) is the most caudal of the circumventricular organs, and as such is highly vascularized and lacks a normal blood brain barrier. In the rat, the AP is a midline structure overlying the nucleus tractus solitarius, at the level of the obex of the fourth ventricle. Although classically thought of as the chemoreceptor in the emetic reflex, the AP has also been attributed with various non-emetic roles, particularly in cardiovascular regulation. Previous studies have demonstrated the existance of neurons in the rat AP which are influenced by increases in blood pressure induced by systemic adrenergic agonists (Papas et al., 1990). The effects of these agonists on neuronal activity may be mediated by adrenergic receptors or, via the baroreceptors.

Electrophysiological experiments have now been done to determine if AP neurons receive input from the baroreceptors. In the rat, the aortic depressor nerve (ADN) carries mainly afferent barorecptor information. Thus, bipolar stimulating electrodes were positioned on either the left or right ADN of anaesthetized Sprague Dawley rats and single unit activity was recorded in the AP. Peristimulus histograms were used to examine the initial effects of ADN stimulation on 17 AP neurons. Thirteen (76%) of the AP cells were excited (Latency 28 ± 0.5 msec, Duration 12 ± 0.4 msec) by stimulation of an ADN. The remaining 24% of neurons were not influenced. These findings suggest that rat AP neurons receive afferent information from the baroreceptors and further support the view that the AP may act as a central regulator of the cardiovascular system.

Acknowledgements: Supported by MRC of Canada and the Heart and Stroke Foundation of Ontario.

10.11

ADRENO SYMPATHETICALLY - MEDIATED HEMODYNAMIC EFFECTS OF AREA POSTREMA STIMULATION. D.K. Hartle and A.S. Soliman*. Dept. of Pharm. and Toxicology, Univ. of GA, Athens, GA 30602.

Blood pressure, heart rate, and regional blood flow in renal, superior mesenteric and hindlimb vascular beds was monitored while graduating electrical frequency during area postrema (AP) stimulation (0-60Hz, 40μA). Blood flows were monitored by Doppler flowmetric techniques. Two groups of pentobarbital-anesthetized Sprague-Dawley male rats were tested 1) adrenals intact:CON, 2) acutely adrenalectomized:ADX. AP stimulation increased mean arterial pressure in a frequency-dependent manner in CON, but caused decreases in MAP in ADX. Renal and mesenteric beds actively constricted in CON, but remained passive in ADX. Renal and mesenteric blood flow decreased significantly in both groups. Hindlimb flow increased markedly in CON, but remained unchanged in ADX. Hindlimb resistance decreased significantly in both CON and ADX. Heart rate was not affected by AP stimulation in either group. We conclude that AP stimulation in CON powerfully augments sympathetic outflow to adrenals causing catecholamine release that produces hindlimb vasodilation and constriction of renal and mesenteric beds. In ADX the overriding effect of massive adrenal catecholamine secretion is eliminated and depression of sympathetic vasomotor efferent activity is unmasked. Renal and mesenteric circulations are rendered passive to blood pressure changes, while hindquarter resistance decreases due to relief of sympathetic tone, which is high in the pentobarbital anesthetized animal. We propose that AP stimulation simultaneously activates both a powerful adrenosympathetic efferent system and a sympathetic vasomotor withdrawal system. ADX unmasks the sympathetic depressor component. HLBI 37705 to DKH.

10.12

CARDIOVASCULAR EFFECTS OF MICROINJECTION OF ANGIOTENSIN III IN RAT BRAINSTEM NUCLEI. C.J. Tseng, L.L. Chou* and C.S. Tung*. Department of Pharmacology, National Defense Medical Center, Taipei, Taiwan, Republic of China.

Angiotensin III (AIII) had been reported to be an equipotent vasoconstrictor as compared to AII. In this study, we investigated the cardiovascular effects of microinjection of AIII into the cerebral ventricle (i.c.v.), the area postrema (AP), and nucleus tractus solitarii (NTS). Male Sprague Dawley rats were anesthetized with urethane and placed in a stereotaxic frame. The animals were prepared for i.c.v. infusion or exposure of the dorsal medulla for intra-AP and NTS administration of AIII. A dose-dependent pressor and bradycardic effect of AIII was observed in the i.c.v. injection as previously reported. However, a dose dependent depressor and bradycardic effects were noticed when low doses of AIII were microinjected into both AP and NTS, and the maximal effect was found in 10 ng. As the doses increased to 100 and 500 ng, a pressor and bradycardic effect was found. These depressor and pressor effects of AIII can be blocked by the selective AIII antagonist, Ile^7-AIII. These results suggest that the cardiovascular role of AIII in the brainstem nuclei need further characterization.

DRUGS OF ABUSE: COCAINE

11.1

DAILY COCAINE TREATMENT REDUCES [^{3}H]DOPAMINE UPTAKE *IN VITRO* IN RAT NUCLEUS ACCUMBENS BUT NOT IN STRIATUM. S. Izenwasser and B.M. Cox. Department of Pharmacology, Uniformed Services University, 4301 Jones Bridge Road, Bethesda, MD 20814-4799.

Male rats were treated daily with cocaine hydrochloride (15 mg/kg ip x 3 days), a regimen which leads to sensitization to the locomotor stimulating effects of cocaine. There was a decrease in [^{3}H]dopamine (15 nM) uptake in chopped rat nucleus accumbens tissue with no change in the striatum when uptake was measured 24 hours after the last injection. The K_m for dopamine uptake was increased in the nucleus accumbens of cocaine-treated rats while the V_{max} was unchanged. Furthermore, cocaine more potently inhibited the *in vitro* uptake of [^{3}H]dopamine in the nucleus accumbens of rats treated with cocaine than in those which had received saline. The IC_{50}s for cocaine in the nucleus accumbens of animals treated with either cocaine or saline were 20 nM and 400 nM, respectively, and in the striatum 400 nM for both treatment groups. There were not, however, any differences in the K_D or B_{max} for binding of [^{3}H]GBR 12935 to the uptake site in either the nucleus accumbens or striatum. In contrast to the effects seen after three injections, twenty-four hours after a single injection of 15 mg/kg cocaine there was no change in either the amount of [^{3}H]dopamine uptake or in the potency of cocaine for inhibiting uptake in either brain region. (Supported by a grant from NIDA).

11.2

EFFECTS OF COCAINE ON SOMATODENDRITIC DOPAMINE AND SEROTONIN RELEASE IN VENTRAL TEGMENTUM:*IN VIVO* VOLTAMMETRIC STUDIES. Patricia A. Broderick and Frank T. Phelan*, Dept. Pharmacol., CUNY Med. Sch., Depts. Biol. & Psychol., CUNY Grad. Sch., Convent Ave. & W. 138th St., Rm. J910, NY, NY, 10031.

Evidence has accumulated on cocaine mechanisms in neuronal circuitry, which points to the presynapse (Goeders, N.E. and Smith, J.E., Pharmacol. Biochem. Behav., 25:191, 1986) and the mesolimbic area (Roberts, D.C.S. and Koob, G.F., Pharmacol. Biochem. Behav., 17:901, 1982) as a fundamental target. *In vivo* electrochemical (ELC) studies were done with stearate electrodes. Fabrication and conditioning are described in detail in Broderick, P.A., Brain Res., 495(1):115, 1989. Indicator electrodes were stereotaxically implanted in ventral tegmentum of chloral hydrate anesthetized rats. Ag/AgCl reference electrodes and stainless steel auxiliary electrodes were placed in contact with the cortex. The results showed that cocaine (20 mg/kg sc) decreased ventral tegmental dopamine release in the first hour after drug administration. The signal returned to basal values during the second and third hour. Serotonin release, on the other hand, progressively decreased, an effect consistent with enhanced brain reward. Taken together with freely moving animal data, which showed increased dopamine release in brain reward substrates (Phelan, F.T. and Broderick, P.A., Soc. Neurosci. Abstr., 15:1234, 1989), cocaine may have direct as well as indirect acting dopaminergic agonist properties in brain reward neuronal circuitry. Supp: DHHS, NIDA R01 DA04755-01 A2 and PSC/CUNY Res. Award 669201 to P.A. Broderick.

11.3

PHOTOAFFINITY LABELING OF THE DOPAMINE REUPTAKE CARRIER PROTEIN USING A NOVEL HIGH AFFINITY AZIDO DERIVATIVE OF GBR-12935. P.Berger[1],R.Martenson[1]*,P.Laing[1]*,A.Thurcauf[2]* B.DeCosta[2]*,K.C.Rice[2]*,S.M.Paul[1]. [1]Sect. of Mol. Pharm., NSB, NIMH; [2]Lab. of Chem., NIADDK, NIMH, Beth., MD. 20892.

In order to develop a photoaffinity probe for the dopamine reuptake carrier protein, a tritiated (Sp. Act 41.8 Ci/mmol) azido derivative of the dopamine reuptake inhibitor, GBR-12935 has been synthesized. Like [3H] GBR-12935 binding, reversible [3H] azido GBR-12935 binding displays complete sodium dependance, and there is a good correlation between the ability of a series of drugs in inhibiting the specific binding of both azido GBR-12935 and [H] GBR-12935 and reuptake of dopamine into synaptosomes. Incubation of striatal (but not cerebellar) membranes with [3H] azido GBR-12935 followed by UV irradiation leads to its covalent incorporation UV into a protein of Mr = 80,000 as assessed by autoradiography following SDS-PAGE. Photo labeling of this Mr = 80,000 polypeptide is sodium dependent, and blocked stereospecifically by (-) but not (+) cocaine. Dopamine (but not serotonin or norepinephrine) reuptake inhibitors also blocked photolabeling. Following WGA lectin chromatography the Mr = 80,000 peptide was detected exclusively in the NAG eluent, indicating that it is highly glycosylated. Purification of the photolabeled dopamine reuptake carries protein using gel, lectin ad HPLC is in progress.

11.4

EFFECTS OF REPEATED COCAINE INJECTIONS ON D1 AND D2 BINDING SITES AND DOPAMINE REUPTAKE SITES IN RHESUS MONKEY CAUDATE. G.M. Farfel, M.S. Kleven, B.D. Perry, W.L. Woolverton and L.S. Seiden. Departments of Psychiatry and Pharmacological & Physiological Sciences, The University of Chicago, Chicago, IL 60637.

In previous studies we have shown that repeated administration of cocaine to rats causes long-lasting decreases in D1 binding sites and transient decreases in D2 binding sites in striatum (Kleven, et al., Brain Res., in press). Evidence of cocaine-induced changes in DA reuptake sites is equivocal. To assess the species generality of these findings, rhesus monkeys were administered either saline (n=4) or cocaine HCl (n=5; 3-4 mg/kg) i.m., q.i.d. for 14 days. One animal did not survive the drug regimen, and the remaining animals were sacrificed 2 wks after the last injection. [125 I] SCH 23982, [3H] spiperone and [3H] GBR 12935 saturation studies were used to label D1, D2 and dopamine (DA) reuptake sites, respectively, in caudate nucleus. The densities of the D1 and DA reuptake binding sites in cocaine-treated animals were significantly decreased (52 ± 13% and 17 ± 19% of control, respectively). There was a small decrease in D2 receptor binding in treated animals (B_{max} = 76% of control, .10> p >.05). Repeated cocaine did not alter the affinity (K_d) of any of the ligands for the binding sites. Analysis of neurotransmitter levels using HPLC-EC showed no significant differences between groups in levels of DA, 5-HT and 5-HIAA. Levels of DOPAC and HVA tended to increase, but the effects were not statistically significant. These results indicate that repeated administration of high doses of cocaine has long-lasting effects on both pre- and post-synaptic elements of the caudate dopaminergic synapse. (Supported by DA-00085 and The Brain Research Foundation of the University of Chicago.)

11.5

[^{3}H]CFT AND [^{3}H]LU 19-005: MARKERS FOR COCAINE RECEPTORS/ DOPAMINE NERVE TERMINALS IN PARKINSON'S DISEASE.
B.K. Madras, M.A. Fahey*, M.J. Kaufman. Harvard Medical School, New England Regional Primate Research Center, Southborough, MA 01772.

[^{3}H]CFT (WIN 35,428) and [^{3}H]Lu 19-005 [(±)-(trans)-3-(3',4'-dichlorophenyl)-N-methyl-1-indanamine] bind to cocaine sites associated with the dopamine uptake system in caudate-putamen of monkey brain. The utility of [^{3}H]CFT and [^{3}H]Lu 19-005 as markers for dopamine nerve terminals was evaluated in membranes prepared from human putamen of control and Parkinsonian brains. Tissue homogenates were incubated with [^{3}H]CFT (0.5 nM) alone or with either mazindol (3 µM) or (-)-cocaine (30 µM) to measure non-specific binding. Levels of bound [^{3}H]CFT and [^{3}H]Lu 19-005 were comparable to those in monkey caudate-putamen. In putamen of Parkinsonian brains, binding of both radioligands was markedly reduced. Autoradiographic studies in striatal sections of control and Parkinsonian brains confirmed the depletion of [^{3}H]CFT (5 nM) binding sites in the putamen of Parkinsonian brains. Low levels of binding were retained in the caudate nucleus, paralleling the progression of dopamine depletion in Parkinson's disease. The results suggest that radiolabeled forms of CFT and Lu 19-005 are useful markers for cocaine receptors/dopamine nerve terminals in the striatum and may have diagnostic utility. (Supported by USPHS Grants DA06303, DA00499, and RR00168)

11.6

COCAINE AND COCAETHYLENE CONCENTRATIONS IN HUMAN POST MORTEM CEREBRAL CORTEX.
S. Rose*, W. Lee Hearn, G. W. Hime*, C.V. Wetli* A. J. Ruttenber* and D. C. Mash. Comprehensive Drug Research Center and Depts. of Neurology and Pathology, University of Miami School of Medicine and the Metropolitan Dade County Medical Examiner Dept., Miami, FL. 33101 and the Center for Environmental Health, CDC, Atlanta, GA. 30333

Recreational cocaine use is frequently accompanied by the consumption of alcoholic beverages. When ethyl alcohol is present, cocaethylene is detected in the urine as a cocaine metabolite. We report here that significant concentrations of cocaethylene are detected in neurological specimens from cocaine-related sudden death cases. Cocaine and cocaethylene were isolated from the cerebral cortex using a liquid-liquid extraction technique with propylbenzoylecgonine as an internal standard. Quantitative analysis of cocaine and its homologue, cocaethylene, was performed using capillary column gas chromatography with nitrogen specific detection. In the cocaine-related sudden death cases, cocaine concentrations ranged in the cerebral cortex from 0.2 to 20 mg/kg. Cocaethylene was detected in significant concentrations in the cerebral cortex in five out of seven cases which had detectable levels of blood alcohol at the time of autopsy. The blood alcohol levels for these cases ranged from 0.01 to 0.3 %. The average cocaethylene concentration measured in brain was 0.3 mg/kg. Studies are currently underway to characterize the pharmacological profile of cocaethylene at neurotransmitter receptors and uptake sites in the human brain. Epidemiological studies of cocaine-related fatalities in Dade County indicate that the combined use of cocaine and ethanol confers an eighteenfold greater risk of sudden death. NIDA grant (DA 06227).

11.7

COCAETHYLENE BINDING TO NEUROTRANSMITTER RECEPTORS AND UPTAKE SITES IN THE HUMAN BRAIN.
D. C. Mash, D. D. Flynn, C.V. Wetli*, and W. Lee Hearn, Departments of Neurology, Pharmacology and Pathology and the Comprehensive Drug Research Center, University of Miami School of Medicine and the Metropolitan Dade County Medical Examiner Department, Miami, FL. 33136

Recreational use of cocaine and alcohol is a common practice, with users reporting an enhanced euphoria. Concurrent cocaine and alcoholic beverage use results in the formation of cocaethylene, the ethyl homologue of cocaine (Rafla, F.K. and Epstein, R.L., J. Anal. Toxicol. 3:59-63, 1979). Cocaethylene has been measured in significant amounts in blood and urine. We have demonstrated the presence of cocaethylene in the human brain post mortem from cocaine-related sudden death cases. The reinforcing property of cocaine is attributed to its ability to block the reuptake of dopamine. We report here that cocaethylene is equipotent to cocaine in inhibiting [^{3}H]-mazindol binding to dopamine transporters. Cocaethylene inhibited [^{3}H]-mazindol binding in human striatal membranes with a Ki of 0.64 µM. Cocaethylene was ineffective in displacing [^{3}H]-raclopride and [^{3}H]-SCH23390 binding to dopaminergic receptor subtypes. Competition studies further demonstrate that unlike benzoylecgonine, cocaethylene has a relatively high affinity for muscarinic and putative sigma receptors. Brain concentrations of cocaethylene are in the same range as cocaine levels seen in cases of preterminal excited delerium and sudden death. Taken together, these results suggest that cocaethylene is a neuroactive metabolite of cocaine. The accumulation of cocaethylene in the central nervous system may contribute to the spectrum and incidence of neurologic and psychiatric complications associated with combined cocaine and alcohol abuse. Supported by a grant from NIDA (DA 06227).

11.8

BRAIN GLUCOSE METABOLISM IN COCAINE DEPENDENCE AND WITHDRAWAL.
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To investigate changes in brain function associated with cocaine dependence we measured brain glucose metabolism with 2-deoxy-2-[^{18}F]fluoro-D-glucose and positron emission tomography in 15 cocaine abusers and 17 normal controls. Patients studied during early withdrawal (< 1 week off cocaine) had significantly higher metabolic rates (ANOVA $p \leq .05$) in the basal ganglia (BG) (8.1±0.4 mg/1000/min) and in the orbital frontal cortex (OFC) (7.5 ± .05) than normals (BG = 7.3 ± 0.9 OFC = 6.3 ± .03) or patients studied during late withdrawal (2-4 weeks off cocaine) (BG 7.4 ± .05 OFC = 6.1 ± 0.5). The increased metabolic activity in OFC and BG was temporary and was not significantly different from that seen in normals 2 weeks after cocaine withdrawal. The dependence of these metabolic changes to the time of cocaine withdrawal was tested with regression analyses which revealed a significant relationship between time "off" cocaine and regional brain metabolism in OFC ($r^2 = .517$ $p \leq .003$) and BG ($r^2 = .389$ $p \leq .02$). The OFC and the BG are part of a brain circuit that regulates initiation and termination of behavior. Its temporary dysfunction in the cocaine abuser could explain the compulsive and episodic pattern of cocaine abuse.

11.9

Cocaine Abuse in Schizophrenia: Effects on Symptoms and Hospital Course

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Cocaine produces profound effects on brain monoamine systems which may be clinically important in schizophrenia including the expression of positive symptoms, negative symptoms, and acute and chronic extrapyramidal side effects. While prevalence of cocaine abuse in schizophrenics is reported to be high, the impact of cocaine abuse in schizophrenia is poorly understood. **Methods**: Inpatient charts were obtained for every fourth patient from the roster of schizophrenics treated in the Schizophrenia Clinic of the West Haven VA Medical Center. Individual hospitalizations were analyzed for substances used and psychosocial functioning prior to hospitalization, presenting symptoms on admission, inpatient clinical management, course of pharmacotherapy, and duration of hospitalization. **Results**: Overall prevalence of history of cocaine use was 21%, other substance abuse 35%, no history of substance abuse 34%, and10% undetermined. Cocaine-abusing schizophrenics (N=16) had greater frequency of hospitalizations compared with non-cocaine using substance abusing (N=22) and non-substance abusing patients (N=20). Within the cocaine group there was increased suicidality and depressive symptoms after cocaine use. Symptoms of paranoia and psychomotor agitation were not different in patients presenting after cocaine use compared to their non-cocaine admissions. Cocaine hospitalizations required markedly higher antipsychotic doses. Overall, the cocaine cohort exhibited higher rates of unemployment and legal problems than non-substance using patients. **Comment**: Cocaine use has significant effects on the course of hospitalization in schizophrenics, including neuroleptic dose.

11.10

WHOLE BODY BINDING AND DISTRIBUTION OF COCAINE.
P. Som*, Z.H. Oster*, N.D. Volkow, D.F. Sacker*, K. Maldonado* and D.A. Weber*. Medical Department, Brookhaven National Laboratory, Upton, NY, 11973.

Whole body distribution of cocaine in the Fischer rat was investigated using carboxy ^{14}C benzoyl cocaine (*C) in conjunction with whole body autoradiography at different times after *C injection (2,4,6,10,15,30 minutes).

Three stages of *C distribution were observed: 1) very fast initial localization in the brain, spinal cord, heart and adrenals (2-5 min), 2) gradual accumulation in the kidneys and liver (5-10 min), 3) excretion into the urine and bowel (15-20 min).

Characterization of cocaine binding was investigated using specific blocking agents; GBR to block the dopamine (DA) transporter and desipramine (DESP) to block the norepinephrine/serotonin (NE/SE) transporter.

In the brain binding of *C was not affected by DESP, and was decreased by GBR pretreatment suggesting that cocaine binds (in the basal ganglia and cortex) to a site associated with the DA transporter. Binding of cocaine in the heart and kidney was decreased by DESP pretrement. Binding of *C to brain, heart, liver and kidneys could account for some of the toxic effects of cocaine on these organs.

11.11

CARDIOVASCULAR (CV) EFFECTS OF NONCONTINGENT COCAINE (C) ADMINISTRATION. TL Smith*, MF Callahan, DC Williams*, SI Dworkin, Dept. Physiol./Pharmacol., Bowman Gray Sch. Med., Winston-Salem, NC 27103.

The CV responses to intravenous C (0.33 mg) in conscious, F344, male rats (291 +/- 20 g, n=6) were evaluated. Rats were instrumented with arterial and venous catheters and EKG electrodes 1-4 weeks prior to study. Three infusions of C were given at either 5 min. or 2hrs. interinfusion intervals (II). Baseline mean arterial pressure (MAP) was 99.3 +/- 2.1 mmHg and heart rate (HR) was 365 +/- 14.1 beats/ min.. C elicited a 52.3 +/- 3.4 mmHg MAP increase and a 15 +/-11.5 beat/min bradycardia. The third dose of C, 10 min later, increased MAP only 7.8 mmHg and decreased HR 43 +/- 8 beats/ min.. Greater than 50% of the animals exhibited arrhythmias following C, and a majority demonstrated a prolongation of the QRS complex. At 2 hr. II, MAP & HR changes elicited by the first C dose were a 60.3 +/- 5.7 mmHg increase and a 38 +/-8 beat/ min. decrease. Changes elicited by the second C dose were not different from the first. The final C dose produced a significantly lower MAP elevation from the first (48.3 +/- 10.8 mmHg) although the HR change was not different. Significant tachyphylaxis, at least in MAP responses, occurs with 5 min. II, and, to a lesser degree with 2 hr. II. Interinfusion interval is therefore very important when evaluating the CV effects of intravenous C. **DA03628**

ALZHEIMER'S DISEASE: AMYLOID I

12.1

AMYLOID NEUROTOXICITY I: MOLECULAR DETERMINANTS. B. A. Yankner and M. Chen. Dept. of Neurology, Harvard Medical School and The Children's Hospital, Boston, MA 02115.

We have shown that a fragment of the amyloid precursor protein (APP), containing the carboxyterminal 106 amino acids, is neurotoxic (Yankner et al., 1989, Science 245:417). The APP sequences necessary for the neurotoxic effect have been examined using purified amyloid-containing proteins with different carboxyterminal extents of APP. The critical carboxyterminal APP sequence has been determined. Amino-terminal regions were inactive. The secreted form of APP was also purified from the conditioned medium of PC12 cells by heparin-sepharose and DEAE chromatography and immunoprecipitation. Purified secreted APP was identified as a 120-125K band on siver-stained SDS gels which could be immunoprecipitated by antibodies against the amino-terminus or Kunitz protease inhibitor domains of APP but not by an antibody against the carboxyterminus. Secreted APP showed a neurotrophic effect on hippocampal neurons but did not show the neurotoxic effect observed with carboxyterminal APP proteins. Our results suggest the possibility that altered patterns of APP processing in Alzheimer's disease can generate neurotoxic amyloid proteins.

12.2

AMYLOID NEUROTOXICITY II: NEURONAL SPECIFICTY AND PATHOLOGICAL CHANGES. M. Chen and B. A. Yankner. Dept. of Neurology, Harvard Medical School and The Children's Hospital, Boston, MA 02115.

The specificity of amyloid toxicity has been examined on primary cultures from different regions of the central and peripheral nervous system. In primary cultures derived from rat E18 hippocampus or forebrain, neurons were selectively killed but glial cells were resistant. Amyloid neurotoxicity was also observed, but to a lesser extent, in cultures from the region of the medial basal forebrain and septum. Highly enriched cultures of cerebellar granule cells and dorsal root ganglion neurons, two neuronal populations which are unaffected in Alzheimer's disease, were resistant to the toxic effects of amyloid proteins. Addition of purified amyloid proteins to susceptible hippocampal pyramidal neurons resulted in a defined sequence of neurodegenerative changes over 48 hours. The earliest changes were observed in the neurites and were characterized by dendritic retraction and loss of axonal integrity followed by later degenerative changes in the cell body. Thus, amyloid neurotoxicity in culture recapitulates some aspects of the neuronal specificity and pathology observed in Alzheimer's disease.

12.3

IMMUNOHISTOCHEMICAL LOCALIZATION OF THE ALZHEIMER AMYLOID PRECURSOR PROTEINS CONTAINING THE KUNITZ-PROTEASE INHIBITOR DOMAIN IN ALZHEIMER'S DISEASE.
B.T. Hyman, R. Tanzi, K. Marzloff and D. Schenk, Dept. of Neurology & Neuroscience, Massachusetts General Hosp, Harvard Medical School, Boston, MA 02114 & Athena Neurosciences, So. San Francisco,CA 94080

Senile plaques (SP) of Alzheimer's disease (AD) contain a core of an amyloid peptide (A4), surrounded by dystrophic neurities. 3 major forms of the A4 precursor protein (APP) are transmembrane proteins of 695, 751, and 770 amino acids. The longer two contain an insert that shares substantial homology with the Kunitz-type serine protease inhibitors (KPI). The KPI forms of APP are relatively preserved in AD brain by Northern analysis, but whether they contribute to SP or tangles (NFT)is unknown. We examined the hippocampal formation in 4 AD and 4 normal control cases using thioflavin S, anti-A4, and anti-KPI domain immunocytochemistry. Tissue was fixed in PLP for 24 hrs, cryoprotected and cut in 50 μ sections. In AD, thioflavin S and anti-A4 showed SP consistently in the molecular layer of the dentate gyrus, in stratum radiatum of CA1, and in the pyramidal and molecular layers of the subiculum. Occasional "tombstone" NFT were also evident. Anti-KPI staining was sensitive to fixation conditions, and diminished with stronger or prolonged exposure to formaldehyde. SP's contain definite anti-KPI staining in the neurites and, to a lesser extent, in the core. As compared to thioS or anti-A4, these SP are a minority of total plaques. Many neurons stained strongly with anti-KPI in both AD and controls. Both neurons that develop NFT (CA1) and those that are less likely to (dentate gyrus granule cells) are strongly stained. These results suggest that the KPI forms of APP are found in neurons and are incorportated into SP. We thank Dr. C. Masters, Melbourne, Australia, for the gift of anti-A4 antisera, the Mass. ADRC tissue bank (Drs. E.T. Hedley-Whyte, N. Kowall and A. McKee) and NIH grant AG08487.

12.4

IMMUNOLOCALIZATION OF AMYLOID P COMPONENT IN THE NEUROFIBRILLARY PATHOLOGY OF ALZHEIMER AND OTHER NEURODEGENERATIVE DISEASES.
Pamela G. Galloway, George Perry and Rajesh N. Kalaria, Dept. of Pathology, Children's Hospital Medical Center, Akron, OH 44308, and Depts. of Neurology and Pathology, Case Western Reserve University, Cleveland, OH 44106.

Amyloid P component, (AP) is an α_1-glycoprotein shown to be consistently present in all types of amyloid deposits. We investigated the extent of AP reactivity in the neurofibrillary pathology of Alzheimer (AD) and related neurodegenerative disorders. The PAP and immunogold cytochemical methods were used to reveal AP reactivity at the light and electron microscope levels. By light microscopy, heavy deposition of immunoperoxidase reaction product was seen in classical amyloidotic lesions of all cortical areas of AD cases examined. The distribution and intensity of staining in neurofibrillary tangles were similar to that of thioflavin S staining in serial sections. Plaques of Creutzfeldt-Jakob disease and Down's syndrome, Pick bodies of a Pick's case and tangles in progressive supranuclear palsy were also stained. At the ultrastructural level, electron dense product was evident in extracellular amyloid fibrils, neuronal filaments and the vascular wall. These results provide evidence for localization of AP in intra-neuronal abnormal filaments in addition to extracellular amyloidotic lesions. AP may be important to pathogenesis of neurofibrillary tangles and cerebral amyloidosis.

12.5

AMYLOID PRECURSOR PROTEIN IN DYSTROPHIC AXONS. B. Bacci*, E. Cochran*, B. Schaetzle*, S. Hite*, L. Sayre*, B.D. Greenberg, D.E. Lowery*, L. Autilio-Gambetti and P. Gambetti. Division of Neuropathology, Case Western Reserve University, Cleveland OH 44106 and The Upjohn Company, Kalamazoo, MI 49001.

Neuritic plaques of Alzheimer disease (AD) are characterized by extracellular amyloid deposits and dystrophic neurites which react with antibodies to ubiquitin (Ub) and amyloid precursor protein (APP). Administration of p-bromophenylacetylurea (BPAU) to rats induces the formation of dystrophic axons with accumulation of tubulovesicular structures which are labeled by antisera to Ub. We now report that these dystrophic axons immunostain with antisera to APP both at the light and electron microscopic level. Both APP and Ub epitopes are located in the membranes which fill the BPAU dystrophic axons. We also detected the presence of the inducible form of heat shock protein 70 and of PGP 9.5, a neuron-specific Ub carboxyl-terminal hydrolase that may be responsible for processing Ub conjugates. The present findings indicate that the dystrophic axons induced by BPAU share antigenic characteristics with dystrophic neurites of AD plaques and are a promising model to study the role of dystrophic axons in amyloid deposition. The presence of Ub and heat shock protein 70 along with APP in BPAU-induced dystrophic axons raises the question of whether the heat shock system is implicated in amyloid deposition. Similar findings have been obtained in axons from a case of human infantile neuroaxonal dystrophy, suggesting that APP may play a role in this familial human disease. Should this be the case, infantile neuroaxonal dystrophy would be the first non-amyloid human disease in which APP is involved. Supported by NIH grants AG 00795 and NS 14509.

12.6

ANALYSIS OF NON-NEURAL DEPOSITION OF AMYLOID β PROTEIN IN ALZHEIMER'S DISEASE (AD) AND AGING. D. Selkoe, C. Lemere*, H. Mori* and C. Joachim. Harvard Medical School, Brigham and Women's Hospital, Boston, MA 02115.

Growing evidence supports the hypothesis that deposition of the amyloid β protein (AβP) in amorphous, non-fibrillar forms is an early event in AD pathogenesis that may precede cellular pathology. Our recent finding of AβP-immunoreactive deposits in certain non-neural tissues of AD and some aged subjects is consistent with this hypothesis. We have now extended this study to >60 human subjects and found that virtually all AD patients examined by skin biopsy showed vascular, perivascular and/or amorphous dermal deposits. About one-quarter of aged non-AD subjects examined to date also showed some amorphous dermal deposits, whereas virtually all non-aged (<63 yr) subjects showed no specific reactivity. Several patients with Down's syndrome and a member of an FAD pedigree linked to chromosome 21q also showed non-neural AβP deposits. Antisera to native AβP detected the non-neural deposits more sensitively than synthetic AβP antisera. Detailed analysis of the distribution of AD dermal staining reveals subepidermal, perivascular and periglandular deposits resembling the sites of cystatin C deposition in patients with Icelandic hereditary cerebrovascular amyloidosis. The discovery of microvessel-associated AβP reactivity in widespread non-neural tissues extends the parallels between AD and certain systemic amyloidoses known to be of circulating origin.

12.7

INCREASED SYNTHESIS OF ALZHEIMER AMYLOID PRECURSOR PROTEIN IN THE CEREBRAL CORTICES OF RATS WITH LESIONED NUCLEUS BASALIS OF MEYNERT. W. Wallace, V.Bragin*, N.K. Robakis, K.Sambamurti*, K.L.Davis, and V. Haroutunian. Dept. Psychiatry and Center for Neurobiology, Mt. Sinai School of Medicine, New York, NY 10029

The nucleus basalis of Meynert was lesioned by administration of NMDA unilaterally in adult rat brain. Seven days postlesion we have observed that polysomes isolated from the cerebral cortex ipsilateral to the lesion synthesized 2.6-fold greater amounts of Alzheimer amyloid precursor protein (AAPP) compared to the nonlesioned side of the same brain. This increase exhibited specificity to AAPP in that overall protein synthesis, as either total 35S-polypeptides or translation products on two dimension gels, was not altered by the lesion. In addition, levels of newly synthesized glial fibrillary acidic protein were not different in the lesion samples. The increase of AAPP did not alter the predominance of the AAPP (695) isotype (which lacks the protease inhibitor insert) in the cortex. These observations indicate that compromise of the nucleus basalis results in the specific synthesis of cerebral cortical AAPP.

12.8

ALZHEIMER-TYPE AMYLOIDOGENESIS. A TRANSGENIC MOUSE MODEL? B.D. Greenberg, S.M. Ali*, R.A. Altman*, D.E. Lowery, P.K. Andrus* and H.G. Polites*. Molecular Biology Research, The Upjohn Company, Kalamazoo, MI 49001.

The lack of an appropriate animal model is an ongoing impediment to the development of treatments for Alzheimer's Disease (AD). While the etiological role for amyloidogenesis in AD remains unknown, transgenic mice expressing the Alzheimer Amyloid Precursor (AAP) in appropriate brain regions would provide a useful system for investigating the role of AAP expression in Alzheimer-type amyloidogenesis, and the role of amyloid formation in AD. To address these issues, we are generating mouse lines transgenic for full length and truncated variants of the AAP cDNA. These cDNAs have been placed under the control of several promoter systems designed to express AAP within neurons in brain regions corresponding to those which are vulnerable in AD. 83 founder mice have been born in our facility. We are analyzing their offspring by in situ hybridization and standard histochemical staining. During the course of our AAP expression work, we also developed several highly specific antisera to recombinant AAP which stain Congophilic and diffuse amyloid, as well as apparent precursor within normal and degenerating neurites and perikarya (Arai et al. (1990) PNAS 87: 2249; Cras et al. (1990) Am. J. Pathol., submitted). We are applying these antisera to brain sections from these mice to investigate whether potentially incipient amyloidotic structures can be visualized. We will report on these studies, and the nature of the transgenic promoter systems employed.

12.9

THE PROMOTER ACTIVITY OF THE GENE ENCODING ALZHEIMER'S β-AMYLOID PRECURSOR PROTEIN (APP) IS REGULATED BY TWO BLOCKS OF UPSTREAM SEQUENCES. D.K. Lahiri and N. Robakis, Mount Sinai Medical Center, One Gustave Levy Place, New York 10029

Sequence analysis of the β-amyloid promoter revealed several regulatory elements including five copies of the GGGCGC sequence, consensus sequences for the transcription factors Sp1 and AP-1, a heat shock element and a GC rich region characteristic of housekeeping genes. To determine the promoter sequence requirements for the expression of the APP gene, we constructed chimeric genes containing different parts of the APP gene promoter linked to the bacterial chloramphenicol acetyl transferase (CAT) gene. Sequences derived from the 5'-flanking region were then tested for their effects on β-amyloid promoter activity by transient CAT expression in PC12 and HeLa cells. Two blocks of regulatory sequences were identified in the 5'-flanking region of the gene. One block extending from -447 bp to -597 bp acts as a positive regulator because its deletion results in a dramatic decrease in promoter activity. A second block of sequences extending from -197 bp to -397 bp acts like a negative regulator as their removal results in increase in promoter activity. A plasmid pAmylE was constructed by inserting 39 bps nucleotide sequence (from -450 to -489) in front of TK promoter of pBLCAT2 plasmid. When PC12 cells were transfected with pAmylE, the CAT activity was found to be five times more as compared to cell transfected with pBLCAT2. Thus the promoter sequence from -450 to -489 may act as a putative enhancer element. These results suggest that by imposing conformational constraints, these regulatory sequences might control the binding of trans-acting factor(s) on the APP gene promoter and thus modulate its expression. The identification of such factors is in progress.

12.10

EXPRESSION, PURIFICATION, AND CHARACTERIZATION OF THE ALZHEIMER'S AMYLOID PRECURSOR PRODUCED BY BACULOVIRUS. [1]D.E. Lowery, [1]P.A. Gonzalez-DeWhitt*, [1]R.A. Altman*, [2]P. Cras*, [2]G. Perry and [1]B.D. Greenberg. [1]Molecular Biology Research, The Upjohn Company, Kalamazoo, MI 49001, and [2]Dept. Pathology, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106.

To facilitate studies on the role of the Alzheimer's Amyloid Precursor (AAP) in Alzheimer's Disease we have developed the baculovirus expression system (Luckow and Summers (1988) Biotechnology 6: 47) as a means of producing milligram quantities of AAP. Baculovirus expression vectors designed to produce secreted 695, 751 and 770 amino acid AAP forms were constructed and used to infect Sf9 cells. Purification of the resulting AAP from the conditioned media results in a >95% pure preparation of AAP. The recombinant AAP has been used to raise highly specific antisera (Cras et al. (1990) Am. J. Pathol., submitted) which stain Congophilic and diffuse amyloid, as well as apparent precursor within normal and degenerating neurites and perikarya. The 751 and 770 amino acid AAP forms are functional protease inhibitors and have been used to screen a variety of proteases. The final preparations of recombinant protein are also devoid of any detectable protease activity, making them ideal for studies on the proteolytic processing of AAP.

12.11

PLATELETS SECRETE AMYLOID β-PROTEIN PRECURSOR (APP). G.M. Cole, D. Galasko, I. P. Shapiro, and T. Saitoh, Dept. of Neurosciences, M-024, UCSD, School of Medicine, La Jolla, California, 92093

The extracellular β-protein deposited in Alzheimer's disease derives from a segment overlapping the membrane-spanning domain of a larger precursor integral membrane protein (APP). Mechanisms for the externalization of this β-protein domain from cells remain hypothetical. Here, we show that human platelets stimulated with thrombin or ionomycin release membranous microparticles which contain ~110kD APP which is both N-terminal and C-terminal anti-APP immunoreactive implying that it contains the entire β-protein sequence. Platelet-derived microparticles are also found in human serum and plasma. This suggests a possible circulating source for β-protein in serum and plasma and a blebbing mechanism for externalization which could also occur in neurons. Stimulated platelets also release soluble, C-terminal truncated APP containing the Kunitz-type serine protease inhibitor domain (nexin II) which has been shown to inhibit part of the prothrombinase complex, Factor Xa. Thus, nexin II derived from platelets and other sources may play a role in regulating the coagulation cascade. This observation provides a potential biochemical connection between Alzheimer's and coronary vascular disease which have been epidemiologically linked.

12.12

DESIGN OF DIAGNOSTIC PROBES FOR ALZHEIMER'S BETA/A4 PEPTIDE. WE Klunk, RJ McClure, K Panchalingam and JW Pettegrew Laboratory of Neurophysics, Dept. of Psychiatry, Western Psych. Inst & Clinic, Univ. of Pittsburgh, Pittsburgh, PA 15261

Neuropathologically, two of the most characteristic features of Alzheimer's disease (AD) are senile plaques and neurofibrillary tangles. The protein core of the senile plaque is composed of a peptide termed the beta/A4 peptide. The definitive diagnosis of AD at present requires neuropathologic quantification of plaques and tangles, usually done post-mortem. Here, work to design and synthesize probes to quantify the beta/A4 peptide before death is described. Congo red is a histologic dye which stains plaques and tangles. We have quantified the interaction of this dye, and similar compounds, with synthetic beta/A4. From this, computer models of the dye-peptide interaction have been developed. The peptide portion of this model is being further refined by two-dimensional NMR studies of the synthetic peptide. Using this data, compounds are being developed to cross the blood-brain barrier and bind specifically to the beta/A4 peptide. Quantification of fluorine labelled probes by *in vivo* NMR spectroscopy or positron emitting probes by PET could then form the basis of an antemortem diagnostic test for AD.

ISCHEMIA I

13.1

SUBCELLULAR MECHANISMS OF ISCHEMIC BRAIN DAMAGE DURING CARDIAC ARREST AND RESUSCITATION. G. Fiskum*, R. F. Chanderbhan*, L. L. Werling and R.E. Rosenthal*. Departments of Biochemistry and Molecular Biology, Emergency Medicine, and Pharmacology, George Washington University School of Medicine, Washington, D.C. 20037.

Membrane lipid degradation and peroxidation and mitochondrial respiratory damage are implicated in the irreversible cell injury that can occur during cerebral ischemia and reperfusion. In this study, mitochondria isolated from the parietal cortex of chloralose-anesthetized female beagles following 10 min of cardiac arrest and 2 and 24 hours of spontaneous circulation exhibited respiratory control ratios that were 50%, 100%, and 75% respectively of those obtained from control animals. Extraction and analysis of lipids from the cerebral cortex indicated the release of free fatty acids following 10 min of ischemia and the generation of lipid peroxides after 2 and 24 hours of reperfusion. These results indicate that delayed post-ischemic mitochondrial damage and lipid peroxidation occur in the cerebral cortex in a clinically realistic model of cerebral ischemia and resuscitation (Supported by a grant from Sigma Tau S.p.A.).

13.2

THE 21-AMINOSTEROID ANTIOXIDANT TIRILAZAD MESYLATE (U-74006F) BLOCKS CORTICAL HYPOPERFUSION FOLLOWING SPREADING DEPRESSION. E. D. Hall and S. L. Smith*. CNS Dis. Res., The Upjohn Co., Kalamazoo, MI 49001.

Cortical spreading depression (SD) has been implicated in the pathophysiology of classical migraine headache and cerebral ischemia. A reduction in cerebral blood flow (CBF), mimicking that seen during the aura and headache phase of migraine, is typically observed following SD in the rat. In the present study, brief cortical exposure to 1M KCl produced a marked suppression of EEG amplitude which persisted 20 min in the rat. Upon normalization of the EEG, cortical blood flow declined 20-30% and remained low for at least 2 hrs, while mean arterial pressure was unaffected. Over the 2 hr. post-KCl time course, arterial pO_2 rose significantly while pCO_2 and pH were unchanged. The increase in pO_2 may indicate a decrease in cerebral oxygen utilization. Treatment with a 1 mg/kg iv dose of the 21-aminosteroid antioxidant tirilazad mesylate (U-74006F), 2 min following KCl application, completely blocked the hypoperfusion while leaving total EEG amplitude and MAP unchanged. The SD-induced rise in arterial pO_2 was also prevented. Tirilazad mesylate is a potent inhibitor of oxygen radical-mediated lipid peroxidation both in vitro and in vivo. Based on this fact and present results, oxygen radicals may play a role in the SD-induced cerebral hypoperfusion.

13.3

AMPA RECEPTORS MEDIATE ISCHAEMIC NEURONAL CELL DEATH. M.J. Sheardown, A.J. Hansen, P. Suzdak, K. Eskesen*, P. Jacobsen* and T. Honoré*. Novo Nordisk CNS Division, Sydmarken 5, DK-2860 Soeborg, Denmark.

NBQX is a selective antagonist at the AMPA subtype of excitatory amino acid receptor that protects against neuronal death evoked by global ischaemia.

Here we administered NBQX (30 mg/kg, i.p.) to Mongolian gerbils 4 and 6 h after 5 min of global ischaemia and found a significant protective effect against hippocampal CA1 cell loss evaluated 4 days later. In comparison, MK-801 had only a marginal effect when given (3 mg/kg i.p.) 0.5 h after the ischaemic challenge and no effect when administered after 2 h. The decrease of 3H-AMPA binding in the CA1 hippocampus produced by ischaemia was also prevented by NBQX. When applied in vitro to hippocampus slices NBQX inhibited Schaffer-Collateral evoked synaptic responses in the CA1 region, whereas MK-801 was without any effect. NBQX had no effect on neuronal phospholipid hydrolysis evoked by excitatory amino acids. The data suggest that stimulation of AMPA receptors by post-ischaemic glutamate release is a key mechanism for ischaemic cell death.

13.4

ELEVATED CYTOSOLIC CALCIUM FOLLOWING OXYGEN-GLUCOSE DEPRIVATION IN CULTURED CORTICAL NEURONS. M.P. Goldberg and D.W. Choi. Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

We examined hypoxia-induced changes of cytosolic Ca^{2+} in vitro using fura-2 fluorescence videomicroscopy. Astrocyte-poor cortical neuronal cultures were loaded with 5 μM fura-2/AM, and then transferred to a defined medium lacking both oxygen and glucose. After 20-50 min at 37°C, cultures were returned to oxygen- and glucose-containing medium, and cytosolic Ca^{2+} was determined by 340/380 nm fluorescence excitation ratio measurement.

Immediately following 45-50 min of oxygen-glucose deprivation, cytosolic Ca^{2+} was markedly elevated (500-2000 nM) in greater than 90% of neurons. These extreme elevations in neuronal Ca^{2+} were sustained for at least 1 hr after exposure, and were associated with morphological evidence of injury, including cellular swelling, rapid leakage of intracellular fura-2, and subsequent neuronal degeneration. Exposure durations of 30-40 min increased Ca^{2+} in a smaller proportion of neurons, whereas 20 min exposure did not raise Ca^{2+} above resting levels (50-100 nM), and produced little subsequent neuronal loss. Increased Ca^{2+} and neuronal death could be blocked by NMDA antagonists 10 μM MK-801 or 100 μM dextrorphan. Ca^{2+} elevation could also be blocked by removing extracellular Ca^{2+} from the exposure medium (with 50 μM EGTA); however, cytosolic Ca^{2+} rose when cultures were subsequently returned to normal Ca^{2+}-containing solutions. These observations indicate that hypoxic neuronal injury is associated with sustained failure of Ca^{2+} homeostasis, and suggest that Ca^{2+} influx via NMDA channels is required for intracellular free Ca^{2+} elevation.

13.5

HIGH CONCENTRATIONS OF DIHYDROPYRIDINES REDUCE NEURONAL INJURY INDUCED BY PROLONGED GLUCOSE DEPRIVATION IN CORTICAL CULTURES. V.M.G. Bruno, K. Rose, S.A. Colamarino*, and D.W. Choi. Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

We investigated the protective action of dihydropyridines against the neuronal injury induced by glucose deprivation in murine cortical cell cultures containing both neurons and glia. Cultures exposed to defined solutions lacking glucose for 6 - 8 hr developed widespread neuronal injury without glial injury when examined 24 hr after insult initiation. As previously reported, both morphological and chemical (lactate dehydrogenase efflux) signs of neuronal injury could be almost fully blocked by the NMDA antagonist MK-801; however, if glucose deprivation was extended to the full 24 hr, this protective effect was overcome. In contrast, 3 - 100 μM concentrations of nifedipine produced concentration-dependent (EC_{50} about 10 μM) injury reduction even with a prolonged 24 - 36 hr insult. Similar although less consistent prolonged neuronal protection was found with 10 - 30 μM nimodipine. Interestingly, the morphinan dextrorphan was intermediate between MK-801 and dihydropyridines in reducing neuronal injury with prolonged glucose deprivation, perhaps reflecting some ability of dextrorphan to block Ca^{2+} influx through voltage-gated channels (Jaffe et al., Neurosci. Lett. 105: 227, 1989).

13.6

THE VULNERABILITY OF CULTURED CORTICAL NEURONS TO GLUCOSE DEPRIVATION-INDUCED INJURY IS INFLUENCED BY GLIAL GLYCOGEN STORES. R.A.Swanson[1] and D.W.Choi[2]. [1]Dept. of Neurology, VAMC and UCSF, San Francisco, CA; and [2]Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

CNS glycogen stores are thought to be almost exclusively in glia. Using murine cortical cell cultures, we tested the effect of altering glial glycogen stores on neuronal vulnerability to glucose deprivation-induced injury.

Cultures were prepared with increased glycogen content by 36 hr pretreatment with either 20 mM glucose, or 1 mM methionine sulfoximine (MSO), a compound which reduces glia glucose utilization and inhibits glial glutamine synthetase. Glycogen depletion was accomplished with 30 min exposure to 0.1 mM dibutyryl cAMP (dBcAMP). One day after exposure to several hours of glucose deprivation, cultures pretreated with either high glucose or MSO exhibited less than 50% of the neuronal death seen in control cultures. The effect of MSO was probably not due to reduced glutamine availability since the bathing medium contained 0.5 mM glutamine. Conversely, glycogen depletion with dBcAMP significantly increased neuronal injury. Glial glycogen stores may importantly influence neuronal vulnerabilty to glucose deprivation, perhaps by maintaining glutamate uptake and reducing excitotoxic injury.

13.7

EXTRACELLULAR ACIDOSIS WORSENS INJURY OF CULTURED CORTICAL GLIA BY COMBINED OXYGEN-GLUCOSE DEPRIVATION. R.G. Giffard, H. Monyer and D.W. Choi. Depts. of Anesthesia and Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

The extracellular acidosis associated with brain ischemia has been hypothesized to contribute to the resultant injury. We studied extracellular acid-induced injury in mouse cortical cell cultures. In mixed neuronal and glial cultures, exposure to a defined PIPES-buffered solution at pH 6.4 - 6.5 for 24 hr produced selective glia injury as demonstrated by trypan blue staining; quantitative experiments with pure glial cultures showed that release of lactate dehydrogenase (LDH) into the medium was already detectable after 6 hr. Controls exposed to the same solution at pH 7.4 were not injured. At pH 7.4, glial cultures tolerated combined oxygen and glucose deprivation for up to 9 - 12 hr before cell death occurred; however at pH 6.4, LDH release was already detectable by 3 - 6 hr. Similar results were obtained in other experiments in which the buffer included 25 mM L-lactate.

We previously showed that extracellular acidity at pH 6.4 can attenuate cortical neuronal injury due to combined oxygen-glucose deprivation (Brain Res. 506:339, 1990). Present results support the suggestion that extracellular acidity may also have a deleterious effect in the same setting, potentiating glial injury.

13.8

CHANGES IN INOSITOL PHOSPHATES DURING 'IN VITRO ISCHEMIA' IN CA1 OF RAT HIPPOCAMPAL SLICES. P. Lipton, J. Suter* and D. Lobner. Department of Physiology, University of Wisconsin, Madison, Wisconsin 53706.

Hippocampal slices were incubated with 3-H myo-inositol for 4 hours, washed for 30 minutes and then exposed to in vitro ischemia (I) for varying lengths of time ranging from 15 seconds to 5 minutes. [In (I), glucose is absent from the buffer and the buffer is equilibrated with $95\%N_2/5\%CO_2$.] In many cases, drugs were included in the buffers; in these cases buffer compositions were changed 10 minutes prior to I. Effects of drugs on control conditions were always measured.

There is a small (to 180%) transient increase in IP_3 between 30 and 60" of ischemia. There is a far larger increase in IP_2 which rises between 1 and 5 minutes of ischemia to about 500% of control levels. IP rises less, with a similar time course. The changes in IP and IP_2 after 5' are unaffected by prolonged incubation in $0\text{-}Ca^{2+}$ and/or low Na^+. They are also unaffected by 1mM ketamine, 1mM atropine, 50μM prazosin, or 1mM AP3.

The mechanism of the increase is thus not known at present. This, and the relationship of the early increases in IP_3 to ischemic damage are currently being investigated.

13.9

KETAMINE BLOCKS EARLY CHANGES IN CELL CALCIUM IN CA1 NEUROPIL DURING "ISCHEMIA". D. Lobner and P. Lipton. Dept. of Physiol., Univ. of Wisconsin, Madison, Wi 53706.

Long-term transmission failure between the Schaffer collaterals and CA1 pyramidal cells is induced by 5' exposure to buffer equilibrated with 95%N2-5%CO2 and devoid of glucose (in vitro ischemia). 1mM ketamine prevents this damage (recovery=100±8%).

Measurements of cell calcium were made using 45Ca. Slices were equilibrated with 45Ca for 45' and then exposed to ischemia for various durations. The table shows CA1 neuropil calcium in the presence and absence of ketamine, presented as % of neuropil calcium just prior to ischemia with no drug added. At 1' ischemia there is a net loss of CA1 neuropil calcium, suggesting a release of calcium from intracellular stores. By 2' ischemia there is an increase in neuropil calcium. 1mM ketamine decreases basal calcium levels, blocks the decrease in calcium at 1' ischemia, and inhibits the increase in calcium at 2 & 3' ischemia. These results suggest that ketamine blocks a release of intracellular calcium early during ischemia and also delays the later increase in total neuropil calcium during ischemia. Either, or both, of these actions may account for the protective actions of ketamine.

Period of ischemia	No drug	1mM ketamine
0'	100±2 (72)	78±2 (66)
1'	87±2 (27)	80±5 (23)
2'	118±4 (20)	70±3 (29)
3'	131±8 (15)	74±4 (15)
5'	137±6 (35)	121±5 (31)

13.10

FAILURE OF KETAMINE TO REDUCE INFARCT VOLUME RESULTING FROM TEMPORARY FOCAL ISCHEMIA IN THE RAT. T.R. Ridenour*, D.S. Warner, M.M. Todd*. Neuroanesthesia Lab, Univ. of Iowa Hospitals and Clinics, Iowa City, IA 52242

To determine if a neuroprotective effect could be observed by using the glutamate antagonist ketamine in a model of focal reversible cerebral ischemia, cerebral infarct volumes were evaluated in male SHR rats weighing between 250-350 gms after middle cerebral artery (MCA) occlusion of 2 hrs duration and a subsequent 4d postoperative survival. Three groups were investigated. 10 rats underwent ligation of the right MCA under ketamine anesthesia (2.5 mg/kg/min with a 50 mg/kg loading dose). 8 rats had the same procedure performed with halothane (0.5-1.0%) used as the anesthetic. A sham group (n=5) was treated as were the animals in the ketamine MCA group, but without ischemia. PaO_2, $PaCO_2$, cranial temp., and mean arterial BP were controlled throughout and plasma glucose levels were assessed as was EEG. 2 animals in the ketamine MCA group and 1 animal in the halothane MCA group died within the first 24 hrs postoperatively. Neurological examinations were performed pre-operatively and 4 d postoperatively before the animals were killed. The rat brains were harvested, sectioned, and stained with triphenyl tetrazolium chloride. Infarct volume was quantified using computerized planimetric analysis. There was no reduction in infarct volume in rats treated with ketamine compared with those treated with halothane during ischemia (134±51 vs 131±64 mm^3). At 4d postoperatively, there was no statistical difference in neurological examination data between the two MCA groups. Performing near baseline, the sham group was superior. The data is not consistent with the belief that ketamine offers an advantage over halothane as a neuroprotective agent during focal temporary ischemia.

13.11

KETAMINE INHIBITS LIPID PEROXIDATION IN ISCHEMIC BOVINE RETINA. M.Zhou*, T.G.Ma*, Z.He*, B.M.Rigor and M.T.Tseng. Departments of Anatomical Sciences and Neurobiology, of Anesthesiology, and of Ophthalmology and Visual Sciences, University of Louisville, Louisville, KY 40292

Lipid peroxidation disrupts membrane integrity and causes structural and functional alterations in ischemic tissues. Ketamine is a known ischemic protectant and it prevents Ca^{++} influx by antagonistic actions on NMDA receptors. However, its effect on lipid peroxidation is unknown. Here we report the influence of ketamine on lipid peroxidation in retinal preparations. First, P_2 membrane fractions from eyes removed minutes after exsanguination were exposed to lipid peroxidation inducer cadmium chloride (200 μM) or L-ascorbic acid (1 mM) with 0-10 mM ketamine. Second, P_2 membranes were prepared from retinas sustained 60 min of ischemia and were then perfused for an additional 60 min with solutions containing 0 or 1 mM ketamine. Lipid peroxidation were determined by thiobarbituric acid assay. In the first group, ketamine inhibited induction of lipid peroxidation by L-ascorbic acid or cadmium chloride. Similarly, 1 mM ketamine reduced ischemia induced lipid peroxidation in the second group. The data demonstrate that ketamine inhibits ischemia induced lipid peroxidation, and suggests an additional mechanism for ketamine's anti-ischemic actions. (supported in part by a grant from Mobil Oil Corp.)

INVERTEBRATE LEARNING AND BEHAVIOR I

14.1

PARALLEL DISHABITUATION AND SENSITIZATION WITH NO INHIBITION OF GILL-WITHDRAWAL REFLEX IN *APLYSIA*. R.D. Hawkins, T.E. Cohen*, V.A. Henzi, & E.R. Kandel. Ctr. Neurobiol. & Behav., Columbia Univ., NYSPI, & HHMI, NY, NY 10032.

Dishabituation and sensitization in *Aplysia* can be dissociated, suggesting that they may reflect different processes (Marcus et al., 1988; Mackey et al., 1988). Alternatively, some of the differences may be due to ceiling effects which limit enhancement of nonhabituated responses. To test this possibility, we investigated the effect of preshock response amplitude on sensitization of gill-withdrawal in a dissected mantle organ preparation. We found that when the preshock response is large, sensitization is modest both 2.5 and 12.5 min after shock (med. increase = 26% and 15%, $p < .01$). However, when the preshock response is small, as it is following habituation, sensitization is robust both 2.5 and 12.5 min after shock (med. = 521% and 554%, $p < .001$). These results indicate that dishabituation and sensitization of the gill component of the reflex are not dissociated when preshock response strength is equated.

These data also do not show any evidence of inhibition, in contrast to previous reports of transient inhibition preceding sensitization of the siphon component of the reflex in intact animals (Mackey et al., 1987; Marcus et al., 1988). To determine whether these differences are due to different preparations or different responses, we measured both gill and siphon withdrawal in unrestrained animals with their parapodia and purple glands removed. Again, we found that whereas siphon withdrawal is inhibited 2.5 min after shock ($p < .01$), gill withdrawal is sensitized ($p < .01$). These experiments demonstrate selective inhibition of the siphon component of the reflex and suggest that, in addition to possible ceiling effects, some of the differences between dishabituation and sensitization of siphon withdrawal may be due to the inhibition.

14.2

THE EFFECT OF INCREASED DIVALENT ION CONCENTRATION ON THE GILL WITHDRAWAL AND THE NEURONAL ACTIVITY IN THE ABDOMINAL GANGLION OF APLYSIA: AN ATTEMPT TO RESTRICT THE NEURONAL SUBSTRATE OF THE BEHAVIOR. Vadim Roshin*, Jian-young Wu, Larry Cohen, C.X. Falk* and Avrum Cohen*. Dept. of Cellular and Molecular Physiology, Yale U. Sch. of Med., New Haven, CT 06510.

Previous experiments have indicated that approximately 300-400 neurons in the Aplysia abdominal ganglion are activated by a light touch to the siphon skin that elicits the gill-withdrawal reflex. We have investigated the possibility that increasing the divalent ion concentration in the sea water bathing the ganglion would reduce interneuronal activity more rapidly than it reduces the gill-withdrawal and thus lead to a preparation that generates the gill-withdrawal reflex with fewer concomitantly active neurons. In three preparations, a mixture of 80% sea water and 20% high-divalent artificial sea water reduced the gill contraction by 50% but only reduced the number of neurons activated by the touch by 10%. In two preparations, a 50% mixture of sea water and high-divalent ASW completely blocked the gill-withdrawal but reduced the number of neurons activated by the touch by only 40%. The gill withdrawal appears to be more sensitive to the divalent ion concentration than is the number of neurons responding to the touch. Thus, our second attempt to develop a preparation which generates a gill withdrawal with substantially fewer neurons was also unsuccessful.

14.3

COMPARISON OF ACTIVITY IN THE APLYSIA ABDOMINAL GANGLION DURING SPONTANEOUS AND TOUCH ELICITED GILL WITHDRAWALS. Jian-young Wu, Vadim Roshin*, Larry Cohen, C.X. Falk* and Avrum Cohen*. Dept. of Cellular and Molecular Physiology, Yale U. Sch. of Med., New Haven, CT 06510.

Gill withdrawals in Aplysia can either occur spontaneously or can be elicited by an external stimulus. The withdrawal elicited by a light mechanical touch lasting 0.6 seconds usually begins during the stimulus and reaches a peak shortly thereafter (fast response). In one unusual isolated-siphon preparation we recorded one spontaneous withdrawal and two instances where a light touch to the siphon skin resulted in a withdrawal that reached a peak in about three seconds and that appeared to be similar to the spontaneous withdrawal. We used optical recordings to compare the neuronal substrate of the fast response and the spontaneous withdrawal. In this preparation, the neuronal activity giving rise to the spontaneous contractions was longer lasting (about 3 seconds) compared to the activity giving rise to the rapid response (about 1 second). The number of neurons and the number of spikes per neuron in the two types of withdrawal were similar. There was overlap in the neurons participating in the two types of withdrawal. About 50% of the neurons were identical in the two kinds of withdrawals; about 50% of the neurons participating in the spontaneous withdrawals had not been active during a preceding rapid response.

14.4

IDENTIFIED CENTRAL MOTOR NEURONS ARE NECESSARY FOR DIRECTIONAL SIPHON RESPONSES IN APLYSIA. C.Hickie and E.T. Walters. Department of Physiology & Cell Biology, Univ. of Texas Medical School, Houston, TX 77225.

Previous studies revealed 3 responses of the siphon to stimulation of different regions of the body: flaring to tail stimulation (T type response), constriction to head stimulation (H type), and longitudinal contraction to midbody stimulation (M type). M responses can be transformed to H or T type by associative and nonassociative training. We now report that a few previously identified central motor neurons account for H and T responses. The H response is largely mediated by a single motor neuron, RDS, hyperpolarization of which eliminates more than 90% of the H response (recorded by a photocell) to stimulation of head nerves (C2). Redriving RDS with a record of its C2-evoked activity causes a nearly identical H response. LDS cells make minor contributions to the H response. Although 3 LFS_A cells also constrict the siphon, they are inhibited by C2 stimulation. The T response is largely mediated by 4-5 LFS_B motor neurons, which are weakly electrically coupled. Hyperpolarization of 4 LFS_B cells eliminates over 90% of the T response to stimulation of tail nerves (P9). Therefore, if peripheral motor neurons contribute to H and T responses, they must be in series with this small number of central motor neurons. These findings will simplify cellular analyses of the selection and plasticity of siphon responses.

14.5

MODULATION OF THE EXCITABILITY OF PERIPHERAL RECEPTIVE FIELDS AND AXONS IN SENSORY NEURONS OF APLYSIA. A.L. Clatworthy and E.T. Walters. Dept. of Physiology & Cell Biology, Univ. Texas Medical School, Houston, TX 77225.

We reported last year that tail pinch reduces the number of sensory neuron action potentials elicited by test shocks to the nerve containing the sensory neuron's axon. We have since found that tail pinch reduces the number of spikes elicited by nerve shocks near spike threshold, but has no significant effect on spikes elicited by nerve shocks 2-3 times spike threshold (n=12 cells). Therefore, tail pinch can reduce the excitability of the sensory axon, but the safety factor for central conduction of sensory spikes is usually (although not always) adequate to prevent conduction block. This also suggests that short-term alterations of axonal excitability might occur within the sensory neuron receptive field (RF). Complex short-term modulation of RF excitability was found using cutaneous test stimuli (0.5 sec taps, 0.5 sec dc shocks, and 20 Hz trains of 5 msec shocks). Most stimuli showed "windup", a decrease in spike latency and increase in spike number when the test stimulus was repeated at 5 sec intervals (n=36). Windup was greater with more intense stimuli. Prolonged testing sometimes caused depression of excitability after windup. Depression of RF excitability (for 2-8 min) was also produced by strong shock inside the RF (n=10), as well as by high frequency activation of the soma (n=5).

14.6

DIFFERENTIAL MODULATION OF EXCITABILITY AND SPIKE DURATION IN THE TAIL SENSORY NEURONS OF DEVELOPING *APLYSIA*. E. A. Marcus and T.J. Carew. Departments of Biology and Psychology, Yale University, New Haven, CT 06520.

Recent evidence from a variety of systems has demonstrated that a single modulatory neurotransmitter can have multiple effects on the firing properties of an individual neuron. For example, as shown by Baxter and Byrne (1990), serotonin (5HT) modulates potassium conductances in the tail sensory neurons of adult *Aplysia* to produce an increase in both excitability (principally through modulation of I_{KS}), and spike duration (principally through the modulation of I_{KV}).

To understand how such complex biophysical effects of modulatory neurotransmitters emerge during development, we have characterized the effects of 5HT on excitability and spike duration in tail sensory neurons of Late Stage 12 juvenile *Aplysia*. We found that 5HT (50 μM) produces a significant increase in excitability ($\bar{x}$ = 332%, $p<0.0025$), which is comparable to that reported for adult animals (Baxter and Byrne, 1990). Thus the ability of 5HT to modulate I_{KS} appears to be fully mature by Late Stage 12. In contrast, 5HT produces only a modest increase in spike duration ($\bar{x}$= 12.81%, $p<0.026$). This increase is significantly smaller ($p<0.001$) than that observed in adults ($\bar{x}$=44.8%, $p<0.0001$). These results suggest that the ability of 5HT to modulate I_{KV} is not fully mature by Late Stage 12.

Since the effects of 5HT on I_{KV} and I_{KS} are known to be mediated by different second messenger systems in adult *Aplysia* (Baxter and Byrne, 1990), it should now be possible to use the tail sensory neurons to examine how complex neuromodulatory effects and second messenger interactions are assembled during development.

14.7

CONTRIBUTION OF INTERNEURONS TO TAIL-SHOCK INDUCED INHIBITION OF THE SIPHON WITHDRAWAL REFLEX IN *APLYSIA*. W. G. Wright and T. J. Carew. Department of Psychology, Yale University, New Haven, CT 06520.

Strong tail shock (TS) produces transient inhibition of the water-jet elicited siphon withdrawal reflex (SWR) in *Aplysia*. Our previous experiments suggested that some of this inhibition may be mediated by interneuronal processes (Wright et al. 1988, 1989). The present study explored this suggestion by examining inhibitory interneurons (cells producing IPSPs onto excitatory interneurons or siphon motor neurons (MNs)) which are activated by water jet stimuli to the siphon.

Using a semi-intact preparation that shows TS-induced inhibition of both the SWR and the water-jet elicited complex EPSP in siphon MNs, we found the following: 1)Intracellular activation of the inhibitory interneuron L16 (Hawkins et al., 1982; Frost, 1990) inhibits water-jet elicited complex EPSPs in MNs; 2)L16 is activated by TS; 3)The inhibitory connection between L16 and the excitatory interneuron L29 is facilitated by intracellular activation of L16 as well as by TS; 4)L16 may play a causal role in TS-induced inhibition, since hyperpolarizing L16 during TS can diminish inhibition of the complex EPSP (2 of 3 preparations); 5)Similar to L16, intracellular activation of another inhibitory interneuron facilitates its own inhibitory connection to L29, and inhibits the complex EPSP. However, this cell does not appear to be activated by TS (we are currently further characterizing this cell).

In summary, we have identified several interneuronal sites in the SWR that may contribute to TS-induced inhibition. Our goal is to quantitatively determine the degree to which these and other sites can account for the magnitude and time course of the behavioral inhibition.

14.8

CHEMICAL CUES MEDIATE INTER-ANIMAL INHIBITION OF SIPHON WITHDRAWAL IN *APLYSIA*. M. Stopfer, X. Chen*, and T.J. Carew. Department of Psychology, Yale University, New Haven, CT.

It has recently become apparent that noxious stimuli such as strong shock can produce significant inhibition of the siphon withdrawal reflex (SWR) of *Aplysia*. We here describe that chemical cues released from animals receiving strong shock produce reflex inhibition in neighboring unshocked animals.

Aplysia were randomly designated Experimental (EXP) or Control (CON). Each animal was then placed into an individual tank. A second (donor) animal was added to each tank, separated from the EXP or CON animal by a perforated partition. After 15 min, the SWR was elicited in EXPs and CONs by mild tail shock in three pre-tests (10 min ITI). Next, in the training phase, a second experimenter delivered strong head shocks to the donor in the EXP tank. Head-shocked donors invariably inked. Neither animal in the CON tank received head shock; rather, to preserve blind procedures, a dye that was indistinguishable from *Aplysia* ink was added to the CON tank water. The first experimenter then conducted blind tests (identical to pre-tests) 4, 10, 20, and 30 min after training. CONs (n=26) showed no significant change from baseline in any test. In contrast, EXPs (n=24) were significantly inhibited relative to their own pre-scores at all times tested (at least $p<0.01$). Moreover, they were significantly inhibited relative to CON animals in the 4 and 10 min tests ($p<0.001$ in each). After the 10 min test, EXP scores trended toward baseline despite the continued presence of chemical factors in the water.

Preliminary evidence from blind experiments using ink obtained from the ink glands of naive, anesthetized animals suggests that at least one inhibitory cue is the ink itself. Since the neural circuits mediating inking and the SWR in *Aplysia* are both relatively well understood, our results raise the possibility of examining inter-animal communication in *Aplysia* at a cellular level.

14.9

SENSITIZATION AND DISHABITUATION IN *TRITONIA*: EXCITATORY EFFECTS OF CHEMICAL STIMULI. G. BROWN AND A.O.D. WILLOWS. Friday Harbor Laboratories, Friday Harbor, WA 98250

We are studying the long-lasting effects of various types and combinations of stimuli on the behavior and neural circuitry of the sea slug *Tritonia*. Previous studies indicated that a chemical stimulus which triggers escape swimming in *Tritonia* will sensitize the animal's response to subsequent presentations of noxious stimuli. We now report that a chemical stimulus which does not elicit the swim response can also produce sensitization. An experimental group of animals received five presentations of a ratfish liver extract at five minute intervals followed in ten minutes by .15 mls of 4M NaCl on the tail. Controls were given only this tail-salt stimulus (that typically triggers the escape swim response). Animals that received sensitization training had increased locomotory behavior and a much longer swim episode (avg. 9.7 cycles vs. 5.6 for controls; N=10, $p<.05$).

A different kind of excitatory effect observed was the restoration of a habituated response by a concentrated salt stimulus to the head. The tail-salt stimulus was given repeatedly at ten minute intervals until the escape swim response was not elicited on five consecutive trials. Experimental animals were then given 1 ml of 4M NaCl to the head to elicit swimming. Control animals were not given this dishabituating stimulus. Experimental animals showed a significantly higher probability of swimming to the next tail-salt stimulus (8/10 vs 1/10 for controls, $p< .02$).

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14.10

5-HT MODULATES TWO DISTINCT K^+ CURRENTS IN *HERMISSENDA* TYPE B PHOTORECEPTORS. J. Acosta-Urquidi and T. Crow. Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, TX 77030.

Serotonin (5-HT) has been proposed to play a role in conditioning of *Hermissenda*. Previous research has shown that 5-HT enhances the peak and plateau phases of the generator potential of identified type B photoreceptors (Crow and Bridge, 1985; Farley and Auerbach, 1986). Two electrode voltage-clamp techniques were employed to investigate the effects of 5-HT on membrane conductances in medial and lateral type B photoreceptors. Here we report two voltage-gated K^+ currents not previously described in medial and lateral type B photoreceptors and their modulation by 5-HT.

With I_A and $I_{K(Ca)}$ removed, the dominant outward K^+ current is $I_{K(V)}$, the delayed rectifier. An inward rectifier, termed I_{IR}, evoked by hyperpolarizing steps is also present in most cells (85% of cells examined under voltage-clamp, n=40). $I_{K(V)}$ activates at around -15mV, and has a τ_{off} between 250 and 350 ms. $I_{K(V)}$ is blocked by TEA and partially blocked by 4-AP (5mM), and exhibits marked twin-pulse inactivation which is quickly removed by conditioning hyperpolarization. The rate of decay of $I_{K(V)}$ was slowed by 5-HT, and twin pulse inactivation was reduced. After the application of 5-HT the average % increase in τ_{off} $I_{K(V)}$ compared to control values was 24±4 (n=5).

I_{IR} activates at potentials around -60 mV and is markedly voltage-dependent. I_{IR} is blocked by 4-AP (5mM) and TEA but not by Ba^{2+} or Cd^{2+}. 5-HT (10^{-4} M) produced a strong enhancement of I_{IR}. Following the application of 5-HT, the average % change in I_{IR} relative to baseline values was 127.3 ± 34 (n=10). The contribution of $I_{K(V)}$ and I_{IR} to the generator potential of type B photoreceptors is under investigation.

14.11

NEURAL CORRELATES OF CONDITIONING IN IDENTIFIED PEDAL NEURONS IN *HERMISSENDA*. T.M. Hodgson and T. Crow. Dept. of Neurobiology and Anatomy, Univ. of Texas Med. Sch., Houston, TX, 77225.

In the course of the investigation of the neural circuitry underlying the expression of behavioral changes associated with conditioning in *Hermissenda*, four light-responsive cell types were identified from intracellular recordings of putative motor neurons in the pedal ganglia. Subsequent experiments showed that the identified pedal cells' light responses were due to input from the photoreceptors (Hodgson and Crow, 1987, 1989). To determine if the light responses of the identified pedal neurons could be modified by conditioning, *Hermissenda* were subjected to one of two procedures: Paired presentations of light and rotation, or random presentations of the two stimuli. The light responses of the identified pedal neurons recorded from animals which received paired stimuli were reduced, as compared with the light responses recorded from random or normal controls. Pedal cell P7, which shows a hyperpolarizing light response, showed a weaker response to 5 min light steps in animals which received paired stimuli (21.8±11.6% decrease in impulse activity compared with baseline activity in the dark;n=4, mean±SEM), as compared to random controls (66.0±8.1% decrease in activity vs. baseline activity in the dark;n=2) and a weaker response to 10 sec light steps in paired animals (37.8±6.11% decrease in activity vs. baseline activity in the dark;n=9) vs. normal controls (48.8±6.4% decrease in activity vs. baseline activity in the dark;n=29). Pedal cell P8, which has a depolarizing light response, showed weaker responses to 10 sec light steps in paired animals (13.8±4.8% increase in activity vs. baseline activity in the dark;n=5) vs. random controls (33.7±8.3% increase in activity vs. baseline activity in the dark;n=3). Cell P9, which also has an excitatory response to light, showed weaker responses to 10 sec light steps in paired animals (14.5±6.6% increase vs. baseline activity in the dark;n=7) vs. normal controls (24.7±3.5% increase compared to baseline activity in the dark;n=29).

14.12

HERMISSENDA PHOTORECEPTORS EXHIBIT SYNAPTIC FACILITATION AS WELL AS ENHANCED EXCITABILITY. E.M. Schuman and G.A. Clark. Program in Neuroscience, Princeton University, Princeton, NJ 08544.

Two forms of neuronal plasticity frequently proposed to underlie learning are changes in synaptic strength and changes in neuronal excitability. The relationship between these two changes remains unclear, in part because they have often been analyzed in different systems (e.g., *Aplysia* and *Hermissenda* respectively). To help clarify these issues, we investigated whether application of serotonin (5-HT) or activation of protein kinase C (PKC), procedures which mimic the effects of conditioned suppression of phototaxis and enhance cellular excitability in *Hermissenda* Type B photoreceptors, also modulate synaptic transmission in these same cells. Acute application of 5-HT dramatically enhanced the size of the monosynaptic inhibitory postsynaptic potential (IPSP) elicited by Type B cell stimulation in Type A photoreceptors (mean ± s.e.m.: pre: .31 ± .09 mV; post: .74 ± .18 mV; $t(9) = 3.81$, $p < .005$). In addition, acute application of phorbol 12-myristate 13-acetate (PMA), a phorbol ester that activates PKC, also produced a marked facilitation of the IPSP produced by B cell stimulation (pre: .37 ± .13 mV; post: 1.31 ± .40 mV; $t(7) = 2.85$, $p < .05$). Control preparations exposed to an inactive phorbol ester or sea water showed no significant changes. Consistent with previous findings, 5-HT and PMA application also produced an increase in the Type B cell input resistance (5-HT: pre: 38.3 ± 4.1 Mohm; post: 46.9 ± 3.6 Mohm; $t(9) = 2.41$, $p < .05$; PMA: pre: 38.5 ± 2.7 Mohm; post: 57.3 ± 4.8 Mohm; $t(7) = 2.96$, $p < .02$), but had no significant effect on Type A cell input resistance, suggesting changes were presynaptic. These results indicate that the same neurotransmitter (5-HT) and intracellular second messenger (PKC) that produce excitability changes in Type B cells also facilitate synaptic transmission. We now plan to investigate whether associative training paradigms also produce synaptic facilitation in addition to enhanced neuronal excitability, and to explore further the functional and mechanistic relationship between these two forms of plasticity.

EPILEPSY: BASIC MECHANISMS I

15.1

GIANT IPSP's ELICITED BY THE GABAERGIC INHIBITORY CIRCUIT IN THE HIPPOCAMPUS
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Feedforward and feedback pathways excite GABAergic interneurons through activation of glutamatergic synapses. We report here a novel mechanism by which GABAergic neurons synchronize in the absence of excitatory amino acid (EAA) neurotransmission.

Transverse slices of guinea pig hippocampus were used. EAA synaptic transmission was blocked with perfusion of CPP (10 uM) and CNQX (10 uM). Under these conditions, 4-aminopyridine (75 uM) elicits giant IPSP's (6-15 mV; 1-4 sec duration, at a resting potentials of -60 mV) in principal cells throughout the hippocampus. Field responses always accompanied giant IPSP's. These hyperpolarizations were significantly larger than baseline unitary inhibitory events (1-3 mV).

Intracellular recordings of dentate hilar neurons revealed a subpopulation of interneurons which exhibited a burst of action potentials riding on a depolarizing wave during the synchronized event. The amplitude of the underlying wave decreased with membrane depolarization, but could not be reversed because it rectified. Unitary ipsp's reversed at -70 mV. Depolarizing bursts in these neurons were still observed when unitary IPSP's were hyperpolarizing. Paired recordings indicated that the giant IPSP in principal cells occurred simultaneously with the bursting response of this subpopulation of hilar neurons. Both the unitary and synchronized IPSP's in all cells were blocked by picrotoxin (25 uM). Picrotoxin also abolished the depolarizing burst responses. Cells that produced burst responses are probably GABAergic interneurons since synchronized discharge of these neurons produced giant IPSP's in projected neurons. We propose that GABAergic interneurons become synchronized via their recurrent connections through a novel excitatory action of GABA.

15.2

EPILEPTIFORM ACTIVITY IN MICROCULTURES CONTAINING SMALL NUMBERS OF HIPPOCAMPAL NEURONS, Michael M. Segal and Edwin J. Furshpan. Department of Neurobiology, Harvard Medical School, Boston, MA 02115

Microcultures containing small numbers of hippocampal neurons were grown for periods of 3 to 7 weeks. The microcultures were made by spraying drops of collagen onto a layer of agarose. Plating of cells onto such dishes resulted in growth of neurons on glial cells attached to the collagen patches. Connections between nearby microcultures were prevented by the properties of the agarose surface. Within each microculture, neurons formed strong chemical synaptic connections, with monosynaptic fast-excitatory, fast-inhibitory and slow-inhibitory synaptic actions, sensitive to blockers of glutamate and GABA transmission (NMDA, non-NMDA, $GABA_A$ and $GABA_B$ blockers). Small networks with as few as one or two neurons generated epileptiform activity that closely resembled the epileptiform activity seen in mass cultures containing thousands of neurons (Furshpan & Potter, 1989). The epileptiform activity was observed when microcultures that were grown for weeks in blockers of synaptic activity (kynurenate and elevated Mg^{2+}) were washed free of these blockers. Such a microculture technique allows study of epileptiform activity in a simplified system and facilitates analysis of the synaptic actions underlying the epileptiform activity.

15.3

LAYER 5 IS THE PREFERRED PATHWAY FOR HORIZONTAL PROPAGATION OF EPILEPTIFORM DISCHARGES IN NEOCORTEX. A.E. Telfeian, M.S. Wehr* and B.W. Connors, Sect. of Neurobiology, Div. of Biology & Medicine, Brown Univ., Providence, RI 02912.

Epileptiform discharges propagate across neocortex when $GABA_A$-mediated inhibition is reduced. We examined the preferred routes of propagation in slices of rat SI *in vitro*. Vertical cuts created bridges comprising single layers, which connected areas of intact cortex. Alternatively, horizontal cuts isolated strips with only a subset of the layers. When bathed with high doses of picrotoxin (35 μM) any small bridge (~350 μm wide) or strip (~500 μm wide) sustained propagation, regardless of laminar position; no particular pathway was necessary. However when picrotoxin was lowered to 2.5 μM, only bridges and strips containing a patent layer 5 supported propagation. After cutting slices along the layers 4/5 border, we recorded and stained layer 5b pyramidal neurons with most of their apical dendrites removed. The proportions of intrinsic burst-firing (33%) and oscillatory (50%) neurons in intact and cut slices were indistinguishable. Apparently intrinsic bursting and oscillations are not dependent upon the apical dendritic tree. Focal ejections of GABA in layer Vb greatly slowed or arrested propagating discharges. Other layers were much less sensitive to GABA. We conclude that the neurons and axonal connections of layer 5 are critical for the horizontal propagation of epileptiform discharges.

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15.4

MAPPING THE PATHWAY OF DIFFERENT SEIZURE MODELS BY USING EARLY INDUCIBLE GENE EXPRESSION. P.Lanaud§, R.Maggio*†, K.Gale†, and D.Grayson§. §FGIN and †Department of Pharmacology, Georgetown University Medical Center, Washington, D.C. 20007.

Using *in situ* hybridization histochemistry for the detection of several early inducible genes (EIG) we examined pathways activated by seizures evoked by a focal application of bicuculline in the limbic system or the inferior colliculus. In situ hybridization was performed on 10 μM coronal sections using cDNA probes encoding c-fos, c-jun, jun-B, and zif/268. 30 min after the initiation of limbic motor seizures from a highly discrete epileptogenic site in the deep prepiriform cortex, each mRNA was markedly increased in the hippocampal formation, amygdala and entorhinal cortex.

Seizures evoked from inferior colliculus were distinct from the limbic seizures both in terms of behavioral activity and anatomic pattern of EIG expression. Within several hours following the seizures, the expression of EIG mRNA returned to baseline but could be re-induced 24 hours later by a second seizure. It appears that expression of EIG can be used to functionally map pathways involved in seizure propagation and that the anatomic pattern of activation is selectively related to the type of seizure evoked.

15.5

ANATOMICAL CHARACTERIZATION OF THE FUNCTIONAL INTERRELATION BETWEEN AREA TEMPESTAS AND SUBSTANTIA NIGRA IN SEIZURE PROPAGATION.
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We performed autoradiographic analyses of brain sections from rats receiving ^{14}C-2-deoxyglucose (2DG), during bilateral motor seizures evoked from unilateral application of bicuculline methiodide (118 pmol) into an epileptogenic site in the deep prepiriform cortex, "area tempestas" (AT). 2DG accumulation occurred selectively in limbic structures ipsilateral to the stimulated AT: entorhinal cortex, ventral hippocampus, amygdala, n. accumbens and basal forebrain, perirhinal cortex, ventromedial caudate, septum and olfactory bulb. Pronounced bilateral activation occurred in mediodorsal and ventrolateral thalamus and substantia nigra; increases were also seen bilaterally in dorsal hippocampus. Bilateral application of the $GABA_A$ agonist muscimol into SN completely suppressed seizures evoked from AT. In these animals, all seizure-associated increases in 2DG accumulation were prevented. Unilateral application of muscimol in SN ipsilateral to the injected AT did not alter seizure activity but eliminated thalamic increases in 2DG, and partially reduced increases in 2DG in other structures. Our results suggest that the anatomic pattern of increase in 2DG accumulation is dependent upon seizure activity and is subject to nigral regulation.

15.6

SYNERGISTIC EFFECTS OF CO-INJECTION OF MU AND GLUTAMATE RECEPTOR AGONISTS INTO THE VENTRAL HIPPOCAMPUS IN PRODUCING SEIZURE ACTIVITY.
L. Thai, X. P. He, W. Q. Zhang and J. S. Hong. LMIN, NIEHS/NIH, Research Triangle Park, NC 27709.

Previous studies from our laboratory showed that injections of mu opioid receptor agonists into the ventral hippocampus produced behavioral convulsions in rats. The purpose of this study was to examine the interactions between opioid and glutamate receptor agonists in the regulation of hippocampal excitability. Coinjection of low doses of kainic acid (3.1 ng) or NMDA (2.5 µg) with a Mu receptor agonist (PL017, 5 µg) into the ventral hippocampus produced severe, continuous behavioral convulsions within 1 to 2 hours after injections. Injection of either compound alone into the same or other areas of the limbic system such as the dorsal hippocampus, substantia nigra, amygdala, entorhinal cortex, or subiculum would not induce status epilepticus. This study provides evidence indicating a positive interaction between Mu and glutamate receptor agonists in the ventral hippocampus in inducing epileptic activity which is consistent with the hypothesis that enkephalin and glutamate might act synergistically in the perforant path terminals to control the excitability of the hippocampus.

15.7

LITHIUM MODIFIES CONVULSIONS AND BRAIN PHOSPHOINOSITIDE TURNOVER INDUCED BY ORGANOPHOSPHATES.
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Inositol-1-phosphate (Ins1P), an index of phosphoinositide (PI) turnover was measured in frontal and piriform cortices, caudate, thalamus, hippocampus and cerebellum from saline or LiCl (5mEq/kg; sc) pretreated rats 60 minutes after exposure to graded doses of paraoxon or soman. Both organophosphates (OP) produced convulsions at lower doses in LiCl than saline pretreated rats. Both soman and paraoxon when given in a convulsive dose range produced changes in regional Ins1P levels that correlated better with the presence or absence of convulsions than the dose or the OP used. This was true both in saline and LiCl pretreated rats. In saline pretreated **nonconvulsing** rats, there was a "cholinergic" increase (1.5 - 2 X) in Ins1P in all regions except cerebellum after OP injection. In saline pretreated **convulsing** rats, there was a marked "seizurogenic" further increase in Ins1P; highest in caudate and cortex. In LiCl pretreated **nonconvulsing** rats, a "cholinergic" increase in Ins1P was significant only in caudate, thalamus and hippocampus. In LiCl pretreated **convulsing** rats, the further "seizurogenic" increase in Ins1P was less than in the saline group except in thalamus and hippocampus. Thus, OP produce both a "cholinergic" and a "seizurogenic" increase in PI turnover. These data suggest that increased PI turnover in the hippocampus may be involved in the lowering of the seizure threshold for OP in the LiCl pretreated rats. Support: DAMD17-83-C-3242.

15.8

DOPAMINE REGULATION OF SEIZURES INDUCED BY LITHIUM+PILOCARPINE.
P.Barone, A. de Bartolomeis*, V. Palma*, G. Muscettola* and G. Campanella*. 2nd Sch. of Med, Univ. of Napoli and Inst. Res. Sanatrix, Venafro, Italy.

Experimental evidence links central dopaminergic mechanisms to the control of seizures. For example, D-1 receptor stimulation reduces, while D-1 receptor blockade elevates the threshold for pilocarpine-induced seizures (Barone et al., *Neurosci.*, 34:209, 1990). The present study evaluates whether dopamine system manipulation may also affect the seizures induced by the combined administration of lithium plus pilocarpine: it is, in fact, well known that lithium chloride (LiCl) may potentiate pilocarpine-induced seizures.

Male Sprague Dawley rats were treated with LiCl (4 mEq/kg; i.p.) 24 hr prior to the intraperitoneal injection of either saline or the D-1 antagonist SCH 23390 (0.01-0.1 mg/kg) or the D-2 antagonist haloperidol (0.5-2 mg/kg). After 5 min all rats were given 15 mg/kg of pilocarpine (i.p.), a dose which induces convulsions in 50% of LiCl-pretreated animals.

At all doses tested, the D-1 antagonist prevented, while the D-2 antagonist potentiated, the convulsive activity as revealed by behavioral, electroencephalographic alterations and widespread brain damage.

This study indicates that the two dopamine receptor subtypes, D-1 and D-2, exert opposing roles in the control of epilepsy propagation. Moreover, these results suggest that the toxic effect of lithium on brain excitability may be mediated by the dopamine system.

15.9

THE TEMPORAL PROFILE OF BRAIN DAMAGE FROM PILOCARPINE-INDUCED SEIZURES.
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Although extensive neuronal damage is known to occur after several hr of pilocarpine (PC)-induced seizures, the time course of appearance of this damage in different brain regions has not been studied. Seizures were induced in male Wistar rats with 400-500 mg/kg PC i.p. Rats underwent brain perfusion-fixation after seizures of 10-20 min, 40 min, 1 hr and 3 hr or were given anticonvulsants after 3-hr seizures and recovered for 24 or 72 hr prior to perfusion. Neuronal necrosis was graded on a 0-3 scale.

Necrotic neurons first appeared in the dentate gyrus (DG) and cerebral cortex 40 min after seizure onset. Mild-to-moderate neuronal damage (grades [gr] 0.5-2) was seen in hippocampal CA1-4 regions, DG, amygdala (A), piriform (PCx), entorhinal and cerebral cortices, and septal nuclei after 1-hr and 3-hr seizures. After 3-hr seizures, thalamus (T), substantia nigra and caudate-putamen (CP) also showed mild damage (gr 0.5-1). Neuronal injury worsened in ventral CA1, A and PCx (gr 3), T (gr 1-2) and CP (gr 1) 24 hr after 3-hr seizures but did not progress at 72 hr. Neuronal necrosis first appears 40 min after seizure onset and is maximal 24 hr after 3-hr seizures. Delayed neuronal death does not occur 24-72 hr after seizures. DG, CA2 and CP neurons, believed to be resistant, are vulnerable to seizure-induced damage.

15.10

EFFECTS OF APPLIED ELECTRIC FIELDS ON EPILEPTIFORM NEURONAL ACTIVITY.
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Constant electrical fields have been shown to be capable of modulating normal neuronal electrical activity with amplitude around 10mV-20mV/mm. These experiments suggested that it should also be possible to modulate epileptiform activity . We analyzed the effect of applied electric fields using monopolar electrodes on penicillin-induced epileptiform activity in hippocampal CA1 neurons in-vitro.

Penicillin (3000 units/ml) was added to the artificial CSF and orthodromic stimulation was applied in order to generate the epileptiform activity. A 50µm diameter electrode was located above the somatic layer and quasi-static (50ms, 0-50µA) current pulses were applied in order to generate electric fields. The results show that the amplitude of the population spikes was largely decreased as the amplitude of an anodic applied current was increased. This effect was observed in all 52 slices tested and complete abolition of the population spikes was observed at a mean current amplitude of 40uA. The mechanism of this large reduction was attributed to a polarization effect of the current producing hyperpolarization at the soma and depolarization at the dendrites. This mechanism was verified with intracellular recordings in 8 cells. The number of action potentials decreased with the application of the current until all action potentials were blocked. The difference between the intracellular and the extracellular voltage showed a net hyperpolarization at the soma correlated with the amplitude of the applied current.

Further increase of the amplitude of the current produced excitation as expected but at current levels averaging 80 uA (N=4). Therefore, these results revealed the existence of a stimulation window in which epileptiform activity can be successfully blocked without simultaneous excitation.

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16.1

SEQUENTIAL AUTORADIOGRAPHIC ASSESSMENT OF TWO FUNCTIONAL/BEHAVIORAL STATES IN THE SAME ANIMAL

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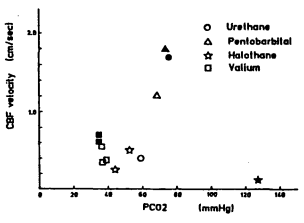
We have incorporated the FDG/PET sequential-study methodology (Chang et al., J Nucl Med 28:652-860, 1987) and the refined DG model (Schmidt et al., J Cereb Blood Flow Metab. 9:290-303,1989) into a sequential-study method with autoradiography. Similar to the described PET method, the proposed tissue autoradiographic method for measuring two sets of lCMRglu values involves two intravenous injections of radiolabeled deoxyglucose, ^{3}H-2DG coupled with ^{14}C-2DG, 40-45 min apart. At 80-85 minutes(T2) after the first injection, the animal is sacrificed and the brain frozen and sectioned. The tracer concentrations of $^3H(C_1^*(T2))$ and $^{14}C(C_2^*(T2))$ are separated and measured at the end of the study using photographic methods. The lCMRglu values of the second experimental state (lCMRglu-2) are obtained directly using the measured tracer concentration from the second injection ($C_2^*(T2)$) and the operational equation of the 2DG method. The fact that the concentration of the first tracer measured in the brain at the end of the study ($C_1^*(T2)$) consist of 1) the tracer that has been taken up during the first experimental state but is still present at time T2 ($R_1^*(T2:T1)$); and 2) the tracer taken up during the second experimental state ($C_1^*(T2:T1)$) from the first injected tracer that is still present in plasma. If the chemical forms of both injected 2DG are identical, $C_1^*(T2:T1)$ can be approximated by the amount of cerebral uptake from the second 2DG injection ($C_2^*(T2)$). Subsequently, $C_1^*(T1)$ can be estimated from its remnant($R_1^*(T2:T1)$) through their relationship described by the kinetic model, and then lCMRglu-1 can be determined. This method should allow us to elucidate the relationship between behavior and regional cerebral metabolism.

16.2

PHYSIOLOGICAL MAGNETIC RESONANCE IMAGING OF BRAIN: BLOOD FLOW AND BLOOD OXYGENATION LEVEL DEPENDENT CONTRAST. S. Ogawa and T.M. Lee*. AT&T Bell Labs., Murray Hill, NJ 07974

Blood Oxygenation Level Dependent (BOLD) contrast of brain MRI at high magnetic fields is sensitive to variations of the physiological state of brain. BOLD contrast, caused by paramagnetic deoxyhemoglobin in the venous blood, depicts the brain vasculature by numerous dark lines in the image, although capillaries are too small to be resolved. Since the venous blood oxygenation level is determined by the supply and demand of oxygen at blood capillaries, we examined the correlation between BOLD contrast and the blood flow velocity measured by MRI at the sagittal sinus when the systemic CO2 level and the oxygen content in the ventilating gas were varied (Fig.1). In addition to BOLD contrast, the image signal intensity of tissue water was dependent to some extent on the venous blood oxygenation level, presumably because the water T2 was influenced by the microcirculation of tissue water around capillaries with deoxyhemoglobin. A simple observation was made on an acute effect of ethanol infusion to an anesthetized rat where induced changes in the brain image differed among various regions of the brain. Brain images with these MRI characteristics can be used to study non invasively effects of CNS drugs or various external stimuli on brain physiology.



16.3

A METHOD FOR RAPID REPEAT MEASUREMENTS OF REGIONAL CEREBRAL BLOOD FLOW (rCBF) WITH POSITRON EMISSION TOMOGRAPHY (PET) AND O-15 WATER. P. Herscovitch, R.E. Carson*, B. Zunkeler*, G. Jacobs* and P. Plasciak*, NIH, Bethesda, MD 20892.

O-15 labelled water is widely used to measure rCBF with PET. Although the half-life of O-15 is short, (123 sec), typically one waits about 12 min for the radioactive background to decay before a repeat rCBF study. We have developed an approach to perform sequential rCBF measurements more rapidly by accounting for residual background radioactivity. Immediately after the initial PET study, a new physiologic state, beginning at time T, can be established. A short scan is obtained to record the brain radioactivity remaining from the previous O-15 injection. Then, after a second O-15 injection, further scan data are collected. Brain radioactivity after time T is described by an equation based on the one-compartment Kety model:

$$Ctot(t) = C(T)\exp[-f(t-T)/\lambda] + f\,Cart(t)^{*}\exp[-ft/\lambda],$$

where C(T) is the residual brain radioactivity at T, f the flow, Cart(t) the arterial time-activity curve, λ the partition coefficient of water, and * the operation of convolution. A rapid least squares method was used to fit values for f, C(T), and λ to the measured scan and blood data after time T. The method was implemented on a Scanditronix PC2048-15B scanner, with automated continuous blood sampling. Its feasibility was demonstrated by repeat rCBF studies obtained within 6-7 min in 2 anesthetized baboons. The correction for residual background ranged from 4-14%, and was greater in lower flow regions. By increasing the frequency at which rCBF measurements with bolus O-15 water can be repeated, our approach provides greater flexibility for brain mapping and other PET studies.

16.4

CEREBRAL BLOOD FLOW (CBF) HETEROGENEITY: COMPARISON OF TWO TRACERS. S.C. Jones, R.M. Bryan, Jr., A.D. Perez-Trepichio and M. Shea*. Div. of Neurosurgery, Hershey Medical Center of Pennsylvania State Univ., Hershey, PA 17033 and Cerebrovascular Research Lab., Cleveland Clinic Foundation, Cleveland, OH 44195.

CBF heterogeneity in terms of ~400 μm columns has been observed under the conditions of quick brain freezing and with less than 15 sec study times using ^{14}C-iodoantipyrine (^{14}C-IAP) or CBF agents that are highly diffusible and chemically trapped such as Ceretec (Amersham, ^{99m}Tc-HMPAO) or Spectamine (Mediphysics, ^{123}I-IMP). This spatial heterogeneity could be due to the temporal (~6/min) heterogeneity of CBF or the vascular architecture of penetrating arteries.

In 8 pentobarbital anesthetized, freely breathing, Sprague-Dawley rats with (mean ± SEM), MABP 129 ± 6; $PaCO_2$ 49 ± 3; PaO_2 86 ± 8; and pH 7.36 ± 0.01, double tracer autoradiography with the indicator fractionation autoradiographic technique using either ^{99m}Tc-HMPAO or ^{123}I-IMP and ^{14}C-IAP was performed. Two time dependent protocols were used: one (n = 5) involved the simultaneous administration of ^{14}C-IAP and ^{99m}Tc-HMPAO 10 sec before decapitation; in the other (n = 3), ^{99m}Tc-HMPAO or ^{123}I-IMP was administered either 15 or 25 sec before, and the ^{14}C-IAP 10 sec before, decapitation. The positions of the columns were compared.

In both sequential and the simultaneous protocols, the distribution of the tracers was similar. In all cases the ^{99m}Tc-HMPAO and ^{123}I-IMP showed higher contrast and sharper edges, whereas the ^{14}C-IAP showed the same pattern, but more diffusely. This distribution is most likely due to transport and retention of ^{99m}Tc-HMPAO or ^{123}I-IMP in the larger arterioles, as opposed to the capillary distribution of ^{14}C-IAP, but could also be due to the post-decapitation diffusion of ^{14}C-IAP. These observations confirm the hypothesis that the columnar distribution of cortical CBF is related to the vascular architecture of the penetrating arterioles.

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16.5

ASSESSMENT OF XE-127 AS A DYNAMIC SPECT rCBF TRACER IN NORMAL SUBJECTS AT REST

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Xe-133 has been the standard for the measurement of rCBF by dynamic SPECT. The need for more usable counts at a lowered patient dose makes the Xe-127 potentially a better isotope. In order to compare actual flow images and quantification we studied eight subjects at rest using both Xe-133 and Xe-127 with a Tomomatic 564 scanner. On each test day the subject had two studies; the first was a sham and the second was the actual study with either Xe-133 or 127. The Xe-133 studies were performed with a high sensitivity five slice collimator (MHS-5) with nominal resolution of 16 mm FWHM. The Xe-127 studies were done with a high resolution (9 mm FWHM) three slice collimator (HR-3), or a medium sensitivity and resolution (12 mm) five slice collimator (MHR-5). The studies were performed one week apart counter balanced for each Xe. Flow was calculated by both the early picture and the sequence of pictures method. Using the SP method gave much more comparable results between the two isotopes. The flow values for Xe-127, though highly correlated with the Xe-133 values, were higher (20%) at the better resolution. Xe-127 with the high resolution collimator clearly produced a more anatomically detailed image with less underestimation due to partial volume in lowered resolution scans. The use of Xe-127 allows higher resolution and thus better quantification.

16.6

AN INEXPENSIVE WORKSTATION FOR THREE-DIMENSIONAL, VOLUMETRIC BRAIN RECONSTRUCTION, DISPLAY AND ANALYSIS. S.B. Berger, R.D. Leggiero*, G.F. March*, J.J. Orefice* and D.J. Reis. Div. Neurobiology, Cornell Univ. Med. Coll., NY, NY 10021.

We sought to develop an inexpensive, computer-based, three-dimensional (3D) volumetric imaging system suitable for quantitative regional densitometric analysis of brain, and compatible with such imaging modalities as immunocytochemistry and autoradiography. This system permits geometric manipulation and display of reconstructed brain models and comparison of regional changes without reference to traditional, cytoarchitectural boundaries. We have designed and implemented data structures and algorithms for fast, efficient 3D reconstruction, display and analysis using (a) octree data compression; and (b) pipeline architecture imaging hardware, to overcome the computational limitation imposed by interpolative operations (e.g. oblique rotations, arbitrary cross sections - "electronic microtomy").

The system, CAVIS (Computer Assisted Volume Imaging Station), which is implemented on an inexpensive hardware platform, performs shading to reveal 3D shape, electronic microtomy, geometric transformations, and animation sequences. Oblique rotations are performed in approximately 187 s, electronic microtomy in less than 1 s and high-quality surface rendering in approximately 3 s (elapsed, not CPU times). This method (a) does not impose *a priori* topographical maps; (b) is unbiased with respect to investigator; (c) is less laborious; and (d) permits the use of alternate statistical methods. As a special application, we have modified and implemented these algorithms on two supercomputers for very high resolution imaging. This system may be useful as an inexpensive laboratory imaging workstation for neuroscience applications.

16.7

THREE DIMENSIONAL SURFACE RECONSTRUCTION OF REGISTERED PET-MRI IMAGES ENHANCES ANATOMICAL LOCALIZATION OF CORTICAL FUNCTION. H.H. Holcomb, H.L. Loats, S.E. Loats C.A. Tamminga, N. Cascella*, P. Loats*, H. Ravert*, R. Dannals,* H. Wagner *. U. Md. Psych. Res. Ctr., Johns Hopkins Medical Institutes, Loats Assoc., Baltimore, and Westminster Md. 21205.

Magnetic resonance images (MRI) provide high resolution of soft tissue boundaries. In contrast, positron emission tomography (PET) reveals local physiological activity. Acquired tomographically, two dimensional images from these diverse methods do not permit easy, accurate identification of cortical surface landmarks. Specific gyri and sulci have been implicated in numerous behavioral repertoires. Subjects performing auditory tone discrimination accurately are likely to exhibit different gyral activty patterns from those detecting differences inaccurately. Accurate gyral localization requires superimposed, registered PET-MRI surfaces.

This pilot study of five normal subjects demonstrates the application of image subtraction using two sets of registered PET-FDG and one set of high resolution, 3 mm, MRI 3-dimensional data sets. These PET studies were acquired in subjects performing either low error (95% correct) or high error (70% correct) auditory discrimination tasks (750 Hz versus 1500 Hz) presented in conjunction with a variable background of white noise. A prospective MRI-PET registration method was used with retrospective fine adjustments (boundary matching optimization). Orothogonal data sets were generated and reconstructed to create serial sets of surfaces. These MRI-PET surfaces were then used to superimpose thresholded PET subtraction images onto their corresponding MR surface. Initial analyses indicate that the right superior temporal gyrus and right middle frontal gyrus exhibit significantly higher metabolic activity in subjects discriminating auditory tones accurately 90% of their trials.

16.8

VIDEOMICROSCOPY OF FLOW IN SINGLE BLOOD VESSELS IN MOUSE BARREL CORTEX *IN VIVO*. T.A.Woolsey, C.M.Rovainen, and O.Robinson* Depts. Neurosurgery and Cell Biology, Washington Univ. Sch. Med., St. Louis, MO 63110

We have implemented optical methods for measuring diameters and velocities in single cerebral microvessels to observe local changes in circulation during neural stimulation. Fluorescent dextrans and 1-2μm latex beads were injected i.v. and were monitored in pial vessels through a cranial window with a microscope and a SIT camera. Transit times were estimated from the differences in time courses of fluorescence between branches of the middle cerebral artery and veins draining the barrel cortex and were similar for the two plasma markers. Measured inner diameters of arteries and veins were 7-8μm larger with fluorescent markers than with absorption of green light by hemoglobin. Root mean velocities of fluorescent beads in venules were calculated from positions of individual beads in sequential video frames recorded at 30 Hz. Cranial chamber fluids at low pH or with adenosine dilated arteries and reduced AV transit times. Vessels were perfused with photographic emulsion, layer IV barrels were stained for cytochrome oxidase in postmortem histology, and the detailed patterns of arteries, arterioles, and veins were related to whisker barrels. With this approach we are mapping the changes in pial vessels suppling capillary plexuses in single barrels with whisker stimulation.

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16.9

VISUALIZATION OF ERYTHROCYTES, LEUKOCYTES, AND BLOOD PLASMA IN THE RAT BRAIN MICROCIRCULATION IN VIVO USING CONFOCAL LASER SCANNING MICROSCOPY (CLSM). A.Villringer*, U.Dirnagl, R.Haberl*, L.Schürer*, U.Büttner, K.Einhäupl*. Dept. of Neurology and Dept. of Neurosurgical Research, University of Munich, 8000 Munich 70, FRG.

To investigate the role of different blood components in the physiology and pathophysiology of the cerebral microcirculation, we established a technique to visualize erythrocytes, leukocytes, and blood plasma within the microcirculation of the outer layers of the rat brain cortex. After implantation of a closed cranial window (dura removed) in anesthetized and ventilated rats the brain microcirculation was observed with a Biorad MRC 500 CLSM system (excitation wavelength 488/514 nm, equipped with appropriate filter blocks) attached to a Nikon Optiphot microscope with a x40 objective (NA=0.75, working distance 1.6 mm). Leukocytes were labelled by injection of Rhodamine G (0.1 ml of a 0.1% solution i.v.), blood plasma was labelled with fluorescein (0.3 ml of a 2% solution, erythrocytes were visualized as sparing of fluorescence. Leukocytes could be observed flowing with the blood stream, as stickers, and as rollers (i.e. adhering to or slowly moving along the vessel walls). Based on the fluorescein-labelled plasma filling of the microvessels 3 dimensional reconstructions of the micro-angioarchitecture of the pia and outer 200 μm of the cortex (including capillaries) were calculated.

16.10

DETERMINATION OF ERYTHROCYTE FLOW VELOCITY IN SINGLE CORTICAL CAPILLARIES OF THE RAT BRAIN USING CONFOCAL LASER SCANNING MICROSCOPY (CLSM). U.Dirnagl, A.Villringer*, R Gebhardt* R.Haberl*, K.Einhäupl*. Dept. of Neurology, University of Munich, 8000 Munich 70, FRG.

In order to study dynamic phenomena of the brain microcirculation in vivo, we developed a method to determine erythrocyte velocity within single capillaries of the rat brain cortex. A closed cranial window (dura removed, distance brain surface-outer surface of the window < 1 mm) was implanted in anesthetized and ventilated rats. After injection of fluorescein (0.3 ml of a 2% solution i.v.) flowing erythrocytes in cortical capillaries down to 200 μm below the brain surface could be visualized as sparing of fluorescence using a Biorad MRC 500 CLSM system attached to a Nikon Optiphot microscope equipped with a x40 water immersion objective (NA=0.75, working distance 1.6 mm). Full frame images were collected at maximum rates of 16/s , and the scan line was placed parallel or perpendicular to a brain capillary for one dimensional single line determinations of erythrocyte flow velocity at a temporal resolution of 500 Hz. We conclude that CLSM can be used to study dynamic phenomena in the brain microcirculation in vivo.

16.11

A MULTIDETECTOR POSITRON EMISSION PROBE FOR STUDIES OF HUMAN BRAIN CHEMISTRY IN VIVO. K.I. Jeffries, H.L. Loats, D.G. Lloyd*, C.A. Tamminga, and H.H. Holcomb. Loats Associates Inc, P.O. Box 528, Westminster, MD 21157; and Maryland Psychiatric Research Center, P.O. Box 21247, Baltimore MD 21228.

The multidetector positron emission probe consists of four pairs of coincidence detectors which can be directed at any selected region-of-interest (ROI) in the brain. Detector positioning is registered to a set of MRI images for the subject. A user friendly computer interface allows the user to select the desired ROI by moving a cursor to the position on the subject's MRI image. A novel gantry design provides for computer controlled automated positioning of the detectors. The focusing of all detector pairs on the ROI in combination with a form of time-of-flight determination allows for quantification of radioactivity within target volumes on the order of 8 cm^3.

Time-of-flight measurements with a FWHM of 3 cm have been obtained with an optimized detector design incorporating BaF_2 scintillation crystals. Novel high speed coincidence detection circuitry has been developed to improve these measurements. A computer model has been developed to simulate the response of the device for various types of studies.

The device provides many ROI measurements comparable to those obtained with positron emission tomography at much reduced cost and radiation dose. Computer simulations and measurements made with the prototype system will be detailed to demonstrate the capabilities of the multidetector probe.

16.12

QUANTIFICATION OF RELATIVE HALOPERIDOL OCCUPANCY IN VIVO WITH A SIMPLE DUAL DETECTOR PROBE. C.A. Tamminga, K. J. Jeffries, H.L. Loats, D.F. Wong*, A.T. Summerfelt*, L.T. Young*, R.F. Dannals*, and H.N. Wagner*. Maryland Psychiatric Research Center, P.O. Box 21247, Baltimore, MD 21228; Loats Associates Inc.; and Johns Hopkins Med. Inst.

A dual detector probe (Bice, A.N., *J Nucl Med* 27:184-191, 1986) makes possible positron emission studies of CNS at much reduced radiation dose and cost in comparison to PET. Coincidence counts obtained from the device can be used either directly or in an MRI-registered computer model to obtain potentially useful clinical information. Studies of the correlation between probe measurements and PET ROI data in humans have been carried out. In these studies, subjects were scanned with the probe immediately following a PET scan. Multiple studies were performed on each subject with varied doses of haloperidol. Blockade of ^{11}C-N-methylspiperone binding was used as a measure of occupancy of D-2 receptors in the striatum by these drugs. Coincidence counts were obtained from various positions through the subjects' brains. Ratios of counts from the position intersecting the striatum with other positions were compared with PET ratios from corresponding anatomical regions. Comparison of probe position ratios with PET ROI ratios in 3 subjects demonstrated a linear dependence: striatum/parietal-frontal ($r=.82$, $p=.045$) and striatum/cerebellum ($r=.72$, $p=.107$). These preliminary data suggest that the probe may provide some information comparable to that obtained with PET. Information which is more anatomically specific obtained with the computer model will be compared with these simple ratios.

17.1

CILIA ARE THE SITE OF OLFACTORY TRANSDUCTION. G. Lowe* and G. H. Gold. Monell Chem. Senses Ctr., Philadelphia, PA 19104.

We investigated the spatial distribution of odor sensitivity in tiger salamander olfactory receptor cells by measuring odor-induced currents across the cell body membrane with a suction electrode. Odor stimulation depolarizes the cell, causing an outward current across the somatic K^+ conductance. The cells were stimulated locally with a puffer pipette, while continuous flow of Ringer's solution, from the cell body toward the cilia, was used to orient the dendrite and cilia along a single axis, and to limit the region of the cell exposed to the stimulus.

Odor sensitivity is restricted to the ciliary region. Starting at the tips of the cilia, response amplitude increased approximately linearly with the length of cilia exposed to the odor stimulus, reaching a maximum at the bases of the cilia. Thus, all portions of the cilia contribute equally to the somatic currents, indicating that the electrotonic length of the cilia is at least several times longer than their physical length. The latency and timecourse of the odor response was independent of the position of the stimulus along the cilia. Thus, transduction is a local process, and does not require the diffusion of odorants or intracellular messengers from the cilia to the dendrite. The distribution of the resting K^+ conductance was measured by local stimulation with a high K^+ solution. K^+ conductance was present in the dendrite and cell body, but absent in the cilia. Exclusion of K^+ conductance from the cilia may serve to maximize the passive spread of ciliary currents to the cell body.

17.2

PRIMARY STRUCTURE AND FUNCTIONAL EXPRESSION OF A CYCLIC NUCLEOTIDE-ACTIVATED CHANNEL FROM OLFACTORY NEURONS. R.S. Dhallan*[1], K.-W. Yau[2,4], K.A. Schrader*[3,4] and R.R. Reed*[2,3,4]. Depts. of Biomed. Eng.[1], Neurosci.[2], Mol. Biol. and Gen.[3], and Howard Hughes Med. Inst.[4], Johns Hopkins Sch. of Med., Baltimore, MD 21205.

A cyclic nucleotide-activated cation channel is now thought to mediate odorant signal transduction in olfactory cilia. We report here the molecular cloning and functional expression of such a channel from olfactory neurons based on structural homology with the cGMP-gated channel in bovine rods (Kaupp et al., Nature 342, 762, 1989). The coding region of the rod channel was used to screen a rat olfactory cDNA library, and a single class of cDNA clones was identified. The longest of these revealed an open reading frame of 664 amino acids that shares extensive homology with the rod channel, including the region implicated as the cyclic nucleotide binding site. Northern blot analysis indicated that the message encoding this putative channel protein is present only in olfactory epithelium, and is reduced upon elimination of the sensory neurons induced by olfactory bulbectomy. Inside-out patches of plasma membrane from human 293 cells transfected with the above cDNA showed the expression of a cation channel that is activated by cyclic nucleotides and has properties broadly similar to those of the native channel in olfactory cilia, including the affinities for cyclic nucleotides and the current-voltage characteristics.

17.3

DELAYED DEPOLARIZING RESPONSES OF *NECTURUS* TASTE CELLS TO CHEMICAL AND ELECTRICAL STIMULI AT THE APICAL PORE OF TASTE BUDS. Douglas A. Ewald & Stephen D. Roper Dept. of Anatomy & Neurobiology, Colorado State U., Ft. Collins CO 80523 and the Rocky Mountain Taste and Smell Center, Denver CO 80262

Taste buds in *Necturus* contain two basic cell types: receptor cells with apical ends extending to the surface of the taste bud and basal cells in the basal region. Chemical synapses have been identified ultrastructurally in the basal region of the taste bud from both types to the afferent nerve and also between the two types (Delay & Roper, J. Comp. Neurol. **277** 268, 1988). We have used intracellular recording from the basal region of taste buds in transverse slices of lingual epithelium (250 um thick) to test for synaptic interactions. For chemosensory stimulation, the apical extremities of receptor cells in each taste bud were transiently depolarized by focal application of 140 mM K+ solution. All cells gave depolarizing responses but in about half the cases the latency of the responses was significantly longer when compared to the receptor potentials recorded at the apical pore. Receptor cells (identified by Lucifer Yellow injection) had responses with latencies <75 msec whereas responses from identified basal cells had latencies >75 msec. For electrical stimulation, the receptor cells were depolarized with a glass stimulating electrode (30 um tip) applied to the pore with gentle suction. One component of the responses was depolarizations that had a time course of secs, had a delay of $\geq$75 msec, were fatigued with repetitive stimulation, recovered with a time course of minutes, and were blocked by the Ca^{++} antagonist Cd^{++}. These slow depolarizations could be reliably ellicited by electrical stimulation of the apical pore, but could not be produced by intracellular depolarization of individual taste cells. The properties of the slow depolarization suggest that it is due to synaptic interactions in the taste bud. Supported by AG06557, DC00374 & PONS20486.

17.4

SUCROSE STIMULATION OF RAT TASTE CELLS CAN INCREASE MEMBRANE POTASSIUM CONDUCTANCE. M.S. Herness. Lab. of Neurobiology & Behavior, Rockefeller University, New York, NY 10021.

Evidence is presented using whole-cell patch clamp recordings that sucrose application to the bath can reversibly increase outward currents in dissociated rat taste cells. These currents appear to be mediated by potassium channels since they are blockable by either TEA or 4-AP. The sucrose-induced increase in outward currents (approx. 1.5X increase) appears to affect both delayed rectifier type and A-type potassium currents as measured in different cells. NaSaccharin, another sweet stimulus, was observed to increase outward currents in some but not all cells. Since a cyclic nucleotide system has been proposed as a sweet taste transduction mechanism, sucrose plus IBMX, which reversibly inhibits cAMP breakdown, was also tested. This application increased outward currents by 5X and was reversible when the sucrose/IBMX mixture was rinsed out of the bath. Collectively these data suggest that sucrose may open potassium channels and that a cAMP dependent mechanism may be operative. These data could account for previously reported hyperpolarizing receptor potentials with increased input resistance or the repolarization phase of a depolarizing receptor potential to sucrose stimulation.

Supported by NIH 5 R29 D00401-04.

17.5

CHORDA TYMPANI SECTION DECREASES ION SPECIFICITY OF SODIUM APPETITE IN RATS. P.A. Breslin*, A.C. Spector, D.J. Luzzi*, and H.J. Grill. Psych. Dept., Univ. of Penn., Phila., PA 19104

The chorda tympani nerve (CT) in the rat is known to innervate highly selective Na^+ and Li^+ taste receptors on the anterior tongue. In the following experiment, sodium depleted rats were tested for their ability to respond selectively to a NaCl solution after these receptors had been denervated by bilateral CT section (CTX). A computer controlled gustometer randomly delivered six solutions sequentially via a single drinking spout: water, 0.1M sucrose, 0.05 & 0.5M NaCl, and 0.05 & 0.5M NH_4Cl. Rats were provided with multiple, five second access periods to each of the solutions, presented one at a time. The computer recorded each lick and reinforced it with 5 ul of solution. The rats received a different solution approximately every 10 sec. Given this design, the preference for any stimulus [stimulus licks / (stimulus licks + water licks)] was most likely guided by afferent signals from the mouth. Intact rats treated with a natriuretic (furosemide) and given Na^+ deficient food selectively increased their preference for NaCl, relative to untreated rats. However, sodium depleted CTX rats increased their preference for both NaCl and NH_4Cl and were thus less selective. The CTX sodium depleted rats expressed different preferences for NaCl and NH_4Cl, demonstrating that they can still discriminate the two salts. Despite their ability to discriminate these salts, the CTX rats failed to increase their sodium preference selectively. These findings suggest that afferent signals in the CT significantly contribute to sodium identification in the rat.

This research was supported by NIMH program project #43787.

17.6

RABBIT GUSTATORY EPITHELIUM EXPRESSES KERATIN 6-LIKE IMMUNOREACTIVITY. B. Oakley, A. Lawton*, and L. Wong*. Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109.

Keratins are the dominant class of intermediate filaments of the cell cytoskeleton. Nineteen soft keratins are present in various combinations in human epithelial tissues. This allows one to identify specific types of epithelial cells by their keratin profile. A monoclonal antibody specific for keratin 6 (MAb KL-1) reacted exclusively with basal cells throughout the non-gustatory lingual epithelium of the rabbit tongue, as indicated by heavy staining with either streptavidin Texas Red or reaction products of the avidin peroxidase complex bound to a biotinylated secondary antibody. One group of basal cells in the lingual epidermis did not show keratin 6-like immunoreactivity---the basal cells directly beneath taste buds (foliate). Moreover, only in the gustatory epidermis did suprabasal cells or flattened squamous cells display keratin 6-like IR. Hence, keratin expression readily distinguishes the gustatory epidermis from ordinary lingual epidermis. The use of other MAbs indicated that fusiform cells of taste buds abundantly expressed keratin 19-like IR that was undetected elsewhere in rabbit tongue epidermis. Keratin 6- and 19-like reactivity was not unique to gustatory tissue as cells comprising the ducts of salivary glands were reactive. We conclude that the gustatory epidermis is highly specialized, since keratin 19-like IR was detected only in taste receptor cells while keratin 6-like IR was observed only in those suprabasal cells adjacent to taste buds. Keratin expression may be a useful way to mark gustatory tissue and assess its state of differentiation.

Supported in part by NIH Grant NS-07072

17.7

GLYCOCONJUGATE DIFFERENCES IN SECRETORY CELLS OF MAMMALIAN OLFACTORY MUCOSAE. J.D. Foster[1,*], M.L. Getchell[2,3] and T.V. Getchell[1,3]; 1, Dept. Physiol./Biophys., & 2, Div. Otolaryngol., Dept. Surg., 3, Assoc., Sanders-Brown Center on Aging, Univ. Kentucky Coll. Med., Lexington, KY 40536.

Distribution of sialic acid (SA), galactose (Gal) and N-acetylgalactosamine (GalNAc) residues in sustentacular cells (SC) and acinar cells of Bowman's glands (BG) of hamster and mouse olfactory mucosae was investigated using fluorescently-labeled lectins. SA binding lectins from Limulus polyphemus (LPA) and Limax flavus (LFA) stained BG only. SA removal by neuraminidase (Nase) resulted in loss of staining. The Gal/GalNAc binding lectins from Peanut (PNA), Soybean (SBA), Bauhinia purpurea (BPA), Dolichos biflorus (DBA), Griffonia simplicifolia I (GSI) & Maclura pomifera (MPA) stained BG differentially. MPA in hamster and BPA in mouse stained most BG. All other lectins except GSI stained a minority of BG, suggesting the presence of a sub-population of BG with glycoconjugates containing terminal Gal and/or GalNAc residues. Following Nase, all the Gal/GalNAc binding lectins in hamster and PNA, BPA and MPA in mouse stained most BG, indicating that SA capped Galβ1,3GalNAC residues in BG of both mammals. In hamster α- or β-GalNAc residues are also capped by SA. None of the lectins stained SC. The different secretions of the two populations of BG may reflect specific functional roles in perireceptor events in olfactory transduction. Supported by NSF BNS-88-21074 (MLG) and NIH NS-16340 (TVG).

17.8

THE EFFECT OF ODORANTS ON ADENYLATE CYCLASE IN PRIMARY CULTURES OF RAT OLFACTORY NEURONS. G.V. Ronnett, L.D. Hester and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

A method for primary culture of neonatal rat olfactory neurons has been developed in order to study the mechanisms of olfactory signal transduction. This method yields a homogeneous population of olfactory neurons which stain for vimentin, neuron-specific enolase and olfactory marker protein. Exposure of cell monolayers to odorants such as isobutylmethylxanthine, isovaleric acid, citralva resulted in rapid transient rises in intracellular cAMP levels as demonstrated by RIA. To ascertain whether this was due to activation of adenylate cyclase (AC), cell monolayers were permeabilized and AC was assayed in situ. Odorants which caused elevation of cAMP activated AC over the appropriate time. This method was also used to study desensitization and sensitization.

HUMAN BEHAVIORAL NEUROBIOLOGY

18.1

WITHDRAWN

18.2

NONWORD PRIMING MAY BE ROBUST DESPITE GLOBAL AMNESIA. I.D.E. Gabrieli, M.M. Keane, and S. Corkin. Dept. of Brain & Cognitive Sciences and Clinical Research Center, MIT, Cambridge, MA 02139.

Despite impaired recall or recognition of words, patients with global amnesia show normal magnitudes of repetition priming on word identification and word completion tasks. Two studies (Cermak et al., 1985; Diamond & Rozin, 1984) reported that amnesic patients show little or no repetition priming of nonwords on identification and completion tasks. The absence of nonword priming indicated that amnesic patients may exhibit repetition priming only for premorbidly known words. We report now that H.M., whose amnesia followed bilateral medial temporal-lobe resection, shows robust nonword repetition priming in studies of nonword identification (presented briefly or emerging from a cover of visual noise) and of nonword reading latency. Nonword priming on the visual-noise task was evident after a 24-hour delay. In all experiments, H.M. had impaired recall and recognition of nonwords. Another study with normal subjects revealed that they do not show nonword completion priming unless they are told that the completion is an explicit test of memory (cued recall). Thus, nonword completion priming depends upon recall and recognition mechanisms in normal memory. These results suggest that, under some circumstances, perceptual and productive components of nonword processing may support nonword priming despite severe damage to the limbic structures that mediate recall and recognition of nonwords. Supported by grants RR00088, AGO5134, and 1P50NS26985.

18.3

DIENCEPHALIC AMNESIA AND SELECTIVE VISUAL AGNOSIA IN CHILDHOOD. F. Vargha-Khadem* and E. Isaacs*, (SPON: European Brain and Behaviour Society), Department of Neurology and Developmental Paediatrics and Hospital for Sick Children, Great Ormond Street, London, WC1, England.

Chronic anterograde amnesia resulting from diencephalic damage in childhood is a rare occurence. We had the opportunity to investigate such a patient with normal development until the age of 13 when a metastasised pineal gland tumour was diagnosed. Tested at age 15, he had bilateral optic atrophy, reduced acuity and tunnel vision. Neuropsychological assessment revealed normal verbal IQ, language, planning, fluency, and visuo-spatial abilities, but severely restricted immediate recall and no delayed recall. Autobiographical memory showed 100% recall of retrograde events but little or none anterograde. The latter, however, could be cued through writing. Due to long term consequences of irradiation, the patient developed selective visual agnosia and pure alexia without dysgraphia. As in the case of the amnesia, visual and word recognition could be cued through the kinesthetic feedback provided by drawing and letter-by-letter writing. Devoid of the kinesthetic cues of drawing and writing, the patient could neither read his writing nor recognise his intricate drawings. Despite the selective visual agnosia, the patient demonstrated normal performance on tests of visual matching, orientation of lines and judgement of rotated abstract objects.

18.4

MEMORY FOR DIFFERENT ASPECTS OF COMPLEX VISUAL SCENES AFTER RIGHT ANTERIOR TEMPORAL LOBECTOMY. S. Pigott and B. Milner. Montreal Neurological Institute, McGill University, Montreal, Canada, H3A 2B4

To examine the nature of the visual memory impairment observed following right anterior temporal lobectomy, delayed recognition memory for different aspects of complex visual scenes was examined in 65 patients with unilateral temporal- or frontal-lobe excisions and 15 normal control subjects. Right anterior temporal lobectomy, irrespective of the extent of hippocampal removal, resulted in an impairment in memory for figurative detail (the visual characteristics of the objects in a scene) and spatial composition (the arrangement of the filled and empty space in a scene). In contrast, only patients with right temporal lobe lesions that included extensive hippocampal removal were impaired at detecting changes in the spatial location of specific objects. A subsequent study provided no evidence that right temporal lobectomy impairs the immediate recognition of these types of visual information, suggesting that the impairments observed represent a failure of retention or retrieval rather than of encoding.

18.5

DEFECTIVE SOMATIC STATE ACTIVATION FOLLOWING VENTROMEDIAL FRONTAL DAMAGE IN HUMANS. D. Tranel and A.R. Damasio. Dept. of Neurol., Univ. of Iowa Col. of Med., Iowa City.

Patients with bilateral ventromedial frontal lobe damage develop severe defects in social conduct. We have hypothesized that this is due to defective activation of somatic states associated with reward and punishment, which deprives the patients of critical markers for behavioral guidance. Preliminary investigation of this theory confirmed that such patients had defective skin conductance responses (SCRs) to visual stimuli charged with social significance, but generated normal SCRs to simple "orienting" stimuli (e.g., startle). Here we test the theory further in the prototypical patient EVR. Target (high social significance) and nontarget (low social significance) stimuli were presented to EVR in different modalities (visual, auditory), forms (verbal, nonverbal), and response conditions (PASSIVE-no response, ACTIVE-verbal description). In PASSIVE conditions, EVR showed a marked failure to generate SCRs to target stimuli, but in ACTIVE conditions, his SCRs were normal. This effect was highly reliable, and it obtained for both verbal and nonverbal stimuli, in both visual and auditory modalities. The findings suggest that EVR does not activate somatic states in response to stimuli whose implications would normally include punishment or reward, and whose experience might trigger states of pain or pleasure.

18.6

A BRAIN-COMPUTER INTERFACE FOR CURSOR CONTROL. D.J. McFarland, G.W. Neat*, C.A. Forneris* and J.R. Wolpaw. Wadsworth Laboratories, NY State Dept. Health and SUNY, Albany, NY 12201.

Previous studies showed that individuals can learn to modulate the 9-12 Hz sensorimotor rhythm (SMR) of the EEG quickly and accurately to move a cursor to a target located at the top or bottom of a video screen (Wolpaw et al 1986 and submitted). This study trained subjects to move a cursor up or down in a graded fashion to a given location.

EEG was recorded from the scalp over the central sulcus of the left hemisphere. SMR amplitude was assessed 3 times/sec by a fast Fourier transform. A cursor began at the midpoint of the right screen edge and moved up or down depending on SMR amplitude. A target began at a random height on the left edge and marched across the screen at that height in 8 sec. The subject's task was to move the cursor so that it intercepted the target.

Performance was evaluated by comparing the vertical distance between the cursor and target at the beginning of the trial to that at the end of the trial. Since the cursor's starting point was, on average, closest to all possible target heights, random performance would have increased this distance. After several weeks of training, three of four subjects reduced this distance to an average of 52% of its initial value ($p<.0001$ for each of the three subjects).

These results show that the SMR can be used to control graded cursor movement. Current efforts are evaluating the possibility of training subjects to control independently SMR from both hemispheres simultaneously. This work may provide a significant new communication and control channel for disabled individuals. (Supported in part by IBM Corporation.)

18.7

SENSORY DETECTION WITH AND WITHOUT AWARENESS, WITH THALAMIC STIMULATION IN MAN B. Libet, D.K. Pearl*, D. Morledge*, C. Gleason*, Y. Hosobuchi* & N. Barbaro, Depts. Physiology & Neurosurgery, Univ. of Calif., San Francisco, CA 94143-0444.

We had demonstrated that stimuli in S-I cortex or n.VPL-thalamus at liminal intensity required minimum train durations (TD) of up to 0.5 sec, to elicit a conscious sensory experience. A "time-on" theory was proposed: Durations of appropriate neural activity less than those required to elicit awareness could subserve mental functions that proceed without awareness; transition from the latter to awareness of the event occurs when durations are sufficiently long. The present study provided a direct test of this proposal.

Patients with electrodes therapeutically implanted in ventrobasal thalamus were given near liminal stimuli (72/sec) with TDs between 0 and 750 msec; TDs were randomly varied among trials. Subjects made a forced choice between which of 2 intervals contained the stimulus even if they were not aware of any sensation. Subjects also indicated their level of awareness after each trial. Detection (correct >50%) occurred even with brief TDs(<10 pulses) and subjects feeling nothing. Any even uncertain awareness required a statistically very significant and substantial increase in TD. Thus, simply increasing the duration of the same repetitive inputs to cerebral cortex can convert an unconscious cognitive mental function to a conscious one. (Supported by U.S.P.H.S. grant NS-24298).

18.8

NEUROMAGNETIC PREMOTOR READINESS FIELDS AND RHYTHMIC ACTIVITY ALTERATIONS PRECEDING SPEECH. T. T. Yang*. C.C. Gallen. S. Hampson*. F. Bloom. Dept. of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

This study measured magnetoencephalographic (MEG) slow surface waves and changes in cortical rhythmical activity preceding speech for detection of language output related cortical function. Topographical activity was bilaterally recorded via serial placements of a 14-channel Neuromagnetometer while subjects said "put" at four to seven second intervals. Simultaneous sound output and lip EMG recording with automatic determination of speech initiation allowed isolation of the two second periods preceding and following the speech trigger for further processing. Epochs were (1) averaged to compute slow waves, (2) filtered for specified frequency bands and studied using a moving window method to assess rates and direction of change over time and (3) spectrally characterized by comparison of the power spectrum in different time periods before and after speech onset. Large amplitude shifts in some subjects began in the time period immediately preceding speech, continued during the time of speech output and in some cases were consistent with a generator source which could be modelled by an equivalent current dipole. Subject responses varied in amplitude and complexity. Alterations in rhythmic activity over time were characterized. Recordings of magnetic premotor waves and reactivity have potential utility for detection of speech related cortical function.

18.9

MODULARITY IN THE VERBAL-VISUAL REPRESENTATION OF SIZE SHOWN BY CORTICAL ELECTRICAL INTERFERENCE. J. Hart*, R.P. Lesser*, B. Gordon. Depts. of Neurology, Neurosurgery, and Psychology, The Johns Hopkins University, Baltimore, MD 21205

Modularity has been proposed as a basic organizing principle of cognitive neuroscience. However, while there is abundant evidence of modularity at the single neuron level from animal studies, and for large-scale modularity of human cognitive functions (e.g., major subcomponents of language) from pathologic conditions such as aphasia, firm evidence as to the existence and the nature of intermediate aspects of modularity of human cognitive functions has been relatively sparse. In a patient undergoing direct cortical electrical interference mapping of language functions as part of a clinical evaluation for epilepsy (Lesser et al., 1987), stimulation of adjacent electrodes over the left (dominant) posterior middle temporal gyrus caused a selective, reversible, abolition of her ability to make verbal judgments of comparative size for objects and animals (e.g., Is a lemon larger than an apple?), without affecting verbal judgments of other visual properties, such as orientation and movement. Visual size comparisons by direct access or by imagery were not affected. These and other data (Moyer, 1973) have three implications: 1) analogue size information is kept apart from language in the visual system, 2) access to this size information, or the information itself, is modularized by this quality, rather than by the particular object with which it is associated, which therefore 3) allows verbal access to size information to be selectively affected by the appropriate interference. The anatomic location of the temporary, 'functional lesion' responsible for this disconnection, at the posterior middle temporal gyrus, is consistent with a block between language functions mediated by the superior temporal gyrus and visual recognition systems mediated by inferior temporal and occipital regions and by other systems.

18.10

Functional Neuroanatomy of Visual Single Word Processing Studied with PET/MRI *Sean Marrett, Daniel Bub*, Howard Chertkow, Ernst Meyer*, Teren Gum*, Alan Evans**. Positron Imaging Laboratories and Department of Neuroliguistics, Montreal Neurological Institute, Montreal, Quebec Canada, H3A 2B4.

The neuroanatomical correlates of visual word and picture processing were investigated with combined PET measurements of regional cerebral blood flow (rCBF) and magnetic resonance imaging studies in six normal right-handed male subjects. PET studies were performed using intravenously administered $H_2{}^{15}O$ followed by a 60 second scan using a Scanditronix PC-2048-15B PET camera. Following the work of Petersen and co-workers, multiple tasks were used to allow for the planned subtraction of activation states associated with the different conditions. High resolution MRI imaging studies were obtained on all subjects, and were subsequently registered in 3-D with PET data from the same individual. The registered MRI volumes were used to derive a set of anatomical parameters from which the PET images were transformed into a stereotaxic coordinate system. This allowed averaging of data from different subjects in the same activation state (*Fox et al, 1988*). In the first task, subjects were asked to stare at a video monitor while single words were presented under computer control. When a fixation condition was subtracted from this state, the most robust response was a bilateral activation of striate and extrastriate cortex. There was also clear unilateral activation of occipito-temporal cortex in the dominant hemisphere, along with a unilateral activation of the anterior temporal lobe. These results are consistent with a specific dominant hemisphere (occipito-temporal) locus activating the orthographic description of words, followed by a second component, more anteriorly located, implicated in the derivation of meaning.

18.11

INTERREGIONAL CORRELATIONS OF REGIONAL CEREBRAL BLOOD FLOW (rCBF) AMONG POSTERIOR CORTICAL BRAIN REGIONS DURING OBJECT AND SPATIAL VISUAL PROCESSING. B. Horwitz, J. Haxby*, C. Grady, M. Schapiro*, R. Carson*, P. Herscovitch, L. Ungerleider, M. Mishkin and S. I. Rapoport. Lab. Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892.

Correlation coefficients between pairs of normalized rCBF values, obtained by positron emission tomography with [15-O]-labeled water, were used to examine functional interactions among brain regions in posterior neocortex in 17 young men during two 2-choice, match-to-sample visual tasks: face discrimination (FD), and dot localization with rotation (DL). Compared with a visuomotor control task, both FD and DL activated lateral occipital and occipitotemporal cortex bilaterally, although the activation during FD was significantly greater than during DL. Bilateral activation of superior parietal cortex occurred during DL, but not FD. Correlational analysis revealed that during FD, occipital and occipitotemporal activations correlated significantly, but only in the right hemisphere. During DL, occipital and superior parietal activations correlated significantly, but again only in the right hemisphere. In the left hemisphere, large significant correlations among many posterior brain regions were found during DL, but not FD. In both tasks, large interhemispheric correlations between normalized rCBF occurred. These results support the view that FD and DL processing are carried out to a greater degree by the right posterior hemisphere than the left, but that the DL task may involve, in addition, left intrahemispheric interactions among posterior regions.

THE AGING PROCESS: NEUROTRANSMITTERS AND ENDOCRINE REGULATION

19.1

MUSCARINIC ANTAGONISTS ON ACETYLCHOLINE RELEASE FROM THE HIPPOCAMPUS OF AWAKE RATS: A PROBE OF AGED BRAIN CHOLINERGIC FUNCTION AND OF ITS AMELIORATION BY ACETYL-L-CARNITINE. L. Angelucci, M.T. Ramacci*§, L. Alivernini*, M.G. Scrocco*, S. Bacchi* and A. Imperato. Farmacologia Medica 2a, Università "La Sapienza", Roma, and § Istituto per la Ricerca sulla Senescenza, Sigma Tau, Pomezia, Italia.

The regulation of acetylcholine (ACh) release by different subtypes of muscarinic receptors in young (3 months) and old (24 months) Fischer and Sprague-Dawley rats was investigated by means of brain microdialysis. Atropine induced a remarkable increase of ACh release in the hippocampus of young rats while the effect was drastically reduced in the old ones. The selective M1 antagonist pirenzepine also stimulated ACh release in young rats although was ten times less potent than atropine. Differently from atropine, the effect of pirenzepine was not reduced but significantly increased in 24 month old rats, in agreement with recent autoradiographic studies showing an age-dependent increase in the hippocampal M1 receptors with parallel reduction in M2 receptors. Six month pretreatment with acetyl-l-carnitine (ALCAR) reduced the significant differences between young and old rats in the receptor-mediated functional release response, suggesting that ALCAR could be utilized to ameliorate cholinergic function in the aged brain.

19.2

PHARMACOLOGICAL CHARACTERIZATION OF THE $GABA_A$/BENZODIAZEPINE RECEPTOR COMPLEX IN RAT HIPPOCAMPUS DURING AGING. D. Ruano*, J. Cano*, A. Machado*, J. Jiménez-Castellanos[1] and J. Vitórica*. Dept. Bioquímica, Bromatol. y Toxicol. Fac. Farmacia. [1]Dept. Ciencias Morfológicas. Fac. Medicina. 41012 Sevilla. Spain.

It has been postulated that the benzodiazepine sensitivity may be increased during the human aging. This increased sensitivity could be explained by age-related changes in either pharmacokinetic and/or pharmacological characteristics of benzodiazepines (Greenblatt et al, J. Clin., Pharmacol., 29:866, 1989). It has also been reported an age-related change in the molecular organization of the $GABA_A$/Benzodiazepine receptor complex in rat cerebral cortex (Erdo S.L. and Wolff J.R., J. Neurochem., 53:648, 1989). In the present communication we have evaluated the pharmacological properties of the $GABA_A$/Benzodiazepine receptor complex in hippocampal membranes from 3 month and 24 month-old rats.

The results indicated the absence of major changes in ^{3}H FNZ or ^{3}H Muscimol binding parameters. Neither the K_ds nor the Bmax for ^{3}H FNZ or ^{3}H Muscimol, determined by Scatchard, were altered during aging in hippocampus. However, in 24 month-old rats the GABA enhancement of 1nM ^{3}H FNZ binding showed a significant increase over 3 month-old rats.

Two Benzodiazepine receptor subtypes can be distinguished by their affinities for the triazolopyridazine C1218-872. In 3 moth-old hippocampus the Benzodiazepine receptor belongs to type II, showing a single low affinity of 2.06±0.55uM (5 of 5 experiments). However, in 3 of 5 hippocampus from 24 month-old rats, two different affinities for C1 218-872 can be discriminate: high affinity, 305±202nM and low affinity, 2.02±0.73uM. In conclusion, the $GABA_A$/BZD receptor binding parameters remains unaltered with aging in rat hippocampus. The increase in the GABA enhancement of ^{3}H FNZ binding could be due to the increase in the heterogeneity of the Benzodiazepine receptor.

19.3

REDUCTION IN IMMUNOREACTIVE CATECHOLAMINERGIC NEURONS IN SELECTIVE BRAIN REGIONS OF OLD FEMALE RATS. L. Givon and A. A. Gerall. Dept. of Psychology, Tulane Univ., New Orleans, LA 70118.

This research identifies catecholaminergic neuronal systems which could participate in age-related prolactin hypersecretion and loss of spontaneous ovulation in rats. Examined in young and old animals were noradrenergic (NA) neurons located in brain stem A1 and A2 groups and dopaminergic (DA) neurons in the rostral ventral periventricular area (AVPv), in the arcuate nucleus A12, and the preoptic area A15 clusters.

Young (5-7 mos) cycling and old (30-32 mos) anovulatory Sprague-Dawley rats were ovariectomized 5-7 weeks before being perfused intra-aortically with paraformaldehyde. Brains were sectioned coronally at 50 µ and immunocytochemically processed using a modified Sternberger PAP methodology. Forebrain sections were treated with tyrosine hydroxylase antiserum (1:1000; Eugene Tech, NJ) and hindbrain sections with dopamine-beta-hydroxylase (DBH) antiserum (1:5000; I. Li Chen, Tulane Univ.). Tissues from paired young and old animals were processed simultaneously.

Old animals consistently had fewer DA neurons in the caudal arcuate nucleus (A12), in the AVPv and in the ventral segment of A15. The number of visualizable DBH-immunoreactive neurons in A1, A2 and TH-immunoreactive neurons in the dorsal A15 was not related to age.

19.4

NOREPINEPHRINE CONTENT AND RELEASE IN CARDIOVASCULAR TISSUES FROM ADULT AND AGED FISCHER 344 RATS. R. Dawson, Jr. and M.J. Meldrum. Dept. of Pharmacodynamics, Univ. Florida, Gainesville, FL 32610

The aim of the present study was to examine cardiovascular tissues and determine if norepinephrine (NE) stores , NE release from sympathetic nerves and α2-adrenergic binding sites declined as a function of age. NE content was determined in adult (8 month) and aged (28 month) male Fischer 344 rats by HPLC with electrochemical detection. [^{3}H]NE release was measured in the caudal artery. [^{3}H]Rauwolscine binding was determined in crude homogenates of whole kidney. Total NE content per organ was not altered in aged rats except for a significant ($p< 0.02$) decline in ventricular content. NE content in aged rats expressed as ng/g wet weight was significantly ($p< 0.02$) reduced relative to adults in whole heart, atria, ventricles, kidney and caudal artery, but was not altered in the renal artery or portal vein. [^{3}H]Rauwolscine binding in the kidney was decreased 22% in aged rats when compared to the adults. Neither basal nor KCl stimulated [^{3}H]NE release was altered in aged rats. Yohimbine augmentation of KCl stimulated NE release was of a similar magnitude in both adult and aged rats. In summary, ventricular NE content and renal α2-adrenergic receptors decrease with aging. Normalization of NE stores by tissue weight in aged rats results in the appearance of a significant decline in NE, however, this is due to an increase in tissue weight and not a decline in NE content. NE release is also also unchanged in the caudal artery which suggests that noradrenergic neuroeffector function is relatively intact in the aged rat.

19.5

INCREASES IN MONOAMINE SYNTHESIS ARE PROPORTIONAL TO THE RATE OF AGE-RELATED NEURONAL LOSS FOR DIFFERENT MONOAMINERGIC POPULATIONS. C.E. Greenwood, W.G. Tatton and D. Holland*. Depts. Nutr. Sci. and Phys., Univ. Toronto, Tor., Ont., M5S 1A8.

Evaluation of age-related changes in neurotransmitter levels in terminal beds must consider the age-related loss of neurons supplying the transmitter. For example in C57Bl mice, striatal levels of dopamine (DA) and its metabolite, DOPAC, show little or no change despite a marked loss of TH+ substantia nigra compacta (SNc) neurons across the lifespan so that striatal DA and DOPAC levels per average remaining TH+ SNc neuron increase progressively (Greenwood et al, Soc. Neurosci. 15:437,1989). To confirm the increased DA synthesis per TH+SNc neuron with aging and to extend the finding to other monoaminergic populations, monoamine synthesis and turnover rates were determined in C57Bl mice following NSD-1015 or pargyline administration and were expressed relative to the numbers of remaining TH+ or 5HT+ neurons (Tatton et al, Soc. Neurosci. 15:160,1989). Striatal DA or DOPAC, cortical NE and spinal cord 5HT or 5HIAA levels were similar at 8, 52 and 104 wks of age. Accumulation of DOPA or 5-OH-TRP following NSD or DA, NE and 5HT following pargyline in these same terminal areas was similar in 8 and 104 wk old mice (expressed relative to tissue wet weight). However, when values were expressed relative to the remaining proportion of TH+ or 5HT+ somata in each nuclei at 104 wks (mean ±SD of 26±1% for locus coeruleus (LC), 30±1% for SNc and 92±3% for medullary raphe neurons), they revealed an approximately 3 fold increase in DA and NE synthesis in remaining SNc and LC neurons at 104 wks in comparison to that at 8 wks of age. In contrast, synthesis of 5HT per neuron was unchanged from 8 to 104 wks of age in raphe neurons. Retinal DA and DOPAC levels showed little or no change, expressed per g protein; however, DA, but not DOPAC, levels per TH+ retinal amacrine increased 1.5 fold from 8 to 104 wks. We hypothesize that monoamine synthesis increases in surviving aging neurons proportionally to the extent of the loss of other neurons in the same population in an attempt to compensate. (MRC Canada)

19.6

IN VIVO ELECTROCHEMICAL STUDIES OF AGE-RELATED CHANGES IN DOPAMINE NERVE TERMINAL FUNCTION: A9 VS. A10. M.N. Friedemann and G.A. Gerhardt Departments of Pharmacology and Psychiatry, University of Colorado Health Science Center, Denver, CO 80262.

The consequences of aging on dopamine (DA) presynaptic elements and their function remains unclear. In order to investigate the effects of aging on DA nerve terminals we studied DA release and reuptake in the striatum of urethane anesthetized Fischer 344 rats. High-speed (5 Hz) in vivo electrochemical measurements of potassium evoked DA overflow were recorded in dorsal and ventral striatum of 6 and 24 month old rats using Nafion-coated multiple carbon fiber electrodes. Average response amplitudes were significantly decreased in the 24 vs. 6 month old animals. The decrease in amplitude was more pronounced in ventral vs. dorsal striatum in the 24 month old rats. While the amplitudes were decreased in aged rats, the average time courses of the responses were not different between the two age groups, suggesting an alteration in the reuptake of DA in the aged animals. The results indicate that there are age-dependent changes in the dynamics of DA release and reuptake and that these changes may be more pronounced in the A10 versus A9 DA system. (Supported by USPHS AG06434, AG00441, and Pharmaceutical Manufacturers Association Foundation.)

19.7

CHANGES IN STRIATAL D_2 RECEPTOR mRNA IN AGED RATS E.R. Mesco, M.J. Blake, J.A. Joseph, G.S. Roth*. Gerontology Research Center/NIA, Baltimore, MD 21224.

The striatal dopamine D_2 receptor displays decreased total binding (B_{max}) of [^{3}H]-spiperone in the aged rodent as well as several other species, including humans. This reduction can be explained by two mechanisms: neuronal loss and decreased net receptor protein synthesis. In order to determine whether the decreased protein synthesis was due to a change in mRNA, the levels of D_2 mRNA were determined in young (6 mo.) and old (24 mo.) male Wistar rats.

A cDNA probe, complementary to bases 4-51 of the mRNA (5'-GTT CTG CCT CTC CAG ATC GTC ATC GTA CCA GGA CAG GTT CAG TGG ATC -3') which encodes the N-terminal of the protein, was used in dot blot and *in situ* analysis of striatal RNA extracts and brain slices, respectively. This probe identifies both subtypes of the D_2 receptor molecule. Total mRNA was ascertained by the use of radiolabeled poly-T probes on duplicate dot blots. No significant age changes in total mRNA were detected. Decreases in D_2 mRNA, averaging 50%, were seen in old striata when compared to the young on dot blot analysis. The decreases in D_2 mRNA observed in the aged animals suggest that transcriptional changes may contribute to the lower levels of the receptor seen during aging.

19.8

THE EFFECT OF AGE ON HALOPERIDOL-INDUCED BEHAVIORAL HYPERSENSITIVITY.
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Rush Medical College, Chicago, IL 60612

Chronic treatment of rats with haloperidol (HAL) leads to an enhanced response to subsequent challenge with dopamine (DA) agonists referred to as behavioral hypersensitivity (BH). Since the incidence of tardive dyskinesia (TD), also a neuroleptic-induced DA BH, is higher in elderly patients, we examined the effect of chronic HAL treatment in young (3 months) and old (20 months) animals to determine if BH was also higher in aged rats. Rats received 24 consecutive daily treatments of haloperidol (HAL; 1.25 mg/kg) or saline (SAL) followed by a 96 hour drug free interval. HAL enhanced apomorphine (0.6 mg/kg) induced stereotypic behavior relative to SAL treatment. The effect of HAL in old animals was also statistically elevated relative to HAL-young. HAL treatment reduced DA and DOPAC content regardless of age. However, these effects were most pronounced and statistically significant in the old animals. Finally, extracts from these animals were evaluated for the presence of a striatal-derived neurotrophic factor (NTF; see Carvey this meeting) in rat E13 mesencephalic cultures. Old animals had less NTF than young animals. HAL treatment enhanced DA neuron growth indices regardless of age, although the effect in old animals was not as pronounced. Taken together, these data suggest that BH is more pronounced in an older animal perhaps as a result of reduced compensatory mechanisms which include alterations in a striatal-derived NTF.

19.9

NMDA MEDIATED RESPONSES DECREASE WITH AGE IN FISCHER 344 RAT BRAIN. R. A. Gonzales, L. M. Brown, and S. W. Leslie. Inst. for Neuroscience, Univ. of Texas, Austin, TX 78712.

N-methyl-D-aspartate (NMDA) receptor-mediated responses were studied in hippocampus, cortex, and striatum of Fischer 344 rats of various ages (3, 12, and 24 months old) to determine whether aging alters the function of NMDA receptors. The inhibition of muscarinic-stimulated phosphoinositide (PI) hydrolysis by NMDA and the stimulation of release of [^{3}H]norepinephrine (NE) or [^{3}H]dopamine (DA) were used as indices of NMDA receptor function. Carbachol (1 mM)-stimulated PI hydrolysis in hippocampal slices was similar in all age groups. NMDA inhibited the carbachol-evoked PI response in a concentration-dependent manner (10-100 μM), although the NMDA-induced (100 μM) inhibition of the carbachol-stimulated response was markedly reduced in an age-dependent manner with losses of 25% and 53% in middle-aged and senescent rats, respectively, compared to young. The maximal response for NMDA-stimulated [^{3}H]NE release in hippocampal slices was significantly decreased from 6.55 fractional [^{3}H]NE release in young to 4.51 and 4.18 in middle-aged and old rats, respectively, with no age-related changes in the potency of NMDA or slope of the curves. In cortical slices, the maximal response was significantly reduced in an age-dependent manner by 23% in the senescent rats compared to the young rats. NMDA-stimulated [^{3}H]DA release from striatal slices was significantly lower in the senescent rats at concentrations of NMDA from 500-2000 μM. These age-dependent decrements in the function of NMDA receptors may underlie, at least in part, some of the cognitive or motor-behavioral impairments seen with advanced age. (Supported by a grant from AFAR and AA08104)

19.10

AUTORADIOGRAPHIC LOCALIZATION OF DECREASES IN NMDA BINDING SITES OCCURRING WITH AGE IN TWO DIFFERENT MOUSE STRAINS. **K.R. Magnusson and C.W. Cotman[1]. Dept. of Anatomy & Neurobiology, Colorado State University, Fort Collins, CO 50623; [1]Dept. of Psychobiology, University of California, Irvine, CA 92717.**

Previous work in this laboratory (UCI) suggests that BALB/c mice exhibit a 55% decrease in N-methyl-D-aspartate (NMDA) binding with age compared with a 17% decrease measured in C57Bl mice (Neurosci. Lett. 104 (1989) 309-313). In these two genetically-different mouse strains it is important to determine whether age-related changes in NMDA binding sites were homogeneous throughout the brain or were restricted to specific brain regions. Quantitative autoradiography was performed on horizontal sections which were incubated with 100nM ^{3}H-L-glutamate and 100μM each of kainate, AMPA and SITS. Significant differences between age groups (3,10,and 30 months) within each strain (BALB/c or C57Bl) for each brain region analyzed were determined by ANOVA of fmol/mg protein values. Old (30 month) BALB/c mice exhibited reductions in binding to NMDA sites, as compared with 3 month old BALB/c mice, in many brain regions (e.g., CA1 stratum lacunosum, caudate nucleus, and frontal, parietal and entorhinal cortex). The percent reduction in binding was heterogeneous between brain regions. Significant reductions in 30 month old C57Bl mice compared to 3 month old C57Bl mice occurred in fewer regions (e.g., entorhinal and frontal cortex, and caudate nucleus). It appears that the strain differences in age-related loss of NMDA binding can be accounted for by the involvement of more brain regions in the BALB/c strain of mice as compared to the C57Bl strain. Furthermore, brain regions, with respect to NMDA binding, do not appear to be uniformly affected by aging-associated changes. Supported by NIA/NIH Physician Scientist Award AG00329.

19.11

TEMPORAL CHANGES IN THE EXPRESSION OF THE GLUTAMATE TRANSPORTER FROM RAT CORTICAL POLY (A)+ RNA IN XENOPUS OOCYTES

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The excitatory amino acid glutamate (GLU) has been implicated in various neurodegenerative processes. The integrity of the GLU transporter is necessary for the proper catabolism and disposal of GLU released by nerve terminals. To permit a molecular characterization of age dependent changes in the GLU uptake system, we studied the expression of the GLU transporter in Xenopus laevis oocytes after injection of poly (A)+ RNA. Poly (A)+ RNA from the cortex of 1, 15, 30 day as well as 3, 6, and 21 month old rats was extracted and injected into oocytes. GLU uptake activity was estimated with [^{3}H]-GLU. GLU uptake was sodium and temperature dependent, similar to that found in neurons. The GLU transporter expression increased with time after birth, and reached adult values by 30 day post-birth. A decline of expressed activity was observed by the 12 month. Similar changes were found when GLU uptake was measured in synaptosomes prepared from cortices of animals of the same ages. This study suggests, that the low GLU uptake found in the brain of aged animals is probably due to a decreased expression of its message.

19.12

SEROTONIN 5-HT$_{1A}$ RECEPTOR DECLINE WITH AGE IN THE FEMALE FISCHER 344 RAT. J. M. Lakoski, E. W. Black and C. Gregg*. Department of Pharmacology & Toxicology, Univ. Texas Medical Branch, Galveston, TX 77550.

We have begun to address the role of specific serotonin (5-HT) receptor subtypes in mediating age-related changes in CNS function, including processes altered with the onset of reproductive senescence. We have previously identified age-dependent changes in the cellular physiology of the 5-HT$_{1A}$ autoreceptor in the dorsal raphe nucleus of young and middle-aged vs reproductively old acyclic female rats. These present investigations assessed the age-related changes in binding site characteristics in the hippocampus, a region enriched with 5-HT$_{1A}$ receptors.

Female Fischer 344 rats (NIA colony), ages 2, 6, 12, 20, and 28 mo, were either ovariectomized under ether or left intact for several weeks prior to decapitation; brain regions were rapidly dissected and frozen at -80°C until assay. Changes in hippocampal 5-HT$_{1A}$ binding sites were assessed by scatchard analysis using the agonist [^{3}H]8-OH-DPAT (8-hydroxy-2-(di-n-propylamino)tetralin; 0.21-6.67nM) with P2 membranes incubated 15 min at 37°, harvested by vacuum filtration, and data analyzed by LIGAND; non-specific binding was defined by 100μm 8-OH-DPAT. Displacement assays with 5-HT (10^{-4}-10^{-12}M) were conducted with 2nM [^{3}H]8-OH-DPAT. Significant age-related decline in receptor numbers (B_{max}) from 2 and 6 mo (1501±222, 1612±167 pmoles/mg protein) vs 12, 20 or 28 mo (1135±260, 927±180, 917±219) was observed. With respect to affinity characteristics, no consistent alteration of K_d values was observed between ages 2 and 20 mo (7.6, 7.8 nM) or IC_{50} values for 5-HT. Thus, these studies have identified specific age-related changes in the numbers of the 5-HT$_{1A}$ receptor subtype which may, in part, mediate the transition to acyclicity observed in the middle-aged female rat.

Supported by AG06017 and AG00450 (JML).

19.13

[35S]TBPS BINDING TO THE GABA IONOPHORE WITH AGE IN RAT BRAIN. P.E. Schauwecker, A.B. Kelly and D.G. Morgan. School of Gerontology and Dept of Biological Sciences, University of Southern California, Los Angeles CA 90089-0191.

The GABAergic system has been examined infrequently in studies of brain aging, in spite of the fact that it is one of the two major transmitter systems in brain. Our initial approach to elucidating age-related changes in this system has been to measure the binding of t-[35S]butylbicyclophosphorothionate (TBPS) to the chloride channel linked to the GABA-A receptor. Membranes from cerebral cortex of Fischer 344 rats aged 6, 15 and 24 months were incubated for 2 hours with 500 mM NaCl and increasing concentrations of TBPS at room temperature (buffered to pH 7.4), and bound and free ligand separated by filtration. The average Kd was 150 nM, and no differences were observed between the age groups. The Bmax values were 4.6, 3.3, and 4.3 pmol/mg protein for the 6, 15, and 24 month groups respectively. ANOVA revealed no significant differences in Bmax as a function of age (n=6 per group). Additional studies are in progress to evaluate effects of aging on [36]Cl uptake into synaptoneurosomes stimulated by brief exposure to GABA. Supported by the Anna Greenwall Award from the American Federation for Aging Research and NIA grant AG07892 to DGM. DGM is an Established Investigator of the American Heart Association.

19.14

ALTERATIONS OF RAT PITUITARY GROWTH HORMONE-RELEASING FACTOR (GRF) BINDING SITES IN AGING: A TIME-COURSE STUDY. T.Abribat, N.Deslauriers*, P.Brazeau* and P. Gaudreau. Neuroendocrinology Lab., Notre-Dame Hospital Research Center, Montreal, Canada H2L 4K8.

We have previously documented a decreased GRF-induced GH secretion in aging rats beginning at 12 mo of age (Deslauriers et al, Int. Symp. on Somatostatin, Montreal, 1989 abst. 51). Therefore we have investigated whether this phenomenon is related to an alteration of pituitary GRF binding sites. Cold saturation studies were performed with [^{125}I-Tyr^{10}]hGRF(1-44)NH$_2$ (35-50 pM) as radioligand in pituitary homogenates of 2, 8, 14 and 18-mo old male Sprague-Dawley rats. [^{127}I-Tyr^{10}]hGRF(1-44)NH$_2$ (0-100nM) was used as competitor and rGRF(1-29)NH$_2$ (2.4 μM) for non-specific binding evaluation. In young animals (2 mo), analysis by the Ligand program statistically ($P<0.02$) revealed the presence of two distinct classes of binding sites (K_{Ds} = 0.86 ± 0.15 and 400 ± 210 nM and B_{MAXs} = 202 ± 35 fmol and 31 ± 14 pmol per pituitary, respectively). In 8- and 14-mo old rats, there was a concomitant increase of affinity (8 mo, $P<0.10$; 14 mo, $P<0.01$) and decrease of capacity (8 mo, $P<0.20$; 14 mo, $P<0.05$) of the high affinity site, and an increase of capacity (8 mo, $P<0.05$; 14 mo, $P<0.10$) of the low affinity site. In old animals (18 mo), the Ligand program no longer statistically analyzed the data with a two-sites model, indicating a severe blunt of the high affinity site. In conclusion, our results show that in aging, alterations of pituitary GRF binding sites occur in parallel to the impairment of GRF-stimulated GH secretion. Supported by MRCC grants.

19.15

EFFECT OF AGE ON RESPONSES TO NERVE STIMULATION IN THE RAT TAIL ARTERY: INFLUENCE OF ALTERED TRAIN LENGTH AND CALCIUM J. Buchholz and S.P. Duckles. Dept. of Pharmacology, Coll. of Medicine, Univ. of California, Irvine CA, 92717.

Previously we have shown that prejunctional control of norepinephrine (NE) release from the rat tail artery declines with age. This results in significantly greater NE release in the presence of deoxycorticosterone and cocaine from nerves in tail arteries of 20 and 27 month old F-344 rats as compared to 6 and 12 month old animals. Furthermore, there is an age related decline in the effectiveness of prejunctional α_2-adrenoceptors. To test the possibility that control of calcium movement across the nerve cell membrane is altered with age, effects of extracellular calcium were investigated with varied stimulation intensity in tail artery segments from 6 and 20 month old F-344 rats. Isometric contractile responses were monitored from arterial segments stimulated transmurally (3 Hz at 3, 9, 15, 30 and 90 sec) in the presence of 1, 1.6 or 5 mM calcium. The effects of altered extracellular calcium were much greater at short train lengths. Furthermore calcium changes had little effect on responses to exogenous NE. The greater sensitivity of contractile responses to TNS to extracellular calcium at low stimulus intensity (short train length) reflects the period of facilitation of transmitter release before peak intracellular calcium levels are achieved. When the effect of age was examined, there were no differences in contractile responses to TNS or NE, regardless of train length or the level of extracellular calcium. These data suggest that the age related increase in NE release may be compensated for at the post junctional site, thus producing no net age related change in contractile response.

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19.16

RELEASE OF ENDOGENOUS ACETYLCHOLINE AND PHOSPHATIDYLINOSITOL TURNOVER IN BRAINS OF YOUNG AND AGED RATS. T. C. Holmes , R. L. Buyukusal*, and R. J. Wurtman, Department of Brain and Cognitive Sciences, MIT, Cambridge, MA, 02139

Release of endogenous acetylcholine (ACh) from superfused striatal slices and the production of inositol phosphates (IP1 and IP2) in cerebral cortical slices were examined in tissues from young (3 month) and aged (24 month) rats. Basal ACh release did not differ between young and aged rats, and the potassium channel blocker 3,4-diaminopyridine (3,4-DAP) increased ACh release in both groups. Electrically evoked ACh release was greater in young rats than in aged rats with or without the addition of 3,4-DAP to the medium. Phosphatidylinositol turnover, measured at 30 sec, 1, 2, 5,and 10 minutes following treatment with carbachol (100 uM) did not differ between young and aged groups. These data indicate that the hypothesized age-related defect in central cholinergic transmission derives from an impairment in evoked ACh release. Supported by NIMH.

19.17

Comparison of Basal Forebrain Cholinergic Neuron Counts in 57 day and 380 day old New Zealand Black (NZB) Mice. S.H. Feldman. Div. of Comparative Medicine, U.T. Southwestern Medical Center, Dallas, Texas, 75235.

NZB/BlNJ mice been proposed as a model of senile dementia of the Alzheimer's type because of an early age-related onset of memory impairment, and age-associated behavioral responses to pharmacologic agents (see Retz et al., 1988; Drug Dev Res, 15:275-295). Because NZB mice are not currently available from Jackson labs (Bar Harbor, ME), this author compared the numbers of basal forebrain cholinergic neurons in 57 day (n=2) and 380 day (n=2) female NZB/NCI mice. Immunohistochemical staining with monospecific polyclonal serum against choline acetyltransferase (donated by L.B. Hersh; see Tago et al., 1987; Brain Res, 415:49-62) was utilized to visualize cholinergic neurons. All cholinergic neurons in alternate horizontal sections were counted. No significant differences in cholinergic neuron counts were detected in the preoptic magnocellular region, nucleus accumbens, horizontal and vertical arms of the diagonal band of Broca, medial septal nucleus, substantia innominata plus nucleus basalis, oculomotor nucleus and trochlear nucleus between the two age groups. A significant difference ($p<0.01$) was detected for striatal interneurons. Also, in horizontal section the preoptic magnocellular neurons were organized into "barrel-like" cytoarchitecture where closely associated with olfactory tubercle and pyriform cortex.

19.18

THREE DIMENSIONAL RECONSTRUCTION OF THE CHOLINERGIC BASAL FOREBRAIN SYSTEM IN YOUNG AND AGED RATS. M.L. Smith and R.M. Booze. Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

Cholinergic neuron dysfunction and/or cell loss has been implicated as one important event associated with age-related cognitive changes. However, the issue of selective vs. diffuse age-related cholinergic cell loss within the basal forebrain remains unresolved. We have produced three dimensional reconstructions of the rat basal forebrain in order to study the selectivity of cholinergic cell loss.

Pairs of young (4-5 month) and aged (24-25 month) Fischer-344 male rats were perfused with an aldehyde solution, and the brains sectioned on a Vibratome (40 um). Serial sections from each pair of young and aged rats were processed simultaneously for ChAT (1:500; Chemicon) using standard ABC immunocytochemical techniques. Cell location and z-value information were used to construct three dimensional maps using a computer-based imaging system (Biographics, Inc).

Three dimensional reconstructions spanning the entire cholinergic basal forebrain system from medial septum through nucleus basalis were generated for each animal. The sensitivity of the reconstruction procedure to detect specific cell loss was examined by mapping the distribution of cholinergic cells in animals with a fimbria/fornix lesion. The fimbria/fornix lesioned animals showed a selective decrease in the number of cholinergic cell bodies located in the medial septal area and showed a correspondingly altered cell distribution. In normal aged rats, the number of cholinergic neurons in the medial septum decreased, suggesting selective age-related cholinergic cell loss. Thus, three dimensional reconstructions provide a sensitive means for studying the selective effects of aging in the cholinergic basal forebrain system.

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19.19

ESTRADIOL-INDUCED REPRODUCTIVE ACYCLICITY: EFFECTS ON LHRH AND TUBEROINFUNDIBULAR DOPAMINERGIC NEURON NUMBER AND MEDIAN EMINENCE MONOAMINE LEVELS. S.G. Kohama, S.A. Brown*, H.H. Osterburg*, C.E. Finch, and T.H. McNeill. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

Orally administered estradiol (E2) induces a premature loss of estrous cycles in rodents by putatively "damaging" neuroendocrine loci. To define possible neural mechanisms sensitive to E2, the number of LHRH and tuberoinfundibular dopaminergic (TIDA, arcuate) neurons was evaluated by immunocytochemistry on free-floating sections. Compared to age-matched intact or ovariectomized (ovx) controls, there was no change in the number of LHRH or TIDA neurons in either young (8 mo) or middle-aged (13 mo) intact virgin C57BL/6J mice, made acyclic by 12 weeks of E2 treatment.

In a second study, the same experimental paradigm was employed, but was conducted on a single age group of mice (7 mo). Subgroups of mice from the ovx and intact+E2 groups were also injected with vehicle or NSD-1015 to test for differences in DA turnover. As an additional control, young (2 mo) ovx mice were treated with vehicle or E2 for shorter periods of 3, 7, or 21 days. Following treatment, median eminences were collected for analysis by HPLC with electrochemical detection. In the chronic study, steady state levels of 5-HT, NE, DA and metabolites did not vary by treatment. However, DA turnover as measured by L-DOPA accumulation, was increased by E2 treatment. Acute treatment with E2 caused a reduction in DOPAC which recovered by day 21. In general, 5-HT and NE levels were greater in the younger animals from the acute study.

19.20

AGE-ASSOCIATED REDUCTION IN PLASMA AND PINEAL MELATONIN LEVELS IN THE RAT IS PREVENTED BY ACETYL-L-CARNITINE. S. Scaccianoce*, G. Cigliana*, G.S. Alemà*, M.T. Ramacci*, J.R. Perez-Polo and L. Angelucci. Farmacologia 2a, Univ. "La Sapienza", 00185 Rome, Italy.

In the rat, as well as in humans, a reduction in plasma and pineal levels of melatonin is associated with the aging process. Experimental evidences show that chronic acetyl-l-carnitine (ALCAR) treatment prevents or extenuatessome age-dependent biological modifications especially of neuroendocrine and hormonal type. On these bases, we investigated the effect produced by an 8-month ALCAR treatment (100mg/kg/day in drinking water) on plasma and pineal immunoreactive melatonin levels at day (1000h) and nightime (0100h), in the old (24 months) male Fischer 344 rat. No differences were found in plasma melatonin levels during the light period between young (3 months) and old rats, while at night a reduction ($p<0.05$) was found in the old group; ALCAR treatment completely prevented this reduction. Pineal melatonin content during the light period was unaffected by aging. On the contrary, at night it was reduced in the old rats ($p<0.05$). This reduction was not present in the old rats treated with ALCAR. The mechanism by which ALCAR produces this effect remains to be studied; we propose, however, that this effect might be involved in the ability of ALCAR to retard age-dependent changes in the CNS and behavioral impairments.

19.21

EFFECTS OF d-FENFLURAMINE (dF) ON AGE ASSOCIATED CHANGES IN ACTH, CORTICOSTERONE (CORT), AND PROLACTIN (PRL). R.J. Handa, M.K. Cross*, L.H. Burgess, T.M. Cabrera, J. Clancy Jr*, S.A. Lorens. Departments of Cell Biology, Neurobiology, Anatomy and Pharmacology, Loyola University Chicago, Stritch School of Medicine, Maywood, IL 60153

Neuroendocrine changes are associated with the aging process in rats. To examine some mechanisms underlying these changes we administered the serotonin releaser and reuptake inhibitor, dF (0.6 mg/kg/day in the drinking water), to young (5 mo) and old (21 mo) male and female Fischer 344 rats for 30-38 days. Animals were sacrificed upon removal from their home cage (HC) or 20 min. after introduction into a novel environment (open field, OF). Significant increases in ACTH and CORT were found in young and old OF animals ($p<0.01$ vs HC groups).In old males, HC ACTH, OF ACTH and OF CORT levels were greater than in young males ($p<0.05$). dF reduced ($p<0.02$) ACTH and CORT in old OF males to levels similar to young OF males. There was no effect of dF in young males. In old OF females, CORT but not ACTH levels were lower ($p<0.05$) than in young OF females, and there was no effect of dF. PRL was elevated in old vs young HC females ($p<0.01$) and was further increased in response to OF. dF treatment reduced ($p<0.05$) the PRL response to OF in old females but not young females. These data support a role for serotonin in some age related neuroendocrine changes and suggest that low doses of dF can ameliorate some of these alterations.

19.22

GLUCOCORTICOID RECEPTOR IMMUNOSTAINING IS INCREASED IN AGED HIPPOCAMPUS. P.W. Landfield, R.M. Booze, S. Vinsant*, L.B. Cadwallader* and J.C. Eldridge. Dept. of Physiol. & Pharmacol., Bowman Gray Sch. of Med., Wake Forest Univ., Winston-Salem, NC 27103.

Glucocorticoids appear to contribute to age-related deterioration of the hippocampus (cf. Landfield *et al.*, Science, 1981; Sapolsky *et al.*, J. Neurosci., 1985). In addition, studies using adrenalectomized rats indicate that hippocampal glucocorticoid receptors (GCR) are decreased with aging. Nevertheless, the brain appears to deteriorate more rapidly with advancing age. This seemingly is a paradox since, if GCR are decreased with aging, brain cells should be protected from further age-related changes. However, recent studies in intact rats indicate that type II GCR may instead be increased with aging (Eldridge *et al.*, J. Neurosci., 1989).

To attempt to resolve this question we utilized immunostaining against type II GCR in intact animals. Pairs of young (4-5 mo-old) and aged (25-26 mo-old) Fischer-344 male rats were perfused with an aldehyde solution, and the brains were sectioned with a cryostat. Alternate sections were stained for Nissl and AChE. Sections for each young and aged rat were processed simultaneously for immunocytochemistry of GCR using standard ABC procedures.

Immunostaining for GCR was consistently more intense in aged animals, notably in hippocampal areas CA1 and CA2, and dentate granule cells, and in the neocortex. CA3 and CA4 stain very little for type II GCR. Qualitatively, these effects were readily apparent, but quantitative analyses are also underway. Thus, in conjunction with previous ligand binding results, the present data indicate that, with aging, there is greater rather than less activation of brain glucocorticoid receptors in intact animals. This observation could have significant implications for mechanisms of accelerating brain cell loss during aging. (Supported by AG04342 and DA03637)

20.1

GENE EXPRESSION DURING RAT BRAIN DEVELOPMENT: EFFECT OF PRENATAL EXPOSURE TO ETHANOL

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We have previously shown that exposure of rats to ethanol in utero affects the postnatal expression of particular brain genes. In those experiments, however, administration of ethanol to young primiparous dams by inhalation resulted in significant differences between treated and untreated dams in weight gain during pregnancy. In contrast, full grown, multiparous dams achieved a much better weight gain in both treated and control animals. As in previous studies, pregnant rats were exposed to ethanol vapor between gestational days 8–21, resulting in BALs of approx. 150 mg/%. At birth, litters from treated and control dams were culled and fostered to normal mothers and RNA was extracted from dissected brain regions at different postnatal ages. There were no differences in the weights of control and ethanol treated pups at birth or during postnatal development, although the cortices of the treated pups were consistently lower in weight. In contrast to previous results, there was little difference in the expression of Tα1-tubulin and proteolipid protein (PLP) mRNAs between ethanol-treated and control pups, suggesting that the delay in normal expression of these genes seen previously in ethanol-treated pups was probably due to nutritional factors. There were differences, however, in the number of cells expressing PLP mRNA, detected by in situ hybridization, during the early postnatal period. The expression of other neural cell-specific mRNAs is under investigation.

20.2

EFFECTS OF PRE- AND POSTNATAL ETHANOL EXPOSURE ON THE L2 DORSAL ROOT OF THE NEONATAL RAT.

E.L. Fagan, R.L. Shew, L.P. Gonzalez and D.L. McNeill. Dept. of Anat. Sci., Univ. of Oklahoma, Oklahoma City, OK 73190

We recently demonstrated that maternal ethanol (ETOH) consumption resulted in a significant reduction in the number of myelinated fibers in the ventral funiculus (VF) of the neonatal spinal cord. Since the VF contains motor and sensory fibers, the present study was designed to focus the effects of ETOH on a pure sensory population of fibers. Dams were fed either a control or an ETOH-containing liquid diet for 2 weeks, then mated. The diets were continued throughout gestation. On postnatal day (PD) 4, the pups were implanted with a gastrostomy tube and artificially reared. Pups from control dams were given an ETOH-free formula while pups from dams on the ETOH diet were given a formula containing ETOH. On PD 10, control and ETOH exposed pups were perfused and their L2 dorsal roots processed for electron microscopy. Quantitative analysis revealed no significant differences between the two groups in dorsal root area, unmyelinated axon number, myelinated axon diameter or myelin thickness. However, in the ETOH exposed pups, there was a significant decrease ($p < 0.01$) in the number of myelinated axons and a significant increase ($p < 0.02$) in the number of axons with a single wrap of myelin. No significant difference in axonal number was observed when myelinated axons and axons with one myelin wrap were summed. These data suggest that ETOH exposure does not affect total axonal numbers. However, as in the CNS, it appears that ETOH delays the acquisition of myelin in this peripheral sensory system. (Supported by the Presby. Hlth. Found., the OU Alumni Res. Assoc. and OCAST.)

20.3

EFFECTS OF DEVELOPMENTAL ALCOHOL EXPOSURES ON MYELIN IN THE DORSAL COLUMN OF THE ADULT RAT SPINAL CORD.

D.E. Phillips, J.E. Rydquist*, H. Brunelle*, Biology Dept., WAMI Medical Education Program, Montana State Univ., Bozeman, MT 59717.

Our previous studies have shown that alcohol exposures for all of gestation plus 10 postnatal days (equivalent to all of gestation in humans) causes a reduction in myelin thickness in adult rat optic nerve. This study was designed to determine if a similar reduction in myelin thickness occurs in other CNS fiber populations from the same animals. Unborn rats were exposed to alcohol via maternal liquid diet, then the artificially reared pups further exposed by diet fed through a gastrostomy tube for 10 days. Control animals were pair fed isocaloric diets. Anesthetized animals were perfused at 90 days with fixative and spinal cord tissues prepared for electron microscopic study. The gross area of the dorsal column in the adult animals was unaffected by the developmental ethanol exposure. The myelin thickness, as a function of axon diameter, was not affected by alcohol, either in the more dorsal, sensory part of the dorsal column, nor in the ventral, cortico-spinal part of the dorsal column. These results indicate that myelin maturation, like neuronal maturation, can be differentially affected in different regions of the CNS by the same developmental alcohol exposures. Supported by NIAAA AA7042 and NIH MBRS S06 08218.

20.4

LHRH NEURON MIGRATION IN FETAL MICE EXPOSED TO ETHANOL IN UTERO.

H.C. Scott*, E. Westling*, W.K. Paull* and P.K. Rudeen. Department of Anatomy & Neurobiology, University of Missouri School of Medicine, Columbia, MO 65212.

The migration of luteinizing hormone-releasing hormone (LHRH) neurons from the medial nasal placode into the forebrain was analyzed in fetal C57BL/6J mice exposed to ethanol (etoh) in utero. Pregnant dams were intubated with 25% etoh solution (0.015ml/kg body weight) or with vehicle at 10 a.m. and 2 p.m. on Day 7 of pregnancy. Animals were sacrificed on Day 18 of pregnancy and the pups were removed and fixed in Bouins. Blood alcohol levels were measured in a seperate group of mice follow-ing a single intubation of ethanol or vehicle. Peak BAC levels were 56mg% within 40 min following ethanol administration. Tissue was processed and serial parasagittal sections were cut at 8um. Immunocytochemical procedures were used to identify LHRH neurons. The brain and nose regions were divided into six neuroanatomical regions: placode, nasal septum, traverse, arch, preoptic area, and caudal preoptic area. The number of LHRH immunoreactive neurons in each area in fetal etoh-exposed and control pups were compared. The data suggest that fetal etoh exposure results in a decrease in the number of LHRH immunoreactive neurons in each neuroanatomical area as compared to vehicle-treated animals. (Supported by NIAAA grants AA07458, AA05893 & AA00107).

20.5

EFFECT OF ALCOHOL EXPOSURE DURING THE BRAIN GROWTH SPURT ON COMPOSITION OF FOUR LIMBIC AREAS IN 40 TO 45 DAY OLD RATS.

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In an attempt to determine the neural bases of changes in behavioral reactivity induced by perinatal alcohol exposure, the chemical composition of the amygdala, the hypothalamus, the septal region and the hippocampus proper was examined. Alcohol exposure occurred between postnatal day (PD) 4 and 10 and was accomplished via an artificial rearing procedure. One group was given 5 g/kg/day alcohol exposure and a second group was given 3 g/kg/day alcohol exposure. A third group was artificially reared but not exposed to alcohol. A final group was reared normally with dams. All artificially reared groups were given milk formula alone from PD 10 to 12 and then fostered back to dams on PD 12. Between PD 40 and 45, the rats were deeply anesthetized and killed. The four limbic regions were quickly dissected free, sonicated in cold 6% trichloroacetic acid, and frozen until the time of assay. Chemical composition was examined using colormetric assays for DNA, cholesterol and protein content (Grant and Samson, *Behav. Toxicol. Teratol.* 4: 315, 1982; Zamenhof et al. *J. Neurochem.* 19: 61, 1972). DNA and cholesterol concentration can be taken as approximate measures of cell number and myelination, respectively. Protein content per DNA can be an indicator of cell size.

Alcohol exposure during the brain growth spurt did not change DNA concentration in any of the neural regions in the female rats but did decrease DNA concentration in the amygdala of male rats. In contrast, cholesterol concentration appeared to be increased in all examined neural regions of female rats exposed to alcohol but no changes in cholesterol concentration were detected in male rats. Protein content per DNA in the hypothalamus was decreased by alcohol exposure in both sexes. (Supported by NIAAA Grant AA08080 to S.J.K.)

20.6

PURKINJE CELL SURVIVAL FOLLOWING ETHANOL EXPOSURE DURING THE BRAIN GROWTH SPURT DEPENDS ON THE TIMING OF EXPOSURE AND LOCATION IN THE CEREBELLAR VERMIS.

K.M. Hamre and J.R. West. Dept. of Anatomy, College of Medicine, University of Iowa, Iowa City, IA 52242.

Purkinje cell loss after ethanol exposure during the brain growth spurt does not occur uniformly in different cerebellar lobules. While the factors contributing to this differential cell loss are unknown, one proposed variable is the degree of Purkinje cell maturity at the time of the ethanol exposure. Previous studies from our laboratory have shown that Purkinje cell dendritic maturity during the early postnatal period can be determined using immunocytochemistry with an antibody against microtubule-associated protein 2, and the cells classified as part of early or late maturing regions. In the earliest maturing regions, lobules IX and X, dendritic outgrowth was initiated on postnatal days (P) 4-5 while in the latest maturing regions, the distal part of lobules VI and lobule VII, dendritic outgrowth was initiated on P9-10. The purpose of this study was to test the hypothesis that varying the ethanol exposure to correspond to these developmental stages would affect the amount or location of Purkinje cell loss. Male rat pups were exposed to 6.6 g/kg/day of ethanol, using an artificial-rearing procedure, for one of the following two-day periods: P4-5, P6-7, P9-10 or P12-13. Purkinje cells were counted in lobules VI, VII, IX and X of the cerebellar vermis on P21. Significant Purkinje cell loss occurred across the four lobules in the animals exposed to ethanol on P4-5 or on P6-7, but not in animals exposed on either P9-10 or P12-13. Slight changes in the timing of exposure e.g., P4-5 versus P6-7, led to changes in the amounts of cell loss, but only in lobules VII and XI. In lobule VII, only the group exposed on P6-7 demonstrated a significant cell loss compared to controls. In lobule IX, pups exposed on P4-5 had significantly greater cell loss than those exposed on P6-7. Within lobules, the loss of Purkinje cells was not uniform across all portions of the lobules. For instance, in lobule IX more gaps in the Purkinje cell layer were seen on the dorsal versus the ventral portion of the lobule. The stage of dendritic maturity is not an accurate predictor of Purkinje cell death, but there do appear to be "windows of vulnerability" which affect the amount of Purkinje cell loss in each region. Support: grant AA05523 from NIAAA.

20.7

DOSE-DEPENDENT DEFICITS IN CEREBELLAR GROWTH INDUCED BY EARLY POSTNATAL ALCOHOL EXPOSURE: DIFFERENCES AMONG INBRED STRAINS OF RATS. C.R. Goodlett, J.M. Nichols* and J.R. West. Department of Anatomy, University of Iowa, Iowa City, IA 52242.

Studies in Sprague-Dawley rats have demonstrated that neonatal alcohol exposure permanently restricts growth of the cerebellum and depletes its neuronal populations when blood alcohol concentrations (BACs) reach 150-190 mg/dl. In an effort to identify genetic influences on the severity of cerebellar growth restriction induced by early postnatal alcohol exposure, six inbred strains were evaluated using three doses of alcohol. The strains [ACI, F344, MR, M520, WN and WKY] were derived from breeders originally established from breeding pairs obtained from NIH. Pups from timed pregnancies of each strain were implanted with a gastrostomy tube on gestational day 27 (usually postnatal day 5), and reared artificially (milk formula feedings every two hours). Treatment groups included 4.4, 5.5 and 6.6 g/kg/day of alcohol, with the alcohol administered in four consecutive feedings each day as a 5.0%, 6.25% or 7.5% (v/v) solution, respectively. Controls included artificially reared controls and suckle controls. On gestational day 32, the pups were perfused intracardially, the brains were carefully extracted and dissected into forebrain, brain stem and cerebellum, and weights were obtained. Alcohol exposure restricted cerebellar growth in a dose-dependent fashion in all strains, but differences among strains in susceptibility were present and depended in part on the dose used. Susceptibility to cerebellar growth restriction based on the 4.4 g/kg dose, which produced mean BACs between 191-243 mg/dl, indicated that two strains were relatively resistant [WN and M520], two strains were relatively vulnerable [MR and F344] and two were intermediate [ACI and WKY]. These strain differences were not dependent on differences in BAC. Across all doses, the MR strain was consistently severely affected, and the M520 strain was relatively less affected. At the higher doses, exceptions to the susceptibility rankings of the other strains were noted, including a disproportionate cerebellar weight restriction by the high dose in ACIs and a relative lack of further growth restriction induced by the two higher doses in F344s. These strain differences indicate that genotype can modulate the neurotoxicity of alcohol exposure during the brain growth spurt. (Supported by grant #AA 07313)

20.8

EFFECTS OF PRENATAL COCAINE EXPOSURE ON ISOLATION-INDUCED BEHAVIORS AND DOPAMINE IN NEONATAL RATS C.B. Boylan* and P. Kehoe. Department of Psychology, Trinity College, Hartford, CT 06106

In the neonatal rat, subcutaneous administration of cocaine during gestation causes alterations in dopamine activity as evidenced by behavioral and neurochemical deficits. To determine the effects of continuous cocaine exposure on isolation-induced behaviors and neonatal brain dopamine levels, studies are underway in which cocaine 5, 10, 20 mg/kg/day or vehicle (0.9% sterile saline) is delivered to pregnant albino rats on gestation day 4 through parturition via surgically implanted osmotic mini-pumps (Alzet). Within 24 hours of birth, pups are cross-fostered to untreated lactating females and on postnatal day 10, tested with pharmacological probes for any deviations in isolation-induced ultrasonic vocalizations (UVs), activity levels and analgesia. Since both the dopamine and endogenous opioid systems are known to mediate these behaviors, pharmacological agonists which act on each system (Cocaine 5 mg/kg on the dopamine system & Naltrexone 0.5 mg/kg on the opioid system) were chosen as appropriate probes. In addition, HPLC with Electrochemical Detection was utilized to determine day 10 striatal dopamine concentrations. In general, pups from cocaine treated DAMs have decreased isolation-induced UVs; don't exhibit either normal cocaine suppression or naltrexone elevation of UVs; have decreased activity levels during isolation and have decreased baseline and isolation-induced pain responsivities. In addition, prenatally exposed cocaine pups demonstrate a trend for decreased striatal dopamine concentrations as compared to controls. These results imply that prenatal cocaine exposure produces an attenuation in dopamine activity which may also correspond to a change in the endogenous opioid system, causing neonatal behavioral alterations.

20.9

COMBINED EFFECTS OF PRENATAL COCAINE AND ALCOHOL EXPOSURE ON OFFSPRING MATURATION. M.W. Church, G.W. Overbeck*, J. Tilak*, and P. Holmes*. Fetal Alcohol Research Center, Dept. Ob/Gyn, Wayne State Univ. Sch. Med., Detroit, MI 48201

While prenatal exposure to cocaine alone can adversely effect the unborn child, pregnant cocaine abusers typically use other harmful drugs as well (e.g., alcohol, marijuana, nicotine, opiates). Consequently, there is a need to investigate the effects of prenatal exposure to various drug combinations involving cocaine. To investigate the combined effects of cocaine and alcohol, pregnant Long-Evans rats were given either cocaine (30 mg/kg, b.i.d., s.c.), alcohol (2 g/kg, b.i.d., p.o.), or both drugs from gestation days 7 to 20. Ad lib and pair-fed control groups and surrogate fostering were also used (N=8 to 14 litters/group). Compared to the other groups, the cocaine-plus-alcohol offspring showed (a) reduced birth weight and postnatal weight gain and (b) delayed maturation regarding pinna detachment, fur growth, ear opening and vaginal opening ($P<0.05$), but no delay in eye opening. These results suggest that the cocaine-plus-alcohol combination has a more deleterious effect than either drug alone. (Supported by DA05536)

20.10

DEVELOPMENT OF BRAIN BETA RECEPTORS CHANGED BY PRENATAL PROPRANOLOL. **Xiao-Ke Gao*, J.C. Miller, K.A. Bonnet and A.J. Friedhoff. Millhauser Labs of the Dept. of Psychiatry, N.Y. Univ. Med. Ctr., NY, NY, 10016.**

Rat cortical beta receptors were determined by ^{3}H-DHA receptor binding assay in rats treated prenatally with propranolol (PRO). PRO was administered either i.p. or orally to pregnant rats on gestational days (GD's) 8-11, a period before the kinetic properties of beta receptors mature. A saline (SAL) injection was used as a control on GD's 8-11 as for PRO. SAL injection caused significant stress to the pregnant rats, and induced a significant decrease in the number of ^{3}H-DHA binding sites in the offspring at 7 days of postnatal age compared to offspring of untreated pregnant dams. PRO injection on GD's 8-11 completely prevented the decrease in ^{3}H-DHA binding sites induced by the stress of injection. Exposure to PRO, given orally to avoid stress, produced a significant decrease in brain ^{3}H-DHA binding sites at postnatal age 7 days. From these observations we conclude that prior to phenotypic differentiation developing beta receptors are vulnerable to perturbation by stress or drugs.

20.11

IN UTERO EXPOSURE TO METHAMPHETAMINE (METH): EFFECTS ON NEURAL, ENDOCRINE, AND IMMUNE FUNCTIONS IN MALE RATS. T.M. Cabrera, M.K. Cross*, M. George*, L. Petrovic*, J. Clancy Jr.*, R.J. Handa and S.A. Lorens. Depts. of Pharmacology and Anatomy, Loyola Univ. Chicago Medical Ctr., 2160 S. First Ave., Maywood, IL 60153.

Gravid Sprague-Dawley rats received (±)METH (5 mg/kg, sc, bid) or saline (SAL) during gestational days 12-20. One SAL subgroup (VC) had ad lib access to the liquid diet. The pair-fed SAL subgroup (PF) received 25-30% less of the diet than the VC group in order to simulate the reduced food intake of the METH treated dams. All offspring were cross fostered to lactating normal females. The METH male offspring had significantly ($p<0.01$) shorter crown-rump lengths and lower (24%) body weights than the VC and PF pups, which weighed less (16%; $p<0.01$) than the VC group. The METH offspring [47-53 days of age (doa)] showed deficits in the acquisition of a food foraging task as evidenced by longer (100%; $p<0.01$) latencies to locate the reinforcer. METH and PF offspring (59-62 doa) showed a decrease (35%; $p<0.05$) in the number of nose pokes emitted during a 9 min open field (OF) test. All groups showed comparable increases in OF activity in response to METH (0.5 mg/kg, sc). Interleukin-2 (Il-2) stimulated natural killer (NK) cytotoxicity was increased (33%; $p<0.05$) in the METH rats (94-103 doa), but no group differences were found in T- or B-cell mitogenesis. Compared to home cage controls, stressed (2 shocks, 10 sec each, given at 1 and 10 min into a 20 min session) PF rats showed higher (34%; $p<0.05$) corticosterone (CORT) levels than the other stressed groups (94-103 doa), and failed to evidence a stress induced increase in medial frontal cortex (MFC) 5-HIAA levels. The METH rats showed a greater ($p<0.05$) stress induced increase (49%) in MFC 5-HIAA levels than the VC rats. Prenatal METH exposure impairs spatial learning, decreases exploration of a novel environment, increases Il-2 stimulated NK activity, and enhances stress induced MFC serotonin turnover. Prenatal malnutrition (PF rats) increases the CORT and inhibits the MFC 5-HIAA response to stress, as well as decreases exploratory behavior.

20.12

THE EFFECTS OF CHRONIC AMPHETAMINE ON GROWTH AND BEHAVIOR IN MALE AND FEMALE RATS EXPOSED TO ALCOHOL IN UTERO. **J.H.Hannigan and M.L .Pilati , Center for Behavioral Teratology, SUNY-Albany, Albany, NY, USA, 12222.**

Fetal Alcohol Syndrome often includes attention deficits treated with CNS stimulants, which can retard growth. Since rats exposed to prenatal ethanol (EtOH) are growth retarded and over-sensitive to acute amphetamine, we tested the biobehavioral impact of daily amphetamine. Subjects were male and female pups of dams fed either EtOH or control diets on gestation days 6-20. Rats received amphetamine (0, 2 or 10mg/kg/day) from postnatal days 22 (PN22) to PN43 and were weighed every other day until PN61. On PN22, PN28, PN36 and PN42, pre- and post-amphetamine behavior was measured.

Prenatal EtOH reduced brain and body weight and increased growth rate. However, EtOH rats never "caught-up" to controls, either during or after amphetamine, which reduced growth rate equivalently for all groups. There were no interactions between prenatal treatment, sex, or amphetamine dose on any growth measure. Behavioral tests will extend findings of EtOH-induced over-sensitivity, plus reduced tolerance to chronic amphetamine. Results suggest that fetal alcohol induced a developmental delay in growth rate but did not change the growth retarding effects of amphetamine.

Supported by RSDA #00111 from NIAAA to JHH.

20.13

PRENATAL METHADONE (M) EXPOSURE AFFECTS ANALGESIA AND NEUROTRANSMITTER CONTENT IN NEONATAL RATS. S.E. Robinson, H.-Z. Guo*, E.K. Enters, U. Pandey*, and K.P. McDowell*. Dept. of Pharmacology & Toxicology, Medical College of Virginia, VA 23298-0613.

On day 8 of pregnancy, female Sprague-Dawley rats were implanted under methoxyflurane anesthesia with 14-day Alzet osmotic minipumps filled with water (W) or M (9 mg/kg/day). There was no difference in food or water consumption or weight gain among unhandled control (C) dams or those treated with W or M. Within 24 h of delivery, pups were weighed, sexed, litter size reduced to 10, and fostered to untreated dams. There was no difference in litter size or number of implantation sites among the 3 groups. M-exposed male pups exhibited an enhanced analgesic response to morphine (0.4 mg/kg, s.c.) as measured by tail-flick on postnatal day 4, but no difference in their analgesic response to saline or naloxone (1 mg/kg, s.c.). On day 4, striatal ACh content, as measured by gas chromatography/mass fragmentography in pups euthanized by focussed microwave radiation, was significantly reduced in male M-exposed pups ($p<0.05$). There were no statistically significant changes in cortical or hippocampal ACh in the M-exposed male pups and nor in the 3 brain areas of M-exposed female pups. Catecholamines and indoleamines were also measured in these areas by reversed-phase HPLC with electrochemical detection. There were no significant changes in dopamine, norepinephrine, or serotonin content in any of these 3 areas. These studies indicate that this method of prenatal exposure to M can produce changes in brain regional ACh and analgesic responses to morphine without substantial maternal or fetal morbidity and mortality. (Supported by NIDA grant DA-05274).

NUTRITIONAL AND PRENATAL FACTORS

21.1

DEGENERATION OF RAT LOCUS COERULEUS (LC) NEURONS IS NOT PARALLELED BY AN IRREVERSIBLE LOSS OF ASCENDING LC PROJECTIONS: EVIDENCE FOR REESTABLISHMENT OF FOREBRAIN INNERVATION BY SURVIVING NEURONS. R. Grzanna and J.-M. Fritschy, Johns Hopkins Univ. Sch. of Med., Dept. Neurosci., Baltimore,.MD 21205.

Systemic administration of the noradrenergic (NE) neurotoxin DSP-4 induces degeneration of LC axons in rats. This degenerative process is followed by profound loss of LC cell bodies. The present study was conducted to determine whether LC neurons surviving DSP-4 treatment are capable of regeneration. The extent of LC neuron loss was compared to the pattern of NE axon staining between 2 weeks and 1 year after DSP-4 treatment (50 mg/kg, i.p.). LC neurons were counted in 30 m Nissl-stained sections. The distribution of NE axons in the brain was visualized by dopamine-ß-hydroxylase immunohistochemistry. After DSP-4 treatment, the number of LC neurons decreased graduallly to reach on average 60% of control at 6 months. In some rats up to 75% of LC neurons were lost. Despite the cell loss, extensive regeneration of LC axons occurred in the forebrain, but not in the brainstem and cerebellum. One year after drug treatment, the LC innervation of the forebrain was largely restored, with some regions exhibiting pronounced hyperinnervation. No reinnervation was observed in the brainstem and cerebellum, even in cases with only moderate LC neuron loss. While the regeneration process was slower in animals with severe LC neuron loss, the pattern of reinnervation was strikingly similar in all cases. The results demonstrate that regeneration of LC axons after DSP-4 treatment is region-specific and occurs in the presence of extensive of LC neuron loss. While the surviving LC neurons exhibit an extraordinary capacity to compensate for cell loss, the pattern of reinnervation appears to be controlled by target regions. DSP-4 represents a promising experimental tool to study cell death and regeneration in the CNS. Support MH41977.

21.2

NORADRENERGIC (NE) AXON DEGENERATION IS ACCOMPANIED BY TRANSIENT AND GENERALIZED INCREASE IN GFAP IMMUNOREACTIVITY IN THE RAT BRAIN. J.-M. Fritschy, C.G. Frondoza, C. Cardona and R. Grzanna, Johns Hopkins Univ., Depts. Neurosci. and Immunology & Infectious diseases, Baltimore, MD 21205.

Glial fibrillary acidic protein (GFAP) is a specific marker of astrocytes in the CNS. Increases in GFAP levels in brain have been reported in response to neuronal injuries. We have previously demonstrated that systemic administration of the NE neurotoxin DSP-4 induces extensive degeneration of locus coeruleus (LC) axons within 4-7 days. The present study was conducted to determine whether degeneration of LC axon terminals increases GFAP levels in the brain and whether such increases are restricted to regions containing degenerating NE fibres. Rats were perfused 1, 4, 5, 7, 10, 14 and 28 days after a single systemic injection of DSP-4 (50 mg/kg). Brain sections were processed for GFAP immunohistochemistry using the avidin-biodin peroxidase method. DSP-4 treatment triggered a dramatic and generalized increase in GFAP immunoreativity throughout the brain, including regions not innervated by LC axons. The response of astrocytes was first detectable 4 days after drug treatment, was maximal after 7 days, and declined progressively thereafter. DMI pretreatment (20 mg/kg) prevented the DSP-4-induced LC axon degeneration and the increase in GFAP staining. The induction of GFAP by DSP-4 is unrelated to transmitter depletion from NE axons, since reserpine treatment did not increase GFAP immunoreactivity. The present results demonstrate that drug-induced degeneration of NE axons is paralleled by a pronounced increase in GFAP levels in brain. Unlike after focal lesions, the increase in GFAP levels is transient and occurs throughout the brain. We propose that GFAP may serve as a useful marker to screen for drug-induced neuron degeneration in the CNS. Support MH41977 and CA 45640.

21.3

EFFECTS OF PROMETHAZINE HCl ADMINISTRATION TO GRAVID RATS ON THE BEHAVIOR OF OFFSPRING. J.H. Patton and M.S. Stanford. Dept. of Psychology, Baylor University, Waco, TX 76798 and Dept. of Psychiatry and Behavioral Sciences, University of Texas Medical Branch, Galveston, TX 77550.

Promethazine HCL (Phenergan®, Wyeth) is a phenothiazine used for its anti-emetic and sedative potential during human pregnancy. Research has indicated that rats exposed to promethazine perinatally have lower steady-state levels of brain serotonin than controls (Stanford & Patton, *Soc. Neurosci. Abstr.*, 15(2), p. 1018, 1989). In the present study seventy-nine rats exposed perinatally (during both gestation and lactation) to promethazine or saline vehicle were evaluated developmentally and behaviorally. The drug was injected into dams subcutaneously at a dose of 3.0 mg/kg per day. Pups were evaluated using a standard developmental protocol including somatic measures and reflexive evaluation. Additionally, animals were evaluated in an open-field and with a conditioned avoidance task of pain sensitivity.

There were no differences in gestation time or litter size, dam or pup weight, nor in pup length. There were no differences in surface righting or avoidance conditioning. There were differences in time to eye opening and incisor protrusion, although these were just short of statistical reliability. There were statistically reliable differences in days to ear extension, cliff avoidance behavior, negative geotaxis, and open-field activity attributable to promethazine treatment.

The results of this study suggest that exposure to promethazine HCl transplacentally and through milk has behavioral consequences in addition to the neurotransmitter effects reported earlier.

21.4

Dietary Copper Deficiency Modified Resident-Intruder Behavior in Mice. E.S. Halas and L.M. Klevay*. Department of Psychology, University of North Dakota and USDA, ARS, Human Nutrition Research Center, Grand Forks, ND 58202.

Thirty-six Swiss-Webster male mice were individually housed with 36 ovariectomized Swiss-Webster females. Each pair was fed an experimental diet deficient in copper. Eighteen pairs were given a drinking solution supplemented with copper (CuS) while the remaining 18 pairs were not so supplemented (CuD). This regimen was maintained for 9 weeks. Once a week for 8 weeks, the female was removed from each cage and was replaced by an intruder male. The two males remained together in the cage for 5 min after the first attack. The interactions between the two males were videotaped. Then the intruder male was returned to its cage. Behavior was scored later by two, independent observers. These procedures were repeated in a second experiment except the drinking water for the deficient group contained 0.5 ppm Cu. In the first experiment, the CuD mice attacked more than the CuS mice during the first week but then attacked less from week 3 through 8. In the second experiment, the CuD mice attacked less from the first week onward. Hematocrits measured in the third week of the second experiment were (mean ± S.D.) 50.3 ± 2.0 and 48.5 ± 3.0 respectively for CuS and CuD mice. A central rather than a peripheral causality is more likely for the passivity of deficient mice as anemia was absent.

21.5

TIMM'S STAINING OF RAT ENTORHINAL CORTEX DECREASED BY SUBCLINICAL DIETARY MICRONUTRIENT DEFICIENCIES. E.J. Root, D.H. Montgomery*, and J. B. Longenecker*. Graduate Nutrition, The University of Texas at Austin, Austin, TX 78712.

Prior studies showed that vitamin and mineral deficiencies too mild to significantly alter weight gain or outward appearance of rats can nevertheless alter brain cell structure & behavior (Nutr. Rep Int. 37:959, 1988; Fed Proc 46:903, 1987). Male weanling rats were fed 6 diets with various combinations of subclinical deficiencies (1/4 the requirement) for 6 months. One rat each from the control group, group 5 (deficient in Mg, Cu, vitamin B6, folacin, & choline), and group 6 (deficient in Mg, Zn, Ca, vitamin B6 & choline) was chosen for Timm's staining by the method of Danscher (Histochem. 71:1, 1981) rather than for conventional electron microscopy.

Group 6 showed a marked decrease of staining in L IV to VI of the entorhinal cortex and also decreased staining of the ventral half of the rhinal sulcus. Group 5 differed little from controls in sulfide-silver staining. Size of neurons and total area of entorhinal cortex were decreased in both groups 5 & 6. Decreased sulfide-silver staining implies decreased numbers of zinc-containing synaptic terminals.

21.6

COMPARISON OF THE BRAINSTEM AUDITORY EVOKED RESPONSE (BAER) DEVELOPMENT IN TAURINE SUPPLEMENTED AND TAURINE-DEFICIENT KITTENS. M-H. Vallecalle, G. Heaney*, E. Sersen*, J. A. Sturman. Inst. for Basic Res. in Dev. Disabil., 1050 Forest Hill Rd., S.I., NY 10314.

It has been demonstrated that the presence of normal taurine (Tau) concentrations in the cat are essential for the normal development of the retina and visual cortex, at least. Very little information is available on its function within the auditory system despite the report of high level of free Tau in the organ of hearing. Brainstem Auditory Evoked Response (BAER) is widely used for the assessment of hearing function, and undergoes characteristic changes during brain maturation. The aim of this study was to assess the development of hearing function in kittens subjected to variable conditions of Tau nutrition (.02%, .05%, 1%) from their period of conception until 12 weeks of age.

The BAERs were elicited by rarefactions clicks at intensities ranging from 30 - 80 dB (p.e. SPL). For each kitten, thresholds and latencies of successive positive and negative peaks were determined at 4 and 8 weeks of age. Our preliminary results are as follows: Most animals younger than 7 postnatal days are unresponsive, and reliable responses are not observed in all animals until early in the second postnatal week. At this age, waves I and IV are identifiable for intensities $\geq$ 50 dB (p.e. SPL). Threshold, determined by the lowest intensity at which PIV is present, does not differ significantly between groups at either age. Analysis of latencies suggests longer brainstem transmission time in the .02% Tau group than in the other two groups.

21.7

PRENATAL MALNUTRITION AND POSTNATAL NUTRITIONAL REHABILITATION ON THE DENTATE GYRUS IN RATS OF FOUR AGE GROUPS. S. Díaz-Cintra, L. Cintra, A. Galván*, A. Aguilar*, T. Kemper* and P. J. Morgane. Inst. Inves. Biomédicas, UNAM, México, D.F. 04510, Neurol. Unit. Boston City Hosp. Boston MA, 02118 & Worcester Found. for Expt. Biol. Shrewsbury MA, 01545.

In a previous study on the hippocampal formation we analyzed morphometrically the effects of protein malnutrition (8% casein diet), instituted before mating and continued during gestation and into postnatal life on the granule cells of the dentate gyrus. The objetive of the present study was to use nutritionally rehabilitated animals that were born from a mother fed with 6% casein diet before mating and during gestation. At born they were cross fostered to a normai mother that was fed in a similar conditions, with a 25% casein diet. Using the rapid Golgi method we studied the dentate gyrus in rehabilitated rats at 15, 30, 90 and 220 days of age. We found significant reductions in the major and minor axes due to diet effect. The number of dendritic spines measured in proximal, medial and terminal segments were significantly reduced by the diet and dendritic density measured by the number of intersections 38 microns apart in 9 concentric rings, showed significanrt reductions on the last five rings. This findings point to a long term effect of prenatal protein malnutrition on the molecular layer of the dentate gyrus in rehabilitated animals. (Supported by CONACyT Fellowships 20518, 27234, México, D.F. and NIH Grants HD-23338 and HD-22539).

21.8

SLEEP DEPRIVATION IN NORMAL AND MALNOURISHED RATS OF 30 DAYS OF AGE. L. Cintra, A. Galván*and S. Díaz-Cintra. Dept. de Fisiología Instituto de Investigaciones Biomédicas. UNAM, México, D.F. 04510.

Sleep deprivation has been employed as an important strategy to unravel sleep functions and recently the homeostatic aspect of sleep regulation was proposed from sleep deprivation experiments, where sleep lost induces a compensatory increase of sleep. However, this strategy as not been used in malnourished animals. The objetive of the present study was to evaluate the effect of protein malnutrition (6% casein diet), instituted before mating and continued during gestation and into postnatal life on the sleep-wake cycle before and after sleep deprivation. A base line-day was followed by one day of sleep deprivation in a continuously rotating cilinder (diam. 32 cm; rotation rate: 1 turn/2.40 min) and three recovery days in normal (25% casein diet) and malnourished rats at 30 days of age. We found a significant increase of slow wave sleep, specially during the activity phase in the second recuperation day. However REM sleep was significantly decreased mainly during the rest phase on the first and third recuperation days. These data reveals that the compensatory increase of REM sleep was more affected by malnutrition in comparison to slow wave sleep. (Supported by CONACyT Grant P219CCOA880341).

21.9

PRENATAL PROTEIN MALNUTRITION ALTERS RAPID KINDLING IN THE DENTATE GYRUS. **R.J. Austin-LaFrance, J.D. Bronzino and P.J. Morgane.** Dept. of Engineering and Comp. Sci., Trinity College, Hartford, CT 06106 and The Worcester Foundation for Experimental Biology, Shrewsbury, MA 01545.

A rapid kindling paradigm was used to investigate the effects of prenatal protein malnutrition on the development of kindling in adult rats. Dietary groups consisted of pups born to dams fed either a low (6%) or control (25%) casein diet. All pups were fostered at birth to lactating dams fed the 25% casein diet and were designated 6%/25% or 25%/25% based on the pre-/postnatal diet. As adults, animals were implanted with a stimulating electrode in the perforant path and a recording electrode in the dentate gyrus. Kindling consisted of 10 sec. trains of biphasic square-waves applied to the perforant path every 5 min. for 1 hour on 5 consecutive days. Results indicate that control animals exhibit more than twice as many stage 5 seizures during the test period as do 6%/25% animals. Additionally, 2 of 6 malnourished animals failed to achieve a single stage 5 seizure. The results parallel our previous findings with a classical kindling paradigm and indicate that rapidly kindled seizures can be effectively used to study the impact of prenatal insults, such as malnutrition, on the development of kindling and the resultant kindled state. **(Supported by NIH Grant # HD-22539)**

21.10

PRENATAL PROTEIN MALNUTRITION RESULTS IN THE LOSS OF BEHAVIOR-MEDIATED THETA FREQUENCY SHIFTING. **P.J. Morgane, K.B. Austin, S.J. Palmer[c], R.J. Austin-LaFrance and J.D. Bronzino.** Worcester Found. for Exp. Biol. Shrewsbury, MA 05145, Dept. of Engineering and Computer Sci., Trinity College, Hartford, CT 06106

We investigated the relationship between hippocampal theta frequency and theta behaviors in prenatally protein malnourished adult rats. Diet groups consisted of pups born to dams fed a low (6%) or control (25%) casein diet. All pups were fostered at birth to lactating dams fed the control diet and were designated 6%/25% or 25%/25% based on the pre-/postnatal diet. Theta frequency measures were calculated from EEG activity recorded in the hippocampus during periods of REM sleep and active waking (AW). AW was defined as periods of exploratory locomotion. 25%/25% animals exhibit a significant shift in theta frequency between these two behavioral states, with the slower frequency seen in AW. 6%/25% animals exhibit no change in theta frequency between these states. No between-group difference was found in theta frequency obtained during REM, suggesting that septo-hippocampal cholinergic systems pacing REM theta remain intact, while non-cholinergic theta systems associated with voluntary movement appear unable to regulate theta frequency shifting to match behavior. **(Supported by NIH Grant # HD-22539)**

21.11

ENVIRONMENTAL ENRICHMENT DURING NUTRITIONAL REHABILITATION INCREASES DENDRITIC SPINE NUMBER. A. Carughi,* L.L. Ring,* K.J. Carpenter* and M.C. Diamond. Dept. of Nutritional Sciences and Dept. of Anatomy/Physiology. University of California, Berkeley, CA 94720.

Environmental enrichment has been reported to aid recovery from behavioral deficits associated with malnutrition in infants and young rats. Our study investigates whether corresponding neuroanatomical changes can be detected. Rats were suckled either by well-fed dams (17% protein) or dams malnourished (8% protein) from the 17th gestational day throughout lactation. At weaning, well-fed male pups were either housed in pairs (standard condition, SC) or 12 per large cage with "toys" (enriched condition, EC) and fed a 17% protein diet. Previously malnourished pups were also fed a 17% protein diet (rehabilitation, "rehab") and housed in SC or EC environments. This treatment lasted for 30 days. Spines with a head and a stalk ("lollipop") and those with equal sized head and base ("nubbin") were counted from segments of oblique and basal dendrites from occipital cortical pyramidal cells. SC-rehab rats had 15% and 16% less "lollipop" spines on oblique and basal dendrites respectively than SC-control ones. However, EC-rehab rats had 30% and 34% more "lollipop" spines than SC-rehab rats on these locations. The interaction diet x environment was significant. Thus, environmental enrichment during nutritional rehabilitation increases "lollipop" spine number. These results support those previously reported by our laboratory (Carughi, A.; Carpenter, K.J.; Diamond, M.C. J. Nutr. 119:2005-2016, 1989) on cortical thickness and dendritic branching.

21.12

DEPLETION OF "OUTSTANDINGLY HIGH" CEREBRAL DEVELOPMENT IN RAT SUBJECTED TO PRENATAL UNDERNUTRITION OR PRENATAL IONIZING RADIATION. S. Zamenhof. Dept. of Microbiology and Immunology, and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024

In previous reports (Life Sci., 18:1391, 1976) we have identified individual rats whose neonatal cerebral parameters (weight, DNA, protein) were "outstandingly high " (OH) (defined throughout as being more than 1 standard deviation (OH_1), or 2 standard deviations (OH_2) above the mean of normal population, i.e., tail end of normal distribution curve). In neonatal human infants we identified individuals with OH_2 head circumferences (correlated with cerebral weights and DNA)(Fed. Proc., 1990, in press). In the present work we have established that pregnant rats subjected to chronic undernutrition ($^2/3$ of ad lib diet) produce offspring in which occurrence of OH individuals (% of total) is depleted:

		cerebral weight	DNA	Protein
OH_1	Control	7%	16%	8%
	Undernutr.	1%	2.1%	0.5%
OH_2	Control	0.8%	0.7%	1.2%
	Undernutr.	0.0%	0.0%	0.0%

The differences between controls (total n = 1597) and undernourished (414) are statistically highly significant. Similar results were obtained for chronic ionizing radiation (tritiated drinking water, 3μ Ci/ml). Possible causes are neuronal death, or deficient mitotic phase. Thus, the harmful effects of these treatments are twofold: 1) Significant reduction of mean values of cerebral parameters in the population as a whole, and 2) depletion of cerebral OH individuals. If these parameters are correlated with behavioral performance, the depletion of individuals with outstandingly high cerebral parameters may be even more harmful than the well know reduction of mean values of these parameters in the population as a whole.

TRANSPLANTATION: GENERAL I

22.1

TRANSPLANTATION OF PREMYELINATING CULTURED OLIGODENDROCYTES INTO RAT BRAIN. M.D.A. Espinosa de los Monteros, M. Aymie*, M.N. Gordon, M.-S. Zhang*, R.A. Korsak*, J. Edmond* and J. de Vellis. UCLA School of Medicine, Mental Retardation Research Center, Los Angeles, CA 90024-1759.

To study myelination by transplanted, premyelinating oligodendrocytes (ol), we used wild-type and myelin deficient (*md*) rats. The *md* rat is characterized by inhibition of ol maturation and almost total lack of CNS myelin. Ol were separated from 12 d glial primary cultures derived from wild-type neonatal rat brains. Such cultures contain >95% ol by ICC for ol specific marker proteins. Premyelinating ol in culture were labeled with fast blue prior to grafting. Ols were suspended in a minimal volume of Hank's medium and 2.5 x 10^5 were stereotaxically injected into the corpus striatum. Grafted ol were identified in brain sections by the presence of the fast blue label and ICC for ol specific proteins at various times after grafting. Implanted ol were able to survive, migrate and express ol markers. In wild-type rats, fast blue$^+$, grafted ol were detectable for at least 3 months after grafting. Grafted, fast blue$^+$ ol were observed primarily ipsilateral to the graft site in the corpus striatum, corpus callosum and cortex. Ol also migrated thru-out the rostro-caudal extent of the corpus striatum and corpus callosum, and into the contralateral corpus callosum. Grafted wild type ol in *md* rats (grafted at P17) also survived, migrated and expressed the specific markers, but were insufficient to ameliorate motor symptoms (tremors and seizures). Future studies will examine grafting into younger *md* rats, and EM analysis of myelin produced by grafted ol. Supported by USPHS grant HD-06576.

22.2

THE ROLE OF MICROGLIA IN TRANSPLANTED RETINAE
R.Sharma and R.D.Lund. Dept of Neurobiology, Anatomy and Cell Science, Sch. of Med., Univ of Pittsburgh, Pittsburgh, PA 15261

We have shown that embryonic retinae transplanted to the brains of newborn rats can mediate pupillary responses in the host. During the transplantation procedure the retinal pigment epithelium (RPE) is typically removed. Since RPE is considered to be an essential component of the visual response, why does the transplant continue to function in its absence?

A previous light microscopic study (Perry and Lund, Neurosci. 31:453, 1989) showed that microglia surround the outer border of the transplanted retinae. Here we have examined the transplant/host interface at the ultrastructural level using pigmented donor retinae (to allow identification of RPE, if present) and a monoclonal antibody (OX-42) to identify microglia. No pigmented epithelial cells were seen associated with the retinal transplants. However, OX-42 positive cells (having the structural characteristics of microglia) were found surrounding the graft with processes interspersed among photoreceptor outer segments. These OX-42 positive cells were often seen phagocytosing outer segment membrane. Occasional astrocytes were seen among these cells. In retinal transplants devoid of RPE, the intimate relationship between OX-42 positive cells and outer segments suggests that microglial cells may assume the functions characteristic of retinal pigment epithelium. (Supported by NIH Grant EY 05283).

22.3

MIGRATION AND DIVISION OF ASTROCYTES FROM XENOGRAFTS IN HOST RAT MIDBRAIN. H.F.Zhou and R.D.Lund. Dept. Neurobiol., Anat. and Cell Sci., Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261

Our previous work has demonstrated that the patterns of astrocyte migration from xenografts are primarily influenced by the local structures of host brain when placed in the cortex, subcortical white matter or hippocampus. In the present study, the patterns of glial migration in the midbrain were investigated by transplantation of CD-1 mouse cerebral cortex (E14) or corpus callosum (P3) to the tegmental region of Sprague-Dawley rats (P1). Host brains were fixed with zinc-aldehyde fixative 3 to 5 weeks post-transplantation (PT). A monoclonal antibody to a mouse astrocyte surface antigen (M2) was used to identify the location of the grafts and the astrocytes migrating from the graft. The majority of astrocytes from both cortical and callosal grafts migrated ventrally and terminated in the substantia nigra (SN). A small number of cells migrated into the medial geniculate nucleus and central grey region.

In order to determine whether the high density of donor astrocytes in the SN was due solely to cell migration from the transplants or to active division of some cells which had migrated to that region, a single injection of ^{3}H-thymidine (10 uCi/gram body weight) was administered at either 6, 12 or 18 days PT. All rats were fixed 25 days after transplantation. Sections stained with anti-M2 antibody were processed for autoradiography, permitting visualization of double labeled cells. In animals that received an injection of ^{3}H-thymidine 6 days PT, a similar number of dividing cells was found in the SN both ipsilateral and contralateral to the transplant. Within the SN ipsilateral to the transplant, a high density of donor astrocytes was seen. Among them, 6.4% (29/452) of the donor cells were labeled by thymidine. This decreased to 3.1% (10/325) in the animals injected at 12 days PT. Dividing donor astrocytes were rarely seen in the animals injected at 18 days PT.

These results suggest that glial cells from the transplant have preferred patterns of migration in the host midbrain and that the migrating donor cells divide mainly at an early stage of PT and at a rate comparable to that seen for host cells. (Supported by NIH grant EY05308).

22.4

FIBROBLAST TRANSPLANTS INTO THE HIPPOCAMPUS. B. P. Vietje, J. Wells and C. J. Cornbrooks. Dept. of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

In order to determine if connective tissue components disrupt CNS sprouting, fibroblasts from embryonic PNS cultures were transplanted into the dentate gyrus. Cultured fibroblasts were suspended in Hanks Balanced Salt Solution (HBSS) and placed in the infragranular cleavage plane. Injections of HBSS alone (no cells) killed the granule cells, caused an astrocytic activation, and initiated a sprouting of AChE fibers into the molecular layer. The sprouting was not enough to rescue the molecular layer which continued to atrophy. The transplants were identified and localized to the infragranular layer by the staining of the collagenous fibers. The fibroblast transplants failed to inhibit the sprouting of the AChE fibers. Moreover, the molecular layer over the highest concentrations of collagenous fibers maintained its thickness. Also in those areas, there was a clear increase in the presence of blood vessels, and astrocytic activation was prominent. At lesion sites away from the transplanted collagenous fibers the molecular layer continued to atrophy. These data suggest that, rather than disrupt CNS sprouting, fibroblast transplants may support axonal growth.

22.5

TRANSPLANTED SCHWANN CELL (SC) CULTURES SUPPORT REGENERATION IN SEVERED NERVES. J.K. Daniloff, D.Kim*[1], A.Smith*, S.Connolly*[1], and D.G.Kline[1]. Dept. of Vet.Anatomy,Louisiana State Univ.Sch. of Vet.Med.,Baton Rouge, LA 70803 and [1]Dept. of Neurosurgery, LSU Sch.of Med.,New Orleans,LA 70112

Return of function in transected nerves sustaining gaps is poor. Entubulization produces limited recovery. Although significant functional recovery occurs with autografts of sensory nerves,sensory loss results. The purpose of this investigation was to test the effectiveness of autografts and SC implants to restore gastrocnemius muscle function. Three surgical procedures were used to repair 1 cm gaps in 48 severed sciatic (ischiatic) nerves of young adult rats: collagen tubes containing confluent embryonic SC cultures (n=24) or collagen gel (n=12) and sural nerve autografts (n=12). Nerve conduction velocity was assessed prior to surgery and function was assessed 60 and 120 days post-surgery. At both survival times, conduction velocities with collagen tubes and autografts were not significantly different. The SC group was more rapid at both times. Enhanced recovery was sustained for 120 days indicating that the improvement in regeneration may be permanent.

Supported by NIH R29 NS25102

22.6

EFFECTS OF CULTURED ADRENAL CHROMAFFIN AND SCHWANN CELL IMPLANTS ON HINDLIMB REFLEXES OF THE 6-OHDA LESIONED SPINAL RAT. B.E.Pulford, A.R.Mihajlov, L.R.Whalen, H.O.Nornes, Dept. Anatomy & Neurobiology, Colorado State University, Ft. Collins, Co. 80523.

The effects of implantation of cultured neuronal and non-neuronal cells on the recovery of neurotransmitter specific reflex activity were studied in the rat spinal cord using an electrophysiological model. Cell suspensions of neonatal adrenal medullary chromaffin (AM) cells, which produce catecholamines, Schwann (Sc) cells, or a mixture of the 2 cell types were implanted into the lumbar region of the rat spinal cord 2 wks after catecholamine (CA) denervation by intracisternal injection of 6-hydroxydopamine (6-OHDA). Cells were taken from 7d neonates and cultured for 10 days in the presence of NGF. Three months after implantation, the extent of implant-associated recovery of reflex activity was determined by electrophysiological measurements of the EMG activity and force of the long latency component of the hindlimb withdrawal reflex, which is CA modulated. After the electrophysiological testing, rats were deeply anesthetized, and the spinal cord was removed and rapidly frozen. Spinal cords were sectioned longitudinally and were stained using immunohistochemical (anti-TH and anti-217c antibodies) or glyoxylic acid techniques. Stained sections were examined to determine the number of implanted cells which survived, and the extent of outgrowth originating from these cells. Results indicated that 1) significant numbers of chromaffin and Schwann cells were able to grow in the segments of the cord into which they had been injected and 2) rats which received AM cells or a mixture of AM + Sc cells had significantly more forceful withdrawal reflexes than those that received Sc cells only or received no implant after lesioning. The level of force was significantly below the level obtained from unlesioned rats.

Supported by NIH grant #NS 21309-04 and APA grant #NA3-8801-2

22.7

ALTERATION OF MOTONEURON PROPERTIES FOLLOWING TRANSPLANTATION OF THORACIC NEURAL TUBE TO THE LUMBAR REGION. Y. Qin-Wei and R. W. Oppenheim. Dept. of Neurobiology and Anatomy, Bowman Gray Sch. of Med., Wake Forest Univ, Winston-Salem, N.C. 27103

Previous studies have shown that transplantation of thoracic spinal cord to the lumbar region at the time of neural tube closure in the chick embryo (E2) results in the initial innervation of the hindlimbs and the survival and maintenance of the ectopic thoracic motoneurons. At later stages of embryonic development (E12-E18), regressive changes occur in the neuromuscular system. However, some thoracic motoneurons continue to survive and maintain contact with limb muscles. The surviving, ectopic thoracic neural tube and motoneurons exhibit a number of changes in morphological properties such that they come to resemble the normal in situ lumbar spinal cord and lumbar motoneurons. These include: (1) changes in the proportion of spinal cord area occupied by white and grey matter; (2) increases in motoneuron size; (3) changes in dendritic arborization and distribution; and (4) the establishment of peripheral intramuscular branching patterns similar to those exhibited by lumbar motoneurons. Because these same changes are also observed in thoracic transplants lacking neuroanatomical continuity with the host spinal cord and brain, we assume that they are not induced by afferent input, but rather that they reflect a response to a target-derived retrograde signal which acts to regulate motoneuron differentiation.

22.8

THE EFFECT OF TRANSPLANTS ON MOTOR BEHAVIOR IN SPINAL KITTENS. D.R. Howland, B.S. Bregman, A. Tessler, and M.E. Goldberger. Department of Anatomy, The Medical College of Pennsylvania, Philadelphia, PA 19129.

The effect of homotypic embryonic transplants on locomotor development was studied. Transplants were placed in the transected spinal cord lesion cavities of one day old kittens. E26 spinal cord survives in the host lesion cavity for as long as eight months. Calcitonin gene related peptide is present in the transplant. The labelled fibers, however, appear abnormal in their pattern and distribution. Serotoninergic fibers are found to descend to the rostral interface. Animals with transplants develop independent quadrupedal treadmill locomotion and conditioned independent overground locomotion. These have traditionally been held to require interspinal and supraspinal communication respectively. Their locomotion has many abnormal characteristics including: a hindlimb stepcycle of increased duration, a broadened base of support, hypermetric hindlimb flexion, postural instability and lack of consistent 1:1 pairing between hind and forelimb stepcycles. The number of falls exhibited during overground locomotion decreases with age by as much as 92.6% from 6 weeks - 8 months The number of full weight supporting hindlimb stepcycles increases with age by as much as 265% from 6 weeks - 8 months. A stepcycle swing analysis suggests that coordination between hind and forelimb stepcycles is present during quadrupedal and overground locomotion. Taken together, these results suggest that embryonic spinal cord transplants are capable of affecting locomotor behavior in the spinal neonatal kitten. (Supported by Grant NS24707 and the Daniel Heumann Fund).

22.9

TRANSPLANTATION OF EMBRYONIC CAT SPINAL CORD TISSUE INTO THE ADULT RAT SPINAL CORD. P. J. Reier* and W. J. Streit* Depts. Neurological Surg. and Neuroscience, Univ. Florida Coll. Med., Gainesville, FL. 32610.

In previous studies of fetal rat spinal cord transplants into the injured adult rat spinal cord (FSC_r) we found such grafts to be capable of suppressing gliosis and of fostering some ingrowth of host fibers. This ability, however, was of limited duration and may have reflected the relatively rapid maturation of these transplants. We tested this possibility in a series of experiments (Group A) in which grafts of more slowly maturing fetal cat spinal cord tissue (E24-28) were made into the spinal cords of adult rats (FSC_{c-r}) immunosuppressed with Cyclosporine A (CsA). We also took advantage of these xenogenic transplants to examine microglial distributions during rejection induced by withdrawal of CsA (Group B). Group A: At 6 wks. post-transplantation (p-t), conventional light microscopy and anti-GFAP staining of vibratome sections revealed a more extensive fusion of donor and host tissue than seen previously with rat homotypic allografts. Although containing matured neurons, these grafts were still relatively immature as reflected by the paucity of myelinated axons. Immunocytochemistry showed, however, that the ingrowth of host serotoninergic fibers was limited. In contrast, CGRP-immunoreactive axons from the host showed a more robust elongation into these grafts. Similar results were obtained at later p-t intervals (up to 10 wks). The ingrowth of these two axonal populations was thus comparable to that seen with FSC_r transplants. Group B: A battery of staining methods were used to demonstrate microglia in immunosuppressed recipients (CsA^+) and in hosts 1 wk. after withdrawal of CsA (CsA^-). No microglia were seen in xenogenic grafts in CsA^+ rats, whereas some were present in the host. Transplants in the CsA^- animals showed extensive lymphocytic invasion; however, no microglia could be demonstrated. In contrast, large and numerous microglia were still seen in the host especially at the graft interface. (Supported by NIH 1PO1-NS 27511).

22.10

FETAL GRAFTS ALTER CHRONIC BEHAVIORAL OUTCOME AFTER CONTUSION DAMAGE TO THE ADULT RAT SPINAL CORD. B.T. Stokes, Dept. Physiology, The Ohio State Univ. and P.J. Reier, Dept. Neurosci., Univ. Florida.

Fetal transplants have been reported to enhance motor recovery in rats when injured and receiving grafts as neonates (Kunkel-Bagden, 1990). Evidence for graft-mediated partial return of certain motor (Buchanan and Nornes, 1986) or sexual reflexes (Privat, 1988) has also been observed in adult rats following transection or neurotoxin-induced lesions. In the present experiments, we have investigated whether fetal neural tissue transplants placed into the contused spinal cord (10 days post-injury) can alter behavioral outcome in a different injury setting. We have used a variety of standard behavioral scoring paradigms (generalized motor activity: open field, inclined plane, and gridwalking) and a modified, sensitive test of motivated behavior (foot-print analysis; Kunkel-Bagden and Bregman, 1990) to examine the results. Rats were extensively pretested in the various trials, anesthetized and injured with an electro-mechanical impactor device. Ten days later they were reanesthetized, transplanted with a suspension of dissociated E_{14} spinal cord cells and then allowed to survive over the next 3 months. In the generalized trials of motor activity, injured controls and transplanted animals showed the same recovery profiles. Footprint analysis, however, revealed statistically significant ($p<.006$; mutivariate ANOVA) improvements in the base of support and right or left stride lengths. This was seen by 7 days post-transplantation and persisted throughout the post-graft survival period. These results suggest that embryonic spinal cord cells may be able to promote long-term sparing of certain functions in a clinically relevant model of spinal cord injury. Supported by NS-10165 and NO1-NS-7-2300.

22.11

EXCITABILITY STUDIES IN THE RODENT SPINAL CORD FOLLOWING CONTUSION INJURY AND NEURAL TISSUE TRANSPLANTATION. Floyd J. Thompson[1], Paul J. Reier[1&2], Gregory W. Schrimsher[1], Christopher C. Lucas[1], Linda R. Ray[1]. Depts: Neurosci[1] & Neurosurg[2], Univ of Fla, Gainesville.

In the present study, hindlimb reflex excitability was studied following midthoracic spinal contusion injury in the adult rat. The injuries were produced by impact of a 10 gm weight dropped from a 2.5 cm height onto the exposed thoracic (T_8) spinal cord, (contusion protocol adapted from Wrathall et al (Exp Neurol., 88:108-122, 1985)). Hindlimb (lumbar) reflex excitability was evaluated at 1 and 2 months following injury.

Contusion injury produced physiological changes characterized by a significant loss of reflex inhibition associated with reflex repetition at low frequency. For example, in normal animals, in response to the frequency test, reflexes were inhibited 54% of the control. Whereas, 28 days following contusion injury, the same test revealed only a 9.2% inhibition of the test reflexes. The mechanism responsible for low frequency depression reflex amplitude is currently accepted to be presynaptic inhibition. The studies on the contused animals form a baseline for evaluation of neural tissue transplantation to address specific physiological deficits.

Observations have been made on animals which received implants of E-14 fetal spinal cord tissue one week following injury. Tested at 1 and 2 month intervals following the initial contusion injury, the loss of presynaptic inhibition of lumbar test reflexes was considerably less in these animals compared to untreated animals. For example, tested at 2 months following the injury, in the animals which had received transplants, the reflexes were inhibited 38 % of control compared to 5 % in the untreated animals. Although preliminary, these observations suggest a sparing function for the treatment of contusion spinal cord injury using transplants of fetal spinal cord tissue.

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22.12

BEHAVIORAL IMPROVEMENT AND TRANSPLANT SURVIVAL AFTER FETAL CELL IMPLANTS IN MPTP LESIONED BONNET MONKEYS. C.J. Hutt*, E.H. Kriek, M.L. Reite*, and C.R. Freed, Depts. of Med., Pharm., and Psych., University of Colorado Sch. of Med., Denver, CO 80262.

To test the value of fetal dopamine cell grafts in the treatment of Parkinson's disease, Bonnet monkeys were made Parkinsonian with systemic or unilateral injections of the neurotoxin MPTP. For systemic lesions, MPTP.HCl 0.5 mg/kg i.m. was given for 5 days (n=8). Unilateral lesions were created with a single carotid infusion of 0.8 mg/kg (n=8). Monkeys were trained to reach for food reward in computer-timed tests and were tested for circling with amphetamine 0.5 mg/kg or apomorphine 0.15 mg/kg. Lesioning reduced the ability to reach for a food reward. Unilaterally lesioned animals showed spontaneous circling ipsilateral to the side of the lesion and apomorphine led to contralateral circling. Mesencephalic dopamine cells from 18-150 mm crown rump length fetus were transplanted in caudate and putamen on one side of brain via 5 needle passes. By 3 weeks after implant, systemically lesioned monkeys showed improved ability to reach. In unilaterally lesioned animals, spontaneous circling contralateral to the side of the lesion was observed and apomorphine circling slowed. At autopsy, tyrosine hydroxylase positive cells were seen along needle tracks of animals transplanted with fetal dopamine cells but not in animals receiving spinal cord implants.

22.13

SURAL NERVE GRAFTS IN FORNIX TRANSECTED MONKEYS. J.H. Kordower1, D.M. Gash2, and M.S. Fiandaca3. 1Dept. of Anatomy and Cell Biology, Univ. Illinois Sch. Med., Chicago Ill. 60612, 2Dept. of Neurobiology and Anatomy, Uni. Rochester Sch. Med. Rochester N.Y. 14642, 3Division of Neurosurgery, Univ. Massachusetts Med. Ctr. Worcester MA 01605.

Following peripheral nerve transection, Schwann cells begin a *de novo* synthesis of trophic factors including nerve growth factor [NGF]. Cholinergic basal forebrain neurons are sensitive to trophic factors such as NGF and consistently degenerate in Alzheimer's disease. Thus peripheral nerve grafts were assessed for their ability to provide trophic influences upon axotomized primate cholinergic medial septal neurons.

Ten Cebus monkeys received a unilateral transection of the fornix. At the time of the lesion, monkeys received either no graft [n=1], a control tissue graft [n=2], or an intraventricular implant of autologous sural nerve [n=3]. Other monkeys received sural nerve grafts 1-3 weeks prior to the fornix transection [n=4]. Comprehensive decreases of acetylcholinesterase [AChE]-containing fibers within the CA3 and dentate gyrus regions of the hippocampus were observed in all animals 30 days post-lesion. Monkeys receiving no graft, control grafts, or sural nerve grafts at the time of the lesion displayed similar reductions in cholinergic medial septal neurons ipsilateral to the lesion. In contrast, monkeys receiving implants prior to the onset of degeneration displayed a reduced cell loss. Furthermore, sural nerve grafted monkeys displayed an AChE-positive sprouting response in the region of the graft. These data suggest that peripheral nerve can provide trophic and neurite promoting effects upon primate cholinergic basal forebrain neurons. [Supported by AHAF and NS 25655].

22.14

CLINICAL FOLLOW UP OF IMPLANTS OF VENTRAL MESENCEPHALON INTO THE CAUDATE NUCLEUS OF SEVERELY AFFECTED PARKINSON´S PATIENTS.

J.J. Lopez-Lozano, G. Bravo*, J. Uria*, J. Dargallo*[1], J. Salmean*[1], J.Cerrolaza*[2] and CPH. Neural Transplantation Group Lab. Neurobiology.Dept. Neurology and Dept. Neurosurgery Clin. Puerta de Hierro, University Autonoma; Dept. Gynecology-Obstectrics. Hosp. Severo Ochoa[1], Leganes; and Hosp. Mostoles [2]; 28035-Madrid. Spain.

Based on our previous experience with autotransplants of adrenal medulla (AM) in Parkinson´s patients (PP), from Oct. 88 to the present, our group has performed 10 implants of human fetal ventral mesencephalon (VM) into caudate nucleus of grade IV-V PP. The fetal tissue (17w and 8-10w old tissue) was obtained from legal abortions and consent for its use and the research procedure follow the corresponding Spanish laws (BOE 42/88, 30/79). The same protocol was used in fetal VM alloimplants as in AM autotransplants (JJLL´90). Patients were maintained with the optimal dose of L-dopa and all dopaminergic agonists were discontinued. The results obtained seem to indicate the existence of a very moderate (but statistically significant) improvement in the overall Parkinson´s disease scale (UPDS) an d int the overall assesment of Parkinson´s symptoms (MYS). The improvement does not follow the course of the AM implants, occurs later (x=7 mo) and the recovery is lesser. The decrease in the amount of L-dopa required begins in month 2, and is less marked than in AM implants. With respect to dykinesia, there is a decrease in the intensity and duration, but not in the frequency or type. This report will discuss the clinical course of the operated patients and the variations observed in the individual Parkinson´s symptoms. (Supported by FIS 90/197, 89/115, CACYT PA86/046). CPH Neural Transpl. Group: B.Brera, J. Gomez-Angulo, M.EL-Barkani and R. Martinez.

TRANSPLANTATION: NEW TECHNIQUES AND CELL LINES

23.1

A NOVEL STEREOTACTIC SURGICAL PROCEDURE FOR TUMOR IMPLANTATION IN RAT BRAIN. V.M. Morreale*, B.H. Herman, V. Der-Minassian*, W. Goldberg, J. Bernstein, M. Palkovits, P. Klubes*, D. Perry & A. Csiffary*. Brain Res Cen, Children's Natl Med Cen, Wash, DC 20010; Dept Psychiat, Ped & Pharm, George Wash Univ Sch Med, Wash, DC; CNS Regen Res Lab, VA Hosp, DC; Lab Cell Biol, NIMH, MD.

A model for implantation of cultured tumor cells into rat brain is presented. Stereotactic implantation reduces tumor variation.

Exp 1: 8 male Wistar rats (225g) were implanted with a permanent caudate cannula using a stereotaxic apparatus. Immediately after cannula implantation, rats were injected(10 ul) as follows:4 with 10^6 cultured glioma cells, 2 with Walker (WL) 256 cells and 2 with culture medium. Two weeks following implantation, brains were cut, and stained with cresyl violet(CV) or for glial fibrillary acidic protein (GFA). In all 4 C6 rats, there was no tumor at AP(+5.7) or AP (-2.3), M±SEM tumor area(Loats,mm^2): AP(4.7)0.5±0.3, AP(3.7) 4.3±0.8, AP(2.7)7.1±1.2,AP (1.7) 11.4 ±1.6, AP (0.7) 9.2±3.4, AP(-0.3) 4.6±3.6, AP(-1.3)0.4±0.4). WL256 rats showed a much reduced AP tumor extension. Similar tumor areas were indicated in GFA compared with CV. Tumor was found in caudate(6/6), but not in n. accumbens, fornix or hippocampus. Exp 2: 5 rats were implanted with C6 glioma cells using 10^5 cells and a reduced volume of 1ul to produce a more defined tumor. Thus, reproducible and circumscribed caudate tumors were produced using this procedure. Funded by Cancer Aid Group, Wash, DC and Discovery Funds of CNMC (BHH).

23.2

A NEW PROCEDURE ALLOWING MULTI-SITE INTRARETINAL GRAFTING

Eliot Lazar and Manuel del Cerro. Department of Neurobiology and Anatomy, University of Rochester, Medical School, Rochester, N.Y.

Since our original presentation on intraocular retinal transplantation (del Cerro et al., ARVO 1984), procedures for retinal grafting have been under constant evolution. All of the techniques described to date require surgically opening of the eye through either a trans-scleral or trans-corneal route. This requirement carries with it all the inherent surgical risks as well as the related complications. As a result, these approaches necessarily limit attempts at multiple implantation into a single eye. We describe here a procedure which avoids these restrictions. A 27 gauge needle, sheathed in plastic, with 1.2 - 1.4 mm of the needle tip left exposed is used for the transplantation. The bevelled tip cleanly enters the tissues while the sheath limits the depth of penetration. Under direct visualization a micro syringeis used to inject dissociated donor retinal cells into numerous sites of the adult rat retina. This procedure which is virtually free of complications, avoids the need for surgically entering the eye and therefore makes it possible to perform multiple simultaneous grafts into the intact globe. Results, using ophthalmoscopy as well as light and electron microscopy, have comfirmed the atraumatic nature of this technique. Histologically, the growth and differentiation of the grafts are comparable with those seen in transplants using a surgical approach (del Cerro et al., 1990). Intraretinal injection of colloidal carbon into control animals using the same technique, dramatically illustrates that a large proportion of the host retina is bathed in material when employing this method. The technique, initially devised for rodent eyes, is presently being used for grafting of cells into monkey retinas.

Supported by NEI grant #05262 and the Rochester Eye Bank.

23.3

HIPPOCAMPAL CELL-LINE TRANSPLANTATION: IMPROVED LEARNING IN RATS WITH HIPPOCAMPUS LESIONS.

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HT4 is neural-precursor cell line derived from embryonic rat hippocampus with a temperature-sensitive conditional oncogene. To evaluate behavioral effects of HT4 transplantation, 13 adult Sprague-Dawley rats were randomized to receive (1) bilateral stereotactic, electrolytic lesions of the dorsal hippocampus followed 2 weeks later by HT4 implantation into the lesion cavity, (2) hippocampal lesions without transplantation, or (3) sham lesions only. Two months later, animals were tested for spatial learning on the Morris water maze (4 trials/d X 15 d). Learning performance of HT4-implanted rats was significantly better than that of lesioned controls ($p < 0.05$) but worse than that of sham-lesioned animals. These preliminary results in a small number of animals suggest that cell-line implants may be functionally beneficial and support the more general strategy of cell-line transplantation for the treatment of human neurodegenerative disorders.

23.4

PRIMARY FIBROBLASTS THAT PRODUCE L-DOPA: LONG TERM SURVIVAL AND BEHAVIORAL EFFECTS WHEN IMPLANTED IN RATS WITH 6-OHDA LESIONS.

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Several investigators have shown that genetically modified cells can survive when implanted within the brain (Gage et al., 1987; Shimohama et al., 1989; Uchida et al., 1989), and exert a functional influence over surrounding tissues (Rosenberg et al., 1988; Wolff et al., 1989; Horellou et al, 1990). The majority of studies to date have described results from the use of established cell lines; cells which have the potential capacity for continued, undesired proliferation in vivo. In the present study, primary fibroblasts were examined as a possible alternative donor cell type for use in grafting in an animal model of neurological disease. Primary fibroblasts were obtained from inbred Fischer 344 rats and infected with a retroviral vector containing a full length cDNA for rat tyrosine hydroxylase (TH). A clone which expressed TH activity and TH immunoreactivity in vitro was selected for grafting. Grafts were placed within the 6-hydroxydopamine denervated striatum of adult Fischer rats. Control lesioned rats were either untreated or received grafts of primary fibroblasts infected with a retroviral vector containing beta-galactosidase. Rats were tested with apomorphine every 2 weeks for 8 weeks after grafting. A pronounced, prolonged decrease in rotational behavior was seen only in those rats with TH-fibroblast grafts. Preliminary anatomical results indicate that all grafted rats contained surviving grafs. Further histological analyses of these grafts is underway.

23.5

EXPRESSION OF GLUTAMIC ACID DECARBOXYLASE GENE IN THE FIBROBLASTS-POTENTIAL USE FOR INTRACEREBRAL GRAFTING.

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The object of the present study is to develop a potential therapeutic approach to focal seizure disorder using a strategy of gene therapy, namely implanting genetically modified cells into the brain. Local application of GABA receptor agonist or GABA transaminase inhibitor into discrete brain areas suppresses seizure in various animal model of epilepsy. Suppressing seizure by permanent increase of GABA concentration locally in the brain regions where seizure generates or propagates might be achieved by grafting the genetically modified, GABA-producing fibroblasts into the brain. To investigate this possibility, glutamic acid decarboxylase(GAD) gene was transferred into fibroblasts.

A retroviral vector containing the full-length feline GAD cDNA (LGADRNL) was derived from the Moloney murine leukemia virus. This vector expresses GAD from the 5' long terminal repeat and the selectable bacterial neomycin-resistance gene from an internal Rous sarcoma virus promoter. Immortalized rat fibroblasts of the Rat1 cell line and primary fibroblasts obtained from Fisher rats were infected with LGADRNL. Neomycin-resistant colonies were e stablished and soluble extract from cell homogenate was screened for GAD activity using $^{14}CO_2$ trapping assay . The GAD activity (nmol CO_2/mg protein/hr incubation) of infected fibroblasts vs non-infected control was 401 vs 3.3 for Rat1 cells and 432 vs 8.7 for primary fibroblasts. Biochemical studies indicated that 35% of GAD expressed in the Rat1 fibroblasts was bounded with endogenous cofactor, pyridoxal-5'-phosphate. In addition, the GAD-producing fibrobalsts was immunohistochemically labelled.

23.6

ULTRASTRUCTURE OF POLYMER ENCAPSULATED PC12 CELLS USED AS BRAIN IMPLANTS AND MAINTAINED IN VITRO.

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The fine structure of PC12 cells growing within polymer capsules was studied because encapsulated cell lines may provide a useful alternative to embryonic neuron grafts for transmitter replacement in the brain. Capsules were prepared from polyvinyl-acrylic copolymer tubing, 800 um in diameter, with a molecular cut-off of 50KDa. Suspended PC12 cells were loaded by ultrafiltration into 3 to 5 mm tube segments, and the ends were closed with liquid polymer. Cell-filled capsules were kept either *in vitro* or implanted in the brains of rats or guinea pigs. Capsules were fixed at 1/2, 1, 3, and 6 months and the ultrastructure of enclosed cells was examined. Structural changes were related to size and distribution of storage granules and were most evident in comparisons of long-term versus short-term encapsulated cell populations. In short-term culture, PC12 cells typically contained abundant quantities of variable sized electron-dense storage granules that were randomly distributed within the cytoplasm and resembled those in PC12 cells grown in monolayer cultures. At periods of ten weeks or more following enclosure, the electron-dense storage granules had a more patchy distribution and were often seen in small cell processes. Increased frequency of 'empty' membrane containers suggested enhanced vesicular release. These morphological changes could reflect alterations of cell/cell contacts due to continued mitosis and crowding of PC12 cells in capsules. The data are relevant for evaluating the usefulness of PC12 cell-filled capsules. Supported by USPHS grant No. NS27694 from NINDS.

23.7

EFFECT OF LONG-TERM POLYMER ENCAPSULATION OF PC12 CELLS IN VITRO.

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The effect of long-term encapsulation of an immortal DA-secreting cell line was studied in order to assess the feasibility of using this approach as a DA-delivery system. PC12 cell suspensions were encapsulated with an acrylic copolymer and maintained in vitro for up to 3 months. DA release from cell-loaded, permselective, cylindrical capsules (8mm long x 0.85mm I.D.) was evaluated after a 15 min. static incubation with Hank's basic salt solution. Twenty-four hours after the static incubation, the following parameters were evaluated for each capsule: total cell number; percent viability; and total capsule DA content. Unstimulated DA release increased with time after encapsulation reaching a maximum of 7.86 ng +/- 0.39 (n=6) after 3 months. Total cell number and DA content also increased with time and reached a maximum after 3 months. The rate of increase in cell number and DA content was comparable to the rate of increase in DA released from the polymer capsules suggesting that the increase in DA efflux over time was related to increased cell proliferation. The percentage of viable cells as determined by trypan blue exclusion declined from 91 to 43 percent over the same time period, however, the total cell number increased with time. Light microscopy revealed the presence of mitotic figures at all time points. A potential problem with encapsulating an immortal cell line is the accumulation of acellular debris within capsules resulting from cellular necrosis over time. Over the 3 month study period, this condition did not appear to compromise DA output from cell-loaded capsules. Whether this observation is true over longer time periods will have to be addressed by subsequent studies.
Supported by grant No. NS27694 from the NINDS-NIH.

23.8

BEHAVIORAL RECOVERY FOLLOWING INTRASTRIATAL IMPLANTATION OF MICROENCAPSULATED PC12 CELLS

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The motor deficits associated with Parkinson's disease may be controlled with intrastriatal placement of dopamine-secreting cells in a polymer capsule. Water soluble polyelectrolytes were utilized for membrane encapsulation of dopamine-secreting PC12 cells. Microcapsules, 600 µm in diameter, were formed by syringe pump extrusion of a sodium alginate/PC12 cell solution into a multivalent cationic bath. A semipermeable membrane was formed by interfacial adsorption of poly-L-lysine over the gelled microspheres. Membrane permeability studies revealed exclusion of radiolabeled 69 KDa albumin, whereas 30 KDa carbonic anhydrase was able to cross the membrane. In vitro, microencapsulated PC12 cells (MEC/PC12) were assessed by quantifying dopamine release and cell viability over time. One month post-encapsulation, dopamine release and viable cell populations reached levels that were maintained for at least 12 weeks. The behavioral effect of intrastriatal dopamine release from MEC/PC12 cells was evaluated in the 6-OHDA unilaterally lesioned rat model. From two to four weeks post-implantation, MEC/PC12 recipients (n=4) revealed a 60% reduction in rotation behavior under apomorphine challenge as compared to animals which received empty microcapsules (n=4). Intact microcapsules containing tyrosine hydroxylase immunopositive PC12 cells were observed after 4 weeks of implantation in animals exhibiting a reduction in turning behavior. Implantation of polymer encapsulated cells may provide a means for long-term delivery of neurotransmitters and growth factors to the nervous system.
Supported by grant No. NS27694 from the NINDS-NIH.

23.9

TRANSPLANTATION OF IMMORTALIZED NEURAL CELLS CULTURED ON DEXTRAN BASED MICROCARRIER BEADS PROVIDE A NOVEL ENVIRONMENT FOR AXONAL INGROWTH INTO THE ADULT MAMMALIAN SPINAL CORD. P. Brittis, M. Zeller*, M. N. Goodman*, J. W. Jacobberger*, J. Rudge*, J. Silver, J. Yoshino* and W. E. Edmonston. Dept. of Neuroscience, Colgate University, Hamilton, NY 13346 and Depts. of Genetics and Neuroscience, Case Western Reserve University, Cleveland, OH 44106.

We have been investigating the use of dextran based microcarrier beads for neural cell transplantation into the mammalian spinal cord. In vivo application of microcarrier beads allows for: accurate placement of cells in the spinal cord, a three-dimensional substrate, contact inhibition of immortalized cells, limited cell migration, and a large transplantable surface area.

Immature (P1) type 1 forebrain astrocytes, neonatal Schwann cells (Tennekoon et al., J. Cell. Bio. 105:2315-2325, 1987), and adult olfactory bulb glial cells (Goodman et al., Soc. Neurosci. Abst., 1989) were immortalized with SV-40 large T antigen via plasmid or retroviral gene transfer. These immortalized cells have been successfully cultured on microcarrier beads and placed in a dorsal root entry zone. We were able to locate the cells up to four weeks after transplantation by allowing them to phagocytize fluorescent microspheres or by labeling the cells with phaseolus vulgaris leucoagglutinin prior to their attachment to microcarrier beads.

Both the internal and punctate forms of laminin as well as the NGF like receptor [Mab 217c] (Kumar et al., Soc. Neurochem. Abst., 1990) have been found to comprise part of the known Schwann and olfactory glial cell antigenic profile, while the astrocytes were GFAP positive and, like the immortalized Schwann cells, sustained neurite outgrowth in vitro. By placing immortalized cells attached to microcarrier beads into the dorsal root entry zone, we have created a potentially conducive pathway for the possible ingrowth of dorsal root fibers into the adult rat spinal cord.

23.10

INTRAOCULAR TRANSPLANTATION AND CULTURE OF SECOND-TRIMESTER HUMAN EMBRYONIC RETINAL CELLS. M. del Cerro, E. Lazar, D. A. Grover*, M. Gallagher*, C. D. Sladek, J. Chu, and C. del Cerro. Dept. of Neurobiology and Anatomy, University of Rochester Medical School, Rochester, N.Y.

This study tested the viability of second-trimester human embryonic retinal cells for transplantation into the eyes of adult hosts. Tissue procurement followed strict ethical guidelines Retinas obtained from electively aborted embryos aged 13 to 17 weeks were dissected and mechanically dissociated into small cell clusters prior to transplantation. Adult albino rats and Cercopithecus aethiops sabaeus monkeys immunosuppressed with Cyclosporine A served as hosts. Anterior chamber and multi-site intraretinal grafts were performed, the later by using our newly developed injection procedure (Lazar and del Cerro, 90). Light (LM) and electron microscopic studies (EM) indicated that the donor fetal retinas had relatively mature neurons and fibers within their inner layers, but they retained an outer neuroblastic layer populated by mitotically active cells. When dissociated donor retinas were cultured in vitro they produced cells endowed with positive immunoreactivity towards the neuronal markers NSE and PGP 9.5, growing over a carpet of flat non-reactive cells. Biomicroscopy allowed us to follow the survival, grow, and vascularization of the grafts. LM and EM showed that many differentiated donor neurons degenerate, while the neuroblastic cells continue their growth and form the mass of the graft. This study indicates that second trimester human fetal retinas are a rich source of viable cells, and retain their capacity to grow and to differentiate morphological and biochemical neuronal characteristics, both in vivo and in vitro. Intraocular grafts of these cells into adult hosts shows vigorous growth and integration with the host. Thus, it appears that the fetal human retina is endowed with a uniquely wide "time window" for succesful transplantation, a crucially important feature behind attempts to use these cells to repopulate damaged adult retinas.

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23.11

NGF PRODUCING FIBROBLAST GRAFTS AND THEIR INTERACTION WITH EXCITOTOXIC STRIATAL LESIONS. J.M. Schumacher, M.P. Short, B.T. Hyman, M.V. Sofroniew, X.O. Breakefield and O. Isacson. Dept. Neurology and Neurosurgery, Harvard Med. Sch., McLean Hospital, Belmont MA 02178 and Massachusetts General Hospital, and Dept. of Anatomy, University of Cambridge, U.K.

Previous studies using pharmacological NGF infusions in parallel with excitotoxic lesions of rat striatum have indicated NGF protective effects (ALoe, 1987). In order to further test this hypothesis we have used a biological delivery system of NGF (Rosenberg et al. 1988), by implanting fibroblasts genetically engineered to produce high levels of NGF into the rat brain, prior to infusing an excitotoxin into the striatum.

A rat derived immortalized fibroblast cell line (208F) was infected by a NGF retroviral vector (N.8) and selected for neomycin resistance. The chosen fibroblasts clones (NGF+) were further selected for highest NGF production by a 2-site enzyme immunoassay (50 ng NGF/mg cell protein). The expanded NGF+ cell-line and and a non-NGF variant (NGF-) of the 208F fibroblast cell line was injected (8 million cells/rat) into the lateral ventricle and mid-line structures. After eight days, quinolinic acid (60 nM in 1µl) was infused into the ipsilateral striatum. Apomorphine induced behavioral rotation (11 days post-lesion) indicated no difference between NGF+ and NGF- grafted groups. Histological evaluation (25 days post-grafting) showed large surviving Nissl stained grafts in the NGF- group, while NGF+ grafts were much smaller. The NGF+ group had slightly smaller lesions than the NGF-group in Nissl and GFAP stained sections. These studies indicate different growth patterns of intracerebrally grafted NGF vector infected (NGF+) as compared to non-infected rat fibroblast cell-lines (NGF-), and suggest that excitotoxic lesion effects in the striatum may be affected by the presence of NGF.

23.12

CHARACTERIZATION OF NEURON-LIKE CELL LINE IMMORTALIZED FROM PRIMARY RAT MESENCEPHALON CULTURES. M. M. Durand,* D. C. Chugani, M. Mahmoudi,*M. E. Phelps. UCLA School of Medicine, Los Angeles, CA 90024

The transplantation of fetal mesencephalic tissue in striatum shows promise as an effective treatment for Parkinson's disease. The limited availability of human fetal tissue as well as complex ethical issues regarding the use of the tissue will prevent widespread implementation of this procedure. Reversibly immortalized mesencephalic dopaminergic cell lines may be the answer to this problem. We have produced reversibly immortalized cells from embryonic day 14 rat mesencephalon using a retroviral vector containing the genes for temperature sensitive large T antigen of SV40 and neomycin resistance (G418). This retrovirus was stably produced by a psi 2 cell line obtained from R. McKay, MIT. Primary cultures were enriched for dopaminergic cells by panning in tissue culture plates that had been coated with the monoclonal antibody, neuron specific protein 4 (Rougon et al., (1983) Neurosci. 10: 511). Following panning, cells were grown with supernatant from psi 2 cell line containing the retrovirus (dilution 1:3). After 48 hr the cells were incubated with G418 to select for cells which had incorporated the virus. Surviving cells, selected by dilution, proliferated at 33°C and displayed flat morphology. At 39°C the cells stopped dividing and some cells changed from flat cells with broad processes to neuron-like morphology. One cell line CSM 14.1, expressed tyrosine hydroxylase and neuron specific enolase immunoreactivity when raised to 39°C for one week. These cells were grown in the presence of various growth factors in attempt to increase neuronal characteristics. The addition of basic fibroblast growth factor produced greater than 90% neuron morphology after one week in culture at 39°C. These cells in addition expressed neurofilament 68 and OL28 (a putative neuronal marker) immunoreactivity. These data suggest that it may to possible to reversibly immortalize mesencephalic dopaminergic cells for transplantation. (Supported by NS 15654 and DOE DE-FC03-87ER60615)

23.13

TWITCH AND MAXIMAL VOLUNTARY CONTRACTION TENSIONS OF NORMAL HUMAN EXTENSOR DIGITORUM BREVIS MUSCLES. J.A. Florendo, D.S. Kirby*, B.C. Schafer*, T.G. Goodwin* and P.K. Law. Depts. of Neurol. and Physiol./Biophys., Univ. of Tennessee, Memphis, TN 38163.

An apparatus was designed and a procedure developed whereby twitch and maximal voluntary contraction tensions of human extensor digitorum brevis (EDB) muscles could be measured. A study of eleven normal adult volunteers was conducted to demonstrate the reproducibility of this technique. Maximal voluntary contraction amplitude, as well as twitch amplitude, maximum rate of rise, and half relaxation time following supramaximal, indirect, electrical stimulation, were determined. A minimum of six measurements of each parameter were obtained from each EDB. All parameters demonstrate relatively low coefficients of variation between individual muscle measurements, but differences exist between right and left feet of some individuals and between different individuals. Therefore, this apparatus and procedure can provide an accurate measurement of muscle function for experimental evaluation.

This apparatus and procedure were used to evaluate muscle function of individual EDBs of eleven boys with Duchenne muscular dystrophy before and after myoblast transfer therapy. Amplitudes of twitch and maximal voluntary contraction tensions were obtained from myoblast- and sham-injected EDBs, to demonstrate functional improvement and efficacy of myoblast transfer therapy. (Supported by MDA, Sandoz and Walgreens.)

23.14

GRAFTING GENETICALLY ALTERED P19 EMBRYONAL CARCINOMA-DERIVED NEURONS INTO RAT STRIATUM

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Treating murine embryonal carcinoma (EC) cells with retinoic acid (RA) causes them to terminally differentiate into neurons, glia, and smooth-muscle cells. The population of neurons formed displays a heterogenous neurotransmitter phenotype reminiscent of rodent forebrain (Staines et al., SFNS abstr.108.6, 1989) and we are now studying the response of these cell to intracerebral tranplantation. To specifically recognize graft tissue after transplantation, undifferentiated EC-cells were transfected with a bacterial gene for beta-galactosidase (B-GAL) detectable by histochemical, immunocytochemical and EM methods.

B-GAL expressing EC-cells were treated with RA for 3 days and grafted into rat striata previously lesioned by ibotenic acid, or deafferented by a 6-OHDA lesion of the MFB. B-GAL expressing cells were demonstrated in vivo for at least 2 wks. post grafting. Through double-labelling using anti-B-GAL in combination with the neuron-specific monoclonal antibody A60 (Mullen et al., SFNS abstr.204.9,1989) we could specifically identify EC-derived neurons within the grafts. Additional double-labelling revealed continued neuropeptide-Y and somatostatin expression by these EC-derived neurons.

A second EC cell line transfected with the rat tyrosine hydroxylase cDNA shows high levels of dopamine production following RA-induced differentiation and results of tranplantation will be reported.

(This work was supported by the MRC of Canada).

24.1

CONTACT-DEPENDENT REGULATION OF PRESYNAPTIC CALCIUM CURRENT DENSITY DURING SYNAPTOGENESIS.
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It is well established that contact between neuronal growth cones and muscle has widespread effects on postsynaptic properties. The reciprocal actions of target cells on presynaptic development are ill defined. We have investigated the effects of postsynaptic target contact on presynaptic development during synaptogenesis between pairs of identified neurons of *Helisoma trivolvis* in cell culture.

To gain direct access to the presynaptic secretory membrane, synapse formation between pairs of neuronal somata was studied. Sustained periods of synaptic contact enhanced both the release of neurotransmitter and the macroscopic calcium current density of presynaptic neuron B5. Thus, in addition to regulating postsynaptic properties, synaptic contact augments the presynaptic calcium current which is responsible for evoking calcium-dependent transmitter release.

To extend these studies, we are currently investigating the effects of target contact on the spatial distribution of sites of calcium influx in neurites and growth cones using the calcium-sensitive dye Fura-2.

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24.2

REACTIVE SYNAPTOGENESIS AT PHOTORECEPTOR FEEDBACK SYNAPSES IN THE FLY'S LAMINA. J.H. Brandstätter, S.R. Shaw and I.A. Meinertzhagen. Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia. CANADA B3H 4J1

Monopolar cell L2 is one of several unique interneurons found in each module or cartridge of the first optic neuropile, the lamina, beneath the fly's compound eye. Its synapses feed back upon the same receptor (R) terminals which provide L2's input at afferent photoreceptor synapses. The number of sites of both photoreceptor afferent and feedback synapses, decreases during normal adult development, after peaking in early adulthood (Meinertzhagen, I.A., *J. Neurobiol.*, 20: 276, 1989). The feedback synapse is usually a dyad L2–>R, T1; in which T1 is a higher-order interneuron. In a study of synaptic plasticity we examined by electron microscopy the structure and frequencies of these feedback synapses upon receptor terminals that were degenerating after a 12-min period of illumination following retinal injections of sulforhodamine 101 in *Musca domestica* (Picaud, S. et al., Neurosci. Lett.,95:24, 1988). Although all the receptor terminals in a photoablated cartridge degenerated, depriving L2 of its chief afferent inputs, degeneration did not progress trans-synaptically within the time period examined (14d), nor was T1 affected by photoablation. Presynaptic degeneration was however accompanied by the generation in L2 of dissociated ribbons of the feedback synapse, which had become separated by a process of invagination from their presynaptic membrane sites, to form free-floating organelles. Although floating synapses seem to be degradation products formed following the degeneration of their postsynaptic (R) targets, the frequency of intact synaptic profiles in the L2 axons of photoablated cartridges in fact increased twofold over 8d, indicating that new synapses had formed during this period of reactive synaptogenesis. The elevated frequency of synaptic sites correlates with and presumably arises from a group of smaller synapses (ribbons < 0.14μm wide), that lies outside the size distribution of synaptic contacts seen in control cartridges. Since the mean size of synaptic contacts increases with age for the population, we infer that these smaller synapses were young ones added early on during the period of reactive synaptogenesis.

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24.3

RETINA CELLS IN CULTURE SEQUESTER MUSCARINIC ACH RECEPTORS TO THEIR DENDRITES A.F.Skorupa, W.L. Klein Northwestern Univ. Inst. for Neurosci. Evanston, IL 60208

Retina cells in situ are known to sequester ACh receptors to their dendrites by the time synapses are first detected (J. Neurosci.8:4225). However, younger retina cells in culture show no distinction in receptor distribution between soma and neurite (J. Biol. Chem. 260:8873). The possibility exists that culture conditions might preclude interactions leading to receptor restriction; alternatively, it may be that previous in vitro studies examined only an initial phase of receptor differentiation. The current data favor the latter2 hypothesis. E9 chick retinas were dissociated and cells were grown in high density (1.35×10^5 cells/cm^2) for 2 and 6 days. Cells were also grown at 10-fold lower density, but fed media conditioned by high density cultures. Culture conditions favored normal development of receptor subtypes previously seen in vivo (PNAS 82:8785). Cells were labeled with [^{3}H]PrBCM and mAChRs were localized by autoradiography. In both types of cultures, cells were observed expressing mAChRs on cell bodies as well as to dendrites. However, with increasing age, there were numerous cells that segregated mAChR to dendrites. This culture system, in which retina neurons can mimic their in vivo restriction of receptors, should be useful in studying the protein trafficking essential to form synaptic signal transducing membranes in the CNS.

24.4

ADHESION COMPLEXES OF SYNAPTOGENIC FILOPODIA H-C.T.Tsui, C.S.Kim*, C.P.Young*, B.Lom*, W.B.Pope* and W.L.Klein. Inst.for Neurosci., Northwestern University, Evanston, Il.

CNS filopodial junctions differentiate into structurally identifiable synapses (PNAS 82:8256). Nascent junctions are held together by "transjunctional adhesion complexes (TACs), multimeric extracellular filaments that contain components of adheron (Dev.Brain Res. 51:205). We now have begun to characterize the individual components of adherons using monoclonal antibodies. Two mabs generated against adherons recognized antigens with marked developmental regulation. Labeling with Mab AD2 showed non-selective distribution in chick retina from E7 to E13, but became segregated to the inner plexiform, ganglion cell and optic fiber layers by P40. The antigen was present in two Mr forms (66 kD and 70 kD) in membrane fractions of E13 retina. However, both forms disappeared in membrane fraction of P28 retinas. In contrast, a 70 kD form was present in the soluble fraction throughout development. Another mab, AD1, labeled distinct bands of the inner plexiform layer transiently during the time of neuritogenesis and synaptogenesis. These antigens were only present on a subpopulation of neurons and were absent in non-neural tissue. When examined with the EM immunogold method, these antigens were found to be present on filamentous materials on the cell surfaces and at some of the filopodia junctions. These result suggest that some of the TACs comprise cell- and stage-specific molecules.

24.5

REGENERATING RETINAL GANGLION CELL AXONS CAN FORM SYNAPSES WITH NEURONS IN FOUR DIFFERENT NON-RETINAL TARGETS IN THE ADULT HAMSTER. T.J. ZWIMPFER*, H. INOUE*, A.J. AGUAYO and G.M. BRAY. Montreal General Hospital and McGill University, Montréal, Québec, Canada, H3G 1A4.

We have previously demonstrated in adult hamsters that regenerating RGC axons can form persistent synapses within the cerebellum (Cb) (Zwimpfer et al., 1989 Soc. Neurosci. Abstr. 15: 458). Regenerative synaptogenesis of RGC axons was investigated in 3 additional targets that do not normally receive direct retinal input. In adult hamsters, regrowing RGC axons were guided by a bridging peripheral nerve graft into either the inferior colliculus, visual cortex, or somatosensory cortex.

RGC axons grew into each of these targets and formed terminals with ultrastructural features (clear vesicles, pale mitochondria and predominantly asymmetric axodendritic synapses) that are similar to those of both regenerated RGC terminals within the Cb and RGC terminals within a normal retinal target, the superior colliculus. Further studies on retinal innervation of the Cb revealed that RGC axons exhibited a marked preference for growth and synapse formation in one region of the Cb, the granule cell layer.

These results suggest either that axons regenerating in the adult mammalian CNS synapse indiscriminantly or that molecular determinants of synaptogenesis are shared by certain neurons in diverse regions of the CNS.

24.6

In vitro maintenance of a neonatal rat spinal cord/nerve/muscle preparation

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We have explored methods for maintaining neonatal rat spinal cord/nerve/muscle (4th deep lumbrical) preparations in culture. Viability was assessed by measuring muscle twitch and tetanic tension in response to peripheral nerve and dorsal root stimulation. A two-chambered organ bath allowed the the spinal cord and the muscle to be exposed to different culture media. Several different media were assessed, including normal physiological saline, DME and an Earle's BSS/BME mixture, with various supplements (e.g., chick embryo extract, neonatal or fetal calf serum, H_2O_2 and insulin) were assessed.

Tension declined first in response to dorsal root stimulation, indicating transmission failure at spinal synapses. This usually began after about 20 hours in culture. Tension evoked in response to peripheral nerve stimulation often lasted 36 hours, and under ideal conditions, for periods as long as 80 hours, with the indirectly elicited muscle tetanus being within 10% of the initial value for the first 45 hours of the experiment.

24.7

GLYCINE RECEPTORS ON RAT MOTONEURONS DEVELOPING IN CULTURE. P.A. St. John & S.L. Stephens*. Dept. of Anatomy and Program in Neuroscience, Univ. of Arizona, Tucson, AZ 85724.

We have used embryonic rat motoneurons developing in culture to study what controls the type, number, and subcellular distribution of neurotransmitter receptors expressed by CNS neurons. Last year, we presented studies of substance P (SP) receptors on developing motoneurons. The results suggested that the intial expression of SP receptors by motoneurons is autonomous, apparently not requiring induction by other neurons and able to precede synapse formation. This year's experiments examined a fundamentally different class of receptors, those for glycine. The time-course for development of glycine receptors, assayed by the binding of radioactive strychnine, differed significantly from that for SP receptors. Neurons in mixed-population cultures had very few, if any, glycine receptors for the first several days in culture. Beginning around day 4, however, the number of receptors increased dramatically to reach a plateau level within 2-3 days. Because the initial expression of glycine receptors coincided with that of SV48, a common synaptic vesicle antigen, it appeared that glycine receptor expression might be induced by other neurons, perhaps through synaptic contact. This question is being addressed using motoneurons purified by cell sorting and cultured in the virtual absence of other neurons. In separate experiments, we used a fluorescent derivative of strychnine to examine the distribution of glycine receptors. In mixed-population cultures of spinal cord neurons, most neurons were labeled on the cell surface. Label was found on both cell bodies and neurites. Much of the labeling was patchy, indicating that many or most glycine receptors were collected in aggregates. Whether these aggregates were sites of synaptic contacts from other neurons is currently being determined. Supported by NSF (BNS-8808506) and the ADCRC (82-9311).

24.8

EXPRESSION OF PRESYNAPTIC AND CYTOSKELETAL PROTEINS FOLLOWING EARLY TARGET REMOVAL IN THE CHICK EMBRYO. O. Braissant*, M.C. Wilson#, W. Wahli* and S. Catsicas. Institut de Biologie animale, University of Lausanne, Switzerland and #Research Institute of Scripps Clinic , La Jolla (CA) USA.

Our aim is to determine whether during neuronal development the expression of specific presynaptic proteins follows an endogenous program of differentiation or is the result of cell-cell interactions. Here we have used antibodies directed against the presynaptic proteins synaptosomal associated protein-25 (SNAP-25), synaptophysin and synapsin I to define the onset and patterns of expression of these proteins in the developing neural tube and retina. We have then determined the effect of early target removal on presynaptic proteins expression by spinal motor and sensory ganglia neurons. As a control, we have used a monoclonal antibody against neurofilament proteins (NF).

SNAP-25 was found to be expressed relatively late during development, at the time of synaptogenesis, whereas synaptophysin and synapsin I seemed already present during initial axonal elongation, well before contact with the target cells occurred. Moreover, preliminary evidence indicate that early limb bud removal does not affect the normal patterns of expression of these proteins, until the target deprived neurons start to degenerate. These results suggest that the expression of presynaptic proteins is independent of neuron/target interactions.

NF proteins were found in subsets of stem cells and most growing axons, but seemed not to be affected by the removal of the target region.

NEURAL PLASTICITY IN ADULT ANIMALS I

25.1

HOUSING ADULT RATS IN ENRICHED CONDITIONS DOES NOT ALTER NEUROGENESIS IN THE OLFACTORY BULB. A.D. York, S.M. Breedlove, M.C. Diamond, and E.R. Greer*. University of California, Berkeley, CA 94720.

Housing adult male rats in enriched conditions increases the normally low rate of neurogenesis in the hippocampal dentate gyrus (York et al, SN Abstr 15: 962). Neurogenesis also continues in adult rats in the main and accessory olfactory bulbs; the effects of enriched housing on the olfactory bulbs have not been previously investigated. The present study looked for changes in adult neurogenesis in the olfactory bulb due to different housing conditions. Nine 60-day-old male Long-Evans rats were housed 30 days in enriched conditions (common large cage with novel objects, changed daily). Eight controls were housed in standard conditions (3 rats per small cage with no objects). All rats were given ^{3}H-thymidine in 5 semi-weekly i.p. injections starting day 1 of differential housing. After autoradiography, all six layers of the main olfactory bulb and the granule cell layer of the accessory bulb were examined for labeled cells. Labeled neurons were seen in the internal granular and glomerular layers of the main bulb and the granule cell layer of the accessory bulb. No differences were seen between the experimental groups on any measure. When compared to the increased neurogenesis found in the dentate gyrus, this finding suggests that enriched condition housing affects the structure of specific cell populations.

25.2

WEANLING AGE AND ADULT RATS EXPOSED TO COMPLEX, SOCIAL AND INDIVIDUAL ENVIRONMENTS SHOW SIMILAR NEURONAL DENSITY AND SIZE PLASTICITY. U. S. Hess*, A. M. Sirevaag, C. S. Wallace and W. T. Greenough. Dept. Psychobiol., Univ. Calif. Irvine, Irvine, CA 92717. Depts., Psych. & Cell & Struct. Biol., & Neuroscience Prog., Univ. IL, Champaign, IL 61820.

Young rats exposed to complex environments (EC) for 30 days have been reported to have 30% larger neuronal nuclei and an 18% lower neuronal density (Nv) than rats reared in individual cages (IC) (Turner & Greenough, *Brain Research, 329*, 195-203, 1985). We now report that adult EC rats (60 days old) exposed to their environment for 30 days exhibit similar neuronal plasticity. Adults exposed to their environments for 10 days did not exhibit nuclear size or density differences. Stereological estimates of neuronal size and density were made using the nucleator (Gundersen, H.J.G. *J. Microscopy, 150*, 1988). EC rats had 20% larger neuronal nuclei and a 20% lower Nv than IC rats. This contrasts with an earlier report based on preliminary data (Hwang and Greenough, *Soc. Neurosci. Abstr. 12*, 1284, 1986). Supported by MH 35321 and HD 07333.

30 Day Exposure Adult Rats

	Nv Neurons	Vv Nuclei	Mean Vol. Nuclei
EC	59,384/mm^3	2.87%	500.0 μm^3
SC	75,760	3.03%	440.7
IC	71,421	3.10%	418.3
EC vs. IC	p<.05	NS	p<.05

25.3

EFFECTS OF FRONTAL LESIONS AND ENVIRONMENTAL ENRICHMENT ON BEHAVIOR AND CORTICAL DENDRITIC ARBOR. B. Kolb and R. Gibb*. Dept. of Psychology, Univ. of Lethbridge, Lethbridge, AB, Canada, T1K 3M4.

Adult male rats were given lesions of the frontal cortex, and, along with normal control animals, were housed for 60 days in either enriched environments or standard cages. The rats were then studied on a battery of behavioral tests before Golgi-Cox staining. The lesions impaired performance on tests of spatial navigation, food hoarding, forepaw use, and tongue extension as well as reducing chronic body weight. Enrichment (ENR) produced only a small reduction of the behavioral deficits although it increased brain weight in both control and lesion groups. Layer II/III pyramidal cells were analyzed in Par 1 (about 1 mm from the lesion site) and in Ocl. These data showed that: (1) frontal lesions increased dendritic arbor relative to controls in Par 1 but not in Ocl; (2) ENR increased arbor in Ocl but not in Par 1 of frontal rats and in both regions of control animals. These data suggest that the cortex may only show plasticity once (either after the lesion or ENR), which may be significant for understanding the effect of therapy in recovery from brain injury.

25.4

OPTICAL IMAGING OF FUNCTIONAL CHANGES IN THE VISUAL SYSTEM OF ANESTHETIZED ADULT MACAQUES AFTER BRIEF MONOCULAR OCCLUSION. R.D. Frostig. Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

The present study was designed to investigated whether functional changes can be detected in the visual system of adult anesthetized and paralyzed monkeys after short term monocular occlusion. Using optical recording of intrinsic signals with a high resolution CCD camera, images of the ocular dominance columns were first obtained from the visual cortex (area 17) under normal conditions. Next, one eye was occluded for a brief period (10-17 h) with non-transparent material. After the occlusion was removed, images of ocular dominance columns were taken and compared to images taken before the occlusion. In 2 of 3 monkeys the difference amplitude of the ocular dominance stripes were greater after the removal of the occlusion, compared to the pre-occlusion amplitude. In 1 monkey the amplitude did not change. This change in amplitude was due to weakening of the deprived eye and possibly in addition strengthening of the non-occluded eye.

In one monkey imaging of the ocular dominance columns continued for 8 h after the occlusion removal. The difference amplitude between columns gradually returned to the pre-occluded magnitude within 5-6 h, suggesting that such changes are reversible.

These preliminary results indicate that although no major changes were observed in the ocular dominance columns' width, differential activation of one set of inputs seems capable of eliciting short term functional changes in the system's response to a given stimulus.

25.5

PLASTICITY IN BINAURAL TUNING OF TECTAL NEURONS IN THE ADULT BARN OWL. J. F. Olsen and E. I. Knudsen. Dept. Neurobiol., Stanford Univ., Stanford, CA 94305.

The tuning of units in the optic tectum to interaural differences in time (ITD) and intensity (IID) changes adaptively when barn owls are raised with altered external ears (Olsen and Knudsen, Abstr. Soc. Neurosci. 15: 290, 1989). Here we report that a similar ear alteration in a fully grown owl led to similar changes ITD tuning, but did not affect IID tuning. The facial ruff and preaural flaps were removed from a sexually mature owl. In ruff-cut owls, ITD varies linearly with the azimuth of a sound source (as in normal owls), but at a rate slightly faster than normal, whereas the pattern of IIDs is radically altered such that IIDs vary with source azimuth rather than with source elevation. The tuning of tectal units for ITD and IID was measured dichotically one month before and three months after ear alteration. To compare tuning before and after ruff-cutting, units were selected from matched tectal sites, based on the locations of visual receptive fields. Before ruff-cutting, the patterns of best ITD and best IID representation were normal (Olsen et al., J. Neurosci. 9:2591 1989). After ruff-cutting, the rate at which best ITD varied with best visual azimuth had increased from 2.4 μsec/deg ($r = .99$; $p < .001$), the normal rate, to 3.2 μsec/deg ($r = .96$; $p < .001$), a rate outside the normal range and similar to that seen in owls raised from birth without a facial ruff. The pattern of best IIDs was essentially normal and unchanged. The lack of effect of ruff-cutting on IID tuning in the adult contrasts with the large change in IID tuning seen in baby owls subjected to the same manipulation. The results suggest that, although large adaptive changes in binaural tuning require experience early in life, an appreciable degree of plasticity persists into adulthood. Supported by NIH grant RO1 DC00155-11.

25.6

ADULT RAT NEOCORTEX CONTAINS A LAMINAR-SPECIFIC DISTRIBUTION OF BOTH CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II (CKII) AND GAP-43 mRNA. K.M. Jacobs, R.L. Neve and J.P. Donoghue Center for Neural Science, Brown University, Providence, RI 02912

CKII and GAP-43 have been implicated in mechanisms underlying synaptic plasticity in the adult nervous system. As part of ongoing studies on experience-dependent reorganization in motor cortex, we examined the distribution of the mRNA for CKII and GAP-43 in normal adult rat cerebral cortex using *in situ* hybridization techniques. CKII may play a role in controlling synaptic structure and generating LTP through action on substrates such as synapsin I, tubulin and MAP2. We identified CKII mRNA using a cDNA clone for the alpha subunit of rat CKII. Labeling was heaviest in the hippocampus and neocortex, especially cingulate and prelimbic cortex, and lowest in the brainstem. Throughout neocortex, neurons in the superficial layers expressed the highest levels of mRNA, while neurons in deeper layers expressed only moderate levels of mRNA. There was also substantial neuropil labeling in the superficial layers, suggesting that neuronal processes from deep or superficial layers may produce CKII independently of the soma.

GAP-43 has been shown to be a marker for neurite extension and axonal growth during development and in response to injury. We examined the distribution of GAP-43 mRNA within sections adjacent to those used for CKII hybridization, using a cDNA clone for human GAP-43 which also hybridizes to rat GAP-43. Levels of GAP-43 mRNA were low throughout the brain of normal animals. The most heavily labeled neurons were found in the stratum pyramidale of the hippocampus and in olfactory cortex. Some large cells within the striatum and many cells within thalamus were also labeled. Within the neocortex, the strongest and most consistent labeling was within large pyramidal cells in layer V. Small cells in the deepest part of layer VI were well labeled, while superficial layers contained only a few labeled cells which had moderate levels of GAP-43 mRNA. These results suggest that layer V neurons may retain a potential for axonal growth, while neurons in superficial layers may contain mechanisms necessary for more rapid adjustments of synaptic strength. Supported by NIH NS 22517 and March of Dimes 1-1169.

25.7

SOMATOSENSORY CORTICAL MAP PLASTICITY: A MODEL AND SIMULATIONS. E. Sklar and M. F. Bear. Program in Cognitive Science and Center for Neural Science, Brown University, Providence, RI 02912.

Plasticity of the cortical somatotopic map in the adult monkey has been described by Merzenich *et al.* (*Neurosci.*, 8:33, 1983) and others. A number of effects have been noted, including map reorganization following the amputation of digits, map regeneration in reversible deafferention, artificial syndactyly effects, and use-dependent map reorganization.

We present a model based on the theory of Bienestock *et al.* (*J. Neurosci.*, 2:32, 1982) which addresses plasticity in the visual cortex of kittens. The model incorporates a Hebb-type process which compares weighted input activity to a 'sliding modification threshold' reflecting time-averaged output activity. Synaptic weights increase or decrease as a product of input activity and output activity relative to this critical value.

Simulation results suggest that the model successfully captures the features of somatosensory cortical map plasticity, *e.g.* the simulated amputation of a digit produces first an unresponsive map region, followed by the expansion of regions representing adjacent digits. The results also predict several experimental outcomes, *e.g.* that the results for surgical versus temporal fusion of digits should converge.

In addition, the current model is compatible with proposed mechanisms of synaptic modification involving the activity of the NMDA receptor complex (Bear *et al.*, *Science*, 237:42, 1987). This suggests a link between the two types of cortical plasticity at the level of synaptic mechanisms.

25.8

EFFECT OF BASAL FOREBRAIN LESION AND INTRAPERITONEAL INJECTIONS OF RESERPINE ON CORTICAL REORGANIZATION IN THE RAT HINDPAW CORTEX. H.H. Webster[1], U.-K.Hanisch[2*], R.W.Dykes[1] and D. Biesold[2]. [1]Dept. de Physiologie, Univ. de Montréal, Montréal, P.Q., Canada and [2]Dept. of Neurochemistry, Karl Marx Univ., Leipzig, D.D.R.

To test if cortical reorganization depends on one or more neuromodulators, the hindpaw representation was mapped in 8 different groups of adult rats: 1) untreated; 2) Day 4 after (D4) sciatic nerve transection; 3) D1 intraperitoneal reserpine injection; 4) D5 reserpine/D4 transection; 5) D11 basal forebrain ibotenic acid (BF) lesion; 6) D11 BF lesion/D4 transection; 7) D11 BF lesion/D1 reserpine; 8) D11 BF lesion/D5 reserpine/D4 transection. Results: in groups 6) and 8) the BF lesion/transection, and the BF lesion/reserpine/transection inhibited the reorganization process. In the reserpine and BF lesion groups: 3) and 5), the response threshold was lowered relative to the transected groups: 4) and 6). The data suggest acetylcholine and one or more of the monoamines have different influences on reorganization of primary somatosensory cortex. (Supported by IBRO/UNESCO and the Ministry of Science & Technology, D.D.R.).

25.9

ACETYLCHOLINE RELEASE IN THE HINDLIMB SOMATOSENSORY CORTEX OF RATS FOLLOWING SCIATIC NERVE TRANSECTION. N. Kabani[1], R.W. Dykes[2], K.M. Brown*[2]. [1]Dept. Surgery, McGill Univ.; [2]Dept. Physiol., Univ. Montreal, Montreal, PQ, H3C 3J7.

The somatosensory cortex contains an organized somatotopic map which may reorganize following deafferentation. Acetylcholine and norepinephrine have been hypothesized to play a role in cortical reorganization. If this is so, the release levels of acetylcholine may be predicted to change during reorganization.

In our study, release levels of acetylcholine were measured following sciatic nerve transection in adult rats using intracerebral microdialysis in combination with high performance liquid chromatography. Preliminary studies show that the acetylcholine release is significantly high ($p<0.01$), 24 hrs after nerve transection (9.56nM/20min) as compared to the sham operated group (5.48nM/20min). Whereas at day 7, the release rate in transected group (3.78nM/20min) return to levels similar ($p<0.05$) to those measured in control animals (4.95nM/20min).

We suggest that cortical reorganization begins shortly after injury and is probably, partially complete within 7 days and that acetylcholine plays an important role in this phenomenon. (Supported by the Medical Research Council of Canada).

25.10

EFFECTS OF AMPHETAMINE ON BIOGENIC AMINE LEVELS FOLLOWING RIGHT SENSORIMOTOR CORTEX ABLATION IN RAT. K.A. Krobert and D.M. Feeney. Depts. of Psych. and Physiol., Univ. of New Mexico, Albuq., NM 87131.

A transient bilateral reduction of norepinephrine (NE) and dopamine (DA) levels has been reported following ablation of the right sensorimotor cortex (RSMCX) in rat (Brain Res. 218:233, 1981). Amphetamine (AMPH) administered 24 hours post-injury produces an enduring facilitation of locomotor recovery assessed on a beam-walk task, considered a result of AMPH increasing NE transmission (CRC Critical Reviews in Neurobiol. 3(2):135, 1987). Using HPLC, this study compared the effect of AMPH or saline upon levels of NE, DA, their major metabolites (3-methoxy-4-hydroxy phenylglycol (MHPG), 3,4-dihydroxyphenylacetic acid (DOPAC)) and the serotonin metabolite 5-hydroxyindole acetic acid (5-HIAA) 2 days after RSMCX aspiration. Compared to sham operates, RSMCX lesion resulted in bilateral depression of NE levels in the frontal pole and posterior SMCX, and DA and DOPAC in the caudate/putamen. Interestingly, a marked increase in 5-HIAA was measured in the frontal pole, posterior SMCX, and caudate/putamen ipsilateral but not contralateral to the injury. Administration of AMPH (2 mg/kg i.p.) 24 hrs after injury reversed the depression of DA, NE, and DOPAC levels, whereas 5-HIAA levels remained unchanged. The NE/MHPG ratio was significantly decreased in the left but not the right cerebellar cortex of injured rats, while the DA/DOPAC ratio in the left caudate/putamen was increased. AMPH returned the DA/DOPAC ratio to that of sham saline controls. These results replicate and extend earlier reports of reduced catecholamine levels in injured rat brain and suggest that AMPH alleviates only some of these consequences of brain injury. Further study of monoamine metabolism in specific brain loci and recovery of locomotion are in progress. Supported by DHHS Grant R01NS20220-03, and funds from the Univ. of New Mexico.

25.11

THE BEHAVIORAL SIGNIFICANCE OF A RAT VIBRISSA IN EXPLORATORY BEHAVIOR. J.G.Johannsen*, K.G.Gallo, R.L. Craik, W.J. Carr* & P.J. Hand. Beaver Coll. Glenside, PA 19038 & U. of Penn, Phila, PA 19104.

Previous research indicates that rats with sub total vibrissae deafferentation sparing one vibrissa (#3 Row C,SC3) have a preferred travel direction in a walled, elevated cylindrical maze. The present study examined whether SC3 or control C3(CC3) was used in exploration. Prior to post natal day 2(PND2), 10 rats underwent unilateral follicle removal sparing SC3 vibrissa. On PND 60, contralateral vibrissae except CC3 were clipped. Animals underwent 4 min trials for 5 days in an elevated circular maze without walls. 8 of 10 experimental animals traveled with SC3 over either inner or outer edges rather than in the center. Animals with R SC3 traveled clockwise(CW) along the inner edge or CCW along the outer edge. The converse occurred for L SC3. No preference was demonstrated by 10 controls with bilateral clipping sparing CC3. 2DG metabolic analysis of SI reveals a 2.2 fold increase($P<.05$)in SC3. An enlarged SC3 barrel accounts for 30% of the 2DG activity. CNS reorganization which accompanies subtotal deafferentation appears to result in a preferred vibrissa for exploratory behavior. Supported by NIH 2283.

25.12

NEW SOMATOSENSORY EPSPS IN REORGANIZED RACCOON CORTEX WITHOUT CHANGE IN THE INCIDENCE OF CORTICOCORTICAL EPSPS. S. Witte*, E. Smits*, P. Zarzecki and D.D. Rasmusson. Dept. of Physiol., Queen's Univ., Kingston, Ontario and Dept. of Physiol. & Biophys., Dalhousie Univ., Halifax, Nova Scotia, Canada.

Stimulation of previously ineffective digits evokes epsps when the representation of the paw in somatosensory cortex is reorganized by altering its sensory inputs. Could newly formed corticocortical connections transfer these new inputs? Intracellular recordings were made in the cortical representation of digit four (D4 cortex). Sensory input to the cortex was altered by amputation of D4 or by joining D3 and D4 to form a syndactyly.

In D4 cortex of normal raccoons, stimulation of D3 or D5 evoked epsps in only 25% of neurons, even though about half of the neurons had monosynaptic corticocortical inputs from the "heterogeneous zone", where multidigit inputs are common. Six to ten months after surgical alterations of the paws, most neurons (70% & 76% in the two groups) had epsps evoked by electrical stimulation of D3, D5 or both. In spite of the dramatic increase in inputs from multiple digits, monosynaptic corticocortical epsps were still found in about half of the neurons in each group. Thus, cortical reorganizations induced by altered sensory inputs do not seem to require the formation of new corticocortical connections, but could instead result from an increased effectiveness of existing pathways. (Supported by MRC)

25.13

UNILATERAL LESIONS TO THE SENSORIMOTOR CORTEX: BEHAVIORALLY-LINKED DENDRITIC CHANGES IN THE CONTRALATERAL CORTEX. T.A. Jones and T. Schallert. Neurosci. Inst. and Psychology Dept, Univ. Texas, Austin, TX 78712.

In recent research, we have reported time dependent anatomical changes in the forelimb representation area (FL) of the sensorimotor cortex following unilateral lesions to the contralateral homotopic area in adult rats (Jones & Schallert, 1989). These changes include an increase in the thickness of the contralateral cortex, an elaboration of astrocytic processes in the vicinity of layer V pyramidal neurons, as well as a behavioral and anatomical vulnerability to damage in this area. These changes were transient, were maximum at 7 days after the lesion, and were specific to the FL area of the cortex. In the present experiment, we examined the possibility that these anatomical events precede changes in neuronal morphology within the FL contralateral to the lesion. At various times following unilateral FL lesions, dendritic arbors of pyramidal neurons were measured in the contralateral cortex. A dramatic increase in the dendritic arborization of layer V pyramidal neurons was found within the FL at 2 to 3 weeks after the lesion, in comparison to sham-operated controls. Between 2 to 4 months, there was a partial reduction in the number of dendritic branches without a complete return to control levels. Furthermore, these anatomical events corresponded to time dependent behavioral asymmetries in the use of the forelimbs for postural support and motor coordination. These findings may be indicative of the initiation and stabilization of a post-injury sequence of behaviorally relevant structural changes evident within the contralateral homotopic cortex of adult rats.
Supported by grants MH 18837 and NS 23964.

25.14

SHORT-TERM VIBRISSECTOMY (VBX) AND NOREPINEPHRINE (NE) DEPLETION EFFECTS ON BARREL CORTEX METABOLISM IN ADULT RAT. A. Dunn-Meynell and B.E. Levin. Neurology Svc., VA Med. Ctr., E. Orange, NJ 07019, Dept. Neurosciences, NJ Med. Sch., Newark, NJ 07103.

Vibrissal stimulation increases local cerebral glucose utilization (LCGU) in the corresponding "barrel" of the contralateral SmI cortex. The metabolic size of a stimulated C3 barrel increases by 30-40% at 2mo after complete VBX with sparing of C3 (VBX/SC3; Levin et al., 1988). To see if this enlargement was due to a delayed sprouting effect versus an immediate removal of inhibitory inputs, LCGU was measured in the stimulated C3 barrels of adult SD rats 24h after unilateral VBX/SC3 using [14C] 2-deoxyglucose (Sokoloff et al., 1977). Neither absolute LCGU nor areal size of SC3 barrels differed from intact C3 barrels at 24h post-VBX suggesting that enlargement seen at 2mo post-VBX was due to delayed sprouting. When bilateral, 24h VBX/SC3 was preceded by a unilateral locus coeruleus lesion, the SC3 barrel was 28% smaller on the NE depleted side ($P<0.01$). Thus, removal of NE's inhibitory input to the "inhibitory surround" of the C3 barrel may reduce the spread of neuronal activity in a VBX/SC3 barrel.

25.15

MK-801 REINSTATES FORELIMB PLACING DEFICITS WHEN ADMINISTERED AFTER RECOVERY FROM UNILATERAL CORTICAL LESIONS IN THE RAT T. M. Barth. Lab. Neurophysiol., NIMH, Poolesville, MD 20837.

There is evidence to suggest that drugs acting as noncompetitive antagonists at the N-methyl-D-aspartate (NMDA) receptor might have both beneficial and detrimental effects on recovery of function following brain damage. For example, MK-801 and PCP are neuroprotective when administered shortly before or after ischemia in rodents. In contrast, these agents induce acute histopathological changes in cortical neurons in intact rats, disrupt developmental plasticity and impair learning in adult animals. A previous experiment has shown that when MK-801 was administered 12-16 hrs after unilateral lesions in the somatic sensorimotor cortex (SMC), there was a slight facilitatory effect on recovery from somatosensory asymmetry (as measured by bilateral-tactile stimulation tests), but there was no effect on the recovery of forelimb placing. The present experiment was designed to investigate the effects of MK-801 administered 14 - 60 days after the lesion when behavioral recovery appears to be complete.

Rats received unilateral lesions of the SMC, were allowed to recover from somatosensory asymmetry and forelimb placing deficits, and then treated with either a single injection of MK-801 (1 mg/kg) or saline. Forty-eight hours after the MK-801 injection, the rats showed a reinstatement of the contralateral forelimb placing deficit. The impairment endured for at least 6 days following the injection, which is similar to the duration of the effects following the lesion. Moreover, there was no impairment in placing the forelimb ipsilateral to the damage, nor did MK-801 affect placing reactions in sham operated control rats. Finally, MK-801 did not reinstate the somatosensory asymmetry as measured by bilateral tactile-stimulation tests. These data support the idea that MK-801 may have detrimental effects when administered after recovery and suggest that different mechanisms may be mediating the recovery from somatosensory asymmetry and forelimb placing deficits.

25.16

EFFECTS OF A GINKGO BILOBA EXTRACT ON FUNCTIONAL RECOVERY AFTER CORTICAL HEMIPLEGIA IN THE RAT. S. Brailowsky, T. Montiel*, E. Hernández*, J. Flores-Hernández*, S. Alvarez* and R.H. Pineda*. Instituto de Fisiología Celular, U.N.A.M., México 04510 D.F., MEXICO.

Interested in the pharmacological treatment of subjects with brain lesions, we studied the effects of chronic treatments with a Ginkgo biloba extract (EGb761-IPSEN) in two animal models of cortical hemiplegia: one induced by motor cortex aspiration and another using a reversible inactivation of the motor cortex through chronic, localized infusion of GABA, via osmotic minipumps.

The elevated beam test was used in water-deprived rats trained to drink saccharin-sweetened solutions (with or without EGb761) and to perform to criteria before the surgical procedures. From the day after the surgery, the rats were daily administered 100 mg/Kg of EGb761 or its vehicle for 7 or 30 days.

In all groups in which EGb761 was administered, a faster and more complete recovery from the motor deficits was observed, which was significantly different from that of rats in which only saccharin was given.

These results suggest a potential use of EGb761 in patients with brain lesions as this product shows little toxicity in man after chronic administration. The active principle(s) and the mechanism(s) for this beneficial effect remain to be elucidated.

25.17

SYNAPTOLOGY OF MONKEY VPL AFTER LESIONS OF THE DORSAL COLUMN NUCLEI. J. Wells, D. D. Ralston and H. J. Ralston, III. Dept. of Anatomy and Neurobiology, University of Vermont and Dept. of Anatomy, U. C. San Francisco.

The synaptology of VPLc was analyzed using EM in 4 control monkeys and 6 lesioned monkeys. Two groups of postlesion survival times were used: 2 - 5 days (n=5) and 5 months (n=1). In the controls, the population density of all synapse types was 14.5 synapses/100um^2 - half the value found in the VPL of rats. Both the lemniscal terminals (LR) and the terminals of the interneuronal dendrites (P) were significantly decreased after the lesion. The terminals from the thalamic reticular axons (F), the cortical axons (SR) and the total number of terminals/100 um^2 remained relatively unchanged 2-5 days after the lesion. Five months after the lesion the LR and P terminals showed few signs of recovery. The values for the LR and P terminals were within the range of the 2 - 5 day group and well below the control group. It appears that there was no reactive synaptogenesis of the principal afferent terminals in the VPL of these monkeys. One suprising finding was that there was little, if any, gliosis at any survival time after the DCN lesion. Supported by NS23347.

25.18

LESION OF THE SUPRAGRANULAR LAYERS OF BARREL FIELD CORTEX IMPAIRS THE ACQUISITION BUT NOT THE PERFORMANCE OF A CORTICALLY DEPENDENT BEHAVIORAL TASK. M.H. Friedberg, S.M. Lee, L.R. Hochberg*, and F.F. Ebner. Center for Neural Science, Brown University, Providence, R.I. 02912.

We investigated the function of the supragranular (SG) layers of barrel field cortex (BF) in adult rats by determining their ability to perform the "gap-cross task" of Hutson and Masterton before and/or after cortical lesions. The high density of NMDA receptors in the SG layers enabled us to destroy consistently the SG layers of BF cortex with a 15 minute epidural application of 150 mM NMDA in dH2O. Nissl and cytochrome oxidase staining indicated that the neurons in layers II, III and the superficial part of IV were destroyed. Multiunit recordings of neuronal responses and spontaneous activity demonstrated that evoked and spontaneous activity were present only in layers IV-VI. The gap-cross task, shown to be dependent upon an intact vibrissae-to-BF pathway, was used to compare vibrissae-dependent performance of animals trained before and after the SG lesion.

The performance of the task was unaffected by SG lesions made after training. In contrast, animals were unable to use their vibrissae to perform the task during the first 30 days of training if the lesions were made prior to training (vs. 3 days for controls). These animals, when retested 60 days later, immediately were able to use their vibrissae to perform the task, indicating that the functional impairment was not permanent.

These results suggest that the SG layers of BF cortex are not required for the performance of a pre-learned task, but are important in the performance of a novel cortically-dependent behavior. We are investigating the mechanisms responsible for the recovery of vibrissae-dependent function seen at 90 days. (Grants NS13031 and the Whitehall Foundation)

25.19

LONG-TERM LOSS OF GABAergic INHIBITION LEADS TO PLASTICITY IN THE RAT THALAMIC VPM NUCLEUS. S.M. Lee, M.H. Friedberg and F.F. Ebner, Center for Neural Science, Brown University, Providence, RI

In rats, the major source of GABAergic inhibition in the thalamic ventro-posteromedial (VPM) nucleus arises almost exclusively from the thalamic reticular nucleus (TRN). The role of this inhibition in shaping the responses of VPM neurons was assessed by mapping the receptive field (RF) following acute iontophoretic application of bicuculline, a GABAergic antagonist, or after chronic excitotoxic lesion of the TRN.

Standard recording and iontophoretic methods were used while the animals were maintained under urethane anesthesia. The lesion of the TRN was made with 0.1 µl injections of KA (0.3 µg/µl) at 3 different sites (D 5.5 mm, ML 4.1 mm and AP -2.1, -2.7, -3.5) and confirmed histologically.

In 4 animals, 14 VPM units were "held" for sufficient length of time to compare the RF and response properties to transient deflections of the mystacial vibrissa before and after iontophoretic application of bicuculline methiodide (5 mM, pH 3.5, 43-109 nA). The primary effect of bicuculline-induced disinhibition in VPM was to alter phasically responding units to ones that responded tonically (28.6% of normal VPM neurons responded tonically vs. 75% in the presence of bicuculline). For all units tested, no statistically significant differences in the size of the RF were seen following the application of bicuculline (2.33 ± 0.31 vs. 2.75 ± 0.53).

To assess the long-term effect of disinhibition, 31 VPM neurons (n = 6) were evaluated 5 days following lesion of the TRN. 76% of the neurons tested had an enlarged RF which ranged from 2 to 17 vibrissa (mean 6.08 ± 0.76), and 72% responded tonically for the duration of the stimulus.

We conclude from these results that inhibition from the TRN serves a major role in determining the temporal response characteristics of VPM neurons. In addition, the long-term effects of disinhibition suggest that the TRN has the capacity to modulate the size of the peripheral RF under certain conditions. (supported by NS13031 and NS25907)

BLOOD-BRAIN BARRIER

26.1

CHLORIDE EFFLUX FROM THE CHOROID PLEXUS OF INFANT AND ADULT RATS. J. E. Preston*, C. E. Johanson and J. T. Parmelee, Program in Neurosurgery, Dept. Clinical Neurosciences, Brown University/Rhode Island Hospital, Providence, RI 02903

The rate of cerebrospinal fluid (CSF) secretion in immature mammals is less than adults on the basis of choroid plexus (CP) weight. Chloride (Cl) and sodium transport across the choroid plexus is a major part of CSF secretion. To study this process, the CP from various ages of rats was isolated in artificial CSF and the efflux of ^{36}Cl from the epithelial cells measured. In young animals, 1-2 weeks of age, the half time ($t_{1/2}$) for ^{36}Cl efflux was 22.9±0.3s (n=15) and was not affected by the transport inhibitors bumetanide, acetazolamide or disulfonic stilbenes (10^{-4} M; all values ± SE). Choroidal tissues from young adult 6-8 week rats, showed a significantly faster efflux, with $t_{1/2}$ = 15.9±1.2 (n=12; p<0.01) which could be inhibited by the above drugs 30 to 40%. In contrast the Cl channel blocker diphenyl carboxylate (DPC) had a significant effect on ^{36}Cl efflux at all ages, with $t_{1/2}$ = 30.3±5.1s (n = 4) at 1 week, and $t_{1/2}$ = 27.9±4s (n = 4) at 6-8 weeks. These pharmacological results indicate that the transporters responsible for Cl efflux in adult rats are not active prior to 2 weeks of age, the major route for Cl movement being via channels. This may explain in part the lower rate of CSF secretion in young rats. (supported by NIH grant NS 27601)

26.2

EFFECTS OF VASOPRESSIN AND ATRIOPEPTIN ON CHLORIDE TRANSPORT IN CHOROID PLEXUS. C. E. Johanson, J. R. B. Nashold,* J. E. Preston, M. Dyas,* N. Knuckey, Program in Neurosurgery, Dept. of Clinical Neurosciences, Brown University/Rhode Island Hospital, Providence, RI 02903

The rate of cerebrospinal fluid (CSF) secretion by adult rat choroid plexus (CP) is affected by neuropeptides such as arginine vasopressin (AVP) and atriopeptin III (AP III). These peptides may alter secretion by modulating choroidal blood flow and/or by changing the transport of chloride and sodium across the CP epithelium. In this study, the CP of adult rats was isolated in artificial CSF and the ^{36}Cl efflux measured. In adult rats, 6-8 weeks of age, the ^{36}Cl efflux rate constant (ERC) significantly decreased by 32% after one hour incubation with AVP, from 0.039±0.006s^{-1} to 0.026±0.002s^{-1} (n = 4; values ±SE). AVP in the presence of a V1 receptor antagonist, however, did not change the ERC. AP III (10^{-9}M) did not alter the ERC, however, 10^{-7} M AP III increased ERC by 13% (p<0.05; n = 5). These results indicate that Cl efflux systems are inhibited by AVP and that this inhibition is blocked by the addition of a V1 receptor antagonist. In contrast, AP III may stimulate Cl efflux. Our electron micrographs of CPs incubated in AVP show increased basal spaces and epithelial fluid retention. This reinforces the concept that AVP has an inhibitory effect on ion transport and CSF formation. (Supported by NIH grant NS 27601)

26.3

CONTROL OF INTRACELLULAR pH (pH_i) IN RABBIT CHOROID PLEXUS EPITHELIUM. S.E. Mayer and E. Sanders-Bush. Dept Pharmacology, Vanderbilt Univ., Nashville, TN 37232.

The amiloride-sensitive and HCO_3^--dependent mechanisms that control pH_i were studied in primary cultures of choroid plexus epithelium (CPE) prepared from rabbit brain. pH_i was estimated from the uptake of ^{14}C-benzoic acid. Control pH_i (7.38 ± 0.03) fell to 6.4 with 20 mM NH_4Cl. Recovery was complete within 15 min after re-exposure to either Hepes (KRH) or HCO_3^--buffered (KRB) Krebs-Ringer solution. Recovery was dependent on $[Na^+]_o$. In KRH, EC_{50} for $[Na^+]$=3 mM. Hexamethylene-amiloride (HxMA) maximally inhibited recovery in 5 mM $[Na^+]$ by 85% (IC_{50} = 180 nM); in 135 mM $[Na^+]$, maximal inhibition was 20-30%. In contrast, in rat CPE HxMA completely inhibited recovery in 135 mM $[Na^+]$ with an IC_{50} of 30 nM. In KRB buffer, the EC_{50} for $[Na^+]$ in rabbit CPE was 18 mM; HxMA maximally inhibited 50% with no inhibition at 135 mM $[Na^+]$. DIDS (diisothiocyanatostilbene disulfonic acid), 10 to 100 μM, caused a small reduction in pH_i from 7.4 to 7.2 regardless of whether or not the cells had previously been acidified. These results indicate the importance of high affinity $[Na^+]$-dependent, but DIDS-insensitive mechanisms for recovery from acidification by rabbit CPE. In Hepes buffer the main component is a Na^+/H^+ antiport, but in HCO_3^- buffer additional mechanisms must come into play, since recovery is incompletely blocked by HxMA. (Supported by USPHS grant MH 34007).

26.4

CALCIUM TRANSPORT INTO CSF WITH ACUTE PLASMA [Ca] CHANGES. Vincent Murphy, Quentin Smith, and Stanley Rapoport. Lab. of Neurosciences, NIA, NIH, Bethesda, MD 20892.

This study was undertaken to determine if CSF Ca transport is saturable with acute plasma [Ca] changes, as was found with chronic changes, and to better analyze kinetic parameters of the transport system, as plasma [Ca] can be varied over a wider range acutely. Unanesthetized adult Fischer-344 rats were infused with solutions of Na citrate to reduce and Ca chloride to elevate plasma [Ca] for 10 min. The infusion schedule was programed to yield constant plasma ionized [Ca]. Ca-45 and Na-22 were injected as an i.v. bolus 3 min before termination. Transfer coefficients for Ca-45 (KCa) and Na-22 (KNa) uptake into CSF were cpm/g CSF divided by the integral of ionized tracer in plasma. Plasma ionized [Ca]s of 0.2, 0.4, 0.7, 1.1, 1.4, 1.9, 2.5, 3.5, and 6.4 mmol/l were obtained. In rats with plasma [Ca] of 0.2, KCa was 1600 nl/g per s. Rats with plasma [Ca] of 6.4 had a KCa of 105 while controls, plasma [Ca] of 1.4, had a KCa of 260. The KCa data was corrected for nonspecific changes in CSF flow and permeability using KNa then fit to Michaelis-Menton kinetics which yielded a Km of 0.02 mmol/l, Vmax of 315 pmol/g per s, and a nonsaturable component of 58 nl/g per s. These results demonstrate that saturable influx of Ca occurs during acute alterations in plasma [Ca] and provide an improved estimate of the kinetic parameters of CSF Ca transport.

26.5

CHARACTERIZATION OF PROTEIN KINASE C IN ENDOTHERIAL CELLS OF BRAIN AND PERIPHERAL VESSELS. H.Kobayashi*, T.Mizuki*, Y.Koda*, M.Okazaki*, N.Yanagihara, A.Wada and F.Izumi. Department of Pharmacology, University of Occupational and Environmental Health, School of Medicine, Kitakyushu 807, Japan.

Protein kinase C is a Ca^{2+}/phospholipid-dependent enzyme which is composed of several isoenzymes. The fact that the expression of protein kinase C subtypes varies from tissue to tissue suggests that each subtype has its own distinct functional role. To study characteristics of protein kinase C of the endothelial cells of the brain and peripheral tissues, subtypes of the enzyme were analyzed. By hydroxyapatite column chromatography, protein kinase C in the rat cerebral microvessels was resolved into two major peaks corresponding to type II and III enzymes with population of 57% and 38 %, respectively. In the endothelial cells of the bovine aorta and adrenal cortex, the protein kinase C was only type III. Thus, the high proportion of type II enzyme in the cerebral microvessels suggests that this type may be involved in specific functions of the cerebral microvessels.

26.6

STIMULATION BY IL-1 AND TUMOR NECROSIS FACTOR (TNF-α) OF VON-WILLEBRAND FACTOR (VWF) SECRETION BY RAT BRAIN MICROVASCULAR ENDOTHELIAL CELLS (RBMEC) PLATED ON MATRIGEL®, A SUBSTRATE WHICH PERMITS SUSTAINED EXPRESSION OF VWF. D.A. Doron, E. Heldman, D.M. Jacobowitz, G. Feuerstein, H.B. Pollard and J.M. Hallenbeck*. Dept. of Neurology, U.S.U.H.S., Bethesda, MD 20814 and Lab. Cell Biol. and Genetics, NIDDK, and Lab. of Clin. Science, NIMH, NIH, Bethesda, MD 20892.

VWF synthesis and secretion is characteristic of endothelial cells (EC), but maintenance of expression of this molecule in a long term culture of RBMEC's has proved difficult. These difficulties are in marked contrast with results for EC cultures from large vessels, where physiologic properties have been more easily studied. Recently we found that RBMEC when cultured on Matrigel®, develop characteristic morphology of EC's and express VWF. We have quantitated the levels of VWF in the culture media using a sensitive ELISA method. We found that when RBMEC plated on Matrigel® are compared to cells plated on uncoated dishes, only the cells plated on Matrigel® released detectable amounts of VWF into the culture media. Secreted VWF was detectable as soon as 5 min after introducing a fresh medium, reaching a maximum concentration after 6 hr. Stimulation of RBMEC with a mixture of IL-1 and TNF-α resulted in a significant increase in secreted VWF. By contrast stimulation of RBMEC with lipopolysaccharide (100 μg/ml) did not increase VWF levels in the culture media above the control. These data indicate that growth of RBMEC's on Matrigel® *in vitro* permits expression and secretion of VWF in a manner similar to the behavior of these cells *in situ*.

26.7

PURIFICATION AND CLONING OF A NOVEL IMMUNOGLOBULIN-LIKE BLOOD-BRAIN BARRIER-SPECIFIC MEMBRANE GLYCOPROTEIN. H. Seulberger*, F. Lottspeich*, U. Albrecht*, R. Jahn, H. Schwarz* and W. Risau*. Max-Planck-Institut für Psychiatrie und Genzentrum, 8033 Martinsried, FRG;

The unique properties of endothelial cells forming the blood-brain barrier (BBB) are reflected by the expression of specific cell surface molecules. We have characterized the monoclonal antibody HT7 raised against an antigen of chick BBB endothelium. The HT7 antigen is first expressed on the surface of endothelial cells at arround the same time as the BBB forms in the chick embryo (day 11). The antigen is absent in blood vessels of the circum-ventricular organs which do not possess a BBB. This indicates that it is a specific molecule of the BBB. Furthermore it is found on epithelial cells of the choroid plexus where the blood-cerebrospinal fluid barrier is localized. We have purified this antigen from the plasma membranes of brains using an immunoaffinity column and reversed phase HPLC. The purified antigen is a highly glycosylated integral membrane protein with a molecular weight of about 45-52 kDa. By using degenerated oligonucleotides and the polymerase chain reaction we have cloned the cDNA encoding the protein. The sequence revealed that it is a new member of the immunoglobulin superfamily. Based on the expression of the HT7 protein on the luminal surface of BBB endothelium, together with the fact that many cell adhesion proteins and receptors belong to this protein superfamily, we suppose that it might be involved in transport or cell surface recognition at the BBB.

26.8

LECTIN BINDING PROPERTIES IN MICROVESSELS IN THE RAT SPINAL CORD. L.J. Noble and J.J. Hall*. Department of Neurosurgery, San Francisco General Hospital and the University of California, San Francisco, CA 94110.

In the central nervous system the role of the endothelial glycocalyx as a barrier to plasma proteins is not clearly understood. As a prelude to a study of structural/functional correlates of permeability to proteins, we have begun to characterize the composition of the endothelial glycocalyx of spinal cord blood vessels. Using lectins that have a high affinity for certain sugar residues, the distribution of manosyl/glucosyl, galactosyl, fucosyl, n-acteyl-galactosaminyl and n-acetyl-glucosaminyl/sialic acid residues was evaluated at both the light and ultrastructural levels.

In general, a homogeneous pattern of lectin staining was apparent in the dorsal horn as compared to the ventral horn and within different regions of white matter. In contrast, lectin staining was more intense in grey matter as compared to white matter. At the light microscopic level all lectins labelled blood vessels. This pattern of lectin binding throughout the microvasculature was confirmed at the ultrastructural level. Staining appeared closely adherent to the luminal aspect of the endothelial cell, following the contours of the plasma membrane including invaginations and endothelial flaps.

In summary, lectin staining was more pronounced in grey as compared to white matter. Both light and ultrastructural findings demonstrate that the endothelial glycocalyx of intrinsic spinal cord vessels is composed of multiple oligosaccharide residues. This composition is similar in arterioles, venules and capillaries. (Supported by NINCDS R01NS23324 to LJN).

26.9

EXTRACELLULAR MIGRATION OF BLOOD-BORNE PROTEIN FROM THE PIAL SURFACE AND FOLLOWING TRANSCYTOSIS THROUGH THE BLOOD-BRAIN BARRIER (BBB). J. Villegas*, R. Broadwell, and M. Salcman. Univ. MD, Balto., MD 21201.

Although evidence from this laboratory suggests blood-borne protein has extracellular entry to the mammalian subarachnoid space and that adsorptive transcytosis of protein occurs thru the BBB from blood to brain (ACTA NEUROPATH.79:117, 1989), spread of these proteins beyond the basal lamina into the neuropil has not been reported. Data are now available from rats and mice injected intravenously with native HRP and lectin conjugated HRP to suggest that movement of blood-borne protein into the neuropil from the pial surface and thru the BBB does occur. HRP fails to cross the BBB but gains access to the pial surface. Through 1hr, HRP is conspicuous initially in the Virchow-Robbins space, suggesting large vessels in this space may be leaky. HRP enters the subpial neuropil through the glial limitans above and from the Virchow-Robbins space laterally. Lectin-HRP undergoes transcytosis thru the BBB globally and at 3hrs is evident beyond the basal lamina both extra- and intracellularly within the neuropil. Extracellular lectin-HRP is particularly conspicuous within the hypothalamic paraventricular nucleus at 12hrs in salt-stressed animals and is most likely attributed to transcytosis thru the BBB and neurosecretory cells within this nuclear group. The data suggest that blood-borne molecules enter the CNS parenchyma thru extra- and intracellular routes to a far greater degree than heretofore appreciated.

26.10

EVIDENCE FOR A RADIALLY ORIENTED PARAVASCULAR/EXTRACELLULAR FLUID CIRCULATION THROUGH THE MAMMALIAN FOREBRAIN. M.L. Rennels. Depts. of Anatomy and Neurology, Univ. Maryland Sch. Med., Baltimore, Md. 21201

The mammalian CNS is permeated by 'paravascular' fluid pathways which include the perivascular spaces (PVS) around penetrating arterioles and veins and the basal laminae (BL) of capillaries (Brain Res., 326:47). Routes of paravascular tracer influx, spread and efflux from the forebrain were investigated by introduction of horseradish peroxidase (HRP) into the subarachnoid space (SAS). An 18 gauge needle was inserted into the cisternae magna of anesthetized cats. Escaping CSF was replaced with an equal volume of 4.0% HRP (Sigma Type II) in Hanks solution. After 4, 5, 10, 15 or 20 minutes, brains were fixed by intravascular aldehyde perfusion and HRP was localized in serial frozen sections using tetramethylbenzidine or 3'-5'-diaminobenzidine. Tracer influx into the forebrain occurs mainly through PVS around penetrating arterioles from the circle of Willis. From these ventral pathways HRP enters the linear extracellular spaces (ECS) of the internal capsule and, within 5 minutes, the tracer is distributed throughout the hemispheres. At numerous sites HRP extends along paravascular pathways from the subcortical white matter through the cerebral cortex to the pial surface, possibly reflecting fluid efflux into the SAS. This rapid and consistent pattern of tracer migration may reveal an on-going circulation of CSF/ECF through the forebrain.

26.11

DEXTRAN-PERCOLL STEP GRADIENT ISOLATION OF CNS ENDOTHELIAL CELLS [ECs] AND ASTROCYTES [As] FROM PRIMATE CORTEX [Ctx], CEREBELLUM [Cbl] AND WHITE MATTER [WM] R. Madden*, S. Bjornsted* and B.R. Brooks. Neurology Svc., Wm. S. Middleton VA Hosp. and Depts. of Neurology and Medical Microbiology, Univ. Wisconsin Sch. of Med., Madison, WI 53792

ECs and As in situ display different cellular binding and pathological characteristics depending on their location in Ctx, Cbl or WM. Purified cell preparations were made from dissected Ctx, Cbl and WM regions in 1-5 yr old male Rhesus and female Cynomologus monkeys as previously described [SNS Abst 15: 1026, 1989; abst 409.7]. Cells were isolated on Matrigel, fibronectin or polylysine in Hams F-10 or DMEM supplemented with ECGS, ITS, Lac/Pyr, Glutamine and Nuserum. Hams F-10 favored initial isolation of ECs which subsequently grew better in DMEM. Cell isolation was independent of SIV/STLV seropositivity. Cells were characterized by FACS with acetylated LDL, enzyme polymorphisms, immunostaining and immunoblotting with specific antibodies and growth in vitro. Terminal cell density [TCD] was similar for Rhesus [1.2 ± 1.0 (SD)] and Cynomologus [1.3 ± 0.7] ECs. However, TCD was decreased in Rhesus [0.5 ± 0.1] compared with Cynomologus [4.0 ± 2.1] As. [Supported by VA Merit Review]

26.12

NITROGEN MUSTARD AMINO ACID WITH HIGH AFFINITY FOR THE LARGE NEUTRAL AMINO ACID CARRIER OF THE BLOOD-BRAIN BARRIER. Y. Takada, N. Greig*, S.I. Rapoport, D. Vistica* and Q.R. Smith. Lab. of Neurosciences, NIA, NIH, Bethesda, MD 20892.

One reason often cited for the failure of brain tumor chemotherapy is inadequate brain delivery of anticancer drugs. Many chemotherapeutic agents are hydrophilic and show only limited transport across the blood-brain barrier. In an attempt to improve brain delivery, an anticancer agent was sought that showed high affinity transport into brain via the large neutral amino acid carrier of the blood-brain barrier. This carrier is located at the brain capillaries and accepts a wide variety of ligands with the alpha-amino acid functional group. Relative affinity of candidate drugs was evaluated from their capacity to inhibit the blood-brain barrier transport of L-[14C]leucine in rats. Affinity for most agents that were examined, including melphalan, acivicin, azaserine, diazo-oxo-norleucine, baclofen and buthionine sulfoxime, was low with transport inhibition constants (Ki; concentration giving 50% inhibition) of 100-4000 uM. In contrast, for a nitrogen mustard derivative of aminotetrahydronaphthoic acid (ATNM), Ki values were only 0.4-0.6 uM and were 10-50 times lower than that for any previously reported compound. The results suggest that ATNM may show improved brain delivery and thus prove of value in the clinical treatment of brain tumors.

26.13

EFFECTS OF ACUTE HYPERTENSION ON THE PHOSPHATASE ACTIVITY OF CEREBROMICROVASCULAR (Na^++K^+)-ATPase. M.L. Caspers, M. Bussone, M.J. Dow, L.J. Ulanski II and P. Grammas. Dept. of Chem., Univ. of Detroit, Detroit, MI 48221 and Dept. of Pathol. Wayne State Univ. Detroit, MI 48201.

Acute hypertension, induced in rats by I.V. injection of angiotensin II, provokes functional alterations of the cerebral endothelium. The phosphatase activity of the cerebromicrovascular (Na^++K^+)-ATPase (CMV-ATPase) from microvessels obtained from hypertensive (HT) and control (NT) rats was determined using p-nitrophenylphosphate. When compared to controls, a 68% decrease ($P<0.02$) in the maximum rate (V_{max}) of the enzyme from HT rats was evident with no change in the Michaelis constant. In contrast, γ-glutamyltranspeptidase was not significantly affected. Sodium arachidonate has been shown to stimulate [^{3}H]ouabain binding to the high affinity isoform of the CMV-ATPase (J. Neurochem.(1988) **50**, 1215). However, the presence of sodium arachidonate, linoleate, oleate or palmitate (100 μM each) produced a 79.7, 88.7, 61.0 or 38.7% inhibition of the phosphatase activity of the CMV-ATPase from NT and HT rats. Thus, these data suggest that the CMV-ATPase ([^{3}H]ouabain binding vs. phosphatase activity) is regulated differently by fatty acids and the enzyme is selectively altered during acute hypertension. (Supported by PHS HL40485, HL 23603, AHA of MI, AHAF, ADRDA and a gift from J. Rose).

26.14

THE BLOOD-BRAIN BARRIER IN ALZHEIMER DISEASE: AN ULTRASTRUCTURAL MORPHOMETRIC STUDY. P.A. Stewart[1], K. Hayakawa[1], M.-A. Akers[2] and H.V. Vinters[2]. 1. Department of Anatomy, University of Toronto, Toronto, Canada, and 2. Department of Pathology (Neuropathology), UCLA Medical Center, Los Angeles, California.

It has been hypothesized that abnormalities of the blood-brain barrier (BBB) contribute to or reflect pathologic change in the brains of patients with Alzheimer disease, or senile dementia of Alzheimer type. To test this hypothesis, we measured ultrastructural characteristics thought to be related to microvessel permeability in brain biopsy specimens from patients with this disorder and age-matched control normal tissue. AD tissue was obtained in the course of intraventricular catheter placement for experimental pharmacologic therapy, while control material was obtained from structurally normal areas of corticectomy tissue derived from patients in the course neurosurgical procedures to treat brain tumors. The following parameters showed no difference between the two endothelial populations: vesicular density, vessel wall thickness, pericyte profile area, and total junction length. In contrast, interendothelial junctional clefts (unfused areas of the junctional complex) were significantly longer in AD brains than in controls. In addition, the number of pericytes was greater in AD capillaries than in controls, although the individual pericytes were of comparable size. These findings suggest BBB leakage in AD patients. Interestingly, the mitochondrial density (percentage of endothelial cytoplasmic volume constituted by mitochondria) was 20% lower in the AD capillaries than in controls. The numbers of mitochondria were the same in both groups, however the individual mitochondria in AD capillaries were significantly smaller. This is consistent with work by others showing alterations in capillary function in AD brains. The role that these structural changes play in AD is unclear; they may contribute to, or result from parenchymal abnormalities commonly observed in this neurodegenerative disorder. (Supported by NS26312, NCIC and MRC)

26.15

LONG TERM EFFECTS OF INTERLEUKIN-1 BETA IN THE RABBIT RETINA. J.A. Martiney, C.S. Raine and C.F. Brosnan. Albert Einstein College of Medicine, Bronx, NY 10461.

We have shown previously that intraocular injection of human recombinant interleukin-1 beta (hrIL-1b) in the New Zealand White strain of rabbits results in a reproducible set of vascular and inflammatory changes in the blood-retinal barrier. In these experiments, the effect of a single injection of hrIL-1b was followed through 48 hours post-injection (PI). We have now studied the effects of a single injection of hrIL-1b up to 5 weeks PI. Histopathological analysis showed that residual hemorrhage, which is first evident at 24 hours PI, is detected up to 3 weeks PI. Inflammation, which peaked at 24 hours PI, is decreased but still significant at 1 week PI. By 2 weeks PI inflammatory cells are barely detectable but plasma cells appear for the first time, persisting until 3 weeks PI. There is apparent gliosis at 2 weeks PI, with an increase in the thickness of the internal limiting membrane and the presence of pronounced astrocyte bridges associated with epiretinal capillaries. These changes persist until 5 weeks PI. Evidence of increased capillary density of the epiretinal vessels is also present. Quantitation of the extent of the reactive gliosis and of the neovascularization are currently under investigation. These observations show that the effects of intraocular injection of hrIL-1b are long lasting and affect the architecture of the retina. Thus altered local IL-1 production may lead to long term dysfunctions associated with acute inflammation of the optic nerve head. Supported by USPHS grant # NS11920.

26.16

INTRACAROTID INFUSION OF LEUKOTRIEN C_4 INCREASES BLOOD-BRAIN BARRIER PERMEABILITY IN ISCHEMIC BRAIN OF RATS. T. Baba, K. L. Black, K. Ikazaki and D. P. Becker. Division of Neurosurgery, U.C.L.A., Los Angeles, CA 90024.

To examine the effects of leukotrien C_4 (LTC_4) on brain ischemia, blood- brain barrier (BBB) permeability in the middle cerebral artery (MCA)-occluded rats was determined by quantitative autoradiography using ^{14}C-aminoisobutyric acid. Seventy-two hr after MCA occlusion, LTC_4 (4 µg total dose) infused into the carotid artery ipsilateral to the ischemia selectively increased the unidirectional transfer constant for permeability, Ki, approximately three-fold within core ischemic tissue and tissue adjacent to the ischemic core. No effect on permeability was seen within non-ischemic brain tissue. This finding suggests that normal brain capillaries resist the vasogenic effects of LTC_4 while LTC_4 increases permeability in capillaries of ischemic tissue. Gamma glutamyl transpeptidase (γ-GTP) activity was decreased in capillaries in the ischemic tissue 72 hours after infarction, compared to high γ-GTP in normal brain capillaries and moderate γ-GTP in capillaries in the ischemic tissue 24 hours after infarction. We speculate that γ-GTP may act as an enzymatic barrier and inactivates leukotrienes in normal brain capillaries.

26.17

CHRONIC BLOOD-BRAIN BARRIER IMPAIRMENT IN CENTRAL DEMYELINATING LESIONS REPAIRED BY SCHWANNIAN REMYELINATION. P.A.Felts,K.J.Smith,E.Tilt*. Dept. of Anat.& Neurobiol.,East. VA. Med. Sch.,Norfolk,VA, 23501

The blood-brain barrier (BBB) arises primarily from tight junctions between endothelial cells, which may be induced by the intimate presence of astrocytes. We examined BBB integrity in central demyelinating lesions which remain chronically free of astrocytes, but which become spontaneously remyelinated by Schwann cells (SC). Nine adult male SD rats received injections of ethidium bromide (1ul, 0.5mg/ml) into the dorsal column, either alone (n=5), or in conjunction with 40Gy of local beta irradiation. Segmental demyelination was initiated by 7-14 days, and SC remyelination was extensive by 16 weeks. New blood vessels were formed at the lesion. At intervals from 11 to 54 weeks the integrity of the BBB was assessed by the intravenous administration of horseradish peroxidase (HRP, 150-250mg/Kg after 5mg/Kg of Benadryl), and the rats were perfused with aldehydes 15 mins later. Adjacent vibratome sections through the lesion were processed for the presence of extravasated HRP, the distribution of astrocytes (by GFAP immunohistochemistry), and light and EM. Eight of the 9 animals showed evidence of significant HRP leakage in the region of SC repair: these regions were negative for GFAP immunoreactivity. Thus BBB integrity is chronically impaired in central demyelinated lesions repaired by Schwann cells.

26.18

The Temporal Relationship Between the Onset of Clinical Signs, Histological Changes, and Increased Permeability of the Blood-Brain Barrier in Experimental Allergic Encephalomyelytis. K.E. Schlageter and D.R. Groothuis* Evanston Hospital, Evanston, IL 60201.

The temporal relationship between the onset of clinical signs, the appearance of histological changes, and increases in permeability of the blood-brain barrier (BBB) was studied in Lewis rats with experimental allergic encephalomyelytis (EAE). Permeability of the BBB was assessed with quantitative autoradiography using ^{14}C labeled α-aminoisobutyric acid as a marker. Increases in BBB permeability in the dorsal gray matter of the lumbar spinal cord were found to precede both clinical and histological changes by 2 days. At the time when clinical and histological signs were most severe, focal areas corresponding to dense cellular infiltration were found to have increases in permeability of up to 10 times those of normal animals. Histological changes and increased permeability were still apparent 30 days after induction of EAE. A model was used to calculate the total "pore" area of the capillary surface that could account for the increased permeability and this value was related to attempts to define the morphological substrate responsible for increased permeability in EAE.

26.19

Bacterial cerebritis: mediators of acute inflammation & blood-brain barrier (BBB) changes
W. Lo, J.D. Mahan,* D. McNeely,* & C. McAllister*

We previously observed in a bacterial cerebritis that the BBB to albumin becomes permeable three days after leukocytes have migrated into the inoculated brain of the rat. We sought to determine whether soluble mediators of inflammation act in this model. We stained frozen sections of brain with FITC tagged rabbit anti-rat antibody to complement component C3, IgG, and albumin at 0.5 h, 6 hrs, and 4 days after inoculation with Staphylococcus aureus. C3 and IgG were detectable at 0.5 and 6 hours after inoculation; by 4 days C3 was not detected and IgG was only weakly present. Extravascular albumin did not appear until 4 days. Our findings indicate that C3 and IgG are deposited soon after inoculation although BBB permeability to albumin does not appear until days later. The sequence of C3 & IgG deposition, and albumin leakage is similar to acute inflammatory changes occurring in non-CNS tissues. Our findings suggest that acute inflammation mediated by C3 and IgG develops in the brain as it does in other tissues, and that in this model the brain is not immunologically privileged.
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26.20

REGIONAL CHANGES IN CNS WATER CONTENT IN LEWIS RATS WITH RECURRENT EXPERIMENTAL AUTOIMMUNE ENCEPHALOMYELITIS. Edward L. Orr and Nancy C. Stanley*. Dept. Anat. & Cell Biol., Texas College of Osteopathic Medicine, Fort Worth, TX 76107

To assess the roll of edema formation and resolution in recurrent experimental autoimmune encephalomyelitis (rEAE), we have determined the regional pattern of the water content in the CNS of Lewis rats during key periods of rEAE. rEAE was induced by inoculation of female Lewis rats with guinea pig spinal cord homogenate (gpsch) in *M. tuberculosis*-enriched complete Freund's adjuvant; control rats received an identical inoculum lacking gpsch. Beginning on day 7 postinoculation (pi), rats were monitored daily for clinical signs of EAE. Groups of control and EAE rats were killed prior to the appearance of clinical signs (day 7 pi), at the peak occurrences of clinical disease (approx. day 12.5 for Peak 1 and day 24 pi for Peak 2), and during remissions (day 17 and 31pi for Remissions 1 and 2, respectively). Wet and dry weights of the whole spinal cord, brainstem, cerebellum, thalamus, hypothalamus, and forebrain were determined, and percent water content calculated.

Significant changes in percent water were observed in the whole spinal cord, brainstem, and cerebellum during rEAE. The percent water in the spinal cords and brainstems of rats with rEAE increased and decreased in concert with clinical severity, while the cerebellum exhibited an increase and decrease in percent water at Peak 1 and Remission 1, respectively, followed by a gradual increase thereafter. These results are consistent with the possibility that edematous changes in the spinal cords and brainstems of rats with rEAE may contribute to the transient clinical symptoms of rEAE, and that elucidation of the mechanisms involved in edema formation and resolution in rEAE may reveal novel methods for modulating the course or severity of rEAE. (Supported by a grant from the National Multiple Sclerosis Society.)

26.21

THE DIFFERENCE IN VASCULAR VOLUME BETWEEN CEREBRUM AND CEREBELLUM IS IN THE PIA MATER. J.A. Holash, K.S. Sugamori*, P.A. Stewart. Dept. of Anatomy, Univ. of Toronto M6E 3C4.

Delivery of nutrients and drugs to specific brain regions depends on several factors including the local density of blood vessels. One measure of vessel density, vascular volume (Vv), is defined as the volume of the vasculature as a percentage of the total tissue volume. Previous studies using intravascular tracers have shown that the apparent vascular volume in the cerebellum is as much as 50% higher than that in the cerebrum. We questioned whether this additional vascular volume in the cerebellum could be accounted for by the vasculature of the pia mater that covers its highly infolded surface. To test this we used a morphometric technique to make two estimates of Vv in cerebrum and cerebellum; one in which only parenchymal vessels were counted and one in which both parenchymal and pial vessels were counted. We found no differences in intraparenchymal Vv between cerebrum and cerebellum. When pial vessels were included, however, the cerebral Vv increased only slightly, whereas the cerebellar vascular volume increased by more than 30%. We suggest that the higher cerebellar Vv measured by others using intravascular tracers is due to inclusion of the pial vasculature. Since pial vessels do not express blood-brain barrier characteristics as prominently as intraparenchymal vessels, we further suggest that estimates of blood-brain barrier permeability in the cerebellum should not be made using simple models developed for cerebrum. (Supported by MRC)

CYTOSKELETON, TRANSPORT AND MEMBRANE TARGETING

27.1

SYNTHESIS, AXONAL TRANSPORT AND POST-TRANSLATIONAL MODIFICATION OF SYNAPSIN I IN MOUSE RETINAL GANGLION CELLS. P. Paggi, P.Macioce°*, M. Cippitelli* and T. Petrucci°* - Dipartimento di Biologia Cellulare e dello Sviluppo, Università "La Sapienza" and °Laboratorio di Biologia Cellulare, Istituto Superiore di Sanità, Roma, Italy.

Synapsin I, a neuronal phosphoprotein associated with small synaptic vesicles and cytoskeletal elements at the presynaptic terminals, is thought to be involved in modulating neurotransmitter release. The state of phosphorylation of synapsin I in vitro, regulates its interaction with both synaptic vesicles and cytoskeletal components, including microtubules and microfilaments. To investigate in vivo, how the phosphorylation state of synapsin I regulates its association with cytoskeletal elements along the axon and at the nerve endings, we have studied the axonal transport of synapsin I in mouse optic system. 35S-methionine or 32P-ortophosphate were injected into the eye vitreous body of anesthetized adult mice. Animals were anesthetized and killed at different times after the labeling injection. Segments from the optic nerve, the optic tract, and the superior colliculus were removed. Labeled proteins were analyzed by 2-dimensional non equilibrium pH gel electrophoresis followed by SDS-PAGE; radiolabeled synapsin I bands were quantified. We observed that the majority of synapsin I is transported down the axon together with the cytomatrix proteins at a rate characteristic of the slow component b of the axonal transport, and it is phosphorylated at both the head and the tail regions. Moreover it seems that after reaching the nerve endings the transported synapsin I is dephosphorylated. These results are consistent with the hypothesis that in vivo, along the axon the phosphorylation of synapsin I prevents the formation of a dense network, which could impair the axonal transport. On the other hand, at the nerve endings the dephosphorylation of synapsin I allows the linkage of small synaptic visicles to cytoskeletal components.

27.2

PROTEIN DEPOSITION FROM RAPID ANTEROGRADE AXONAL TRANSPORT OCCURS WITHOUT DIMINUTION OF VESICLE TRAFFIC. R. S. Smith, X. Chen* and R. E. Snyder. Depts. of Anatomy and Cell Biology, and Applied Sciences in Medicine, University of Alberta, Edmonton, Canada T6G 2G3.

Deposition of protein from the moving phase of rapid anterograde axonal transport to a relatively stationary phase is a well-described but poorly understood phenomenon. We have tested the hypotheses that the deposition may be caused by: 1) loss of protein from transport vesicles, or 2) loss of vesicles from the transport system, possibly by fusion with axonal membrane sytstems. We used isolated sciatic nerves from the amphibian Xenopus laevis; where appropriate, the 8th and 9th dorsal root ganglia were retained with the preparation. Fast transported proteins were labeled with ^{35}S-methionine and the transport dynamics of a pulse of labeled protein were studied in living preparations with a position sensitive detector of ionizing radiation. Organelle traffic in isolated myelinated axons was studied using computer enhanced, differential interference-contrast, video microscopy.

Protein transport studies showed that 1.2-1.6% of the protein in a labeled pulse was deposited to a stationary phase in each millimeter of nerve (distance required for half amplitude loss = 40-60 mm). Anterograde organelle traffic was estimated, at locations 60 mm apart, as the number of organelles that crossed, in a unit time, a unit diameter of axon in the focal plane of the objective. There was no statistical evidence of any difference in organelle traffic at the two locations (proximal mean ± SEM, 8.07 ± 0.46 organelles/min/µm, n axons = 46; distal, 8.81 ± 0.51, n = 52) We conclude that protein deposition is likely to represent protein loss from moving organelles.

Supported by the AHFMR and the MRC, Canada.

27.3

EVIDENCE FOR THE RECYCLING OF AXONALLY TRANSPORTED ORGANELLES. R.E. Snyder and R.S. Smith. Depts. of Applied Sciences in Medicine, and Anatomy and Cell Biology, University of Alberta, Edmonton, Canada T6G 2G3.

Membrane-bounded organelles are presumed to transport molecules synthesized in the cell body of the neuron into its processes. These organelles, or derivatives of them, are known to be returned to the cell body following reversal of transport direction in the axon terminal and at axonal lesions. We ask the question here whether, following their return to the cell body, the organelles can serve to carry newly synthesized molecules into the axon. All studies were performed using sciatic nerve of *Xenopus laevis*. Organelle traffic in isolated myelinated axons was studied using computer enhanced, differential interference-contrast, video microscopy. Transport of molecules labeled with ^{3}H-leucine, $^{32}PO_4$ (>30% phospholipid) or ^{35}S-methionine was studied with either a position-sensitive detector or by liquid scintillation analysis.

Cycloheximide (125 µg/ml), applied to the cell bodies, was found to reduce export of ^{3}H and ^{32}P labeled molecules to <1% of their control values during a 20-h period, but organelle traffic, both anterograde and retrograde, and in the same proportion, to only 20-25%. In another experiment, >56% of anterogradely moving organelles reversed direction of transport at a distal lesion compared to <25% of ^{35}S labeled and <10% of ^{32}P labeled molecules. It is concluded that organelles may be exported from the cell body without the need of recently synthesized molecules and do not depend upon recently synthesized phospholipids for their return, consistent with the hypothesis that retrogradely transported organelles may be recycled to anterograde transport within the cell body. (This work was supported by the Medical Research Council of Canada and the AHFMR.)

27.4

THE SPATIAL-TEMPORAL PATTERN OF DELAYED AXONAL DEGENERATION IN THE C57BL/6 MOUSE SCIATIC NERVE. J.D. Glass*, D. Archer*, and J.W. Griffin

The C57BL/6/Ola mouse has previously been shown to exhibit prolonged survival of axons distal to an axotomy. This phenotype appears to be related to a deficient macrophage response. This prolonged axonal survival contrasts with the usual explosive axonal breakdown seen in mammaljan peripheral nerves. In standard C57BL/6 mice, axoplasm is reduced to granular and amorphous debris throughout the distal stump within 48 hours of nerve transection. The C57BL/6/Ola model thus offers a unique system to examine the fate of axons disconnected from their cell bodies. We examined the axons of the distal stump at intervals after axotomy. Most axons survived more than 14 days, and many for more than 21. They underwent some redistribution of axoplasm along individual internodes, forming narrowed segments containing predominantly microtubules, and varicosities containing densely packed neurofilaments. These changes appeared to reflect a local redistribution of cytoskeletal constituents. We did not find a proximal-to-distal sequence of axonal breakdown, as would be predicted if much of the axoplasm is undergoing continued slow anterograde transport in the distal stump. These data favor models of axonal organization in which much of the axonal cytoskeleton is relatively stationary, rather than undergoing somatofugal transport.

27.5

Mechanisms of Degeneration in Anucleate M-axons of Goldfish.
J. Moehlenbruck, M. Wakefield*, M.E. Smeyers*, G.D. Bittner
Dept. of Zoology, Univ. of Texas, Austin, Tx. 78712

The severed distal (anucleate) segments of giant Mauthner axons (M-axons) of goldfish remain morphologically and physiologically intact for months at 10-30°C, but ultimately degenerate after 30 days at 30°C, 60 days at 20°C, and 160 to 250 days at 10°C. Light microscopic studies suggest that anucleate M-axons degenerate in a proximal to distal (P-D) direction (Blundon et al., 1990 J. Comp. Neurol., in press). We are investigating whether this P-D degeneration results from slow transport of cytoskeletal elements within anucleate M-axons. In particular, the density and integrity of microtubules and neurofilaments have been examined in electron micrographs from intact M-axons, from anucleate M-axon segments immediately distal to the severance site, and from anucleate M-axon segments more distal to the severance site. Electron micrographs show a greater depletion of microtubules and neurofilaments in areas near (within 10-20 mm) the severance site, compared to more distal (30-40 mm) segments or to intact M-axons. We are now studying the slow transport of radiolabelled proteins in intact and severed M-axons in order to compare slow transport rates with P-D rates of degeneration. Supported by TAT grant #194 to G.D. Bittner.

27.6

FUSION OF THE AMINOTERMINAL 10 AMINO ACIDS OF GAP-43 TO BETA-GALACTOSIDASE TARGETS THE CHIMERIC PROTEIN TO NEURONAL PROCESSES.
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Dept. of Psychobiology, Univ. of California, Irvine, CA 92717 and Dana Farber Cancer Inst., Boston, MA 02115.

The neuronal protein GAP-43 is thought to be attached to the growth cone membrane via fatty acylation of the protein's only two cysteine residues (J Cell Biol 108: 613, 1989). These cysteine residues are found within a 10-amino acid domain at the amino terminus of the molecule. To determine whether this domain alone is capable of transporting a protein to the neuronal process membrane, and to develop a method for targeting molecules to the neuronal growth cone, we constructed a chimeric clone in which the first 10 amino acids of human GAP-43 were fused to beta-galactosidase (GAPlac) in a defective Herpes Simplex Virus (HSV-1) vector. PC12 cells were infected with GAPlac virus and pHSVlac virus, which expresses the unmodified beta-galactosidase. Beta-galactosidase activity was detected predominantly in the cell body of PC12 cells infected with pHSVlac virus, whereas it was detected prominantly in the cell processes as well as the soma of cells infected with GAPlac virus. These results suggest that the first 10 amino acids of GAP-43 are sufficient to direct the protein to the cell membrane, and provide a means of targeting recombinant molecules to the neuronal growth cone. Further experiments involve infection of primary hippocampal neurons with the recombinants to determine whether the chimeric protein is directed specifically to the axon.

27.7

DIFFERENTIAL SUBCELLULAR LOCALIZATION OF mRNA'S FOR NEURONAL PROTEINS IN HIPPOCAMPAL NEURONS IN CULTURE
R. Kleiman, G. A. Banker and O. Steward. Department of Neuroscience, and the Neuroscience program, Univ. of VA, Charlotesville, VA 22908.

Recent studies of in situ hybridization patterns in tissue sections suggest that neurons are able to sort mRNA molecules into different subcellular compartments. Some mRNA's are found only in the cell body while others are found in neuropil regions containing axons and dendrites (Garner et al., 1988, Nature, 336:674-677). However, in tissue sections it is very difficult to determine the actual subcellular distribution of mRNA's that may be present in neuronal processes. In the present study, we used in situ hybridization to evaluate the subcellular distribution of mRNA's in hippocampal neurons grown in low density cultures, where it is possible to visualize the entire extent of axonal and dendritic arborizations of single neurons. ^{35}S labeled riboprobes complementary to the mRNA's for GAP-43 (F1, B-50), neurofilament-68, MAP2 and beta tubulin were prepared. Autoradiographic analysis revealed that the mRNA for neurofilament-68, beta tubulin and GAP-43 were localized primarily within neuronal cell bodies. For neurofilament and tubulin, the localization was cell body specific. However, hybridization with the GAP-43 riboprobe led to labeling that extended a short distance into the proximal portions of larger dendrites. In contrast to the other probes, the mRNA for MAP2 was localized both in cell bodies and throughout the dendrites. These results demonstrate that there is a differential distribution of mRNA within neurons, suggesting a selective transport of some mRNA's into dendrites. Supported by NIH NS23094 to GB and OS. RK was the recipient of a predoctoral fellowship from NIH HD07323.

27.8

A COMPARISON OF LOW MOLECULAR WEIGHT GTP-BINDING PROTEINS IN AXONS AND SYNAPTIC TERMINALS OF SQUID NEURONS.
G. J. Chin. Lab. of Developmental Neurobiology, NIH, Bethesda, MD 20892

Recent work in mammalian cells and in yeast suggests that low molecular weight GTP-binding proteins may be involved in the traffic of vesicles among cellular compartments, such as occurs in the constitutive exocytosis of proteins and in the transport of ligands taken up by receptor-mediated endocytosis. As a first step in studying whether this family of proteins might also function in regulated exocytosis in neurons, GTP overlays were used to identify and characterize these proteins in two spatially segregated regions, the axon and the synaptic terminal. Squid giant axon contained five GTP-binding proteins with molecular weights of 19-28 kDa; all were localized mainly to soluble and particulate fractions of extruded axoplasm. Squid optic lobes contained a similar set of GTP-binding proteins; some of these were enriched in a partially purified synaptic vesicle fraction, as assayed by synaptobrevin immunoreactivity. Like axons, optic lobe synaptosomes contained both soluble and particulate GTP-binding proteins.

27.9

ANTI THY-1 IgG UNDERGOES TRANSCYTOSIS FOLLOWING RETROGRADE AXONAL TRANSPORT.
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Dept. Neurology and Marine Biomed. Inst., U.T.M.B., Galveston, TX 77550-2778.

There has been increasing interest in the possible involvement of antineuronal IgG in the pathogenesis of motor neuron disease. Since other exogenous proteins have been noted to undergo transcytosis following retrograde axonal transport to neurons, we investigated an anti-Thy-1.2 monoclonal IgG (30-H12, American Type Culture Collection) for this phenomenon at the ultrastructural level.

Purified 30-H12 IgG was biotinylated using sulfosuccinimidyl 6-(biotinamido) hexanoate and injected into the mystatial vibrissae of anesthetized mice (N = 12). Following survival intervals of 12, 24, and 48 hours, the mice were deeply anesthetized with Halothane inhalation and perfused with 3% glutaraldehyde in cacodylate buffer. Vibratome sections of the medullae were stained for the presence of biotin using streptavidin-peroxidase and then prepared for ultramicrotomy. Observations at the ultrastructural and light level revealed that biotinylated IgG moved quickly though neurons following retrograde axonal transport to become localized diffusely in the neuropil.

These results support the hypothesis that antineuronal IgG is capable of neuronal transcytosis. This pathway may bring antineuronal IgG into contact with motor neurons despite the presence of the blood brain barrier.

Supported by NIH 11255.

27.10

A SYNTHETIC LEADER PEPTIDE FOR RAT PRE-ORNITHINE TRANSCARBAMYLASE (pOTC) INHIBITS THE MITOCHONDRIAL TARGETING OF HUMAN MONOAMINE OXIDASE (MAO).
C. Titlow, J. Hendrick*, W. Weyler*, and X.O. Breakefield. Prog. in Neuroscience, Harvard Med. Sch., Boston, MA 02115 and Mol. Neurogenetics Unit, Mass. General Hosp., Charlestown, MA 02129.

MAO is the primary enzyme involved in degradation of neurotransmitters such as dopamine, norepinephrine, and serotonin. MAO exists as A and B forms which can be distinguished by genetic, biochemical, and immunological criteria. Both forms are located in the mitochondrial outer membrane. We have placed a 2 kb cDNA clone containing full-length coding sequences for human MAO-A into a Bluescript vector to allow transcription of sense message under control of a T7 promoter. This RNA generates a full size MAO-A protein when translated in vitro in a rabbit reticulocyte lysate system using ^{35}S-methionine to label the polypeptide. We have examined the binding of ^{35}S-MAO-A to freshly isolated rat liver mitochondria. Binding of ^{35}S-MAO-A is blocked by a synthetic peptide corresponding to the N-terminal, targeting signal of the precursor protein, pOTC, for the mitochondrial matrix protein, OTC. Thus MAO-A appears to share the same recognition site on mitochondria as other proteins targeted to this organelle.

Supported by NIH grant NS21921.

27.11

EXPRESSION OF THE NEURONAL INTERMEDIATE FILAMENT PROTEIN (IFP) PERIPHERIN IN THE RAT CNS AND OLFACTORY NEURONS. J.D.Gorham, H.Baker*, and E.B.Ziff Howard Hughes Med. Inst. and Dept. of Biochem., New York Univ. Med. Ctr., NY, NY 10016 and +Lab. of Mol. Neurobiol., The Burke Rehabilitation Ctr., Cornell Univ. Med. Coll., White Plains, NY 10605.

Peripherin is a type III IFP expressed by all PNS neurons, and by somatic and visceral motoneurons. Using peroxidase immunohistochemistry, peripherin expression was demonstrated in a subset of rat CNS neurons, including the cranial nerves, as well as isolated cortical neurons. The cortex also showed rather extensive networks of small-diameter peripherin-containing fibers, running both parallel and perpendicular to the pial surface. The olfactory bulb (OB) showed a similar network of peripherin-containing fibers. Interspersed among these OB fiber tracts were occasional peripherin-containing cell bodies, which morphologically resembled neurons, not glia. These results indicate that peripherin expression in the brain is considerably more widespread than previously appreciated.

The IFP expression pattern of olfactory receptor neurons (ORNs) was examined. ORNs did not stain for neurofilament light chain, but did react with antisera to vimentin or peripherin. Vimentin immunoreactivity was seen in cell bodies, axons, and some, but not all, termini within OB glomeruli. In contrast, peripherin expression was seen only in axons and not in cell bodies or termini. Thus, ORNs co-express two type III IFPs, but distribute them within the neuron in an overlapping but non-identical pattern. This differential distribution implies that peripherin and vimentin perform different functions in ORNs. The unusual IFP isotype combination expressed by ORNs may have implications for their regenerative potential.

27.12

Microtubule Polymerization Equilibria and Membrane Depolarization in Rat Sympathetic Neurons. C. H. Keith, Department of Zoology, University of Georgia.

We have developed methods that allow us to measure the polymer/total tubulin ratio locally in the neurites of cells in culture. We inject cells with a fluorescent analog of tubulin and allow it to equilibrate into microtubules throughout the neuron. We then digitize the fluorescence of this cell on a 512 X 512 raster, and store the digitized image. We then extract the cell in a Triton X100-containing microtubule-stabilizing buffer, and re-record the fluorescence. By aligning the two images and integrating blockwise out the neurite, we can define a ratio of intensities out the neurite in the extracted image (microtubules) to intensities in the unextracted image (total tubulin)

We have been applying this methodology to measure microtubule/tubulin ratios in rat sympathetic neurons that have been exposed to a medium containing 50 mM K^+-HEPES. We find that in control, undepolarized cells, measured microtubule/tubulin ratios are of the order of .8-1 throughout the neurite. However, after exposure to K^+-HEPES for one hour, microtubule/tubulin ratios drop to 0.50 or less, with a tendency for the growth cone ratio to be lower than than near the cell body. This drop in microtubule / tubulin ratio is probably due to a rise in $Ca^{++}{}_i$, caused by the opening of plasma membrane voltage-dependent calcium channels on chronic depolarization.

Supported by NIH grant NS25101.

27.13

NEUROFILAMENTS AT THE NODE OF RANVIER ARE DIFFERENT FROM INTERNODAL NEUROFILAMENTS. M.Mata, N.Kupina* and D.J. Fink. VAMC and U.Michigan, Ann Arbor, MI 48104.

In order to study the organization of the cytoskeleton along the length of the peripheral nerve fiber, we performed post-embedding electron microscopic immunocytochemistry with colloidal gold using antibodies specific for different neurofilament (NF) epitopes and for microtubules (MT).

Monoclonal antibodies specific for the 160 kD and 200 kD NF subunits (Boehringer-Mannheim) and for phosphorylated epitopes on the 160 and 200 kD NF subunits (SMI-31, Sternberger-Meyer) showed a reduction in the density of immunostaining at the node of Ranvier. An affinity purified polyclonal antibody to microtubules (BTI) showed essentially constant density of gold particles along the axon, with a slight increase in the region of axoplasm underlying the node of Ranvier, as did an antibody specific for the 68kD NF subunit (Boehringer-Mannheim).

Because previous data has shown that the density of NF along myelinated axons is constant, these results suggest that the organized NF cytoskeleton at the node of Ranvier is different from the internodal cytoskeleton. It is possible that post-translational modification of NF epitopes may occur at the node of Ranvier.

27.14

EFFECT OF PHOSPHORYLATION AND DEPHOSPHORYLATION ON GFAP FILAMENT ASSEMBLY. Y.Nakamura*, M.Takeda*, K.J.Angelides*, T.Tanaka*, K.Tada*, E.Senba, S.Hariguchi*, T.Nishimura*. Dept.of Neuropsychiatry Osaka Univ.Med.Sch., Osaka, 553.

The dynamic nature of GFAP as well as neurofilament core protein can be studied by measuring fluorescence energy transfer. Fluorescence-labeled GFAP was shown to be assembled into 10 nm filaments. The fluorometric assay was designed to measure the degree of assembly of fluorescence-labeled GFAP. Among factors modulating GFAP filament assembly, phosphorylation seems to be important. Phosphorylation of soluble GFAP by cAMP dependent protein kinase inhibited filament assembly in proportion to the phosphorylation level, and the assembled GFAP filament was disassembled by phosphorylation. Dephosphorylation of GFAP by unweaned calf intestinal ALP increased the critical concentration of assembly, and vanished its Mg^{2+} sensitivity. Dephosphorylated GFAP formed thicker filaments (Ø:15-20 nm). The possible sites, which were phosphorylated or dephosphorylated, were likely to locate in the HEAD region. HEAD region of GFAP is essential for the modulation of filament assembly.

27.15

ORIGINS OF ACTIN AND TUBULIN IN ANUCLEATE CRAYFISH MEDIAL GIANT AXONS. R.A. Sheller, M.E. Smyers* and G.D. Bittner. Dept. of Zoology, University of Texas, Austin, TX 78712.

In the crayfish, *Procambarus clarkii,* the distal stumps of severed medial giant axons (anucleate MGAs) exhibit long term survival (LTS) for 50-200 days post severence (G.D. Bittner, Amer. Zool. 28:1165, 1989). LTS of MGAs, previously characterized morphologically and electrophysiologically, was analyzed biochemically in this study. Axoplasmic proteins were collected by perfusing MGAs with an internal saline, electrophoresed on SDS-PAGE gels, and silver stained. The resulting silver stained proteins and the immunological detection of actin and tubulin on Western transfers were the same for anucleate MGAs severed for minutes or months.

If such axoplasmic proteins in anucleate MGAs originated in the soma and were transported to the axon prior to severence, these proteins were not degraded for at least 6 months in the anucleate axon. Alternatively, these axoplasmic proteins could have been synthesized in adaxonal glia and subsequently transferred to anucleate MGAs. Data supporting the latter hypothesis were obtained when 35S-methionine was placed in the bath surrounding an anucleate MGA and radiolabelled proteins were detected in perfused axoplasm. Radiolabelled proteins in the perfused axoplasm (analyzed by SDS-PAGE and fluorography) were very similar to radiolabelled proteins in the glial sheath.

Newly synthesized proteins in MGA axoplasm might replenish some, but not all, existing axoplasmic proteins. Our data suggest that the actin of anucleate MGAs may be synthesized by glia and then transferred to the axons, whereas tubulin apparently originates from some source other than protein synthesis in glia. Supported by TAT Grant #194 and NSF grant # ECS 8915178.

28.1

PSEUDORABIES VIRUS: A HIGHLY SPECIFIC TRANSNEURONAL CELL BODY MARKER IN THE THE SYMPATHETIC NERVOUS SYSTEM. A.M. Strack and A.D. Loewy. Dept. of Anatomy and Neurobiology, Washington University, St. Louis, MO 63110

The present report presents a series of experiments in rats using Bartha's K strain of pseudorabies virus (PRV) that demonstrate specificity of retrograde transneuronal viral transport in the sympathetic nervous system. Three experiments were performed. First, an injection of PRV was made in the anterior chamber of the eye, followed by a second injection of wheat germ agglutinin-horseradish peroxidase (WGA-HRP). PRV infected neurons in the superior cervical ganglion (SCG) always contained WGA-HRP. This dual labeling of SCG neurons suggests that the PRV cell body labeling in the SCG remains specific. Second, after PRV injections into the pinna or eye, a specific segmental distribution of the transneuronal cell body labeling occurred in the intermediolateral cell column (IML) and related spinal areas. After eye injections, the majority of transneuronally labeled neurons were in the T1-T3 segments. After pinna injections, the majority of labeled cells were in the T2-T5 segments. Since the SCG cells innervating these two end-organs lie in close proximity to each other within the ganglion, these results suggest that a specific transfer of the virus occurred, possibly via a trans-synaptic mechanism. Third, virally infected glial cells were rarely found in the SCG and spinal cord using a dual immunohistochemical staining procedure with antibodies against glial fibrillary acidic protein or galactocerebroside and PRV was used. In summary, Bartha's K strain of PRV can be used as a specific transneuronal retrograde marker in the sympathetic nervous system.

28.2

SYNAPTIC UPTAKE AND TRANSPORT OF RECOMBINANT TETANUS TOXIN FRAGMENT C (rTTC) IN THE RAT VISUAL SYSTEM
T.J.Cavanagh, D.S.Grega, G.Bowker* and F.Dwulet*. Indiana University School of Medicine, Program in Medical Neurobiology and Boehringer Mannheim Biochemicals R&D, Indianapolis, IN 46250

Tetanus toxin is rapidly taken up and transported trans-synaptically within the nervous system, making it an ideal marker for tracing neuronal circuits. The health hazard assumed by working with this molecule has been somewhat ameliorated by the use of an enzymatically derived atoxic fragment (fragment C) of the native tetanus toxin (TTC). However, enzymatically digested native toxin may present two problems: 1) It may not be a homogenous preparation, and 2) A percentage of the impurities may comprise intact (undigested) toxin. Neither of these problems is encountered with recombinant tetanus toxin C fragment. The rTTC was 93% pure as assessed by gel filtration on a TSK G3000SW column. Elution time was consistent with a MW of approximately 50,000. We present evidence that CNS uptake and transportation of rTTC appears identical to that seen with enzymatically-derived TTC. As described for TTC, intraocularly-injected rTTC is taken up and transported anterogradely by retinal ganglion cells to retinorecipient areas, then taken up and retrogradely transported to neurons which innervate retinorecipient areas.

28.3

INVESTIGATION OF DISYNAPTIC PATHWAYS IN A DOUBLE-LABELING PARADIGM. K. Bruce* and I. Grofova. Dept. of Anatomy, Michigan State University, E. Lansing, MI 48824.

Major problem in the investigation of the synaptic arrangement of nerve terminals derived from a specific source with neurons projecting to anatomically defined targets has been the lack of tracers that can be used for both, light and electron microscopic studies, and are compatible as to the rate of transport as well as fixation procedures. In efforts to determine the termination of efferent fibers from the pars reticulata of the substantia nigra (SNR) in the rat on neurons projecting to the pontomedullary reticular formation and/or the cervical cord, we have developed a double-labeling technique that combines retrograde transport of cholera toxin B (CTB) with anterograde transport of Phaseolus vulgaris-leucoagglutinin (PHA-L). Both anterogradely PHA-L labeled fibers and terminals, and retrogradely CTB labeled somata and primary/secondary dendrites can be visualized in the same section using a sequential immunostaining protocol yielding different colored reaction product. Although the color difference is lost following postfixation in osmium, the two tracers can be readily recognized in ultrathin sections. The CTB reaction product is generally more discrete, and it is associated mostly with the Golgi apparatus in the cell somata, and with the microtubuli in dendrites. On the other hand, the PHA-L yields a very dense reaction product that fills the matrix of nerve terminals and appears only slightly lighter in unmyelinated and myelinated fibers.

Due to its reliability, relative technical simplicity and a high degree of selectivity, this approach is considered an appropriate tool to analyze disynaptic pathways. (Supported by N.I.H. grant NS25744).

28.4

FLUOROGOLD TRACING AFTER CHEMITRODE INJECTIONS OF DORSAL RAPHE: AFFERENTS SUPPORTING BRAIN STIMULATION REWARD. M. P. Vachon, W. Staines and E.Miliaressis. Lab. of Neurophysiology and Department of Anatomy, University of Ottawa, Ottawa, Canada K1N 6N5.

The dorsal raphe (DR) supports a robust level of brain stimulation reward (BSR). We have oberved that some afferents to the dorsal raphe either support BSR themselves or modulate the efficacy of dorsal raphe BSR. As a prelude to a more detailed study of the effects of DR afferents on BSR elicited from this nucleus we have studied the distribution of neurons labelled by retrograde transport of Fluorogold injected into the DR using a novel, behaviorally-controlled technique. Injections of .02 to 0.1 µL of Fluorogold (4% solution) were made via a chemitrode implanted in DR areas highly supportive of BSR. Despite the small target size, behavioral evaluation allowed for very accurate injection placements. Brains were processed after 2 to 3 days survival time and fluorescence microscopy revealed densely retrogradely labeled neurons in various diencephalic, mesencephalic and myelencephalic areas of the brain. Specific nuclei containing labelled neurons included the diagonal band of Broca, septum, interpeduncular nucleus, lateral preoptic area, lateral habenula, ventral tegmental area, other raphe nuclei, parabrachial nucleus and the prepositus hypoglossal nucleus. These results are in accordance with already known DR afferents and highlight structures that could influence the DR BSR system.

28.5

EXTRACELLULAR LABELING OF UNMYELINATED PRIMARY AFFERENTS AFTER INJECTIONS OF WGA-HRP IN DORSAL ROOT GANGLIA AND PERIPHERAL NERVES. D.J. Tracey, R.J. Weinberg and A. Rustioni. Dept. of Cell Biology and Anatomy, University of North Carolina, Chapel Hill, NC 27599.

In anesthetized rats, 0.25 to 0.5 µl of 2% WGA-HRP were injected into L5 ganglia. Spinal cord sections and dorsal roots were reacted for HRP, using TMB with tungstate stabilization. Label in the spinal cord after 8 h was concentrated in the substantia gelatinosa, where unmyelinated primary afferents terminate. EM observations of substantia gelatinosa revealed labeled dendrites and glia as well as axons and terminal boutons. Label was most prominent, however, in the extracellular space surrounding presumed terminals of unmyelinated fibers. Tracer was also concentrated in superficial laminae after injections in peripheral nerves, although the extracellular labeling was not as prominent. EM observations on dorsal roots after ganglion injections revealed that the tracer is inside unmyelinated and myelinated fibers and in Schwann cells. The evidence suggests that WGA-HRP reaches the spinal cord by fast axoplasmic transport and is then rapidly released at terminals and possibly along unmyelinated fibers. WGA-HRP might also be transported along a chain of Schwann cells, and then extruded into the extracellular space. Binding of released WGA-HRP to axolemmal sialoglycoproteins of unmyelinated fibers may contribute to apparent greater affinity of the tracer for these fibers.

28.6

COMBINED TRACING OF KITTEN RETINOGENICULATE AXONS *IN VITRO* AND LABELLING OF LATERAL GENICULATE NEURONS IN FIXED SLICES. L-A. Coleman and M.J. Friedlander. Neurobiology Research Center, University of Alabama at Birmingham, Birmingham, Al 35294.

As part of our ongoing studies of retinogeniculate development in the cat, we have developed a technique for combining anterograde tracing of retinogeniculate axons and intracellular labelling of geniculate neurons in the target area. In deeply anesthetized, postnatal kittens between one and six weeks of age, the region of the brain containing the LGNd was removed, immersed in ACSF and maintained in a tissue chamber for up to 6hrs to allow for anterograde transport of HRP-Texas Red (Mol. Probes) placed in the optic tract. Following immersion fixation with 4% paraformaldehyde and 0.1% glutaraldehyde the LGNd was sectioned at 400µm on a Vibratome. Selected cells were injected intracellularly under visual control with a glass micropipette containing 1% Lucifer Yellow (Mol. Probes) and 5% Biocytin in Tris/HCl buffer (pH 7.6). Injection of Biocytin enables cells to be permanently labelled by reacting sections with the ABC complex (Vector), followed by heavy metal intensified diaminobenzidine processing. Axons containing HRP were simultaneously revealed by this processing. Our preliminary results show that retinogeniculate axons and their LGNd target cells can be successfully labelled using this technique. We are currently investigating whether apparent contacts at the light level can be confirmed as sites of synaptic contact at the ultrastructural level. To date, the axonal arborizations of 5 retinogeniculate axons and dendritic morphology of 10 LGNd neurons have been studied. Similar experiments are currently being performed using adult cats. This technique offers the potential to evaluate synaptic convergence in young animals where the use of the anesthetized *in vivo* preparation is problematic.

Supported by NSF Grant BNS8720069

28.7

TRANSNEURONAL DIFFUSION OF DiI IN THE VISUAL SYSTEM OF THE HORSESHOE CRAB, *LIMULUS*. [1]I. Kelly Johnson, [2]Robert N. Finks*, and [1,2]Steven C. Chamberlain. [1]Institute for Sensory Research and [2]Department of Bioengineering, Syracuse University, Syracuse, NY 13244.

Crystals of DiI (1,1'-dioctadecyl-3,3,3',3'-tetramethylindocarbocyanine perchlorate, Molecular Probes) were applied to the cut ends of the lateral optic nerve of dissected brains fixed and stored in 4% paraformaldehyde in 0.1 M phosphate buffer (pH 7.2). Tissue was maintained in fixative in darkness at room temperature or at 37°C for 1-4 months. Serial 100-µm sections were cut with a vibratome, collected on subbed slides, and coverslipped with Gelmount.

Dense fluorescence was noted in the trunk of the lateral optic nerve, the laminar and medullar neuropils, the optic tract and adjacent central body neuropil, and the ocellar ganglion. These are all sites of optic nerve fiber terminations previously demonstrated with cobalt chloride and Lucifer yellow and were labelled by direct dye diffusion.

Additional structures were clearly transneuronally labelled. Many or all laminar somata were stained. Somata in the medullar group and dorsal medial group were stained. However, some of these somata must belong to circadian retinal efferents (Invest. Ophthalmol. Vis. Sci., 1990, 31(4):286). A few fibers in the median and ventral optic nerves were lightly stained near their central terminations. Two tracts of fibers from medullar neurons, one crossing the midline in the central body neuropil and one running from the posterior medullar neuropil toward the circumesophageal, were stained.

The mechanism underlying this transneuronal staining is unclear. Although these various structures could be interconnected via gap junctions, it seems equally possible that direct diffusion might occur across small distances including synaptic clefts. In conclusion, transneuronal diffusion occurs in, and is apparently restricted to, the visual system in the brain of *Limulus*.

Supported by NIH grants EY06064 and EY03446.

28.8

STAINING COMPLETE AXON ARBORS IN PRE-FIXED MAMMALIAN NERVOUS TISSUE: OPTIC FIBERS IN RATS AND MARSUPIAL EASTERN QUOLLS (DASYURUS VIVERRINUS). M. Scheel* and W. L. Weller, Univ. of Tasmania, Hobart, Tasmania 7001, Australia.

To be able to study retinogeniculate endings in quolls, we have adapted, for use with mammals, Kageyama and Meyer's method (J Histochem Cytochem 35:1127) of filling pre-fixed neurons and their processes with horseradish peroxidase (HRP) through a severed optic nerve.

Following standard EM fixation by vascular perfusion of mixed aldehydes (1% paraformaldehyde and 2.5% glutaraldehyde) in 0.1M phosphate buffer (PB), pH 7.4, at 4°C, brains are removed and the lateral geniculate bodies (LGBs) are dissected, with optic nerves and tracts attached. Tissues are immersed in a 30% sucrose-PB solution at 4°C until they sink, then washed in PB. A 7mm length of plastic tubing is fitted over an optic nerve's stump and filled with 15µL of 30% HRP in water. Through this solution the nerve's cut end is punctured once with a fine pin. The blocks of tissue, with tubes attached, are stored at 4°C for 11 days.

Light microscopy of HRP demonstrated in Vibratome sections with the metal-intensified DAB protocol revealed apparently complete, dense filling of optic fibers in both rats and quolls. The pattern we saw in the dorsal LGB of rats conformed to the pattern seen after in vivo HRP injections into the optic tract (Brauer et al., Exp Brain Res 69:481). Using 2% dimethylsulfoxide as the solvent, instead of water alone, produced weaker staining of axons and staining of LGB glia and neurons. Ultrastructurally we found electron-dense HRP reaction product in the LGB in association with myelin sheaths (around and within the myelin) and, in axons, along outer mitochondrial membranes and neurofilaments, and at retinogeniculate synapses.

Our results indicate that uptake, movement and deposition of HRP in pre-fixed tissues reveal the same structural relationships and level of detail as they do in living tissues. Therefore we are encouraged to apply this method to studying the detailed pattern and synaptology of retinogeniculate terminals in quolls.

Supported by the BMBW (F. R. G.) and the NH&MRC (Australia).

28.9

SELECTIVE "ARGENTAFFIN" STAINING OF NERVE FIBERS AFTER MERCURIC ACETATE POSTFIXATION. A.Pellegrino de Iraldi and C.J. Tandler*. Instituto de Biología Celular, Facultad de Medicina, Paraguay 2155, 1121 Buenos Aires, República Argentina.

A novel type of argentaffin histochemical technique (Hg-Ag)(Tandler, Com.Biol.,7,1-8) based on the reduction of diammine silver nitrate by mercuric acetate postfixed tissues has been shown to stain selectively proteins localized within the lateral elements of triads/diads in striated muscle cells (Tandler and Pellegrino de Iraldi, Histochemistry,92,15-22). In this work the Hg-Ag technique was applied to glutaraldehyde-fixed gray and white matter of the brain, cerebellum and spinal cord, retina, sciatic nerve and sympathetic fibers of the rat. Strongly reactive sites appeared related to nerve fibers. Silver grains were localized and unevenly distributed inside the axons. The amount of reactive material was found to be variable within the same or the different tissues studied. In longitudinal sections linear tracks of silver grains were evidenced, strongly suggesting the staining of cytoskeleton structures such as neurofilaments and/or neurotubules and/or their associated proteins. This conclusion is strenghthened by the finding that pretreatment (15 min) of fresh tissues with 1% Triton X-100 did not extract the silver reducing axonal components.

Work supported by grants from CONICET and UBA,R.Argentina.

28.10

Activity-dependent fluorescent staining of living rat motor nerve terminals with RH dyes

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Exposure of rat 4th deep lumbrical (4DL) muscle for 60 min to the fluorophores RH414 or RH795 (170 uM; Molecular Probes, Inc.) stained motor nerve terminals weakly. However, terminals stained strongly after 15 min exposure to 44 uM RH dye if the muscle nerve was electrically stimulated, or after only 5 min exposure to 60 mM [K] in the presence of dye. The dyes were imaged best with the Leitz H3 cube (excite 420-490 nm; emit >510 nm).

The 4DL muscle receives innervation from two nerves. Electrical stimulation of only the smaller (sural) nerve caused only a few end plates to be stained. Subsequent electrical stimulation of the other nerve (or exposure of the muscle to high [K]) caused many more end plates to become visible. Replacing Ca with Mg and adding 4 mM EGTA during dye exposure did not block staining. Denervation of the muscle produced a loss of end plate staining with high [K] loading within 1-2 days. These data were obtained with adult rats; neonatal preparations gave similar results.

Some structures were stained in an activity-independent fashion. These included myelinated axons and sensory nerve endings (especially IA endings in muscle spindles, but also free sensory endings in tendons).

STAINING, TRACING AND IMAGING TECHNIQUES II

29.1

AUTOMATED DELINEATION OF NEUROANATOMICAL STRUCTURES. J. Nissanov, A. Waks*, D.L. McEachron, C.R. Gallistel, O.J. Tretiak*. Image Processing Center, Drexel University, Philadelphia, PA 19104 & Dept. of Psychology, University of California, Los Angeles, CA 90024

A major source of inaccuracy in the analysis of autoradiograms is trial-to-trial and inter-individual variability in demarcation of anatomical boundaries from the corresponding histology. To reduce this error, we have designed an algorithm which reproducibly locates the boundary of neuroanatomical structures on digitized images of histological sections. This rule-based expert system selects the optimal border for the structure of interest from the set of all smooth boundaries in the vicinity of the expected border location. The boundary selected is that which overall traverses across the steepest grey-scale gradient. The expected boundary location in the vicinity of which the search area is confined is derived from a manually delineated computerized atlas or from the boundary located in one section and projected to subsequent sections in a series of consecutive sections (Supported by NIH Grant # 2 P41 RR01638)

29.2

CAJAL MEETS FRACTALS: FRACTAL ANALYSIS OF CAJAL'S NEURONAL IMAGES J. Clay Goodman. Department of Pathology (Neuropathology), Baylor College of Medicine, Houston, Tx 77030

Fractal geometry is the study of objects possessing non-integer dimensionality. Such entities are known as fractals and they occur frequently in nature including such things as trees, clouds, snowflakes, and neurons. They are characterized by visual complexity and self-similarity. A fractal of dimensionality 1.0-2.0 has geometry lying in between that of a Euclidean line and plane whereas a fractal of dimension 2.0-3.0 lies between a plane and a solid. Fractal dimensionality then is a measure of a fractal's ability to fill space.

The fractal dimensions of Cajal's neuronal images were measured using Kaye's structured walk technique to construct Richardson plots. Images were photocopied onto paper bearing grids of varying sizes, and the number of grids through which the perimeter of the neuronal image passed counted. The log of the perimeter was plotted against the log of the grid size. The slope of the line formed by connecting the log perimeter points corresponds to the fractal dimension of the image. The fractal dimensions of Cajal's images correspond to the visual complexity of the neurons. For example, Cajal's studies of pyramidal neurons in various vertebrates revealed increasing complexity with phylogeny. The fractal dimensions of these neurons climb steadily with phylogeny and complexity.

Fractal analysis can be performed manually or can be computer assisted, and can be applied to previously acquired images. Fractal analysis provides a numerical method of expressing image complexity permitting comparision between objects. The technique has limitations in that it does not express asymmetry or scale so that fractals of different sizes and shapes may have the same fractal dimension.

29.3

AUTOMATED SYSTEMATIC-RANDOM THREE-DIMENSIONAL SAMPLING IN STEREOLOGICAL STUDIES OF GEOMETRICALLY COMPLEX COMPARTMENTS L.M.Bookman*, L.T.Malmgren,Dept. of Otolaryngology and Communication Sciences,Head and Neck Morphology Lab.,SUNY Health Science Center, Syracuse,N.Y.13210

Stereological techniques rely on geometrical probability to provide statistically unbiased, precise and highly efficient estimates of 3-dimensional morphological parameters (eg. volume, surface, number, length, curvature). The use of "optical sections" (Acta Path Mic Immun Scand 96:857) and systematic random sampling greatly increases the efficiency and versatility of these techniques. However, the "optical section" technique generally requires the use of high power, high numerical aperture, oil immersion objectives to achieve a narrow depth of field (eg. 0.15 microns for Nikon 100X Plan Apo). It can be difficult to accurately confine sampling within the limits of the compartment of interest due to the loss of overall perspective at high magnification. In addition, the efficiency of systematic random sampling relies on the use of a randomly distributed, but regularly spaced lattice of sampling fields along the X,Y and Z axes, which is not practical to do manually. In order to further improve the efficiency and versatility of stereological techniques based on the use of "optical sections", a system has been developed which uses robotic control of X,Y,Z movements of the microscope stage to achieve a systematic random sampling field distribution within compartment borders of any shape traced by the user at low magnification via an optically-linked illuminated cursor. This system also greatly facilitates stratified, systematic random stereological sampling since sample fields can be robotically positioned within geometrical constructs with respect to reference structures (eg. a specified depth from a traced surface of any shape with sampling intervals at regular, but randomly positioned intervals along the reference surface). This equipment and software provides a powerful and general purpose tool for efficient, quantitative morphological analysis in a wide variety of applications.

29.4

COMPUTED IMAGE ALIGNMENT FOR OBJECTIVE 3D RECONSTRUCTIONS FROM ELECTRON MICROSCOPY. L.S. Hibbard, B.J. Dovey-Hartman*, and R.B. Page. Washington University School of Medicine, St. Louis, MO; UMDNJ/The Robert Wood Johnson Medical School, Piscataway, NJ; and The Pennsylvania State University College of Medicine, Hershey, PA.

Successful reconstruction requires accurate alignment of serial section images. In principle, alignments can be obtained from cross-correlation (CC) of image features (Frank, et al., Meth. Enzymol., 164:1, 1988) but in practice, correlation may be confounded by abrupt changes in the sizes and shapes of features, and by noise and sectioning artifacts. We have devised empirical solutions involving correlation of thresholded edges of variable weights obtained over variable-sized supports. For a more robust solution, and we are examining the effects of various image filters on the structure of the CC function peak density, since it is from that peak's location that the rotation and translation values are derived. Fourier measures of image similarity suggest (Van Heel, Ultramicroscopy, 21:95, 1987), for example, that for disparate features, alignment may be achieved by iterating over low-pass filtered images with increasing cutoff radii to obtain a least squares best fit.

These methods are applied in 3D reconstructions of the median eminence (ME) of the hypothalamus in support of studies of neurohypophyseal blood flow.
(Support: NIH NS15926)

29.5

METHODOLOGY FOR PLASTIC EMBEDDING OF WHOLE BRAIN SPECIMENS AND EVALUATION OF ADJACENT SECTIONS BY ROUTINE HISTOLOGIC STAINS AND BY ELECTRON MICROSCOPY. **D.L.Feeback,* R.A. Brumback, R.W.Leech, J.L.Ketring* Departments of Anatomical Sciences and Pathology, University of Oklahoma College of Medicine and Veterans Affairs Medical Center, Oklahoma City, OK 73104.**

We have previously reported a technique for embedding whole brain specimens using methyl methacrylate, a low viscosity xylene-soluble ester that readily infiltrates large tissue blocks (Brumback, et al., Ann. Neurol., 26:301, 1989). After polymerization, the block can be cut using a tungsten carbide knife on a sledge microtome. Sections picked up onto slides and dried can be stained (after xylene immersion to remove the plastic) with modifications of most routine paraffin staining techniques. We have now found that for detailed analysis, particular areas of the block can be cut out using a fine-toothed saw and sectioned using a glass knife on a rotary microtome. Semithin sections can be stained with toluidine blue. Thin sections of the same area prepared on an ultramicrotome, picked up onto Formvar or carbon-coated grids, and stained with uranyl acetate and lead citrate can be viewed with an electron microscope. Thus, this technique permits whole brain embedding, serial sectioning and staining of the whole block to identify specific areas of interest, and subsequent detailed evaluation of those areas in adjacent sections utilizing routine stains at the light microscopic level as well as with electron microscopy.

29.6

REAL TIME MULTI-WAVELENGTH FLUORESCENCE IMAGING OF LIVING CELLS. Stephen J. Morris, Division of Molecular Biology and Biochemistry, School of Basic Life Sciences, University of Missouri-Kansas City, Kansas City MO, 64110-2499.

A new fluorescence video microscope design for simultaneous, real-time capture of intensified images of cells containing dual wavelength "ratio" dyes or multiple dyes at video frame rates (30 frames/sec or faster) has been described (BioTechniques **8**:296-308 (1990)). Each emission wavelength is imaged by one of two cameras, then digitized, background corrected and combined on a single frame to be stored at high resolution on video tape or digital disk for further off-line analysis. Image intensifiers and CCD cameras produce stable, high contrast images at ultra-low light levels. The design has no moving parts, which overcomes a number of technical difficulties in designs using rotating filter wheels for multiple imaging. Coupled to compatible image processing software, the new design can be built for a significantly lower cost than presently available commercial systems. For ratio imaging, the software can calculate the ratio of the fluorescence intensities pixel by pixel at 30 ratios/sec, and generate false-color maps of ion concentrations as well as other calculations.

Examples are presented for the kinetics of rapidly changing intracellular calcium detected by the calcium indicator probe indo-1 and emission from multiple vital dyes placed in cells undergoing cell fusion.

29.7

OBSERVATIONS OF NODE OF RANVIER STRUCTURE DURING SUSTAINED IMPULSE ACTIVITY IN SINGLE FROG AXONS. Stephen A. Raymond, Scott C. Steffensen, Mark H. Ellisman. Department of Neurosciences, Laboratory for Neurocytology, University of California San Diego, School of Medicine, La Jolla, California 92093.

Repetitive action potential propagation has been associated with ultrastructural alterations in peripheral nodes of Ranvier (Wurtz & Ellisman, J. Neurosci. 6:3133-3143, 1986). In these earlier experiments it was not possible to observe the structural changes directly and concurrently with the physiological events produced in the fiber during repetitive activation. Here we introduce a recording system and preparation that allows the observation of changes in structure in the nodal complex during electrophysiological monitoring of activity dependent changes in conduction properties of individual axons. The system resolves latencies to 1μs and digitizes and plots action potential waveforms (12 bit samples @ 50K samples/s) while recording video enhanced light microscopic images of nodal regions of the fibers being recorded electrically. The optical recording system employs video enhanced Nomarski DIC with image digitizing to 8 bits @ 512x512 pixels for obtaining and reviewing time lapse sequences.

Although diffuse structural changes occur in the nodal complex of frog sciatic nerve fibers that are independent of stimulation, we observe specific sub micron structural changes at densities corresponding to the paranodal loops even with mild levels of simulation (e.g., @ 1Hz). More remarkable changes are observed when the fiber is subjected to stimulation paradigms that elicit more substantial activity dependent slowing of impulse propagation (20-30%). As depression of excitability (Raymond, J. Physiol. 290:273-303, 1979) and impulse slowing develop, the density that emerges in the region of the paranodal loops during tonic stimulation marches toward the node. We are currently engaged in characterizing activity dependent structural dynamics of nodes.

29.8

THRESHOLD FOR BEADING OF MYELINATED NERVE FIBERS PREPARED WITH COLD-FIXATION.
S. Ochs, R. Pourmand and R. A. Jersild Jr. Depts. Physiology/Biophysics, Neurology, and Anatomy, Indiana Univ. Sch. of Med. Indianapolis, IN 46202.

Nerves subjected to a mild stretch and freeze-substituted are beaded, this seen as a series of constrictions and swellings along the fibers. In the constrictions the cytoskeleton is compacted (Ochs and Jersild, Neurosci. 22: 1041, 1987) and this was used to show that slow transport carries cytoskeletal proteins down as soluble subunits (Ochs and Jersild, Neurosci. 33: 421, 1989). We recently found that fibers fixed with glutaraldehyde and paraformaldehyde at 0-5^{0}C also retain beading (Ochs and Jersild, in prep). This was used to determine the tension needed to bead the fibers. Pieces of rat sciatic nerve were mounted so that the bands of Fontana, the wavy disposition of the fibers at rest, were seen. The bands disappeared when the nerves were lengthened 14-15% beyond their resting length at which point a tension of 0.6 gm caused a small degree of beading as seen in cold-fixed nerves. Tensions of 3-5 gm gave optimal beading with no further lengthening or beading seen with tensions up to 40 gm or more. Exposure to collagenase allowed a further small elongation with lower tensions of 0.3 gm giving rise to an exaggerated beading, a "hyperbeading" with longer constrictions and rounder swellings than usual. Fibers may show beading under physiological tensions, the collagen fibrils acting to prevent excessive beading.

29.9

INTENSIFIED VIDEO IMAGING OF NEURONAL POPULATION ACTIVITY. J. S. George and J. C. Fowler.
Los Alamos National Lab. MS-M882. Los Alamos, NM 87545.

Neuronal membranes contain Ca^{2+} channels gated by ligand binding or membrane voltage which can serve as useful correlates of cellular excitation. We have employed the Ca^{2+} indicator Fura-2 together with intensified video techniques to visualize spatial and temporal patterns of neuronal population activation in the rat hippocampal slice. Slices were preincubated at 30° C in Ringer's containing Fura-2 AM. Optical measurements used a photomultiplier or a Philips intensifier coupled to a CCD video camera via a pair of photographic lenses or a tapered fiber optic image guide. Bipolar electrical stimulation was delivered by twisted wire electrodes and field potentials were measured with a glass micropipette mounted on the motorized stage of an inverted microscope. Electrical stimuli were delivered every 4-6 sec, timelocked to the vertical sync of the video signal. Up to 256 8-bit images were summed, a baseline image was subtracted from each frame in the sequence, and 340/380 nm sequences were ratioed. The fluorescence transient had a peak latency of 20-40 msec with a 100-300 msec return to baseline and was typically 1% of total fluorescence. Images demonstrated focal activation of CA1 and dentate gyrus. Mn^{2+} quenched 30-40% of total fluorescence; difference imaging at higher magnification disclosed periodic intensity variations consistent with expected cellular structure. Responses were obtained with antidromic as well as orthodromic stimulation and were not eliminated by APV suggesting that the Ca^{2+} transient was associated with voltage-gated Ca^{2+} channels.

29.10

PKC LOCALIZATION IN HERMISSENDA CNS. J.Farley, Prog. in Neural Science, Indiana Univ., Bloomington, IN 47405.

Previous research has implicated PKC activation as playing a critical role in the induction and maintenance of learning-produced changes in neuronal excitability in H.c. Confocal scanning microscopy, image analysis, and fluorescent derivatives of phorbol esters were used to monitor PKC in living whole-mounted H.c. nervous systems. When incubated with 10^{-6}M NDB-TPA-1 at 19-20°C, virtually all neurons within the pedal and cerebropleural ganglia rapidly accumulated the dye, reaching maximum fluorescence within ~ 20 min. In central neurons, NDB-TPA-1 fluorescence was primarily localized within somatic plasmalemma membranes and as yet unidentified subcellular organelles. Considerably less staining in nerves and axonal fiber tracts was observed. Fluorescence was also observed within somatic membranes of ocular photoreceptors and statocyst hair cells, with little staining observed in photoreceptor rhabdomeres. Pre-incubation with unlabeled TPA decreased the fluorescence of NDB-TPA-1 stained neurons in a dose-dependent manner. Incubation of nervous systems with lower concentrations of NDB-TPA-1 (100-500 nM) resulted in a pattern of staining which was both less intense and less localized to the membrane. Under these conditions, treatment of nervous systems with the calcium ionophore A23187 resulted in fluorescent staining of membranes, reflecting an apparent translocation of the PKC-bound fluorophore.

29.11

EXTRACELLULAR DYE INDUCES PHOTO-DEGENERATION AND PHOTO-PERMEABILIZATION OF VERTEBRATE AND INVERTEBRATE NEURONS

S. Picaud*1,2, H. Wunderer*1, L. Peichl 2 and N. Franceschini*1; (1) Lab. Neurobiology, CNRS, 13009 Marseille, FRANCE; (2) Dept. Neuroanatomy, MPI für Hirnforschung, 6000 Frankfurt, FRG.

Local irradiation of neural tissue in the presence of an extracellularly applied dye was found to trigger neuronal degeneration and permeabilization with interesting applications in neuronal tracing and neuronal ablation. This phenomenon, first reported in fly photoreceptors (Picaud et al., Neurosci. Lett. 95: 24, 1988), has now been extended to (non photoreceptive) rat retinal neurons. A dye was applied to the extracellular space of the retina. A selected patch of neurons was irradiated in vivo for 30 minutes within the absorption band of the dye with the light of a conventional epi-fluorescence microscope. Immediately after the irradiation, neurons and fibers displayed degenerative features and became permeable to Lucifer Yellow. After several days survival, degenerating neurons were phagocyted whereas en passant fibers showed retrograde degeneration or neuronal regeneration. Control experiments demonstrated the involvement of dye-induced photosensitization in this photo-lesion technique which holds great potential as anatomical and physiological tool for investigating various nervous systems.

29.12

DOUBLE-LABELLING FLUORESCENCE FOR SIMULTANEOUS IMAGE ANALYSIS OF BBB-INTEGRITY AND MICROVASCULAR PERFUSION IN FOCAL BRAIN INJURY. **Lindsberg P J, Sirén A-L, Hallenbeck J M*. Dept. of Neurology, USUHS, Bethesda, MD 20814.**

Since brain injury and ischemia involve localized areas of neuronal vulnerability and microvascular failure, methodology with high spatial resolution is mandatory to monitor the hemodynamic microenvironment of discrete neuron populations. Two fluorescent dyes (Evans-Blue albumin [EBA], FITC-dextran) were infused to map simultaneously microvascular perfusion deficits and BBB-leakage in progressive focal Nd-YAG laser-induced (1.0s, 20W) secondary brain damage in rats. EBA was allowed to circulate for 30 min and mark BBB-damage and FITC-dextran was infused 20 s before decapitation. Fresh-frozen sections were laser-excited at 488 nm, emissions were optically split, individually detected, quantitated and recombined with computerized image-analysis. In an acutely progressive lesion, the ongoing BBB-damage and capillary perfusion deficit coexisted in the penumbral zone surrounding neuronal necrosis in this model. Acute regional perfusion impairment without BBB-opening was observed (0.5-2 hrs after injury) in the CA1 hippocampal region, an area of delayed neuronal death at 24 hrs. This technique permits high-resolution temporal and spatial correlation of focal microvascular derangements to neighboring neuronal damage.

29.13

NONINVASIVE MEASUREMENT OF INTRACELLULAR/EXTRACELLULAR WATER RATIOS IN SPATIALLY-DEFINED NEURONAL MICROVOLUMES USING MAGNETIC RESONANCE IMAGING. M.A. Martin, W.G. Tatton, C. Lemaire*, R.L. Armstrong*. Departments of Physiology and Physics, University of Toronto, Toronto, Ontario, Canada. M5S 1A8.

To non-invasively study the time course of fluid shifts between extracellular and intracellular (E/I) water compartments in the intact, living brain, we produced a method based on magnetic resonance (MR) imaging. T1 relaxation times from localized tissue subvolumes as small as 2.5 μL were measured using an extracellular relaxation-modifying probe (0.5 mM gadolinium-DTPA) assuming a fast exchange model (Martin et al., J. Mag. Res. 15;1, 1990) to provide tissue water ratios. This approach was tested in the central neuropil and the region of the fast flexor motoneuron (FFMN) giant somata within living, excised abdominal ganglia from crayfish (P. balangii). E/I water shifts were induced by changing extracellular osmolarity via sodium ion levels in a graded series between 270->546 mOsm (norm.=400 mOsm). The shifts were completed in 20 minutes and were measured from the differences in the steady state levels for one hour before and one hour after the change. T1 recovery curves were found to be monoexponential in concert with the compartmental model we applied. The T1-derived measures were compared with: 1) the average cross-sectional area through the central ganglionic neuropil obtained from either MR images or computer measurement plastic sections immunoreacted for neuron specific enolase as a marker of neuronal cytoplasm. 2) E/I ratios obtained in the FFMN region reconstructed from serial sections. The MR cross-sectional area measures slightly underestimated the histological volumes but linear correlation coefficients between the direct and the T1 relaxation measures ranged from 0.96 to 0.98. The T1 relaxation technique should be readily applicable to the intact rodent brain where nuclear or laminar dimensions are similar to the crayfish ganglia. It should allow measurements of the time course of water shifts that accompany the actions of excitotoxins in the intact, functioning brain. (Supported by MRC grant MT5218)

30.1

WITHDRAWN

30.2

M-CURRENT SUPPRESSION AFTER MANIPULATION OF MEMBRANE PHOSPHORYLATION. J.A. Zidichouski, H. Chen* and P.A. Smith. Dept. of Pharmacology, Univ. Alberta, Edmonton, Canada, T6G 2H7.

There is considerable interest in the possible role of cytosolic second messengers in agonist-induced M-current (I_M) suppression. This mechanism would involve phosphorylation (or perhaps dephosphorylation) of the channel protein. We tested this possibility using the whole-cell patch-clamp technique to study I_M suppression by 2μM muscarine in Rana pipiens sympathetic neurones. Inclusion of 1 or 2mM ATP-gamma-S in the pipette, which either reduces steady-state membrane phosphorylation (by antagonising ATP) or promotes irreversible 'thiophosphorylation', decreased steady-state I_M to 56.1±11.5% (n=8) of control after 10min. Despite this, muscarine always produced the same percentage depression of the remaining I_M. Also, inclusion of the phosphatase inhibitor, diphosphoglyceric acid (1mM) failed to alter the amplitude or duration of muscarine-induced responses. These results are inconsistant with the involvement of phosphorylation and/or dephosphorylation mechanisms and cytosolic second messengers. Receptors may instead be 'directly coupled' to M-channels via a G-protein.

30.3

COMPOUND 48/80 INCREASES NEURONAL EXCITABILITY WITHOUT MAST CELL ACTIVATION. T.J. Heppner and J.F. Fiekers. Dept. Anat. and Neurobiol., Univ. of Vt. Coll. Med., Burlington, VT 05405.

The action of compound 48/80 was examined in sympathetic ganglion cells located in the 9th and 10th ganglion of Rana catesbeiana. When treated with compound 48/80, intracellular recordings obtained from B-type ganglion cells showed a reversible reduction in: 1) the duration of the AHP from (mean+S.E.) 282+28.0 ms to 129+17.1 ms (n=7), 2) the current underlying the AHP from 0.89+0.144 nA to 0.29+0.075 nA (n=8) and, 3) the amplitude of the EPSP following an evoked spike.

The action of compound 48/80 was primarily postsnayptic, because puffer applications of ACh showed a concentration-dependent (0.25-25 ug/ml) decrease in the amplitude of the ACh potential. Compound 48/80 increased the number of spikes evoked during depolarizing pulses both in intact and dissociated ganglion neurons. These findings suggest that compound 48/80 has actions on ganglion neurons, which include a direct action to increase neuronal excitability. Supported by NIH grant #NS27319 to JFF.

30.4

EFFECT OF CCK ON GUINEA PIG CELIAC GANGLIA NEURONS IN PRIMARY CULTURE. S.R. Knoper, T.L. Anthony*, D.L. Kreulen. University of Arizona, Department of Internal Medicine and Pharmacology, Tucson, AZ 85724.

Pressure ejection of cholecystokinin (CCK) depolarizes guinea pig sympathetic neurons in intact ganglia. Our goal was to analyze CCK's effects in primary cultured sympathetic neurons. Dissociated neurons from adult guinea pig celiac ganglia were maintained in primary culture for 1 to 4 days prior to whole cell recording. Cell attached patch recording (n=4) revealed spontaneous channel activity during control periods. Pressure ejection of CCK (100 ms, 10^{-4} M) elicited an increase in open time. In whole cell recording mode CCK produced an inward current at a holding potential of -100 mV which reversed at -30 to -50 mV (n=2). Peak amplitude ranged from -400 pA at -100 mV to 500 pA at +20 mV. Two cells showed only outward currents at potentials from -100 to +100 mV. These results suggest that there exists a non-specific cation channel mediating the CCK induced slow depolarization in prevertebral sympathetic neurons. Supported by DK36289, HL27781.

30.5

CHARACTERIZATION OF VESICULAR CARRIERS AT NERVE MUSCLE CONTACTS. S.Bursztajn, Y.J. Jong, and S.A. Berman, Baylor College of Medicine and M.D.Anderson Hospital, Houston, Texas 77030

Our previous studies have shown that both acetylcholine receptors (AChRs) and acetylcholinesterase (AChE) are transported via coated vesicles some of which accumulate beneath the neuromuscular synapse where AChRs cluster. To investigate the mechanisms by which these proteins are transported and the remodeling which the postsynaptic membrane undergoes, we have purified coated vesicles and have raised monoclonal antibodies to the epitopes of the vesicular membranes. We isolated vesicles and purified them on Sephacryl S-1000 columns. We checked fractions for purity by assaying for 1) AChE (coated vesicle enriched) 2) hexosamindase (lysosomal contaminants) and 3) NADH cytochrome C reductase (mitochondrial). We obtained 95% pure coated vesicles, as judged by electron microscopy using negative staining with 2% uranyl acetate, and used them (after removing their coats) as immunogens to prepare monoclonal anitibodies. We characterized three monoclonal antibodies: C-33, C171 and F-22. Immunoblots of purified vesicles revealed that mAb C-33 stained a 180K band and immunofluorescence revealed punctate staining. C-171 stained a 100K band and also gave punctate immunofluorescence. F-22 stained 50K and 55K bands. Immunoblots of cultured skeletal muscle showed that C-33 stained a 180K band and C-171 stained a 100K band. The distribution of punctate fluorescence appears to depend upon the stage of myotube development. Myotubes stained with C-33 or C171 3-4 days after plating show punctate fluorescence throughout the myotube, whereas those stained 8 days after plating show a punctate perinuclear distribution. Myotubes innervated by ciliary neurons show punctate fluorescence limited to the nuclear periphery and most concentrated around nuclei which are located beneath the neurons. We suggest these vesicles may be involved in membrane insertion and removal at the neuromuscular synapse.

30.6

EFFECTS OF AMMONIA ON PRESYNAPTIC AND POST-SYNAPTIC GLUTAMATERGIC FUNCTION

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In order to elucidate the mechanisms involved in the inhibition of synaptic transmission by ammonium ions, the effects of pathophysiologic levels of NH_4Cl on glutamate release and on synaptic transmission from Shaffer collaterals to CA1 pyramidal cells was measured in hippocampal slices from control and portacaval shunted (PCS) rats. Synaptic transmission in control slices was reversibly depressed by NH_4Cl levels in excess of 1 mM. Firing of CA1 pyramidal cells evoked by electrophoretically applied glutamate was inhibited by 2 mM NH_4Cl. 5mM NH_4Cl had no effect on Ca^{2+}-dependent release of glutamate evoked by electrical stimulation of slices. PCS rat hippocampal slices superfused with NH_4Cl showed significantly increased glutamate release. This latter finding probably results from decreased glutamate uptake into damaged perineuronal astrocytes following portacaval shunting. Taken together, these observations suggest direct toxic effects of ammonia on glutamate release as well as on postsynaptic glutamatergic transmission in the central nervous system. Such effects could be involved in the neuronal dysfunction associated with acute or chronic hyperammonemic states.

(Funded by the Medical Research Council of Canada)

30.7

EFFECTS OF CHANGES IN EXTRACELLULAR pH (pH_o) ON MEMBRANE PROPERTIES OF RAT CA1 HIPPOCAMPAL PYRAMIDAL NEURONS *IN VITRO*. J. Church. Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C., Canada V6T 1W5.

The effects of changes in pH_o, elicited by changing $[HCO_3^-]_o$ at a constant P_{CO2}, on membrane properties of CA1 pyramidal neurons in hippocampal slices prepared from adult rats were examined using intracellular recording techniques. Reducing pH_o to 6.9 attenuated high-threshold Ca^{2+} spikes, the slow after-hyperpolarization following a current-evoked train of action potentials, spike frequency adaptation, depolarizing after-potentials, and the rebound depolarization following a current-evoked hyperpolarization to >-80 mV. Raising pH_o to 7.9 produced qualitatively opposite changes. The results suggest that changes in pH_o within the pathophysiological range might be an important mechanism whereby transmembrane Ca^{2+} flux and/or cytosolic free $[Ca^{2+}]$ is modulated and indicate at least one mechanism whereby carbonic anhydrase inhibitors such as acetazolamide might exert anticonvulsant activity and how mild acidosis might exert a neuroprotective effect. Supported by the Medical Research Council of Canada.

30.8

LACK OF OUTWARD Cl TRANSPORT AND LATE DEVELOPMENT OF HYPERPOLARIZING IPSPs IN HIPPOCAMPAL CA1 NEURONS OF THE IMMATURE RAT. L. Zhang & P.L. Carlen, Playfair Neurosci. Unit, Toronto Western Hosp., Dept. of Physiology, University of Toronto

We studied the mechanisms underlying the late development of $GABA_A$-mediated IPSPs in postnatal 2-5 (P2-5) and 7-14 (P7-14) day old rats, using whole cell patch recordings in hippocampal slices. Slices were perfused with standard ACSF ([Cl]=130 mM). Patch pipettes were filled with KGluconate, and the pipette [Cl] was adjusted as required. Postsynaptic potentials were evoked orthodromically from stratum radiatum. GABA was applied onto the soma by pressure injection in the presence of TTX. The table summarizes the resting potentials and reversal potentials for IPSP (E_{IPSP}) and GABA currents (E_{GABA}) in the two groups of neurons. The E_{IPSP} and E_{GABA} were 6 to 14 mV positive in P2-5 neurons compared to those of P7-14 neurons at each different internal [Cl]. When pipette solutions contained [Cl] of 15-30 mM and the extracellular [K] was increased from 2 to 15 mM, there was no change in E_{GABA} in P2-5 neurons (n=10), whereas a depolarizing shift of 11.8±6.1 mV (n=13, mean±S.D.) was found in P7-14 neurons. We conclude that a lack of outward Cl transport may account for the absence of the hyperpolarizing IPSP in P2-5 neurons. Supported by the MRC of Canada.

internal [Cl]		P2-5 (n)	P7-14 (n)
2 mM	RP (mV)	-56.6±6.0 (16)	-53.5±5.8 (24)
2 mM	E_{IPSP} (mV)	-56.7±4.8 (9)	-63.1±5.0 (15) *
2 mM	E_{GABA} (mV)	-55.3±5.0 (15)	-67.1±1.8 (11) *
8 mM	E_{GABA} (mV)	-41.0±6.1 (4)	-55.1±5.9 (5)
15 mM	E_{GABA} (mV)	-38.9±1.3 (6)	-49.5±6.9 (8) *
30 mM	E_{GABA} (mV)	-30.6±8.5 (12)	-38.6±8.2 (12) *

* $P < 0.05$, Student's t test, two tailed.

30.9

GABAERGIC SYNAPTIC CURRENTS IN SLICES OF NEOCORTEX ANALYZED WITH WHOLE-CELL AND CELL-DETACHED PATCH-CLAMP TECHNIQUES. A.R. Kriegstein and J.J. LoTurco. Dept. of Neurology and Neurological Sciences, Stanford Univ. Sch. of Med., Stanford, CA 94305

In order to study fundamental properties of GABAergic inhibition in the neocortex we have made both whole-cell and outside-out patch-clamp recordings from pyramidal neurons in slices of rat neocortex.

Whole cell recordings were obtained as described by Blanton et. al. (1989). Spontaneous synaptic currents (mean conductance = 457 pS) were shown to be mediated by GABAa receptors by their sensitivity to bicuculline methiodide and picrotoxin (PTX). As little as 0.5 uM bicuculline was effective at blocking these spontaneous currents, indicating that an effective GABA concentration of below 5 uM is reached at the synaptic cleft. The decay of spontaneous GABAergic synaptic currents increased with depolarization from a time constant of 34 msec at -70 mV to 150 msec at 40 mV. After recording synaptic currents in the whole-cell configuration, electrodes were slowly removed from the slices to form outside-out patches. The patches were then moved rapidly between different concentrations of GABA. The resulting macroscopic currents had an EC50 of 1 uM and exhibited desensitization that became faster with increasing GABA concentration (tau= 3 sec in 1 uM GABA and 830 msec in 1 mM GABA). Following the removal of GABA, macroscopic currents decayed exponentially. This decay largely reflects the off rate of GABA from receptors, and similar to the decay of synaptic currents, increased with depolarization from 65 msec at -70 mV to 210 msec at 40 mV. In patches with few active channels we observed channel events with a major unitary conductance of 23 pS and rarely occurring minor conductances of 7 and 16 pS.

The results obtained in these experiments indicate that at the peak of a spontaneous GABAergic synaptic event approximately 20 channels are open, and the shape of the synaptic currents are largely determined by activation and deactivation and not by desensitization.

30.10

ANTIFACILITATION AT A RECTIFYING ELECTRICAL SYNAPSE. D.H. Edwards, W.J. Heitler & E.M. Leise, Department of Biology, Georgia State University, Atlanta, GA 30303.

Mechanosensory interneurons (MSIs) in the crayfish abdominal nerve cord make electrical connections with the Lateral Giant (LG) interneuron, which is the command neuron for tailflip. Single impulses in one MSI, interneuron A (Int A) evoke 1 mV EPSPs in the proximal segment of LG. Stimulation at 300 Hz produces summating PSPs that rapidly decrease in amplitude as a 2 mV plateau depolarization is achieved. The first PSP in the train is of normal size, but the fifth and all subsequent PSPs are reduced by 90%, to 0.1 mV.

Because PSPs from ohmic electrical synapses will sum linearly in passive dendrites, we considered the possibility that synapses between MSIs and LG were rectifying rather than ohmic electrical connections. We found that hyperpolarizing, but not depolarizing current will spread from LG to Int A, whereas depolarizing, but not hyperpolarizing current will spread from Int A to LG. Orthodromic PSPs increase in amplitude when LG is hyperpolarized and decrease when LG is depolarized; antidromic PSPs in Int A from LG impulses increase when Int A is depolarized and decrease when it is hyperpolarized.

We conclude that the antifacilitation results from the postsynaptic depolarization created by the summed PSPs. The depolarization will reverse-bias the rectifying electrical synapse and thereby limit further entry of synaptic current. It may also activate any existing dendritic membrane conductances that would shunt the postsynaptic current. We are investigating the relative importance of these two mechanisms. Supported by NIH Research Grant NS26457.

30.11

INTRADENDRITIC RECORDINGS FROM IDENTIFIED SITES IN CA3 HIPPOCAMPAL PYRAMIDAL CELLS. K.L. Smith,* J.N. Turner, D.H. Szarowski* and J.W. Swann. Wadsworth Center for Labs and Research, NY State Dept. of Health, Albany, NY

Recent reports suggest that ion channels are not uniformly distributed in the membranes of dendrites of CNS neurons. Previous indradendritic recordings in hippocampal slices have been assumed when impalements were made some distance from the cell body layer. However, precisely where in the dendritic tree an impalement was made was unknown. In experiments reported here recordings were made from stratum radiatum of the CA3 subfield with microelectrodes filled with either lucifer yellow or carboxyfluorescein. After electrophysiological characterization of the impaled element the cell and electrode were imaged by video microscopy. This was achieved by using a SIT camera mounted on a stereomicroscope, equipped with an epifluorescence illuminator. Framed averaged images were stored on VCR tape. Afterwards slices were fixed and the cell was again imaged. This time by confocal laser scanned microscopy. A graphics workstation was employed for three dimensional reconstruction. By direct comparison of images from video and confocal microscopy, the precise location in dendrites where recordings were obtained could be identified in 3 dimensional space. Such methods should allow a detailed examination of the differential distribution of voltage gated conductances in dendrites. Support NS18309, RR02984, RR01219.

30.12

ADENOSINE DECREASES CALCIUM ENTRY IN RAT LOCUS COERULEUS NEURONS. W.J. Pan, S.S. Osmanovic, and S.A. Shefner, Dept. of Physiology and Biophysics, University of Illinois College of Medicine, Chicago, IL 60612.

We have previously shown that adenosine may activate a K^+ conductance that leads to hyperpolarization in locus coeruleus (LC) neurons. In the present study, the effect of adenosine on the shape of the action potential was examined. Intracellular recordings from LC neurons were obtained in a submerged rat brain slice preparation. All drugs were administered by bath application. LC neurons fired spontaneously at rates of 0.1 to 3.5 Hz. Adenosine (100 μM) reduced the peak afterhyperpolarization (AHP) following spontaneous spikes in 57% of cells (n=7). The reduction of the peak AHP was 1.5±0.2 mV (mean±S.E.M.). In 86% of these cells, the late phase of the AHP was increased, which was associated with a decrease in firing rate. 2-chloroadenosine (2CA) (1-2 μM) reduced the peak AHP in all cells tested (1.4±0.2 mV, n=5). Ba^{2+} (1 mM) was added to the superfusate to enhance the calcium component of the spike. Adenosine (100 μM) reduced the duration of the Ba^{2+} spike measured at 33% of the peak amplitude (12.7±6.8%, n=3). 2CA (1 μM) also reduced the duration of the Ba^{2+} spike (10.6±1.2%, n=7). The effect of 2CA on the duration of the spike was concentration-dependent. Adenosine (100 μM) reduced the amplitude of TTX-resistant "Ca^{2+} action potentials" by 5.2±1.3 mV and also reduced the peak AHP by 1.8±0.3 mV (n=4). The adenosine-induced reduction of the amplitude of TTX-resistant "Ca^{2+} spikes" and the duration of Ba^{2+} spikes suggests that adenosine decreases Ca^{2+} entry in LC neurons.

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30.13

MODELLING CHANGES IN $[Ca^{2+}]_i$ IN HIPPOCAMPAL CA3 NEURON BY VOLTAGE-GATED Ca^{2+} CHANNELS. *D.B. Jaffe and D. Johnston*, Div. of Neurosci., Baylor College of Medicine., Houston, TX 77030.

Recent work in our laboratory indicates that the induction of mossy fiber LTP in area CA3 of the hippocampus is dependent upon postsynaptic depolarization *and* an increase in $[Ca^{2+}]_i$, but independent of NMDA receptor activation. Our working hypothesis is that voltage-gated Ca^{2+} channels provide a source for raising $[Ca^{2+}]_i$. A multi-compartmental model of a CA3 pyramidal cell was created using CABLE (Hines, J. Biomed. Comput. 24:55, 1989) to examine changes in $[Ca^{2+}]_i$ produced by postsynaptic depolarization. Low- and high-threshold, inactivating Ca^{2+} conductances and a high-threshold, non-inactivating Ca^{2+} conductance were added to CABLE. Ca^{2+} conductances were located on spines as well as on dendrites and somata. $[Ca^{2+}]_i$ was regulated by the three Ca^{2+} currents, Ca^{2+} buffering, a Ca^{2+} extrusion pump, and by both radial and longitudinal diffusion. Depolarization was achieved either through a change in synaptic conductance with appropriate kinetics at the spine head or by a comparable somatic depolarizing current injection. There was no difference between peak $[Ca^{2+}]_i$ achieved by synaptic current versus somatic current injection. Peak $[Ca^{2+}]_i$ was higher at the spine head than in dendrites due to larger surface to volume ratios. However, $[Ca^{2+}]_i$ decayed faster in spines than in dendrites due to longitudinal diffusion. In addition, we examined the effect of two Ca^{2+} chelators, EGTA and BAPTA, on $[Ca^{2+}]_i$ transients. Both EGTA and BAPTA at low concentrations (10–20 μM) were effective at buffering Ca^{2+} *following* depolarization. During depolarization, however, a concentration of EGTA 100 times that of BAPTA was required to comparably buffer $[Ca^{2+}]_i$. Changes in $[Ca^{2+}]_i$ within small, distal distal dendritic spines and larger, proximal thorny excrescences was also compared.
(Grants NS11535, MH44754, and AFOSR 88-0142)

30.14

A TRANSIENT ELEVATION OF INTERNAL CALCIUM IS ASSOCIATED WITH THE ONSET OF A LONG-LASTING CHANGE IN EXCITABILITY OF BAG CELL NEURONS
T.E.Fisher, S.Levy and L.K.Kaczmarek. Dept. of Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06510 and Dept. of Physiology, Boston Univ., Boston, MA 02118

Following a train of action potentials stimulated via an afferent nerve, the bag cell neurons of *Aplysia* depolarize by 20-30mV and begin to generate action potentials repetitively. This afterdischarge continues for about 25 minutes while the membrane potential gradually repolarizes. We have measured internal calcium (Ca_i) in bag cell neurons during the afterdischarge using calcium selective (double-barrelled) electrodes. There is a large but transient (30 to 120 sec.) increase in Ca_i during the onset of the afterdischarge. This is followed by a smaller, gradual build-up of Ca_i over the remainder of the afterdischarge. When calcium in the external medium is replaced with barium an early increase in Ca_i still occurs, indicating that this elevation involves release of Ca from intracellular stores. In external media lacking both calcium and barium, stimulation results in neither the increase in Ca_i nor the depolarization that underlies the afterdischarge. This depolarization is not prevented by removal of sodium from the external medium. Our results suggest that release of calcium from intracellular stores accompanies the afterdischarge and that an increase in Ca_i is necessary for the initiation of the afterdischarge.

30.15

ELECTROPHYSIOLOGY OF NICOTINIC ACTION IN RAT MEDIAL PONTINE RETICULAR FORMATION IN VITRO.
U. Gerber, D.R. Stevens, R.W. McCarley, and R.W. Greene.
Harvard Medical School/VAMC, Brockton, MA 02401

The medial pontine reticular formation (mPRF) receives cholinergic afferents from pontine cholinergic nuclei. Previous studies have characterized muscarinic cholinergic effects in the mPRF which have been implicated in the generation of REM sleep. The nicotinic agonist, diphenylpiperazinium (DMPP), was bath applied (5-40 μM) for 1 minute to neurons of the mPRF during intracellular recording using an in vitro brainstem slice preparation. In 8 of 13 neurons, DMPP caused an increase in membrane conductance associated with an inward current (0.1-0.5 nA). The reversal potential for this response was extrapolated to be close to 0 mV. This would be consistent with a non-specific cation channel. In 3 of 13 cells, DMPP caused an increase in conductance associated with an outward current. The reversal potential for this response was examined in two of these three cells and was found to be -85mV in $[K]_o$ of 5mM. The conductance, which was blocked by 10mM TEA, showed marked inward rectification at membrane potential positive to -70 mV. Nicotine (400 μM) evoked the same response as DMPP. With both types of response to DMPP, exposure to tetrodotoxin had no effect consistent with a postsynaptic site of action. DMPP had no effect on two cells which did respond to muscarinic agonists. Three cells in the median raphe nucleus in the same slice from which mPRF neurons were recorded also did not respond to DMPP.

30.16

EFFECTS OF APAMIN ON CHOLINERGIC AND NON-CHOLINERGIC MEDIAL SEPTAL/DIAGONAL BAND (MS/DB) NEURONS OF THE GUINEA PIG *IN VITRO*. R.T. Matthews and W.L. Lee, Department of Anatomy, Texas A&M University, College Station, TX 77843.

The MS/DB region contains a variety of electrophysiologically distinct types of neurons. One cell type recorded *in vitro* is slow firing with a broad action potential and a slow (200-800 msec) afterhyperpolarization (S-AHP) seen after single action potentials. These neurons stain positively for acetylcholinesterase and are presumed to be cholinergic (*Neurosci. Lett.*, **71**:169-174, 1986). The present experiments were done in an *in vitro* brain slice preparation of the MS/DB region. We tested the hypothesis that S-AHPs regulate the firing rate of cholinergic MS/DB neurons. The bee venom toxin, apamin, was used to selectively block the Ca^{2+}-dependent K^+ conductance responsible for the S-AHP.

In extracellular unit recording experiments where firing was maintained by microiontophoresed glutamate, apamin at 3-30 nM increased firing of cholinergic neurons in a dose-related fashion (250 ± 55% increase at 30 nM, n=5), but had much less effect on fast firing non-cholinergic neurons (35 ± 7% increase, n=9). In intracellular current clamp experiments, apamin (30 nM) blocked the S-AHP but not other AHPs in cholinergic neurons (n=6) and increased firing induced by current injection from a constant holding potential (>100% increase, n=4). Some, but not all, non-cholinergic MS/DB neurons had a 20-150 msec AHP which was blocked by apamin (30 nM, n=5). Firing rate after current injection was unchanged or moderately increased (<100%) by apamin in these cells. In all cell types, the percent increase in firing rate caused by apamin was dependent upon the size of the depolarizing current pulse.

Our results suggest that the S-AHP found in cholinergic MS/DB neurons is important for their characteristic slow firing rate.

30.17

BURSTING AND NONBURSTING NEURONS IN THE RAT DORSOLATERAL SEPTAL NUCLEUS. M. J. Twery, K. D. Phelan, and J. P. Gallagher. Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550

Electrical activation of neurons in the dorsolateral septal nucleus (DLSN) entrains the activity of medial septal neurons and facilitates expression of hippocampal rhythmical slow wave (theta) activity in a frequency dependent manner. In order to characterize the firing properties of spontaneously active DLSN neurons, we have examined membrane potential (V_m)-dependent changes in repetitive firing using intracellular recording techniques and a submerged slice preparation of septum in vitro.

DLSN neurons were spontaneously active at rest (V_m=-67mV) and exhibited a rhythmic pattern of either single spike (n=9) or burst (n=25) firing. In the majority of nonbursting neurons, single spikes were followed by a prominent afterhyperpolarization. In bursting DLSN neurons, an initial fast spike was followed either by a prominent shoulder giving rise to 1-2 high threshold spikes or by a train of spikes arising at increasingly depolarized threshold levels. When hyperpolarized (5-10mV) by current injection, neurons which fired in bursts at the resting V_m exhibited single spike activity (50%). On the other hand, hyperpolarization of nonbursting neurons from the resting V_m produced burst firing (60%). Both of these effects were reversed when V_m was restored to resting levels.

The present study demonstrates DLSN neurons have burst and nonburst modes of spontaneous activity and suggests the existence of neuronal subtypes with distinct intrinsic membrane properties. Similar burst discharges from DLSN neurons in vivo may underlie facilitatory effects of DLSN stimulation on hippocampal theta rhythm.

30.18

OSCILLATORY ACTIVITIES OF THALAMOCORTICAL CELLS: PHYSIOLOGY, PHARMACOLOGY AND LOW-DIMENSIONAL DYNAMICS. V. Crunelli, N. Leresche*, D. Jassik-Gerschenfeld*, A. Aszodi* and I. Soltesz*. Dept. Visual Science, Inst. Ophthalm., London; MRC Unit, Dept. Pharm., Oxford and Inst. Neurosci., Univ. P. & M. Curie, Paris.

Thalamocortical cells in vitro (0.5-0.8mM Mg^{2+} and 2.2-3.0mM Ca^{2+}) are capable of generating four types of membrane potential oscillations that are unaffected by TTX, $GABA_A$, $GABA_B$ and non-NMDA excitatory amino acid antagonists: i) the "pacemaker" oscillations (0.5-2.5Hz, present between -60 and -70mV) that consist of rhythmic, low threshold Ca^{2+} potentials; ii) the "NMDA" oscillations (2-4Hz, present only in 0mM Mg^{2+}) which lack rhythmic, pacemaker properties and show additional depolarizations on the falling phase of the low threshold Ca^{2+} potentials; iii) the "spindle-like" oscillations, which are similar to the "pacemaker" type but occur in discrete periods every 5-20sec; iv) an oscillation, consisting of a slow (3mV/sec) hyperpolarization followed by a slow and a faster depolarization that bring the membrane potential back to its original level (full cycle: 35-90sec).

The dynamics of the "pacemaker" and the "NMDA" oscillations is low-dimensional and has properties indicative of chaos. Selective blockade of NMDA receptors, which transform the latter into the former type of oscillation, result in reproducible changes of the calculated dimensions.

30.19

ANTIDEPRESSANTS ACTION SITES IS ON THE α SUBUNIT OF G PROTEIN. H.Yamamoto*[1],U.Tomita*[2],M.Mikuni[1],A.Kagaya*[1],I.Kobayashi*[2],T.Katada*[2]& K.Takahashi*[1] (SPON:M.Mikuni). Div. Mental Disorder Res., Natl. Institute of Neurosci.,NCNP,Tokyo 187, Dept. Life Sci.,Tokyo Institute of Technol.,Yokohama 227

Our previous study showed that antidepressant (AD) affects the in vitro [^{3}H]GTP binding in the rat crude cortex membrane, in a pertussis toxin (PTX) sensitive manner, suggesting an important role of Gi/Go. To investigate the direct action of AD on the function of G protein, we examined the effect of various ADs on the GTPase activity using purified Go. A dose-dependent activation of the GTPase activity was observed in the presence of typical or atypical AD except MAO-I.Lineweaver Burk analysis of the GTPase activity with AD revealed a decreased affinity and an increased catalytic rate for GTP. These results suggest that one of the action sites of AD is on the Go, and that AD causes dissociation of αo subunit from βγ. The enhancement of GTPase activity by AD was moderately prevented by PTX-induced ADP-ribosylation. In addition, AD antagonized the effect of receptor-resemble activator. Taken together, these results lead to provide a hypothesis that AD may decrease the efficiency in transmembrane signalling to shorten αo-GTP complex state.

30.20

EARLY AND LATE ALTERATIONS OF TRANSDUCTIONAL GTP-BINDING PROTEINS IN THE CENTRAL NERVOUS SYSTEM OF DIABETIC ANIMALS. M.P. Abbracchio, F. Cattabeni, A.M. Paoletti*, A.M. Di Giulio^, M.L. Malosio*^, B. Tenconi*^ and A. Gorio, Inst. Pharmacol. Sciences., School of Pharmacy, and ^ Dept. Med. Pharmacol., School of Medicine, Univ. of Milano, 20133 Milano, Italy

Alterations of cell-to-cell communication seem to develop in the CNS of alloxan treated diabetic rats as a consequence of the pathology. We have shown that Gs- and Gi-mediated transductional processes are altered in late diabetes, i.e. 14 weeks after alloxan-injection (Abbracchio et al., J. Neurosci. Res. 24:517-523, 1989). In this study, we have monitored the onset of such transductional alterations in various CNS tissues at early and late stages of the pathology (5 and 14 weeks after diabetes induction, respectively) by measuring Gs and Gi modulation of adenylate cyclase activity and by direct 32-P-ADPribose radiolabelling of G protein subtypes with pertussis (PTX) and cholera (CTX) toxins. Results show that the retina is precociously compromized in diabetic animals. In 5 week diabetes, when no consistent alterations were detected in the other examined CNS regions, an early reduction of retinal PTX-sensitive G-proteins was observed, which was causally related to the pathology, being reversed by treatment of diabetic animals with insulin. At later stages of diabetes, also Gs proteins were markedly affected, as shown by both adenylate cyclase studies and CTX-radiolabelling. These data therefore suggest that an early defect of Gi/Go proteins might be responsible for the detected transductional changes, which is later followed by involvement of also Gs proteins.

SYNAPTIC TRANSMISSION

31.1

TPA, FORSKOLIN AND BACLOFEN CHANGE THE SUBCELLULAR DISTRIBUTION OF SMALL GTP-BINDING PROTEINS IN PRIMARY CULTURES OF RAT CEREBELLAR NEURONS. R.E. Paulsen, E. Costa and D.R. Grayson FIDIA-Georgetown Inst. for Neurosciences, Georgetown University, Washington, DC 20007

Two classes of GTP-binding proteins have been identified in mammalian tissue, based on differences in molecular weight. G proteins are heterotrimers transducing receptor-mediated signals across plasma membranes to specific effector systems. The functions of the "low molecular weight GTP-binding proteins" (20-30 kDa) or ras-related proteins, are still unknown. The subcellular distribution of small (low molecular weight) GTP-binding proteins was examined using a [α-^{32}P]GTP blot binding assay on protein fractions prepared from primary cultures of rat cerebellar granule cells. At least 4 different sizes of GTP binding proteins (25 to 29 kDa) were detected, differing in subcellular distribution. Small GTP-binding proteins with similar molecular weights were detected in the membrane fraction and in the high speed (100 000 g) supernatant from adult rat cerebellum. The forskolin elicited increase in cAMP or the TPA induced increase in protein kinase C activity lead to an increased GTP binding activity of certain bands in the 25-29 kDa region in the membrane fraction prepared from rat cerebellar granule cells. Treatment of these cells with baclofen, which has been reported to inhibit adenylate cyclase, resulted in a decrease in GTP-binding to the membrane fraction, preferentially to the P1 fraction (800 g pellet containing nuclei and sheets of plasma membrane). These results suggest that protein kinases A and C may be involved in the regulation of the subcellular distribution of certain members of the 25-29 kDa GTP binding proteins.

31.2

PROPOFOL FACILITATES POSTSYNAPTIC RESPONSE TO SUBSTANCE P IN THE GUINEA PIG INFERIOR MESENTERIC GANGLION.

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Propofol (PF) is a new intravenous anesthetic agent whose mechanism of action is unknown. The present study was designed to determine the effects of PF on nicotinic fast EPSPs and on noncholinergic slow EPSPs in a sympathetic ganglion *in vitro*. Inferior mesenteric ganglia (IMGs) were dissected from guinea pigs and superfused with Krebs solution at 35-37°C. Intracellular recordings were obtained from IMG neurones. Fast and slow synaptic inputs were evoked by electrical stimulation of lumbar splanchic (LSN) and lumbar colonic (LCN) nerves, respectively. PF (5μg/ml) had no effect on fast EPSPs evoked by LSN stimulation. In contrast, PF increased slow EPSPs evoked by LCN stimulation by 35% ($P<0.05$). Since slow EPSPs are known to be mediated by substance P (SP) and VIP, effects of PF on exogenous SP and VIP were tested. PF increased the postsynaptic depolarization to exogenous SP by 43% ($P<0.05$), whereas responses to exogenous VIP remained unchanged. When tested in IMGs obtained from animals depleted of SP by prior capsaicin (100 mg/kg) treatment, the SP dependent slow EPSP was abolished and PF had no effect on the remaining slow EPSP. These data suggest that PF: 1) does not function as a ganglionic blocking agent; 2) specifically facilitates SP-mediated slow EPSPs by a postsynaptic mechanism of action.

(Supported by NIH DK 17632 and Mayo Foundation).

31.3

NORADRENERGIC MODULATION OF SYNAPTIC RESPONSES IN RAT PREFRONTAL CORTEX NEURONS IN VITRO. J.C. Hirsch & F. Crépel*, CNRS URA 1121, Bat. 440, Université Paris- Sud 91405 Orsay, France.

Considerable evidences suggest that norepinephrine (NE) may increase the signal/noise ratio in the cerebral cortex. This modulatory influence could be of significance in controlling the use-dependent changes in synaptic efficacy we observed in prefrontal cortex neurons maintained in vitro (Hirsch & Crépel, J. Physiol., 1990, in press). In the present investigation we examined the effect of NE on the postsynaptic potentials (PSP) evoked in layer V pyramidal cells following electrical stimulation of layer II. Seventeen neurons were recorded with Kacetate QX314-filled micropipettes (12 in the presence of bicuculline (0.5-1 uM) and 5 without). The input resistance was 65 + 20 M (mean + SD) at a resting potential of 61 + 5mV. Suprathreshold stimulation activated polysynaptic responses peaking around 100ms after the early EPSP- IPSP sequence. Superfusion with NE (10 uM) reduced the amplitudes of all the PSPs. However, the decrease was most prounounced for the polysynaptic responses i.e. 76 + 19% at 100ms (N=10/10); 34 + 15% for the early EPSP (N=14/17) and 42 + 23% for the disynaptic IPSP (N=12/13). A similar reduction of the late components occurred in 4 cells subsequently tested with APV (20μM), an NMDA channel blocker. In these cells, no further reduction was noted when the 2 drugs were simultaneously applied .

Overall, these data indicate that NE has a general depressant effect on synaptic efficacy, but still selectively favors mono or paucisynaptic inputs over polysynaptic ones.

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31.4

AN ANALYSIS OF THE MUSCARINIC DEPRESSION OF SYNAPTIC TRANSMISSION AT THE CA3 MOSSY FIBER SYNAPSE.

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Acetylcholine generally acts to excite mammalian CNS neurons, primarily acting through muscarinic receptors. These actions can be attributed to reduction in various postsynaptic potassium conductances. However, in many parts of the CNS muscarinic agonists also depress synaptic transmission. We have studied this latter action at the mossy fiber CA3 synapse of the hippocampus in the *in vitro* slice preparation. Standard single-electrode voltage clamp techniques were used and picrotoxin (10μM) was routinely added to the saline to block inhibition.

Both 1 and 10μM muscarine significantly increased the input resistance of CA3 cells. However, while 1μM muscarine had little effect on synaptic transmission, 10μM caused a significant *decrease* of the measured EPSP and EPSC. The actions of 10μM muscarine were compared in two groups of cells, one recorded with KCl electrodes, the other with CsCl. The synaptic depression was very similar in both groups. In the Cs^+ loaded cells the I-V relation of the synaptic current was measured before and after muscarine application. 10μM muscarine depressed the synaptic conductance by 33 ± 6% ($n = 4$), but did not significantly alter the reversal potential of the current. There was no significant effect of muscarine on the half decay time of synaptic current.

The muscarinic depression of synaptic transmission cannot be accounted for by changes in postsynaptic membrane resistivity or alteration of synaptic reversal potential. While a postsynaptic locus of action cannot be entirely excluded, it seems likely that muscarine acts presynaptically.

(Supported by grants MH44754, NS11535, and AFSOR 88-0142).

31.5

LOW Ca^{2+} - HIGH Mg^{2+} BLOCKS TRANSMITTER RELEASE NOT RECEPTORS IN MOUSE NIGRO-STRIATAL BRAIN SLICE. J.A. Wilson. Creighton Univ. School of Medicine, Omaha, NE 68178

Release of neurotransmitter from brain slices is blocked by decreasing the Ca^{2+} concentration in artificial cerebrospinal fluid (ACSF). In practice, Ca^{2+} concentrations below 0.5 mM can produce a non-reversible inhibition of transmitter release. Increasing the Mg^{2+} concentration in conjunction with decreases in the Ca^{2+} concentration may prevent the non-reversibility. However, high Mg^{2+} concentrations are known to block the responsiveness of NMDA receptors, making it difficult to distinguish whether a decrease in the amplitude of a field potential is due to the action of Ca^{2+} on release or the action of Mg^{2+} on the receptor. This study was carried out to determine whether a low Ca^{2+} - high Mg^{2+} ACSF which blocked synaptic transmission was acting pre- or postsynaptically. Cortico-striate transmission is reversibly blocked by lowering Ca^{2+} concentration from 2.4 mM to 0.3 mM and raising Mg^{2+} concentrations from 1.2 to 3.3 mM. The field potential's amplitude and latency did not change when Mg^{2+} was raised from 1.2 mM to 3.3 mM while Ca^{2+} concentration was held constant at 2.4 mM. This indicates that low Ca^{2+} - high Mg^{2+} ACSF blocks release of transmitter without significantly altering the sensitivity of postsynaptic NMDA receptor channel complex.

Supported by a grant from the Health Future Foundation.

31.6

PATCH-CLAMP RECORDING OF SPONTANEOUS SYNAPTIC CURRENTS FROM CA3 PYRAMIDAL NEURONES IN RAT HIPPOCAMPAL SLICES. C. J. McBain and R. Dingledine. Dept.of Pharmacology, University of North Carolina at Chapel Hill NC. 27599.

Whole-cell patch recordings were made from CA3b/c pyramidal neurones maintained in the in vitro hippocampal slice (350-450μm). On cell resistances of up to 4GOhm were typically encountered using CsCl filled microelectrodes (electrode resistance of <4Mohm). The input resistance of neurones was 213 $\pm$ 15 Mohm; (mean $\pm$ s.e.m, n=13) at a resting membrane potential of -54 $\pm$ 3.1mV (n=13). High frequency (<11Hz) spontaneous synaptic inward currents 20-150pA, 5-30msecs in duration were observed. The majority of inward currents were blocked on bath application of 5μM bicuculline methobromide, those events remaining were abolished by CNQX (10μM). Tetrodotoxin (1μM) eliminated most synaptic currents. In three cells however, bicuculline sensitive unitary ipscs (20-50pA; $\leq$5Hz) persisted in the continued presence of TTX. Elevation of $[K^+]_o$ to 8.5mM, a manipulation known to induce interictal burst firing in CA3 neurones, resulted in a persistent inward current of 92 $\pm$ 8.6pA (n=5), an event not observed with conventional current clamp recordings (Korn et al J. Neurophysiol. 57; 325-340, 1988). Potassium elevation however did not influence the rate of spontaneous ipscs, epscs or TTX resistant unitary currents, but did induce spontaneous interictal bursts. Interictal events were abolished by TTX (1μM) or CNQX (10μM) with no effect on the persistent inward current.

31.7

THE DUAL EFFECTS OF DAGO ON GLUTAMATE MEDIATED SYNAPTIC TRANSMISSION OF TRIGEMINAL NEURONS IN THIN RAT MEDULLARY SLICES. L. Chen and L.-Y.M.Huang, Marine Biomed. Inst., Univ. TX Med. Br., Galveston, TX 77550.

The effects of DAGO (D-Ala^2,N-Me-Phe^4,Gly^5-ol-enkephalin) on the evoked synaptic activities and glutamate responses were studied in neurons located in the subnucleus of trigeminal caudalis of thin medullary slices. Under whole cell patch recording conditions, afferent fiber stimulations evoked excitatory postsynaptic current (EPSC) which was blocked by NMDA antagonist, APV, and non-NMDA antagonist, CNQX. We found that 1 μM DAGO reduced or completely abolished the EPSC evoked by afferent fiber stimulations. The EPSC recovered rapidly once DAGO was washed away. The inhibitory effect of DAGO was reversed by naloxone. To determine whether the effect of DAGO was presynaptic or postsynaptic, we have examined the action of DAGO on the pressure applied glutamate responses. In Mg-free solution, glutamate induced a large APV-sensitive inward current. Postsynaptically, DAGO potentiated the glutamate responses. This enhancement took a few min to develop and often lasted more than 20-30 min after DAGO was washed away. This enhancing effect of DAGO was also naloxone reversible. Thus DAGO had dual effects on the trigeminal cells. Presynaptically it inhibited the glutamate release and postsynaptically, it enhanced the NMDA response. Supported by DE09508, NS01050 and John Sealy Mem. End. Fund.

31.8

FREQUENCY-DEPENDENT CHANGES IN EFFICACY OF THE HIPPOCAMPAL RECURRENT INHIBITION. B. Esplin and R. Capek. Dept. of Pharmacology and Therapeutics, McGill University, Montreal, Quebec, H3G 1Y6, Canada.

We have examined the dependence of inhibition on the frequency of activation of the recurrent inhibitory pathway in rat hippocampal slices using extracellular recordings of field potentials. Population spikes recorded in the CA1 pyramidal cell body layer were evoked by orthodromic stimulation of stratum radiatum. They were inhibited by conditioning antidromic stimulation by a single pulse or by a train of 20 pulses to the alveus at different intervals before orthodromic stimulation.

Antidromic stimulation by a train of pulses at low frequencies (10 and 20 Hz) produced less inhibition at various time intervals after the last stimulus in the train than a single antidromic stimulus. Antidromic stimulation at high frequencies (100 and 200 Hz) produced more and much longer inhibition than a single pulse of the same intensity. Picrotoxin (3 to 10 μM) attenuated preferentially the inhibition produced by a single pulse, whereas 2-hydroxysaclofen (100 μM) had a greater disinhibitory effect on that produced by high frequency trains. These results suggest a greater involvement of the $GABA_B$ receptor activation in the prolonged inhibition produced by repetitive activation of the inhibitory pathway at high frequencies.

(Supported by the MRC of Canada).

31.9

DIRECT EFFECTS OF 17β -ESTRADIOL ON THE MEMBRANE EXCITABILITY OF CA1 PYRAMIDAL NEURONS IN THE RAT HIPPOCAMPUS. M. Wong and R.L. Moss. Dept. Physiology, University of Texas Southwestern Medical Center, Dallas, TX 75235.

The gonadal steroid, 17-βestradiol, has been found to exert direct, short-term effects on the electrical activity of neurons in the hypothalamus, amygdala and hippocampus. These rapid actions of estradiol have been attributed to non-genomic mechanisms. In the hippocampus, estradiol causes an increase in amplitude of the extracellular CA1 field potential (Teyler et al., Science 209:1017, 1980). In the present study, we utilized intracellular recording to investigate further the direct effects of estradiol on membrane excitability of CA1 pyramidal neurons in the rat hippocampal slice preparation. Hippocampal slices were prepared from 12 Sprague-Dawley rats (9 female, 3 male, 75-125 g) using a Lancer vibratome and were superfused with artificial cerebrospinal fluid. Intracellular recordings were obtained from 27 CA1 neurons with resting membrane potentials of at least -50 mV and action potential amplitudes of at least 60 mV, using glass microelectrodes filled with 4 M potassium acetate. The biologically active 17β -estradiol or the inactive control analogue, 17α-estradiol, in concentrations of 10^{-10} M to 10^{-8} M were applied to the tissue by switching a valve in the superfusion system. 17β -estradiol, but not 17α -estradiol, caused a depolarization and increased firing rate in 8 out of 27 (29.6%) CA1 neurons tested. These effects occurred within a minute after application of 17β-estradiol in all 8 cases and were completely reversed within five minutes after washout of the drug in 5 out of the 8 cells. Neurons that were excited by 17β-estradiol were found in both female (6 out of 19 cells, or 31.67%) and male (2 out of 8 cells, or 25 %) rats. Furthermore, estradiol-responsive cells were found in prepubertal females and adult females in various stages of the estrous cycle. 17β -estradiol had no observable effect on accommodation and after-hyperpolarization of CA1 neurons. In summary, these findings provide evidence for a short-term, direct membrane action of 17β -estradiol on CA1 hippocampal neurons. Supported by NIH grants HD09988 & MH44591.

31.10

BINDING OF INHALATION ANESTHETICS TO THE NICOTINIC ACETYLCHOLINE RECEPTOR. L. Lin*, D. D. Koblin* and H. H. Wang. Dept. of Biology, Univ. of California, Santa Cruz, CA 95064, and Dept. of Anesthesia, Univ. of California., San Francisco, CA 94121 (DDK).

The most likely targets of general anesthetic action are the synaptic receptors in the brain. The nicotinic acetylcholine receptor from the Torpedo californica was used as a model to test the hypothesis that inhalation anesthetics are able to act directly on synaptic receptor proteins at clinical concentrations. If the hypothesis is correct, we expect to observe specific binding of inhalation anesthetics to the acetylcholine receptor. The receptor is a chemically gated, cation channel which is blocked by non-competitive inhibitors such as phencyclidine (PCP). PCP at concentrations below 20 μM has been shown to bind to a single class of sites in the acetylcholine receptor at high affinity (K_d = 0.4 μM) with a stoichiometry of one per receptor monomer. Nitrous oxide which has a minimum alveolar concentration (MAC) in humans at 105% was used to inhibit the specific binding of ^{3}H-PCP (at 10 nM) to the acetylcholine receptor. The results showed that, in acetylcholine receptors pre-treated with carbamylcholine, equilibration with 100% nitrous oxide led to an approximately 20% decrease in specific ^{3}H-PCP binding. In the control, 100% ^{3}H-PCP binding was determined from the identical preparation but equilibrated with 100% nitrogen. This finding is comparable to the inhibition of ^{3}H-PCP binding at the minimum alveolar concentration of halothane (MAC = 0.75%). These findings indicate that both halothane and nitrous oxide bind to the non-competitive inhibitor site in the acetylcholine receptor with affinities which correlate with their anesthetic potencies.

31.11

CATECHOLAMINE-MEDIATED COMPONENT OF THE SLOW-INHIBITORY POSTSYNAPTIC POTENTIAL IN THE SUPERIOR CERVICAL GANGLION OF THE RABBIT. C. G. Acosta, B. J. Kolls* and J. H. Ashe, Neuroscience Program, Dept. of Psychology, University of California, Riverside, CA 92521

The mode of generation of the slow-inhibitory postsynaptic potential (s-IPSP) is a matter of controversy. A central issue is whether the s-IPSP is mediated via a disynaptic pathway involving catecholamine-containing interneurons. Data are presented in support of a catecholamine mediated pathway for the generation of the s-IPSP.

Paired superior cervical ganglia of rabbits were excised and mounted in sucrose gap chambers. Micromole amounts of norepinephrine (NE), dopamine (DA) and the specific D1 agonist, SKF-38393, were applied by injection into the superfusion medium and resulting changes of ganglionic potential were recorded. Synaptic potentials, the s-IPSP, the fast-excititory postsynaptic potential (f-EPSP), and the slow-excititory postsynaptic potential (s-EPSP), were elicited by supramaximal stimulation applied to the preganglionic nerve (d-tubocurarine: 35μM).

SKF-38393, NE and DA all induced a hyperpolarization (HP) of the ganglionic potential; the amplitudes varying with NE>SKF-38393≥DA. Subsequent applications of SKF-38393 either failed to elicit a HP or elicited a reduced amplitude HP. Following exposure to SKF-38393, the amplitude of the s-IPSP was reduced (by 20-50%) as was the amplitude of the NE-induced HP (by 30-70%). The amplitude of the DA-induced HP was also reduced, however, the magnitude of the effect was less (10-30%). The amplitudes of the of the s-EPSP and the f-EPSP were unaltered.

The HP typically induced by the initial exposure to SKF-38393 was not observed when the alpha-receptor antagonist, yohimbine, was present at concentrations sufficient to partially reduce the amplitudes of the catecholamine-induced HPs and of the s-IPSP. Yohimbine did not alter the amplitudes of the s-EPSP and the f-EPSP.

Exposure to small amounts of SKF-38393 resulted in the reduction of subsequent SKF-38393-mediated HPs. Following exposure to SKF-38393, the amplitude of the s-IPSP was selectively reduced as were the amplitudes of HPs induced by catecholamines. These results are consistent with the hypothesis that the s-IPSP is generated via a pathway requiring the effect of catecholamine. These data, of course, do not exclude the possibility of acetylcholine acting directly upon principal cells of the ganglion and thereby contributing in part to the generation of the s-IPSP.

31.12

COMPARISON OF STATIC AND DYNAMIC STIMULATION PARADIGMS: K^+-STIMULATED AMINO ACID RELEASE FROM CULTURED CEREBELLAR NEURONS. K.L. ROGERS[1], R.A. PHILIBERT[1], G.R. DUTTON[2], Department of Psychiatry[1] and Department of Pharmacology[2], University of Iowa, College of Medicine, Iowa City, IA 52242.

Two stimulation paradigms were employed to investigate the amino acids released from rat neuronal cultures following K^+ exposure at varying concentrations with and without Ca^{2+}. An increased sensitivity in stimulated release of the neurotransmitters aspartate (ASP), glutamate (GLU) and GABA at a given $[K^+]$ in the static vs. dynamic paradigm was seen. ASP, GLU and GABA were released in a dose- and Ca^{2+}- dependent manner, while rates of proline (PRO) efflux were not. Release rates of taurine (TAU) and the nucleoside adenosine (ADN) increased with depolarization, but removal of Ca^{2+} caused a marked increase in basal release such that efflux rates fell during depolarization. Basal alanine (ALA) and serine (SER) release rates were increased in Ca^{2+}-free conditions, but further increases were seen over and above this change with elevated $[K^+]$. Results suggest a number of different classes of efflux: neurotransmitter-type release (ASP, GLU, GABA), depolarization-insensitive release (PRO), an osmoregulatory, possibly neuromodulatory-type, release (TAU), a Ca^{2+}-insensitive neuromodulatory-type release (ADN) and a class partially Ca^{2+}-sensitive (SER, ALA). Supported by NS20632.

31.13

DUAL EFFECTS OF THE PROTEIN KINASE INHIBITOR H-7 ON CA1 RESPONSES IN HIPPOCAMPAL SLICES. J. C. Leahy and M. L. Vallano. Dept. of Pharmacology, SUNY Health Science Center, Syracuse, NY 13210.

An attractive neural model of learning and memory is long-term potentiation (LTP) in the hippocampus. In the pathway from CA3 to CA1, LTP can be observed as an enhancement in CA1 responses following high frequency stimulation of the Schaffer collaterals. Recent studies employing protein kinase inhibitors (300μM H-7) support a role for calcium-dependent kinases in EPSP potentiation (e.g. Malinow et al., 1988). However, both a negligible effect on EPSP potentiation (Muller et al., 1988), and an enhancement in CA1 responsiveness (Corradetti et al., 1989) have been observed with lower concentrations (30-100μM) of H-7. The goal of this study was to examine the effects of low (50μM) and high (300μM) concentrations of H-7 on CA1 responses.

Experiments were performed using the *in vitro* rat hippocampal slice preparation. Two bipolar stimulating electrodes were placed in stratum radiatum, on either side of an extracellular microelectrode positioned in stratum pyramidale for recording the CA1 population spike. LTP was induced in one of the two pathways followed by perfusion with medium containing either 50μM or 300 μM H-7. The CA1 responses in the presence of 50μM H-7 were characterized by the onset of multiple spikes (2-4) and an increase in the population spike amplitude, particularly in the previously unpotentiated (control) pathway. In the presence of 300μM H-7, the population spike amplitude was inhibited, particularly in the previously potentiated (LTP) pathway. These results, in conjunction with pharmacological studies using GABAergic compounds, suggest that H-7 exerts concentration-dependent effects in different pathways. At low concentrations, H-7 disinhibits CA1 neurons by reducing inhibition from local circuit neurons. At high concentrations, H-7 inhibits CA1 neurons either presynaptically or postsynaptically in the CA3-CA1 pathway.

31.14

SELECTIVE REDUCTION OF INHIBITION AT THE CRAYFISH OPENER NEUROMUSCULAR JUNCTION BY LOW CONCENTRATIONS OF ETHANOL. J.A. Blundon and G.D. Bittner. Department of Zoology and Institute for Neurological Sciences Research, University of Texas, Austin, TX, 78712.

We have examined the effects of ethanol (EtOH) exposure on behavior, central nervous system activity, and synaptic transmission in crayfish (*Procambarus clarkii*). We have previously reported that crayfish show behavioral changes we interpret as sedation within 6-24 hours after adding 75-150 mM EtOH to aquarium water (Friedman *et al.*, 1988, JPET 246:125-131). However, within minutes of EtOH exposure at these concentrations, crayfish first show signs of increased excitability. Coincident with this initial behavioral change is an increase in spontaneous activity recorded within the crayfish ventral nerve cord.

The opener muscle of the crayfish walking leg receives excitatory input from one axon only (the opener excitor or OE). This muscle also receives direct inhibitory input from the opener inhibitor axon (OI). In addition, the OI makes direct contact with the OE, thereby providing presynaptic inhibition to the opener muscle. We now report that 60 mM EtOH reduces inhibition of the opener muscle but has no effect on excitatory postsynaptic current. Further, 120 mM EtOH reduces inhibition of the opener muscle but also decreases excitatory postsynaptic current and muscle fiber membrane resistance as well. Selective reduction of inhibition by low concentrations of EtOH may in part be responsible for behavioral excitability in crayfish seen within minutes of exposure. Supported by NIAAA NRSA # AA05282 to JAB and NIAAA grant # AA007746 to GDB.

31.15

EPSPs in rat ambigual oesophageal motoneurons *in vitro*.
R.S. Neuman, Y.T. Wang and D. Bieger, Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3V6

The major if not principal afferent pathway to the oesophagomotor of the ambigual complex originates from the subnucleus centralis of the nucleus tractus solitarii and has been characterized with retro-and anterograde tracing methods. Based on this anatomical information, we have developed a sagittal brainstem slice containing both oesophagomotor neurons and the solitarial efferent pathway. In this investigation we report monosynaptic responses elicited by electrical stimuli applied along the trajectory of the solitario-ambigual fibres. Both simple and complex EPSPs were recorded, the latter having three discernible components based on activation thresholds. With few exceptions, kynurenate blocked both the simple and complex EPSPs, whereas AP-7 suppressed a high-threshold, rapidly-rising component in complex EPSPs, but failed to decrease the simple EPSP.

In conclusion, the use of appropriately cut brainstem slices permits examination of synaptic inputs to ambigual motoneurones which are thought to be involved in swallowing and oesophageal peristalsis. Supported by MRS (Canada)

31.16

GLUTAMERGIC EXCITATORY SYNAPTIC TRANSMISSION IN THE RAT SENSORIMOTOR CORTEX. G.G.C.Hwa & M.Avoli. MNI, McGill Univ., Montréal, Qué., Canada.

In the present study, intracellular recording techniques were used to characterize the excitatory postsynaptic potential (EPSP) evoked in cells located in layers II/III of the rat sensorimotor cortex. The EPSP-inhibitory postsynaptic potential (IPSP) sequence was evoked by focal stimulation of the white matter or layers IV/V. The voltage behaviour displayed by the EPSP was dependent on the stimulus intensity. At above threshold intensity, the EPSP usually displayed conventional voltage characteristic. At lower intensity, the EPSP sometime increased and decreased in size during membrane depolarization and hyperpolarization respectively. This type of anomalous volatage behaviour 1) was observable following the blockade of GABAergic IPSP 2) was insensitive to NMDA antagonist CPP 3) could be mimicked by a brief pulse of depolarizing current. The non-NMDA antagonist CNQX (0.5-2.5 μM) reduced the EPSP in a concentration-dependent manner. Our results suggest that the EPSP in the rat sensorimotor cortex is primarily mediated by non-NMDA receptors. In addition, the shape of this EPSP is also contributed by intrinsic voltage-dependent currents whose expression is controlled by the GABAergic inhibitory mechanism. This work is supported by the MRC and the Savoy Foundation.

31.17

INTERACTIONS OF TETRAHYDROAMINOACRIDINE AND PHENYLMETHYLSULFONYL FLUORIDE ON ACETYLCHOLINESTERASE. K.A. Skau. U. Cincinnati, College of Pharmacy, Cincinnati, OH 45267.

Recent results suggest that phenylmethylsulfonyl fluoride (PMSF) selectively inhibits acetylcholinesterase (AChE) associated with membranes. Thus, brain AChE, which is primarily membrane bound, was inhibited more than the enzyme in tissues with a large proportion of cytosolic AChE. Tetrahydroaminoacridine (THA) has been reported to concentrate in brain and inhibit AChE-an action that may be beneficial in Alzheimer's disease. However, THA inhibition of AChE in vivo is difficult to demonstrate. To show such an action, rats were injected with THA (3.2 mg/kg ip) or vehicle and 15 min later PMSF (85 mg/kg in sesame oil) was administered by gavage. The rats were sacrificed 24 hr later, various tissues were harvested and AChE activity was assayed. PMSF had the greatest effect on brain and bladder; THA protected these tissues to the greatest extent. Salivary gland and muscle (both skeletal and smooth) AChE were also protected by THA. In vitro, THA (10μM) appeared to enhance the irreversible inhibition of AChE by PMSF (5mM) but prevented AChE inhibition by echothiophate (5μM). These results provide evidence that THA does interact with AChE in vivo and can protect the enzyme from irreversible inhibition.

31.18

PHARMACOLOGY OF EXCITATORY POSTSYNAPTIC POTENTIALS OF PURKINJE CELLS AND NEURONS OF THE DEEP NUCLEI IN OLIVO-CEREBELLAR CO-CULTURES. E. Audinat, B. Gähwiler and T. Knöpfel. Brain Research Institute, University of Zürich, CH-8029 Zürich.

The olivary climbing fibers which form synapses on both Purkinje cells (PCs) and neurons of the deep cerebellar nuclei (DCN), have been proposed to use an excitatory amino acid as neurotransmitter. We have compared the pharmacology of the excitatory postsynaptic potentials (EPSPs) evoked by the stimulation of the inferior olive (IO) in morphologically identified PCs and in neurons of DCN. Slices of cerebellum and IO were obtained from 1 to 3 old day rats and co-cultured for 2-4 weeks. In PCs, all or none climbing fiber responses evoked by field stimulation of the IO were reversebly abolished by bath application of CNQX (10 uM) but were unaffected by D-APV (20 uM) or by superfusing the slices with magnesium-free solution. In neurons of the DCN, stimulation of the IO induced sequences of excitatory and inhibitory postsynaptic potentials. After blockade of the inhibition with bicuculline (20 uM), CNQX (10 uM) abolished only the fast rising component of the EPSPs. The remaining, slowly rising, CNQX-insensitive part of the EPSPs was blocked by D-APV (10 uM) and also by magnesium in a voltage-dependent manner. We conclude that in PCs climbing fibers responses are mediated by the activation of non-NMDA excitatory amino acid receptors while, in neurons of the DCN, both NMDA and non-NMDA receptor subtypes are involved in the EPSPs evoked by IO stimulation.

31.19

MUSCARINIC RESPONSES OF CELLULAR SUBTYPES IN RAT BASOLATERAL AMYGDALA *IN VITRO* M.S. Washburn and H.C. Moises Neuroscience Program and Dept. of Physiology, Univ. of Mich, Ann Arbor, MI 48109.

We recently reported that pyramidal-type neurons in the rat basolateral amygdala (BLA) respond to pressure-application of carbachol or muscarinic agonists with an initial hyperpolarization followed by a prolonged depolarization (Washburn and Moises, Neurosci. Abstr., 1989). The hyperpolarization reversed near E_{Cl}, and was blocked by GABA antagonists, TTX and Cd^{++}. These results suggest that the initial inhibitory response was indirect in nature and mediated by short-latency muscarinic excitation of intrinsic GABAergic interneurons.

In this study, we examined muscarinic responses of presumptive intrinsic interneurons in BLA using intracellular recordings from rat brain slices. These cells were distinguished from the predominant pyramidal type by their short duration action potentials (0.6 $\pm$ 0.1 ms, mean $\pm$ SEM, n=7), short time constants (4.9 $\pm$ 0.7 ms) and absence of spike frequency accomodation in response to a depolarizing current pulse. In contrast to pyramidal cells, presumed interneurons depolarized immediately to local application of carbachol or muscarine. The depolarization was associated with an increase in R_{input} and was blocked by atropine (0.1-1 μM). An additional class of cells (n=32) was identified by their negative V_m (-80 mV) and distinct synaptic responses. Unlike pyramidal cells and interneurons, these cells did not fire action potentials during the initial phase of the depolarization evoked by a current pulse. This property appeared to be due to a large intrinsic A-current since it was blocked by 4-AP (100 μM) or by holding the membrane potential to a depolarized level. While these cells depolarized immediately to pressure application of carbachol or muscarinic agonists, Lucifer yellow labelling revealed a morphology distinct from that of interneurons.

Our ability to demonstrate short-latency muscarinic excitation of presumptive interneurons provides a neuroanatomical substrate for the initial hyperpolarization recorded in pyramidal type cells. Thus, a common mechanism apppears to underlie the inhibitory muscarinic action seen in pyramidal cells in BLA, hippocampus and cortex. (Supported by NIDA grant 03365)

31.20

MUTUAL ANTAGONISM OF THE ENHANCING EFFECTS OF INDOMETHACIN AND CAPTOPRIL ON VASCULAR NORADRENERGIC NEUROTRANSMISSION. **W.H. Cline and L.L. Stephenson. Dept. of Pharmacology, Southern Illinois Univ. Sch. of Med., Springfield, IL 62794-9230.**

We previously reported that captopril (CAP), enhanced periarterial nerves stimulation (PNS)-induced release of endogenous norepinephrine (NE) when infused into isolated, perfused mesenteric vascular-intestinal loop preparations from 13 to 16 week old normotensive Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) . We have also reported that indomethacin (IND), enhanced PNS-induced release of endogenous NE from WKY but not SHR preparations. Since it had been reported that IND releases mesenteric vascular renin and that CAP releases prostacyclin in the mesenteric vasculature, the ability of IND and CAP to antagonize one anothers effects on PNS-induced NE release was determined in isolated, Krebs-perfused mesenteric vascular-intestinal loop preparations of 13 to 16 week old WKY and age-matched SHR. The effect of concurrent infusion of CAP at 5 μM and IND at 3 μM on PNS-induced NE release was determined at PNS of 4 to 14 Hz in WKY and SHR preparations. PNS-induced NE release did not differ from untreated control values during this combined treatment in WKY preparations. PNS-induced NE release from WKY preparations during this combined treatment was significantly less at 4 to 14 Hz than that observed during IND treatment alone and was significantly less at 10 to 14 Hz than that observed during CAP treatment alone. PNS-induced NE release did not differ from untreated control values during this combined treatment in SHR preparations. The significant increases in PNS-induced NE release observed at 4 to 14 Hz during CAP infusion alone were prevented at all PNS frequencies during IND infusion in SHR preparations. These finding suggest that the ability of IND to enhance PNS-induced NE release may be the result of activation of the mesenteric vascular renin-angiotensin system. These finding also suggest that the enhancement of PNS-induced NE release which occurs during CAP infusion may result from the production of a cyclooxygenase product in the mesenteric vascular wall. **Supported by the American Heart Association, Illinois Affiliate.**

NEUROCHEMISTRY OF TRANSMITTER SYSTEMS

32.1

TAURINE AND THE DEVELOPMENT OF CAT-AND MOUSE-CEREBELLUM IN CULTURES. E. Trenkner, Ph.D. Institute for Basic Research in Developmental Disabilities, 1050 Forest Hill Road, Staten Island, NY 10314.

Two lines of evidence suggest the importance of taurine in early postnatal cerebellar development of cat and mouse. Female cats, when raised on a taurine-deficient diet produce offspring with severe brain abnormalities, particularly in the cerebellum, (1). Similar morphological defects were described for the autosomal recessive neurological mutation weaver (wv/wv), (2). The function of the wv/wv gene is unknown but it appears to be inherent to cerebellar granule cells, (3). The wv/wv defect might be related to reduced concentrations of taurine at early stages of postnatal development (4), in this respect resembling taurine-deficient kittens.

Taurine is synthesized in rodents but not in cats and primates, including man, (5). Thus cat cerebellum as well as the wv/wv cerebellum seem to be ideal systems to investigate the function of taurine during early postnatal development. This study compares the effect of taurine on survival and behavior of cerebellar cells in culture of the taurine deficient mutant mouse weaver and in cerebellar cultures of cat under taurine deficient conditions. We found that in the presence of high concentrations of taurine the survival rate of weaver cerebellar cells was up to 80% of that of normal mice. Surprisingly, however, cultured cat cerebellar cells died in the presence of taurine but thrived in the absence of taurine as well as in the presence of taurine-uptake blockers, suggesting a qualitatively different mechanism from that postulated for mouse. Implications and possible mechanisms will be discussed.

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32.2

EFFECTS OF MEMBRANE ω6 POLYUNSATURATED FATTY ACIDS ON ADENOSINE UPTAKE AND 5'-NUCLEOTIDASE ACTIVITY IN N1E-115 NEUROBLASTOMA CELLS. M.G. Murphy and Z. Byczko*, Department of Physiology & Biophysics, Dalhousie University, Halifax, Nova Scotia B3H 4H7

We have examined the effects of increasing the membrane polyunsaturated-fatty-acid (PUFA) content of N1E-115 neuroblastoma cells on processes that could regulate the availability of extracellular adenosine (Ado). Measurement of uptake of [3H]Ado (1.3 - 20 μM) for 20 sec (+ NaCl) demonstrated that PUFA-enriched N1E-115 cells have an $\geq$2-fold higher capacity (V_max) to take up the nucleoside than do control cells (e.g., 0.66 & 0.30 nmol/mg protein, respectively). Values for K_T were also higher in the PUFA-rich cells (e.g., 25 vs 9 μM). Assays of 5'-nucleotidase activity indicated that conversion of [3H]5'-AMP to [3H]Ado was not affected greatly by increasing the ω6 PUFA content of the cells. Apparent values for V_max were 6 & 7 nmol/mg protein, and for K_A were 15 & 23 μM in the control and PUFA-enriched cells, respectively. The results of this study suggest that increases in extracellular Ado in PUFA-enriched cells can not be explained by PUFA-dependent changes in Ado uptake or in 5'-nucleotidase activity. (Supported by the MRC)

32.3

EFFECTS OF BROFAROMINE ON TRACE AMINE LEVELS IN THE RAT STRIATUM. L. E. Dyck and A. A. Boulton.
Neuropsychiatric Research Unit, Dept. of Psychiatry, Univ. of Saskatchewan, Saskatoon, Canada S7N 0W0

Previous studies have shown that endogenous phenylethylamine (PE) in the rat striatum is a substrate for type B MAO, while tryptamine (TRA), m- and p-tyramine (mTA and pTA) appear to be substrates for both types A and B MAO. In the following study, brofaromine (BFO), a reversible type A MAOI, and tranylcypromine (TCP), an irreversible A and B MAOI, were injected i.p. into male Wistar rats and the trace amine levels in the striatum quantified. Four h after the injection of 10 mg/kg TCP the levels of PE were increased 25-fold, TRA 69-fold, mTA 3.3-fold and pTA 6.7-fold compared to saline controls. By contrast, 4 h after the injection of 10 mg/kg BFO, the levels of PE and TRA did not change, but mTA increased 1.9-fold and pTA 4.7-fold. 100 mg/kg BFO did not alter PE levels. This lack of effect on striatal PE by the high dose of BFO is further evidence for the specific inhibition of type A MAO by BFO. The magnitude of the increases in the levels of TRA, mTA and pTA permitted the detection of significant increases in these amines at lower doses of BFO than is possible with serotonin and the catecholamines. For those reversible MAOI's which do not bind to MAO, then, measurement of their effects on endogenous TRA and TA levels would be superior to ex vivo assessment of MAO activity. Supported by Saskatchewan Health and Ciba-Geigy Canada Ltd.

32.4

COMPARATIVE EFFECTS OF MAO-A AND MAO-B INHIBITORS ON STRIATAL DOPAMINE STUDIED BY *IN VIVO* VOLTAMMETRY. M. Hong, B. Milne* and K. Jhamandas. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Drugs that inhibit the disposition of dopamine (DA) inhibitors of monoamine oxidase (MAO) or monoamine re-uptake - have the potential to enhance the actions of L-DOPA on striatal DA function. To evaluate their effects on DA function, *in vivo* voltammetry was employed. Catechol oxidation current (CA·OC), reflecting accumulation of DOPAC and DA, was measured using carbon fibre electrodes implanted in the striatum of halothane anesthetized rats. Administration of clorgyline (1.0 and 2.5 mg/kg i.v.), a MAO-A inhibitor, produced a dose-dependent decrease in CA·OC, peak effect occurring 45 min following the drug. Deprenyl (2.5, 5.0 and 10 mg/kg i.v.), a MAO-B inhibitor, and pargyline (5.0 mg/kg i.v.), a mixed MAO inhibitor, also decreased CA·OC, but their effect was weaker than that of clorgyline. Following inhibition of CA·OC, L-DOPA (200 mg/kg i.p.) produced a large and sustained increase in CA·OC which was blocked by 3-hydroxybenzylhydrazine (25 mg/kg i.v.), a centrally-acting decarboxylase inhibitor. It appears that in the rat MAO-A inhibitors produce a more effective decrease in DA metabolism and may have a greater potential to augment the action of L-DOPA.
[Supported by the Parkinson's Foundation of Canada]

32.5

SYNTHESIS OF 3,4-DIHYDROXYPHENYLGLYCOLALDEHYDE, THE BIOGENIC ALDEHYDE OF NOREPINEPHRINE. M.A. Javors*, M.S. Sabaratnam*, T.S. King, and C.L. Bowden. Departments of Psychiatry, Pharmacology, and Cellular-Structural Biology, University of Texas Health Science Center, San Antonio, Texas 78284-7792.

Although they are implicated in various physiological or pathological processes, the role of biogenic aldehydes in these processes has yet to be studied in depth. Since these aldehydes are not commercially available, the purpose of this study was the synthesis of the biogenic aldehyde of norepinephrine (NE), 3,4-dihydroxyphenylglycolaldehyde (DHPGA). We synthesized DHPGA from NE using partially purified monoamine oxidase-B (MAO-B) from human platelet membranes. DHPGA was extracted from the reaction mixture with alumina due to the catechol structure of DHPGA. The conversion of 1 mM NE to DHPGA was 100% complete within 5-6 h after the start of the reaction. DHPGA was stable in storage at 0-4^{o}C or -80^{o}C with or without 1 mM sodium metabisulfite for up to 14 days. DHPGA was quantitated using reverse phase HPLC (3 micron, C18) with coulometric detection. DHPGA was sensitive coulometrically and the voltage at half-maximal oxidation was approximately 40 millivolts. We have identified this aldehyde in human CSF and urine using an alumina extraction procedure. These results indicate that DHPGA can be synthesized in quantity and stored with stability for use as a standard in studies that require the measurement of DHPGA in biological tissues.

32.6

ELEVATION OF TAURINE IN HIPPOCAMPAL EXTRACELLULAR FLUID AND CSF IN ACUTELY HYPOSMOTIC RATS: EXTRACEREBRAL ORIGIN? A. Lehmann[1] and C. Carlström*[2], [1]Inst. of Neurobiol. and [2]Dept. of Neurol., Univ. of Göteborg, Göteborg, Sweden.

Previous studies have demonstrated a selective increase in extracellular taurine in the pyriform cortex of water-intoxicated rats. The present work examined whether this response could be due to influx of the amino acid from blood. Urethane-anesthetized rats were injected (i.p.) with distilled water (150 ml/kg) and plasma osmolality and amino acids were determined. CSF was collected for measurement of amino acids, osmolality and albumin, an indicator of the integrity of the blood-CSF barrier. In separate experiments, water or 0.9% saline (150 ml/kg of each) were administered, and hippocampal amino acids (intracellular amino acids) and specific gravity were determined. Finally, amino acids were measured in hippocampal microdialysates after water administration. CSF osmolality decreased less than plasma osmolality which reflects the osmoconserving capacity of the CNS. Taurine, phosphoethanolamine (PEA) and ethanolamine (EA) increased in microdialysates, CSF and plasma during acute hyposmolality. CSF albumin was transiently elevated after water administration. The level of taurine in hippocampal tissue decreased with respect to the volume increase (8%) whilst there was a significant diminution in PEA and an increase in EA. Opening of the blood-CSF barrier in parallel with a 25-fold increase in plasma taurine during water intoxication indicates that the augmented levels of the amino acid in cerebral extracellular fluids may partly be caused by influx from blood. The origin of the increase in plasma taurine is unknown, and dilution of blood with water in vitro showed that only a small fraction derives from lyzed blood cells. The results indirectly suggest that acute hyposmolality alters phosphatidylethanolamine metabolism which may affect membrane composition and, eventually, transmembrane fluxes of ions and water.

32.7

DEPOLARIZATION INDUCED ATP RELEASE FROM MOUSE BRAIN SYNAPTOSOMES: CALCIUM-DEPENDENT AND INDEPENDENT MECHANISMS. J.L. Fiedler , H.B. Pollard and E.Rojas*. Lab. Cell Biology and Genetics. NIDDK. NIH. Bethesda, MD. 20892.

ATP is stored in secretory vesicles of presynaptic nerve endings. A sudden elevation of K^+ causes membrane depolarization and secretion of ATP together with specific neurotransmitters. We demonstrated here that ATP is secreted both in the presence of physiological external Ca^{2+} concentration and in the absence of this divalent cation. The aim of our work has been to characterize the kinetics of both modes of ATP secretion from highly purified preparation of mouse brain synaptosomes. We meaured the time course of ATP release using luciferin-luciferase included in the external solution. The rate constant of the ATP secretion increases with $[Ca^{2+}]_o$. In the absence of Ca^{2+}, the release process is slow. This calcium independent modality of ATP release could be induced by either KCl or RbCl but not by CsCl. These results are consistent with the idea of different permeabilities for K^+, Rb^+ and Cs^+ across K^+-channels known to be present in the membrane of synaptososmes. Moreover, experiments conducted with impermeant anion isethionate keeping the product $[K^+]_o x [Cl^-]_i$ constant showed that ATP release was as in controls, indicating that ATP release was not due to osmotic lysis. Using potenciometric dye ($DISC_3(5)$) we obtained an empirical relationship between $[K^+]_o$ and membrane potential. The extent of ATP release follows a linear relationship with the membrane depolarization.

32.8

APOMORPHINE-INDUCED ALTERATIONS IN DOPAMINE RELEASE AND METABOLISM IN THE NEOSTRIATUM OF DEVELOPING RATS. R.A. Gazzara and S.L. Andersen*. Center for Developmental Psychobiology and Dept. of Psychology, SUNY-Binghamton, Binghamton, NY 13901.

Apomorphine (APO) has been shown to inhibit dopamine (DA) release and synthesis in the neostriatum of adult rats, and to inhibit DA synthesis in the neostriatum of rat pups 14 days of age or older. This action of APO has been attributed to its activity as a DA autoreceptor agonist.

The study reported here analyzed the effect of APO on spontaneous and K^+-evoked DA release in the neostriatum of urethane-anesthetized rat pups aged 10-11 days, 21-22 days, and 35-36 days, using in-vivo microdialysis. Samples were collected every 15 min and extracellular levels of DA, DOPAC, and HVA were measured by HPLC-EC. Rats were injected s.c. with 0.05, 0.1, or 1.0 mg/kg APO or vehicle. APO produced a dose-dependent decrease in spontaneous DA release in all three age groups at all three doses. Similarly, APO produced a dose-dependent decrease in K^+-evoked DA release in all three age groups at all three doses. In addition to these APO-produced effects, age-related trends were found in both pre-drug spontaneous DA release and pre-drug K^+-evoked DA release. These data suggest that DA autoreceptors in the neostriatum are functional by 10 days of age in the rat, but that DA release mechanisms mature at a later age. Supported by USPHS BRSG SO7RR07149 and SUNY-Binghamton Faculty Grant.

32.9

MULTIPLE KINETICALLY DISTINGUISHABLE COMPONENTS OF ^{3}H-DOPAMINE RELEASE FROM NERVE TERMINALS. S. Katragadda*α, M. L. Brown*+, T. J. Turner*α and S. M. Goldinα. α Dept. Biol. Chem., Harvard Medical School, Boston, & Cambridge Neuroscience Research Inc., Cambridge, MA and +Dept. of Math., Simmons College, Boston, MA.

^{3}H-dopamine (DA) release from rat brain synaptosomes was characterized at ~60 msec time resolution by a rapid superfusion method (c.f. Turner et al. [1989] Anal. Biochem. 178, 8). The level of ^{3}H-DA uptake and subsequent K^+-stimulated release were ~6-fold higher in striatal vs. whole brain synaptosomes. A large, decaying Ca-dependent component of DA release was superimposed upon a small, persistent Ca-independent component. The decay of DA release was analyzed by a variety of power and logarithmic transformations as alternative models. A biphasic exponential decay model best fit the data, with time constants for the 2 components of decay of 0.12 sec and 1.8 sec.

As compared with ^{3}H-GABA release studied by the same method, maximal Ca dependent DA release required a stronger depolarization (60 mM K^+ vs 30 mM K^+). The Ca-independent component of DA release was much smaller than the corresponding component of GABA release (8% vs 25% of maximum rate). DA release can be restimulated following decay by repolarization for at least 0.3 sec; 10 sec of repolarization produced 80% recovery of DA release amplitude. DA release was not blocked by nimodipine.

32.10

EFFECTS OF TETRAHYDROBIOPTERIN (BH_4) ON DOPAMINE (DA) AND SEROTONIN (5-HT) SYNTHESIS IN RAT BRAIN SLICES.
P.Z. Anastasiadis[1], E. Ziaja*[2], W.A. Wolf[2], D.M. Kuhn[2], and R.A. Levine[1]. Cellular and Clinical Neurobiology Program, Department of Psychiatry, Wayne State University, and [1]Laboratory of Molecular Neurobiology, [2]Laboratory of Neurochemistry, Lafayette Clinic, Detroit, MI, 48207, USA.

BH_4 has been shown to influence the metabolism of DA and 5-HT, presumably through regulating the activities of tyrosine and tryptophan hydroxylase (TPH). We previously showed that BH_4 was devoid of effects on 5-HT metabolism in rat brain synaptosomes (Wolf et al., J. Neurochem., 1990). This is in contrast with other reports that showed a BH_4-induced stimulation of tryptophan hydroxylase (TPH) and 5-HT metabolism in vivo and in rat midbrain slices. These data suggest that BH_4 may have a greater effect on 5-HT synthesis in cell body regions, or that stimulation of 5-HT synthesis is not related to its role as hydroxylase cofactor. Indeed, BH_4 has been shown to enhance DA release into dialysis media from rat striatum (Koshimura et al., J. Neurochem., 1990), which could account for some of the effects of BH_4 on striatal DA metabolism. The present studies are examining the anomaly between our synaptosomal data and other in vitro and in vivo data, and the mechanism of BH_4 action in influencing 5-HT and DA metabolism. In preliminary experiments, we have been able to show that BH_4 can stimulate 5-HT synthesis in rat midbrain slices (0.5 mm). Control slices produced a total 5-HTP + 5-HT accumulation of 0.58 pmol/min/mg prot. Incubation of slices with 200 uM BH_4 increased 5-HTP + 5-HT accumulation to 1.33 pmol/min/mg. We are currently examining the effects of BH_4 on 5-HT and DA metabolism in slices enriched with aminergic cell bodies or terminals.

32.11

IN VITRO INHIBITION OF [^{3}H]5-HT, [^{3}H]NOREPINEPHRINE (NE), AND [^{3}H]DOPAMINE (DA) UPTAKE BY AN EXTRACT OF THE GINKGO BILOBA PLANT (EGB761 Ipsen). J. E. Taylor. Neuropharmacology Laboratory, Biomeasure Inc., 9-15 E Ave, Hopkinton, MA 01748.

A standardized extract of the leaves of Ginkgo biloba (EGB761) known to contain flavonglycosides, proanthocyanidins, terpenes, and organic acids has been used clinically to treat peripheral and cerebral vascular insufficiency, and behavioral deficits associated with aging. In addition, pre-clinical studies in rodents have also indicated that EGB761 may be effective in the treatment of acute mechanical damage to the frontal cortex (Attella et al., Exp. Neurol. 105:62, 1989). Previous studies in isolated vascular tissues and *in vivo* regarding the biochemical mechanism(s) of EGB761 have suggested that a potentiation of NE activity and down-regulation of β-adrenergic receptors may underlie, in part, some of the pharmacological actions of the extract (Auguet et al., Gen. Pharmacol. 13:225,1982; Gen. Pharmacol. 13:169, 1982; Brunello et al., Pharm. Res. Comm. 17:1063, 1985). To further explore these observations using *in vitro* techniques, the actions of EGB761 on [^{3}H]NE, [^{3}H]5-HT, [^{3}H]DA uptake into isolated synaptosomes has been assessed. Initial experiments employing a single concentration (1 mg/ml) showed that EGB761 inhibited NE uptake by 99%. DA uptake was inhibited 95% and 5-HT 60%. Dose - response curves yielded the following Ki values (mg/ml ± SEM):

NE = 0.12 ± 0.01 DA = 0.21 ± 0.01 5-HT = 0.44 ± 0.02

These results provide evidence that EGB761 contains constituents which are active *in vitro* as inhibitors of biogenic uptake, and this information may contribute to an understanding of the *in vivo* behavorial effects of the extract. Additional studies are required to determine which components of the extract produce this effect.

32.12

IMPROVED METHODS FOR THE ASSESSMENT OF HUMAN PLATELET FUNCTIONING AND SEROTONIN-RELATED PHYSIOLOGY. G.M. Anderson*, W.C. Horne*, D. Chatterjee*, L.M. Hall*, A. Chatterjee*, B.A. Shaywitz, and D.J. Cohen*. Child Study Ctr., Yale Univ. Sch. of Medicine, New Haven, CT 06510.

An improved method for the isolation of platelet dense granules has permitted the first observation of affinities and capacities of granular 5-HT uptake (Kd 1.07 uM, Vmax 1.4 nmol/mg/min) and granular tetrabenazine (TBZ)-displaceable [^{3}H]-ketanserin binding (Kd 9.4 nM, Bmax 5.4 pmol/mg). Observation of reserpine-blockable 5-HT uptake and of [^{3}H]-ketanserin binding in crude membrane/organelle fractions indicated that the crude fraction can be used for determining capacities when blood volumes are limited.

The responsivity, temporal stability, and intra-subject variability of ADP-induced platelet aggregation were improved by anticoagulating with 60 uM PPACK instead of 11 mM citrate. The extents of aggregation (1-1.5 uM ADP, N=9) at 1.5 and 4.5 hours with PPAK were 37.8 ± 5.8% and 37.2 ± 4.4%, with citrate 19.1 ± 4.3% and 14.5 ± 4.4%, respectively. Stable and highly responsive (ED50 20-50 nM) 5-HT-induced platelet shape change was also observed in PPACK-anticoagulated, syringe-stored, plasma.

Release of endogenous platelet 5-HT could be assessed in PRP (25ul) or whole blood (50ul) using glass fiber filtration and HPLC analysis. Dose-response curves for release induced by thrombin and A23187, and very rapid (< 1 sec) stoppage, could easily be obtained.

32.13

INTERACTION OF METAPHIT, AN ISOTHIOCYANATE ANALOG OF PHENCYCLIDINE, WITH VOLTAGE-DEPENDENT SODIUM CHANNELS. I. Zimanyi[1], A.E. Jacobson[1,2], K.C. Rice[3] and M.E.A. Reith[4] [1]Dept. Vet. Pharm. Tox. UC Davis, Davis, CA 95616; [2]NIDDKD, NIH, Bethesda, MD 20892; [3]Center for Neurochemistry, NKI, Ward's Island, New York, NY 10035.

Our previous work on the effect of metaphit on electrically induced release of [^{3}H]dopamine from rat striatal slices suggested an action on the voltage-dependent sodium channels. Indeed, the binding of [^{3}H]batrachotoxinin A 20-α-benzoate (BTX-B) to voltage-dependent sodium channels in rat striatal synaptoneurosomes was inhibited by the presence of metaphit (IC_{50}=7 μM) or phencyclidine (PCP) (IC_{50} = 6 μM). This effect was due primarily to an increase in the dissociation rate of [^{3}H]BTX-B binding: a 2-fold increase in k_{-1} was observed with 10 μM metaphit or 3 μM PCP. Treatment with metaphit at 36 °C followed by its removal by washing, reduced [^{3}H]BTX-B binding (IC_{50}=22 μM),in contrast to the lack of effect of PCP under these conditions. Finally, tetrodotoxin (TTX)-sensitive, veratridine(30 μM)-induced influx of [^{14}C]guanidine ion was inhibited by metaphit (IC_{50}=11 μM) and PCP (IC_{50}=26 μM). At a lower concentration of veratridine (5 μM) both metaphit and PCP became more potent (7 and 18 μM). Unexpectedly, also the TTX curve shifted to the left (from 88 to 11 nM), suggesting a possible interaction between site 1 and 2. The present results indicate that the voltage-dependent sodium channel should be added to the growing list of sites of action of metaphit. Supported by grant DA 03025 to M.E.A.R..

32.14

WIDESPREAD REDUCTIONS IN KYNURENIC ACID CONCENTRATIONS IN HUNTINGTON'S DISEASE. M.FLINT BEAL, WAYNE R. MATSON*, KENTON J. SWARTZ, PAUL MILBURY*, ELIZABETH A. RYAN*, ELSDON STOREY, TATSUO OGAWA AND EDWARD D. BIRD. Neurochemistry Laboratory, Mass. Gen. Hosp., Boston, MA 02114 and ESA Inc., Bedford, MA 01730.

Huntington's disease (HD) is characterized by gradually evolving selective neuronal death. Several lines of evidence suggest that an "excitotoxin" mechanism may play a role. Tryptophan metabolism leads to both the excitatory amino acid agonist, quinolinic acid and the excitatory amino acid antagonist kynurenic acid. We recently found increased kynurenine/kynurenic acid rations in HD postmortem putamen. In the present study we used HPLC with 16 sensor coulometricelectrochemical detection to measure kynurenic acid and 20 other electrochemically active compounds in 8 cortical regions, caudate and cerebellum from controls, HD, Alzheimer's disease (AD) and Parkinson's disease (PD) patients. Kynurenic acid was also measured in CSF with HPLC with fluorometric detection. Significant reductions in kynurenic acid concentrations were found in the following cortical regions: Brodmann areas 4, 6, 9, 17, 20, 21, and 22. Reductions were as large as 75%. Kynurenine was also reduced in a few regions. Tryptophan showed no significant changes. Smaller reductions in caudate, cerebellum and Brodmann area 10 did not reach significance. No significant reductions in kynurenic acid were found in the AD and PD patients. Kynurenic acid was significantly reduced in HD CSF. Significant changes in guanosine concentrations and tyrosine/xanthine ratios were found in several regions amongst the various disorders. These findings show a widespread deficiency of kynurenic acid in HD. Since kynurenic acid is an antagonist of NMDA receptors, a deficiency could contribute to the pathogenesis of neuronal degeneration in HD.

32.15

DIFFUSION CHARACTERISTICS OF INTRASTRIATALLY INJECTED β-ENDORPHIN ANALYZED BY IMMUNOCYTOCHEMISTRY AND CONFOCAL LASER MICROSCOPY WITH 3-DIMENSIONAL RECONSTRUCTION: RELATIONSHIP TO VOLUME TRANSMISSION

B.Bjelke and K.Fuxe, Dept.of Histology & Neurobiology, Karolinska Institutet, 104 01 Stockholm, Sweden.

In previous work using intrastriatal adenopituitary transplants (Bjelke.B et al. Neurosci. Lett. 93:1988, 107:1989) it was evident that extracellular concentrations of dopamine could inhibit the release of prolactin from the transplant. Prolactin diffused into the striatal neuropil for distances up to 1.5 mm. In the present study immunocytochemistry show that intrastriatally injected (inj) β-enorphin (β-End) (1 μg/1 μl) not only diffused into the adjacent neuropil but was also taken up into discrete nerve cell populations in neostriatum and far away located nerve cells in the basal layers of the piriform cortex. β-End immunoreactivity (IR) was present within the cytoplasm of these cellbodies and their dendrites. Following inj of β-End into the external capsule diffusion was observed along the extraneuronal space surrounding the myelinated axons radiating into the cerebral cortex. Some scattered pyramidal cellbodies were surrounded by strong β-End IR immediately adjascent to the nervecell membrane probably located within the extrapericaryal space. Following an intraventricular inj of β-End (0.1 μg) only some tanycytes of the median eminence (ME) became strongly IR with visualization of their processes all the way from the ventricular wall into the external layer of the ME. Thus, diffusion of peptides is not only dependent upon tortuosity and diffusion coefficient of the peptide but also on the ability of certain nerve cells to take up and inactivate peptides. -The extracellular space surrounding the fiber bundles appear to give rise to anisotropic diffusion patterns. -The tanycytes in the ME may participate in the transport of large peptides from the CSF into the primary capillary plexus of the ME and also into the surrounding medianosomes. -The present results give new aspects on the transport pathways for volume transmission.

32.16

HEMICHOLINIUM-3 BINDING IN SYNAPTOSOMAL PROTEINS FROM LIMULUS CENTRAL NERVOUS TISSUE C. Torrence-Campbell, G.A. Lartey*, J.G. Townsel, Department of Physiology, School of Medicine, Meharry Medical College, Nashville, TN 37208.

In our effort to isolate and characterize the high affinity choline transporter we report here on hemicholinium-3 (HC-3) binding in protein fractions prepared from a crude synaptosomal preparation from *Limulus* central nervous tissue. HC-3 is a specific inhibitor of the High Affinity Choline Uptake System (HAChUS). Our laboratory has demonstrated a HAChUS in *Limulus* that is significantly inhibited by micromolar concentrations of HC-3. This has been demonstrated in both whole tissues and synaptosomes (Sukumar,**et al.,Comp.Biochem.Physiol.75**:317,1983, Ivy,**et al,Comp.Biochem.Physiol.86**:103,1987). Since HC-3 specifically binds but is not tranpsorted by the HAChUS, the HC-3 binding site is thought to be on or very closely associated with the high affinity choline transport protein. Ammonium sulfate concentrations from 30 to 70 per cent were used to fractionate proteins from crude synaptosomal homogenates prepared from pooled circumesophageal ring glands and abdominal ganglia. The fractions containing bound [^{3}H]HC-3 were seperated by polyacrylamide gel electrophoresis and visualized by fluorography. Labeled bands corresponding to HC-3 binding sites had a molecular mass similar to that of the HC-3 binding proteins described by Knipper **et al.,(Neurochem.Int.14**:211,1989). We also demonstrate that binding of [^{3}H]HC-3 can be blocked N-ethylmaleimide (NEM). This HC-3 binding site will be utilized in protocols aimed at isolating the high affinity choline transport protein for molecular characterization. (Supported by the MBRS Grant RR08037 and the NSF/MRCE Grant 871485).

32.17

ANALYSIS OF RATES OF ELECTRON TRANSFER BETWEEN ASCORBIC ACID AND CYTOCHROME b_{561} IN SECRETORY VESICLES. Patrick M. Kelley, Vishram Jalukar* and David Njus*, Dept. of Biological Sciences, Wayne State Univ., Detroit, MI 48202.

Chromaffin vesicles from the adrenal medulla and secretory vesicles from the neurohypophysis contain monooxygenases involved in the synthesis of catecholamines and/or neuropeptides. These enzymes require reducing equivalents which, *in vivo,* are provided by ascorbic acid. Internal ascorbate is maintained by transferring reducing equivalents from external ascorbate across the vesicular membrane through a transmembrane protein, cytochrome b_{561}. Electron transfer between the cytochrome and ascorbate/semidehydroascorbate seems to occur via bimolecular reactions characterized by first-order rate constants. The rate constant for the reduction of cytochrome b_{561} by external ascorbate measured by stopped-flow is 450 (±190) $M^{-1}sec^{-1}$ at pH 7.0 and increases slightly with pH. The rate constant for oxidation by external semidehydroascorbate is 1.2 (±0.5) x 10^6 $M^{-1}sec^{-1}$ at pH 7.0 and decreases strongly with pH. The strong pH-dependence of the rate constant for oxidation can be explained by a pH-dependent membrane surface charge. The pH dependence of the rate constant for reduction can be rationalized by the greater reducing power of the ascorbate dianion. The rate constants are consistent with the midpoint reduction potential of cytochrome b_{561} and suggest that the cytochrome is maintained in a reduced state poised to reduce internal semidehydroascorbate as it is formed by intravesicular monooxygenases. Supported by NIH grants GM30500 and GM33849.

32.18

STIMULATION OF ^{45}Ca INFLUX IN FRTL-5 CELLS BY MONENSIN. M.L. Koenig, I.D. Gist*, and R.C. Smallridge* Divisions of Neuropsychiatry and Clinical Physiology, Walter Reed Army Inst. Research, Washington, DC 20307.

It has been reported recently that Na^+ currents can alter the Ca^{2+} homeostasis of isolated cardiac myocytes by a Na^+-Ca^{2+} exchange-mediated increase in Ca^{2+} influx (Science 248, 372-376, 1990). We have found that at least part of the Ca^{2+} uptake into FRTL-5 cells which have been exposed to the Na^+ ionophore monensin can be attributed to a similar reversal of the Na^+-Ca^{2+} exchange process. Under physiological conditions (135 mM NaCl; 1.3 mM $CaCl_2$), monensin has no measurable effect on ^{45}Ca influx in suspended FRTL-5 cells. However, if the Na^+-Ca^{2+} exchange protein is "primed" by reducing the extracellular Na^+ concentration to 67.5 mM (with an equimolar substitution of choline chloride), monensin (500 uM) promotes a 296 ± 33 percent increase in the uptake of ^{45}Ca uptake measured after 10 min. When extracellular Na^+ is completely removed, monensin (500 uM) is still able to elicit a significant increase in the amount of ^{45}Ca influx. This suggests that the effect of monensin on Ca^{2+} fluxes in FRTL-5 cells is not solely due to the promotion of Na^+ entry. We are investigating the possibility that monensin-induced changes in intracellular pH (BBA 1051, 242-249, 1990) may also play a role in the alteration of Ca^{2+} homeostasis in these cells.

OTHER BIOGENIC AMINES AND PURINES

33.1

cDNA CLONING AND SEQUENCE DETERMINATION OF OCTOPAMINE LIGAND BINDING PROTEINS. L. Kantham,* E. Hunnicutt, and J.A. Nathanson. Dept. of Neurology, Harvard Medical School and Neuropharmacology Research Lab., Massachusetts General Hospital, Boston, MA 02114.

Octopamine (OA) functions in invertebrates as a neurotransmitter and hormone with actions analogous to those which norepinephrine and epinephrine manifest in vertebrates. We have recently designed a photoaffinity probe for OA receptors which specifically labels three proteins in the firefly light organ, a tissue highly enriched in OA receptors. These are: A1 (MW 79 kDa), A2 (MW 75 kDa), and B (MW 73 kDa). A2 has recently been isolated and the first 20 amino acids of its N-terminal determined. Using oligonucleotides synthesized according to this sequence, a lambda GT10 cDNA library constructed from firefly tail mRNA was screened and 8 positive clones were isolated from 80,000 plaques. All of these contained identical inserts of approximately 2200 bp. On Northern blots, the common insert labeled an RNA band of 2300 bp from firefly lanterns but not from firefly abdomen, thorax, head, or from rat liver or brain. The insert was cloned into pCDM8 and sequenced, nucleotides 17-76 corresponding to the original protein sequence of the A2 band. The structure of the A2 clone will be described and its relationship to OA receptors discussed. In addition, the isolation of other putative OA receptor clones will be described.

33.2

AGE AND STRAIN RELATED CHANGES IN THE RAT MELATONIN RECEPTORS. J.T. Laitinen, M. Viswanathan and J.M. Saavedra. Section on Pharmacology, Lab. Clin. Sci., NIMH, Bethesda, MD 20892.

Apart from GTP and cation modulation of agonist binding to melatonin (MT) receptors, little is known about factors regulating these receptors. We have therefore assessed MT receptor status in the rat, using several experimental models and quantitative autoradiography in vitro.

Experiments planned to clarify short term homologous regulation (pinealectomy or MT injections) revealed no changes in MT receptor status in the area postrema (AP) and suprachiasmatic nuclei (SCN). On the contrary, age-related changes in MT receptor expression were evident in the AP, SCN, pituitary/pars tuberalis and in two recently identified (Viswanathan, M. et.al., this issue) MT receptor sites, the caudal artery and the arteries forming the circle of Willis.

A role for water and salt balance in the regulation of MT receptors in the AP is suggested based on selective upregulation of these receptors in spontaneously hypertensive rats only after manifestation of hypertension.

These and additional experimental models are likely to deepen our understanding on the factors regulating mammalian MT receptors.

33.3

PURIFICATION OF HUMAN BRAIN A_1 ADENOSINE RECEPTOR. H. Nakata, Lab. of Clinical Science, NIMH, Bethesda, MD 20892

A complete purification of A_1 adenosine receptor from rat brain membranes was recently reported using a novel affinity chromatography system (Nakata, H.,J. Biol. Chem. 264: 16545, 1989). In this study, purification of A_1 adenosine receptor from human brain membranes has been performed in order to obtain more knowledge about human adenosine receptors. The A_1 adenosine receptor was solubilized with digitonin from human cerebral cortex membranes and then purified more than 18,000-fold by sequential use of chromatographies on XAC-agarose, hydroxylapatite, re-XAC-agarose and TSK-3000. As assessed with a specific A_1 adenosine receptor antagonist [^{3}H]DPCPX (2nM), the membrane preparation, the digitonin-solubilized preparation and the final purified preparation showed the specific binding activity of 0.7, 1.3 and 13,000 pmol/mg of protein, respectively. SDS-PAGE of the final receptor preparation showed one predominant protein band of approximately 35 kDa with silver stain. Antibodies raised against purified rat brain A_1 adenosine receptor were also shown to react with the 35kDa band of the purified human A_1 adenosine receptor on the western blot. The ability to obtain highly purified human brain A_1 adenosine receptor should facilitate the precise screening of various drugs which act on adenosine receptors in human tissues.

33.4

A DISCRETE HIGH MOLECULAR WEIGHT COMPLEX CONTAINING THE ADENOSINE A_1 RECEPTOR SOLUBILIZED FROM RAT CEREBRAL CORTEX. M.R. Sherman, F.B. Tuazon* and M.N. Martin*. Dept. of Biological Sciences, Rutgers University, Newark, NJ 07102.

There is general consensus regarding the binding specificity, inhibition of adenylate cyclase and electrophysiological responses mediated by adenosine A_1 receptors. Reported values of the physicochemical parameters, however, differ widely. Values of M_r vary from 34-38 kDa for the purified ligand-binding unit to *c.* 60 kDa for receptors studied by radiation inactivation *in situ*. We have characterized the A_1 receptors solubilized from rat cerebral cortex by glycerol gradient centrifugation and Agarose gel filtration in buffers containing 2.5 mM $MgCl_2$, protease inhibitors and various detergents. Receptors were labeled with ^{3}H-*R*-phenylisopropyladenosine and stripped of unbound ligand on Sephadex LH-20 columns. Solubilization in 24 mM CHAPS and analysis in 4 or 24 mM CHAPS revealed highly aggregated receptor forms ($s_{20,w}$ = 24-28 S). Combinations of CHAPS and digitonin (Dig.) released mixtures of receptor forms (4-11 S). Solubilization in 1% Dig. and analysis in 0.2% Dig. revealed a single discrete complex ($s_{20,w}$ = 14 S, Stokes radius = 6.1 nm). For a protein of normal density, these parameters correspond to M_r = 360 kDa. Others have shown that Dig.-solubilized receptor forms contain G-protein(s). Our results indicate that the 14 S complex must contain more than one receptor and one G-protein (*c.* 88 kDa). The discrete form and the stability of this complex suggest that it is not the product of adventitious association following solubilization.

33.5

CHARACTERIZATION OF ADENOSINE A_1-RECEPTORS IN BOVINE RETINAL MEMBRANES C. Blazynski and C.L. Woods.* Depts. of Biochemistry & Molecular Biophysics, Washington University School of Medicine, St. Louis, MO 63110

Using retinas prepared from freshly dissected bovine eyes, we have characterized the binding of the A_1- selective agonist, [^{3}H]-PIA. Specific binding was linear over a range of membrane proteins from 0.10 to 1.0 mg, and accounted for an average of 90% of the total binding. At room temperature (24° C), binding reached equilibrium at 60 minutes, and was reversible upon addition of an excess of cold ligand. Saturation analysis and Scatchard transformation revealed two apparent populations of receptor binding sites. The higher affinity site exhibited a K_d of 0.134 ± .007 nM and B_{max} of 26.18 ± 3.06 fmole/mg protein. The lower affinity site exhibited a K_d of 21.83 ± 4.39 nM and B_{max} of 53.94 ± 15.8 fmole/mg protein. Using 0.3 nM [^{3}H]-PIA, kinetic analysis of association and dissociation rates yielded a calculated affinity constant of 0.2 nM, in agreement with saturation experiments. Competition studies with a number of purine nucleoside agonists and antagonists were performed, using radioligand concentrations of 1 nM or less to examine binding at the high affinity site, and revealed a rank order of potency consistent with the reported pharmacology of A_1 receptors. We have also assayed for adenylate cyclase activity in this same preparation and determined that PIA inhibited forskolin-activated adenylate cyclase in a dose-dependent manner. Maximum inhibition (40%) was observed with 1 nM PIA, and 10 μM CPDPX completely antagonized this modulation by PIA. Similar results have been reported previously for homogenates of rabbit retina (J. Neurosci. 7:2522).
Supported by EY02294.

33.6

DIFFERENTIAL EFFECTS OF A GUANINE NUCLEOTIDE AND A CATION ON ADENOSINE RECEPTOR AGONIST AND ANTAGONIST BINDING. J.Deckert, P.F. Morgan, J.C.Bisserbe*, K.A.Jacobson*, K.L.Kirk*, J.W.Daly* and P.J.Marangos . NIMH and NIDDK, NIH, Bethesda, Md. 20892.

Guanine nucleotides have been demonstrated to decrease adenosine receptor agonist binding, cations in general increased it in brain membranes. Antagonist binding appeared not affected (Goodman, R.R. et al., Mol.Pharmacol., 21:329, 1982).
With the availability of high affinity antagonist radioligands (Jacobson, K.A. et al., PNAS U.S.A., 83:4089, 1986) we decided to investigate the effects of 100 uM Gpp(NH)p and 2 mM $MgCl_2$ on adenosine receptor antagonist and agonist binding in brain slices using as radioligands (^{3}H)XAC and (^{3}H)CHA.
As expected Gpp(NH)p decreased and $MgCl_2$ increased (^{3}H)CHA binding while Gpp(NH)p had no effect on (^{3}H)XAC binding. Surprisingly, however $MgCl_2$ decreased (^{3}H)XAC binding.

33.7

NEONATAL CAFFEINE EXPOSURE ALTERS ADENOSINE A1 RECEPTOR KINETICS DURING A CRITICAL DEVELOPMENTAL PERIOD. R. Guillet. Dept. Psychol., Univ. of Rochester, Rochester, NY 14627.

Neonatal caffeine exposure of rats over postnatal d2-6, which mimics therapeutic caffeine exposure in the premature human infant with apnea of prematurity, has been shown in this laboratory to result in lasting effects on adenosine A1 receptor kinetics and on behavioral responses to acute treatment with caffeine, an adenosine antagonist, and with D-phenylisopropyl adenosine (D-PIA), an adenosine agonist. Specifically, control 18d pups responded to acute caffeine injection with an increase in activity, whereas neonatally caffeine-exposed rats did not. Initial kinetic studies done on tissue from 14, 21, 28d (and older) rats suggested no change in binding affinity but an increase in receptor density in cortex (CTX), cerebellum and hippocampus at these ages.

In the present study,cortical tissue from 18d rats that had been unhandled (NH) or exposed to caffeine (C) over postnatal d2-6, at doses yielding serum caffeine levels of 4-15 mg/L over the 24h period, was assayed for specific binding of 3H-cyclohexyl adenosine. While specific binding in 18d NH CTX was not different from 14d or 21d NH CTX, specific binding was significantly lower (p=.006) in CTX from 18d C pups compared with that in CTX from 14 and 21d C pups or that in 18d NH CTX. Furthermore, kinetic analysis demonstrated that this effect was due to a significant decrease (p<.0001) in binding affinity (Kd: NH=.78±.07; C=3.63±.20). It thus appeared that the A1 adenosine receptors in 18d C pups were primarily in a lower affinity state than in 18d NH pups. Analysis of other brain regions is currently underway.

The developmental period between 15 and 18d appears to be a critical period for the appearance of a stimulatory effect of caffeine in control rats and earlier caffeine exposure may interfere with receptor changes that take place over this period.

Supported by NIH grant no. HD22782.

33.8

TENTATIVE SUBCLASSIFICATION OF THE ADENOSINE A1 (AD A1) RECEPTOR IN MAMMALIAN HIPPOCAMPUS. H.L. Wiener, B. Craddock-Royal* and S. Maayani. Coll. Pharmacy, St. John's Univ., Jamaica, NY 11439 (H.L.W.), and Depts. Anesthesiol. (H.L.W., B.C.-R., and S.M.) and Pharmacol. (S.M.), Mt. Sinai Sch. Med., CUNY, NY, NY 10029.

The reported species-related diversity of the adenosine A1 (AD A1) receptor may be accounted for by the existence of different receptor subtypes across the species. Classification of receptor subtypes by pharmacological methods can be accomplished by determination of affinity constants of antagonists (but not agonists) as they should display the same affinity for the multiple receptor affinity states. Dissociation constants (Kd) of 8-cyclopentyltheophylline (CPT) and 8-cyclopentyl-1,3-dipropylxanthine (CPX) were determined in membrane preparations from bovine, rat, and guinea pig hippocampi. Three populations of the AD A1 receptor affinity states were assayed: 1. the low affinity agonist state (L) by assaying adenylyl cyclase (AC) activity: AD A1 agonists inhibited forskolin-stimulated AC activity and Kd values of antagonists were calculated from the dextral shifts of concentration-response curves. 2. High-affinity, 5'-guanylylimidodiphosphate-insensitive agonist state (H) by [3H]R-PIA binding. Binding curves of antagonists for these sites exhibited simple competitive behavior. 3. L and H affinity states by [3H]CPX. Antagonists competed for these sites in a simple competitive manner while agonists exhibited a shallow curve, best described by two affinity states. In a given species, the Kd values of CPT and CPX were independent of the population of the affinity state. Across species the Kd values varied over a log unit, being the lowest in the bovine. These data suggest that the observed species related diversity of the AD A1 receptor can be explained by subclasses that exist across species. (US PHS GM 34852).

33.9

MODULATION OF MOSSY FIBER INPUT TO CA3 HIPPOCAMPAL PYRAMIDAL CELLS BY ADENOSINE AND HIGH FREQUENCY STIMULATION
C. Janusz[1], M.F. Jarvis[2] & R. F. Berman[3]. Depts. [1]Physiol. & [3]Psych., Wayne State Univ., Detroit, MI and [2]CIBA-GEIGY Corp., Summit, NJ.

The hippocampal mossy fiber system provides a major excitatory synaptic input to CA3 pyramidal neurons. The aim of the present study was to examine adenosinergic modulation of synaptic input into area CA3 of the hippocampus using the hippocampal slice preparation. Extracellular field potentials were recorded from CA3 pyramidal cells while stimulating the stratum radiatum of CA3 in the region of the hilus. Current-source density analysis showed regional differences in the appearance of the field potentials elicited from each of the subdivisions of CA3. Also, high frequency mossy fiber stimulation elicited at least two forms of LTP; one rapid and one slowly developing. Superfusion with adenosine produced a dose-dependent reduction in the amplitude of the evoked EPSP and of the population spike (PS). Addition of theophylline to the perfusate produced a dose-dependent enhancement of the field EPSP and PS. Application of the A2 specific agonist CGS-21680 using the interface slice preparation also resulted in a dose-dependent reduction of the field EPSP and PS which could be reversed by application of theophylline. These results suggest both A1 and A2 inhibitory modulation of hippocampal synaptic activity. (Supported by NIH Grant No. RR-08167).

33.10

INHIBITION OF SYNAPTIC POTENTIALS BY ENDOGENOUS ADENOSINE IN HIPPOCAMPAL SLICES FROM YOUNG AND AGED FISCHER-344 RATS.
Lisa A. Bauman and Valentin K. Gribkoff. CNS Pharmacology. Dept. 404, Bristol-Myers Squibb Pharmaceutical Research Institute, 5 Research Parkway, Wallingford, CT 06492.

Endogenous adenosine has been suggested to play a potentially important tonic inhibitory role in the rat hippocampus. Little is known, however, about the integrity of this system in the hippocampus of aged rats. In these experiments, the degree of endogenous adenosinergic inhibition was assessed in hippocampal slices from young (1-3 mo) and aged (24-27 mo) F-344 rats. In normal medium, CA_1 population responses from slices from young rats were single peaks that increased in amplitude with increased stimulation currents. In slices from aged rats, high current levels produced multiple peaks, suggesting that cell excitability was enhanced. The specific adenosine A_1 receptor antagonist, 8-cyclopentyltheophylline (8-CPT; 250 nM-2 μM) produced an increase in the amplitude of synaptic responses in slices from both young and aged rats, but appeared to enhance excitability to a greater extent in slices from aged rats. Multiple peaks were observed at relatively low stimulus levels in slices from aged rats in 8-CPT, but the antagonist did not produce this response profile in slices from young rats even at high stimulus currents. These data suggest that aged rat hippocampal neurons may be more excitable than young counterparts and that the tonic inhibitory role of adenosine may be particularly important in the aged hippocampus.

33.11

CHARACTERIZATION OF EXTRACELLULAR ADENOSINE IN THE RAT STRIATUM WITH *IN VIVO* MICRODIALYSIS.
D.J. Fontana, A. Mele, M. Clark, R.M. Post and A. Pert. Biological Psychiatry Branch/National Institute of Mental Health, Bethesda, MD, 20892.

Although adenosine (ADO) is an important catabolite of ATP there are also indications that it may serve as a neuromodulator or neurotransmitter. The origin and processes underlying its extracellular levels in brain are not fully understood, however. The purpose of this study was to characterize the extracellular levels of ADO in the the rat striatum utilizing *in vivo* microdialysis. *In vitro* studies indicate that recovery of ADO through a 3mm microdialysis probe (CMA) perfused with artificial CSF at 2 μl/min was approximately 3%. The ADO levels in the dialysate from striatal perfusion were 41.4 nM immediately following probe insertion and decreased gradually over 3 hours. From 3-7 hours ADO levels remained stable at approximately 2.5 nM in the dialysate. These data suggest the basal levels of ADO in the striatal extracellular space are approximately 83 nM. Basal levels of ADO were not altered following pefusion with artificial CSF containing 10 uM TTX or with Ca^{++}-free aCSF containing high levels of Mg^{++} or Co^{++}. Addition of high concentrations of K^+ to the perfusion medium enhanced striatal ADO levels by 439%. This K^+-evoked ADO release was attenuated by both TTX and Co^{++} treatments. These findings suggest that whereas basal levels of striatal ADO are not derived from exocytotic release, the K^+-evoked release of ADO may be.

33.12

ADENOSINE AGONISTS AND DOPAMINE ANTAGONISTS BLOCK THE CONTRAVERSIVE CIRCLING BIAS INDUCED BY UNILATERAL INTRASTRIATAL CAFFEINE.
S. A. Josselyn and R. J. Beninger. Dept Psychol, Queen's University, Kingston, K7L 3N6, Canada.

Although caffeine is generally classified as a psychomotor stimulant, the neurotransmitter systems mediating its behavioral effects have not yet been established. Evidence suggests possible involvement of adenosinergic and/or dopaminergic (DA) systems. Previous evaluation has shown that unilateral intrastriatal microinjections of caffeine (10.0 ug) produced a contraversive circling bias in otherwise intact rats (Beninger and Josselyn, Soc. Neurosci. Abstr., 1989). To evaluate the neurotransmitter systems involved in this, three experiments examined the effect on circling behavior of 1) 2-chloroadenosine (2-CADO; an adenosine agonist) alone, 2) caffeine and 2-CADO co-injection and 3) caffeine and co-injection of the DA receptor antagonist, cis-flupenthixol. Each experiment consisted of seven test sessions; the first and seventh were preceded by no treatment, the second and sixth by a control microinjection (either saline or cis-flupenthixol) and the third, fourth and fifth by drug microinjections. Results showed that 2.0, 5.0 but not 1.0 ug doses of 2-CADO produced an ipsiversive circling bias, opposite in direction to that produced by caffeine. Rats pre-treated with central 2-CADO or cis-flupenthixol (in doses that did not influence circling bias when administered alone) prior to caffeine (10.0 ug) failed to exhibit a contraversive bias. Results strongly suggest that the effects of intrastriatal caffeine on motor behavior are mediated by antagonism of endogenous adenosine which, in turn, functionally increases DA. *Supported by NSERC.*

33.13

THE CONTRIBUTION OF ADENOSINE TO BRADYKININ-INDUCED PLASMA EXTRAVASATION IN THE RAT KNEE JOINT.
Paul G. Green, Allan I. Basbaum and Jon D. Levine*
Dept. Medicine, UCSF, Box 0452A, San Francisco, CA 94143.

The potent inflammatory mediator bradykinin (BK) produces plasma extravasation (PE) in the knee joint of the rat that is dependent on intact sympathetic postganglionic neuron (SPGN) terminals (*J. Neurophysiol.* **62**:48, 1989). Although BK releases a variety of mediators from SPGN terminals, it is not known which of these mediators contributes to PE. We have examined the contribution of adenosine, a co-transmitter stored in and released from SPGNs, to BK evoked PE in the rat knee joint.

Male rats were anesthetized and injected i.v. with Evan's blue dye. The knee was continuously perfused with saline, and baseline PE levels were established. BK alone or with adenosine agonists or antagonists were then added to the perfusion fluid. PE was quantified by measuring Evan's blue concentration in the perfusate (absorbance at 620nm).

The A_2 selective agonist, CGS21680, enhanced BK PE; the A_1 agonist, CPA, antagonized BK PE. Inhibition of adenosine metabolism (with deoxycoformycin) and uptake (with dipyridamole) also enhanced BK PE. We conclude that SPGN-derived adenosine contributes to neurogenic inflammation. *Supported by NIH grant AM32634*

33.14

IDENTIFICATION OF MULTIPLE H_3 HISTAMINE RECEPTOR SUBTYPES.
R.E West, Jr., A. Zweig*, M.I. Siegel*, R.W. Egan* and M.A. Clark*. Department of Allergy and Immunology, Schering-Plough Research, 60 Orange Street, Bloomfield, NJ 07003.

We have characterized binding to the rat brain H_3 histamine autoreceptor with the agonist [^{3}H]N$^\alpha$-methylhistamine ([^{3}H]NAMHA). [^{3}H]NAMHA saturation binding and NAMHA inhibition of [^{3}H]NAMHA binding were consistent with an apparently single class of receptors (K_D = 0.37 nM, B_{max} = 73 fmol/mg protein). Competition assays with other agonists and the antagonists impromidine and dimaprit also disclosed only a single class of sites. In contrast, the specific, high-affinity, H_3 antagonist thioperamide revealed two classes of sites (K_{iA} = 5 nM, B_{maxA} = 30 fmol/mg protein; K_{iB} = 68 nM, B_{maxB} = 48 fmol/mg protein.) The distinction between H_{3A} and H_{3B} receptor subtypes, the former a high-affinity and the latter a low-affinity thioperamide site, draws support from published in vitro data.

33.15

THE EFFECTS OF HISTAMINE ON THE MEMBRANE POTENTIAL OF RAT MAGNOCELLULAR SUPRAOPTIC NEURONS RECORDED INTRACELLULARLY *IN VITRO*. B.N. Smith and W.E. Armstrong. Dept. of Anat. Neurobiol., Univ. Tenn., Memphis, The Health Science Center, Memphis, TN 38163.

The effects of histamine on magnocellular neurons in the rat supraoptic nucleus were investigated using intracellular recording techniques from the hypothalamo-neurohypophysial explant. Exogenous application of histamine depolarized 75% of the neurons and hyperpolarized the remaining 25%, with no predictable effect on input conductance. Interestingly, the neurons which were depolarized had resting potentials more negative than -45 mV (-45 to -80 mV), whereas the hyperpolarized neurons had resting potentials between -40 and -45 mV. Depolarizations exhibited a rapid onset and slow recovery, while hyperpolarizations generally exhibited a slower onset and were occasionally preceded by a small, transient depolarization. Tetrodotoxin (1 µM) failed to block histamine's effect on membrane potential.

Neurons which depolarized in response to histamine exhibited a decrease in the amplitude of the afterhyperpolarizing potential following individual spikes or current-evoked trains of spikes, and an increase in the depolarizing afterpotential following current-evoked trains of spikes, when measured at the pre-histamine membrane potential. Opposite effects on afterhyperpolarizing potentials and depolarizing afterpotentials were exhibited by neurons which were hyperpolarized by histamine.

The present findings indicate that histamine directly depolarizes or hyperpolarizes magnocellular supraoptic neurons and affects the afterpotentials which shape the firing patterns of these neurons. Supported by NIH grant #NS23941 and the Neuroscience Center of Excellence.

33.16

Voltage-dependent, Ca2+-activated Outward Potassium Currents in Explanted Cells of Neonatal Swine Carotid Arterial Smooth Muscle. Hossein Hashemzadeh-Gargari, Yong I Kim and Christopher M. Rembold From the Division of Cardiology, Departments of Medicine, Biomedical Engineering, and Physiology, University of Virginia Health Sciences Center, Charlottesville, VA 22908, USA

Using the patch-clamp technique, we measured whole-cell K+ currents (Ik) in neonatal (1-5 days) swine carotid arterial smooth muscle explanted cells and determined their biophysical and pharmacological characteristics. The identity of the cultured smooth muscle cells was determineed by immunocytochemical staining of the cytoskeleton using rhodamine-phalloidin and antibodies against smooth muscle myosin, desmin and actin. The cell length of explanted smooth muscle in M199 medium ranged 150-200 µm in cell culture and had a resting potential of about -40 mV in different passages. K+ currents from the cells held at -80 mV and depolarized to -30 to +120 mV with a pulse duration of 60 ms exhibited exponential rise with little inactivation. The total outward K+ current was variable, but reached up to 3 nA at 120 mV. These K+ currents appeared to at least partially consist of Ca2+-activated currents since replacement of Ca2+ with 1 mM Co2+ and 11 mM EGTA resulted in an approximately 40% reduction in Ik. The outward K+ current was reduced by external bath application of tetraethylamonium (10 mM) or 4-AP (5 mM). Histamine (100 µM) increased the total amplitude of the K+ currents in neonatal explanted smooth muscle cells. Histamine also transiently increased intracellular [Ca2+] as measured with INDO-1 at 37 0C. These data suggest that a Ca2+-dependent outward K+ current exists in swine carotid cells and this current is activated by histamine.

33.17

RECOGNITION OF H_2 AGONISTS AND ANTAGONISTS BY [^{3}H]HISTAMINE-LABELED SITES IN GUINEA-PIG CEREBRAL CORTEX. William G. Sinkins* and James W. Wells. Faculty of Pharmacy, University of Toronto, Toronto, Ontario, Canada M5S 2S2.

Tritiated histamine and H_2-specific ligands distinguish between at least two states of putative H_2 receptors in membranes from guinea-pig cerebral cortex. GMP-PNP promotes a conversion of sites from higher to lower affinity for histamine, with no change in the equilibrium dissociation constant for either state (log K_1 = -8.46, log K_2 = -6.54). The labeled sites thus resemble G-linked receptors. Among 12 agonists tested, apparent binding affinity for the appropriate state correlates with H_2 functional potency in guinea-pig right atrium ($P = 0.0045$); the labeled sites thus reveal a pharmacological specificity characteristic of H_2 receptors. There is no correlation in a similar comparison with H_2 antagonists, which nevertheless appear to recognize the labeled sites as H_2 receptors: inhibitory behavior at two concentrations of [^{3}H]histamine shows that four antagonists and the weakly efficacious agonist impromidine reveal the reverse preference ($K_2 < K_1$) to that of histamine and four other agonists ($K_1 < K_2$) for the labeled sites. Such a reverse preference is typical of those G-linked receptors that reveal a dispersion of affinities for antagonists. Among H_2 antagonists, the ratio of affinities for the two states (K_1/K_2 = 4–250) is comparable to that observed for agonists (K_2/K_1 = 4–600); in other systems, the ratio for antagonists typically is much smaller than that for agonists. Ligand-dependent differences in the inferred affinity of histamine and in the apparent distribution of sites between the two states suggest that inhibition of the radioligand is at least partly noncompetitive.

33.18

BRAIN L - HISTIDINE DECARBOXYLASE (HDC) MEASUREMENT BY CO_2 TRAPPING. H. Kishikawa,* S. Willinger,* S. Jackowski,* and L. B. Hough, Dept. Pharmacol. Toxicol. Albany Medical College, Albany, NY 12208.

A convenient and sensitive method for measuring HDC in crude brain homogenates was developed by modification of existing CO_2 trapping methods. Crude homogenates prepared from male Sprague-Dawley rat hypothalamus containing 0.1 M sodium phosphate, pH 6.8, 0.2 mM dithiothreitol, 0.1 mM disodium EDTA and 1% polyethylene glycol - 300 were incubated (37°) in the presence of ^{14}C-L-histidine (0.1 uCi, 2.9 nmol) and pyridoxal phosphate (1 nmol) in a total of 0.1 ml. $^{14}CO_2$ was trapped and counted as described by Beaven et al.(Analyt. Biochem. 84:638, 1978). Hypothalamic homogenates (8 mg, 3.5 hr) typically yielded 630 - 860 cpm, with buffer blanks and boiled homogenates giving 80 - 125 cpm. Enzyme activity was linear with incubation time (0.5 - 4 hr) and tissue content (2 - 10 mg), and the crude enzyme was stable for up to 10 weeks at -80°. A-fluoromethylhistidine and a-hydrazino-histidine (HDC inhibitors, 10^{-4} M) inhibited activity by 95%, whereas chelidonic acid, difluoromethylornithine and a-methylDOPA (inhibitors of glutamate, ornithine and DOPA DC, respectively) had little or no effect. Results from substrate-velocity and brain regional studies are similar to previous findings with brain HDC measured by other methods (supported by DA-03816).

CATECHOLAMINES I

34.1

TYROSINE HYDROXYLASE mRNA IN THE ARCUATE NUCLEUS IS MORE ABUNDANT IN THE FEMALE THAN THE MALE RAT. S.L. Cottingham, K.J. Lookingland, and K.M. Moore. Dept. Pharmacology, Michigan State University, East Lansing, MI 48824.

The activity of tuberoinfundibular dopamine (TIDA) neurons is greater in female than in male rats. This difference may lead to differential expression of the gene for tyrosine hydroxylase (TH), the rate-limiting enzyme in catecholamine synthesis. The following experiment tests the hypothesis that TH mRNA is more abundant in TIDA neurons in female than in male rats. Males (n=4) and diestrous females (n=4) were decapitated and brains frozen on dry ice. Twelve µm frontal sections were taken from the rostral periventricular hypothalamus through the arcuate nucleus. In situ hybridization histochemistry was performed using a ^{35}S-labelled oligonucleotide probe against TH (gift from Brian Martin, NIMH). Emulsion autoradiograms were obtained from labelled sections and grains/cell were counted manually, at 400X magnification. For analysis, cell bodies in the arcuate nucleus (A_{12} cell group) were classified by anatomical location as periventricular or ventrolateral. The amount of TH mRNA in the periventricular arcuate nucleus was significantly higher in female than in male rats (30.9+1.8 v. 20.9+2.0 grains/cell). There were no female-male differences in the ventrolateral arcuate nucleus (22.9+3.8 v. 18.6+2.6 grains/cell) or the rostral periventricular hypothalamus (A_{14} cell group; 25.0+2.6 v. 23.8+1.8 grains/cell). These data suggest that the gender difference in the regulation of the TH gene may be due to a difference in the rate of activity of TIDA neurons. Ongoing studies will explore the role of prolactin in regulating TH gene expression in TIDA neurons. (Supported by ADAMHA grant MH 42802.)

34.2

EFFECT OF AGING ON TYROSINE HYDROXYLASE PROTEIN CONTENT AND THE NUMBER OF DOPAMINE NERVE TERMINALS IN HUMAN CAUDATE. M.E. Wolf, P.A. LeWitt, M.J. Bannon, L.J. Dragovic, and G. Kapatos. Center for Cell Biology, Sinai Hospital, The Cellular and Clinical Neurobiology Program, Wayne State Univ. Sch. of Med. and The Wayne County Medical Examiner's Office, Detroit, MI 48235.

To examine the effect of aging on dopamine (DA) nerve terminals in human caudate nucleus, two measurements were performed on post-mortem tissue obtained from normal subjects (1 mo to 63 yrs). First, a synaptosomal fraction was prepared and dopaminergic synaptosomes were selectively labeled with a monoclonal antibody to tyrosine hydroxylase (TH) and a fluorescent secondary antibody. Fluorescence-activated cell sorting was then used to identify dopaminergic synaptosomes and to quantify the percentage of caudate synaptosomes which were dopaminergic. Second, quantitative immunoblot studies were used to examine the concentration of TH protein in the same synaptosomal preparations. Our findings indicate that the normal aging process does not alter either the relative number of DA nerve terminals or the concentration of TH protein and therefore, by inference, the amount of TH protein per DA nerve terminal. The immunoblot studies identified 3 forms of TH monomer differing in molecular weight (60.6, 61.7 and 65.1 kDa), providing the first evidence that mRNAs coding for high molecular weight forms of TH may be actively translated in human brain. No age-related differences in the relative abundance of these forms were found. Supported in part by the United Parkinson Foundation (GK) and NS 27892 (PAL).

34.3

DOPAMINE RECEPTOR ANTAGONISTS RAPIDLY INCREASE AROMATIC L-AMINOACID DECARBOXYLASE (AADC) ACTIVITY, IN RAT STRIATUM. M. Y. Zhu, A V. Juorio,I. A. Paterson and A.A. Boulton, Neuropsychiatric Research Unit, M.R. Bldg, University of Saskatchewan, Saskatoon,Saskatchewan, Canada. S7N 0W0

Decarboxylation of phenylalanine by AADC is the rate limiting step in 2-phenylethylamine (PE) synthesis. Since neuroleptics increase striatal PE levels it suggested that this effect may be mediated by a change in AADC activity. It has been reported that D1 receptor antagonists increase AADC activity in the retina and recent investigations suggest that DA receptor blockers increase the rate of accumulation of PE in the striatum. The object of the present study was to investigate the effects of some DA receptor antagonists on AADC activity and to examine the interaction between AADC activity and PE synthesis in the striatum and nucleus accumbens. AADC activity was determined in rat brain homogenates by measuring the amount of DA formed from L-DOPA using HPLC-ED. The drugs SCH 23390 and pimozide were administered intraperitoneally 2 hours before death. SCH 23390, at doses of 0.01-1 mg kg^{-1} significantly increased AADC activity in the striatum (16-33 %). Pimozide (0.1-3 mg kg^{-1}) increased AADC activity in the striatum (25-41 %) and similar increases were observed in the nucleus accumbens. These findings suggest that AADC activity in the striatum is modulated by D1 and D2 receptors and indicate that the increase in the rate of accumulation of PE after administration of DA receptor blockers, may be related to the activation of AADC and this could have implications with respect to the mechanism of the effect of some neuroleptic drugs. Supported by Saskatchewan Health, Saskatchewan Health Research Board and the M.R.C. of Canada.

34.4

CHARACTERIZATION OF TWO cDNA FORMS OF BOVINE AND RAT DOPAMINE β-HYDROXYLASE. K.T. Kim, T. Wessel, K.S. Kim, C. Carver and T.H. Joh. Lab. Molec. Neurobiol., Cornell Univ. Med. College, The Burke Rehabilitation Center, White Plains, NY 10605.

Dopamine β-hydroxylase (DBH) is one of the key enzymes in catecholamine biosynthesis, catalyzing the conversion of dopamine to noradrenaline. This enzyme serves as specific marker protein for noradrenergic neurons. We have cloned DBH cDNA from bovine adrenal medullary cells (Soc. Neurosci. Abstr. 15:818, 1989) and rat PC-12 cells (FASEB J. 4:A318 1990). Both the bovine and rat DBH cDNAs have two forms that differ in the 3'-terminal region, as prevoiusly described for the human DBH cDNA (Kobayashi et al., Nucl. Acid. Res. 17:1089,1989). The bovine DBH messages were of 2.8 kb and 4.4 kb size, compared to the rat cDNA forms of 2.5 kb and 2.8 kb, seen on Northern blot analysis. The ratio of small and large message in bovine and rat adrenal gland was approximately 10:1. The two forms of DBH cDNA share the same coding sequences but have different 3'-untranslated regions that possess alternative polyadenylation sites. It is presently unknown whether the different mRNA sizes represent cytosolic and membrane-bound forms of DBH. Twenty-four hours after a single injection of reserpine (10 mg/kg) comparable increases in mRNA for both sizes were observed in the rat adrenal gland. In situ hybridization using a rat cDNA probe for DBH specifically localized to the locus coeruleus in central nervous system as well as the adrenal gland. Supported by MH44043.

34.5

INTERACTION OF NUCLEAR PROTEINS WITH THE PROMOTER REGION OF THE TYROSINE HYDROXYLASE GENE. A. W. Tank, L. H. Fossom, J. Best*, E. Lynd and R. Patil*, Department of Pharmacology, University of Rochester, Rochester, NY 14642.

The transcription rate of the tyrosine hydroxylase (TH) gene is influenced by a large number of extracellular stimuli including nicotinic receptor agonists, glucocorticoids and drugs that elevate cyclic AMP. These stimuli are thought to regulate TH gene expression by influencing the DNA binding properties and/or transcriptional regulatory activities of intracellular trans-acting protein factors that interact specifically with sequences within the TH gene. We have investigated the binding of nuclear proteins isolated from rat pheochromocytoma PC18 cells to the promoter region of the TH gene extending 149 bp upstream from the transcriptional initiation site. Using a gel shift assay we have identified PC18 cell nuclear proteins that apparently bind to three segments within this region of the gene; two proteins bind to the sequences between bp -149 to -106 (this region contains a consensus SP1 binding site); two proteins bind to sequences between bp -108 to -58; and three proteins bind to sequences between bp -60 to -27 (this region contains a consensus cyclic AMP response element). Competition studies suggest that at least 4 of these proteins bind specifically to these regions of the TH gene. Furthermore, treatment of the PC18 cells with cycloheximide for 4 hr prior to isolation of the nuclear proteins results in a decreased amount of binding for a number of these nuclear proteins. Since cycloheximide treatment also decreases basal transcription of the TH gene, these protein factors may be involved in maintaining the basal rate of transcription of the gene.
(Supported by AHA grant 89-050G and DA 07232.)

34.6

NICOTINE INCREASES TYROSINE HYDROXYLASE GENE TRANSCRIPTION IN RAT ADRENAL MEDULLA. L.H. FOSSOM AND A.W. TANK, Department of Pharmacology, University of Rochester, Rochester, NY 14642.

The amount of tyrosine hydroxylase (TH) increases in rat adrenal medulla after prolonged stress or treatment with catecholamine-depleting drugs. Recent studies have shown that TH-mRNA levels increase prior to the increase in TH enzyme levels. This induction of TH is blocked by pretreatment with nicotinic antagonists, suggesting that stimulation of nicotinic receptors on adrenal chromaffin cells may be a necessary step in the trans-synaptic induction of TH. In the present study the effects of acute treatment of rats with nicotine on TH enzyme activity, TH-mRNA levels and TH gene transcription rate have been investigated. The transcription rate of the TH gene (as measured by *in vitro* nuclear run-on assay) increased 2-3-fold 10 min after injection of 3.3 mg/kg nicotine, remained elevated for at least 1 h and returned to control level by 3 h. There was a dose-related increase in TH gene transcription rate 20 min after administration of 1.0-3.3 mg/kg nicotine. A single dose of nicotine (0.33-3.3 mg/kg sc) failed to alter TH enzyme or TH-mRNA levels, although doses of 1.0-3.3 mg/kg resulted in a dose-related activation of TH. Our results indicate that a single dose of nicotine rapidly stimulates TH gene transcription in rat adrenal medulla, but that the stimulation is transient. Apparently, an increase in gene transcription that persists for longer than 1 h is required to induce TH-mRNA and enzyme.
(Supported by AHA grant 89-050G and DA 07232.)

34.7

VIP, SECRETIN, AND PHI EFFECTS ON TYROSINE HYDROXYLASE GENE EXPRESSION IN PC12 CELLS. M. Wessels-Reiker, A.C. Howlett, and R. Strong. Departments of Pharmacology and Medicine, St. Louis University School of Medicine, and GRECC, St. Louis VA Medical Center, St. Louis, MO, 63125

As the rate limiting enzyme in catecholamine biosynthesis, tyrosine hydroxylase (TH) is important in determining sympathetic tone. The activity of TH is regulated in the short term by changes in the phosphorylation of the enzyme and in the long term by changes in expression of the tyrosine hydroxylase gene. Vasoactive intestinal peptide (VIP) has recently been established as a neurotransmitter within the splanchnic nerve which innervates the adrenal medulla (Wakade,89) and both VIP and secretin, homologous peptides within the glucagon family, have recently been demonstrated to elicit short term activation of tyrosine hydroxylase (Zigmond,89). We therefore undertook a series of studies to determine whether VIP, secretin, and peptide histidine isoleucine (PHI), another related peptide, could induce long-term changes in gene expression which parallel their short term effects on TH activity.

Experiments were performed in PC12 cells, a rat chromaffin cell line. Preconfluent cells were incubated 6 hours with various concentrations of hormone. Following total RNA extraction, Northern and slot blots were performed using a full length TH.36 cDNA probe. We found secretin to be more potent at low doses while VIP had a greater maximal effect. Our findings with these neuropeptides parallels what has been reported for their effects on short term activity, suggesting that TH phosphorylation and gene expression may be regulated by the same intracellular signals.

34.8

MULTIPLE NEUROTRANSMITTER SYSTEMS MEDIATE THE REGULATION OF TYROSINE HYDROXYLASE BY SPLANCHNIC NERVE ACTIVITY. J. Haycock[1], R. Shukla[2*], T. Wakade[2*] & A. Wakade[2]. [1]Dept. Biochem., LSU Med. Ctr., New Orleans, LA and [2]Dept. Pharmacol., Wayne State Univ. Sch. Med., Detroit, MI

Splanchnic nerve activation, which releases ACh and VIP, increases the phosphorylation and activity of tyrosine hydroxylase (TH) in rat adrenal medulla. Because TH is phosphorylated *in situ* at multiple sites (ser8, 19, 31 and 40), the site-specificity of the increases was determined and correlated with alterations in TH activity. Nicotinic, muscarinic and VIP receptor involvement was also studied.

Isolated rat adrenal glands were perfused retrogradely with Krebs solution (± 2 mCi $^{32}P_i$/ml) for 85 min, treated during the last 30-300 s (300 pulses at 1 Hz or 10 Hz; 3 μM nicotine, 100 μM muscarine, or 1 μM VIP for 180 s), and frozen on dry ice immediately thereafter. For ^{32}P incorporation, TH was immunoprecipitated from solubilized samples and isolated by SDS-PAGE. ^{32}P-TH was normalized to ^{32}P-total cellular protein and to TH protein levels, and ^{32}P-tryptic peptides were analyzed by HPLC. TH activity was measured in desalted extracts and normalized to TH protein levels. At 10 Hz, stimulation increased ^{32}P incorporation into ser19 and ser40 whereas at 1 Hz, ^{32}P incorporation into ser19, ser31, and ser40 was increased. VIP selectively increased ser40 phosphorylation. Nicotine increased ser19 and muscarine increased both ser19 and ser31 phosphorylation several fold; neither cholinergic agent produced comparable effects on ser40 phosphorylation.

All treatments increased TH activity (pH 7.2) at either 30 or 300 μM BH_4. VIP and 1 Hz stimulation produced the largest increases. 10 Hz stimulation produced an intermediate activation whereas nicotine and muscarine produced smaller increases. In pH-activity profiles (pH 6.0-7.1), activation by electrical stimulation and VIP was larger at the more neutral pHs.

Both cholinergic and VIP receptors mediate effects of splanchnic nerve activity on TH phosphorylation, but VIP may be more important in regulating TH activity.

34.9

INFLUENCE OF LONG-TERM NICOTINIC CHOLINERGIC STIMULATION OF ISOLATED CHROMAFFIN CELLS ON THE ACETYLCHOLINE-MEDIATED SHORT-TERM REGULATION OF TYROSINE HYDROXYLASE AND DOPAMINE SYNTHESIS. G.L. Craviso, J.B. Redell* and J.C. Waymire. Dept. Neurobiology and Anatomy, Univ. Texas Medical School, Houston, TX 77225.

We have previously reported that exposing isolated bovine adrenal chromaffin cells in culture to the nicotinic agonist dimethylphenylpiperidinium (DMPP; 1 uM) for 3 days leads to a 2-fold induction of tyrosine hydroxylase (TH) immunoreactive protein and enzyme activity, that this induction enhances the dopamine synthesis capacity of the intact cell severalfold and that dopamine synthesis rates are enhanced even further by acute stimulation of these induced cells with acetylcholine (Ach). To explore the basis of the effects of Ach on dopamine production after prolonged DMPP treatment, we examined the phosphorylation and activation of TH in induced cells in response to Ach and compared these results to those obtained in non-induced cells. As in our earlier studies characterizing the short-term cholinergic regulation of TH in these cells, stimulation of non-induced cells with 100 uM ACh for 30 sec led to the rapid activation of TH and increased phosphorylation of the enzyme on 3 RP-HPLC tryptic phosphopeptides (serines 19 and 40). This rapid response was followed by a later phosphorylation of 2 other RP-HPLC phosphopeptides (serine 31) which peaked by 4 min. In induced cells, neither the rapid activation of the enzyme nor the enhanced phosphorylation of serines 19 and 40 in response to Ach were apparent. In contrast, the later increased phosphorylation of the TH subunit on serine 31 following Ach stimulation was observed in induced cells. These results suggest the presence of two separate phosphorylative mechanisms which enhance dopamine synthesis in response to Ach and that chronic nicotinic cholinergic stimulation of the chromaffin cells results in only one of these mechanisms being expressed. Supported by USPH NS 11061 and NS 27550.

34.10

INFLUENCE OF OKADAIC ACID ON TYROSINE HYDROXYLASE ACTIVITY AND PHOSPHORYLATION IN ADRENAL CHROMAFFIN CELLS: COMPARISON WITH ACETYLCHOLINE (Ach) STIMULATION. R. Van Horn*, G.L. Craviso, and J.C. Waymire. Dept. Neurobiology and Anatomy, Univ. Texas Med. Sch., Houston, TX 77225.

Our recent studies have shown that the enhanced phosphorylation of tyrosine hydroxylase (TH) in bovine adrenal chromaffin cells in response to Ach occurs on serines 19, 31, and 40. The report by Haavik et al. (FEBS Letters 251:36, 1989) that treatment of chromaffin cells with the protein phosphatase 2A and 3A inhibitor, okadaic acid, leads to the increased phosphorylation of TH, prompted us to examine which TH sites are affected by this compound in the intact cell. Using 2 uM okadaic acid, we observed increased phosphorylation of serines 19, 31 and 40, those phosphorylated in response to Ach, as well as an increased phosphorylation of one additional site tentatively identified as serine 8. The phosphorylation of each of these serines occurred in a time-dependent manner, maximally increasing 4-fold over basal levels by 60 minutes. When measured at this time, in situ catecholamine synthesis rates were elevated 5-fold and TH catalytic activity 2-fold. Kinetic analysis showed an increased affinity of TH for the pterin cofactor with no change in the Vmax of the enzyme. This contrasts with the characteristics of the Ach-mediated phosphorylation of TH in which changes in both cofactor affinity and Vmax occur. These studies suggest that phosphatases 2A and 3A may be the major enzymes in the chromaffin cells which are responsible for the dephosphorylation of TH following the phosphorylation of the enzyme in response to Ach. Further, the kinetic properties of the enzyme under conditions in which serines 19, 31 and 40 are maximally phosphorylated, as observed in this study using okadaic acid treatment of the cells, are similar to those observed for the cAMP-mediated phosphorylation of serine 40, alone, rather than for the Ach-mediated phosphorylation of these three serines. That Ach causes a greater increase in the phosphorylation of serines 19 and 31 relative to serine 40 would explain this difference in the kinetic characteristics of the enzyme.

34.11

Amino acid sequence analysis of endogenously phosphorylated tyrosine hydroxylase (TH). J.C. Waymire[1], L. Denner[2], G.L. Craviso[1] and R. Van Horn[1*]. [1]Dept. Neurobiology and Anatomy, Univ. Texas Medical School, and [2]Dept. Cell Biology, Baylor College of Medicine, Houston , TX 77030.

Previous studies have shown that TH in bovine adrenal medullary chromaffin cells is phosphorylated on 7 tryptic phosphopeptides and that acetylcholine produces a phosphorylation of 5 of these (Waymire et al., JBC 263:12439, 1988). In the present studies amino acid sequence analysis of these tryptic peptides has been used to establish the number and location of phosphorylated serines within the TH subunit. Bovine chromaffin cells in culture were treated with 8-Br-cAMP for 3 days to increase the quantity of TH per cell, followed by a 60 min incubation with 2 uM okadaic acid to maximize the phosphorylation of the enzyme. TH was isolated using a 20-35% ammonium sulfate fraction of the chromaffin cell supernatant, subjected to SDS-PAGE, transferred to nitrocellulose, and digestied with trypsin (3:50 ratio trypsin:TH). Each tryptic phosphopeptide was purified using a combination of RP-HPLC with 0.1% TFA/acetonitrile and 0.1% HCl/acetonitrile. Comparison of the sequence of each phosphopeptide with the published sequence of bovine TH (De Mello et al., J. Neurosci. Res. 19:440, 1988) showed that the peptides eluting in the 3rd and 5th positions are serine 19, the peptide eluting 4th is serine 31, and the peptide eluting in the 6th position is serine 40. Peptides eluting in the 1st, 2nd and 7th positions were of insufficient quantity to be sequenced. These results are of particular interest when taken together with our previous analysis of the influence of cholinergic stimulation of TH showing that acetylcholine stimulates a large increase in the phosphate labelling of peptides 3, 5, 4, and 7 and only a small increase in peptide 6. This implies that serines 19 and 31 are involved in the cholinergic regulation of TH while serine 40 plays only a minor role. The protein kinase responsible for the phosphorylation of serine 19 is Ca/CaM kinase II. The protein kinase responsible for phosphoryating serine 31 remains unknown.

34.12

TYROSINE AND PHENYLALANINE AS SUBSTRATES FOR DOPAMINE SYNTHESIS IN PC12 CELLS. R.P.S. Kwok, F. R. DePietro*, J.C. Byrd, and J.D. Fernstrom. Department of Psychiatry, University of Pittsburgh, Pittsburgh PA 15213.

Phenylalanine (PHE) has been reputed to be both a substrate for and an inhibitor of tyrosine (TYR) hydroxylase. Previous work in striatal synaptosomes suggested PHE is an important substrate, with ≈1/3 of all dopamine (DA) deriving from PHE. However, only single TYR and PHE concentrations were studied, well below the enzyme K_ms and in vivo concentrations. We are therefore examining DA synthesis from ^{3}H-TYR and ^{3}H-PHE over a range of amino acid concentrations, initially in PC12 cells. The cells are grown under standard conditions, and used at 2x10^6 cells/ml. For an experiment, the growth medium (RPMI 1640, containing 10% horse serum/5% fetal bovine serum) is replaced with Krebs-Ringer bicarbonate buffer, pH 7.4, supplemented with 1-10 μCi ^{3}H-TYR or ^{3}H-PHE and different concentrations of cold TYR and/or PHE (0.1-100 μM). The cells are incubated at 37°C for 0-150 min, and then analyzed for DA by HPLC. Endogenous DA is detected by on-line electrochemical detection; fractions are collected to count ^{3}H-DA. ^{3}H-TYR conversion to ^{3}H-DA was found to be linear for 60-90 min. Using the 45 min timepoint, and varying cold TYR level, ^{3}H-DA synthesis increased with increasing TYR level up to 100 μM (the highest concentration thus far tested). ^{3}H-PHE was also clearly converted to ^{3}H-DA in the PC-12 cells; preliminary estimates indicate DA synthesis rate from PHE to be 15-20% that from TYR. The results suggest that, at least in this cell line, DA synthesis from PHE is clearly evident, and may be an important component of overall DA synthesis.

34.13

EFFECTS OF γ-AMINOBUTYRIC ACID RELATED DRUGS ON TYROSINE HYDROXYLASE ACTIVITY IN CULTURED BOVINE ADRENAL CHROMAFFIN CELLS. A. NAKANISHI, N. WEINER Dept. Pharmacol. University of Colorado Health Science Center., Denver, Co 80262.

We examined the effects of γ-aminobutyric acid (GABA) related drugs on tyrosine hydroxylase (TH) activity in order to elucidate the regulation of catecholamine biosynthesis by GABA receptors in cultured bovine adrenal chromaffin cells. Chromaffin cells were isolated by the method of Waymire et al. (J. Neurosci. Methods, 7:329, 1983) and were cultured for 3-7 days. GABA which has been reported to have a stimulatory action on the secretion of catecholamines from bovine adrenal chromaffin cells (Kataoka et al. J. Neurochem. 50:1765, 1988.) decreased the activity of TH in cultured bovine adrenal chromaffin cells (60% inhibition with 1mM of GABA). 4,5,6,7-tetrahydroisoxazolo [5,4-c] pyridin-3-ol (THIP) which has been reported to increase the release of enkephalin from intact adrenal gland (Fujimoto et al, J. Pharmacol. Exp. Ther. 243:195, 1987) also decreased the activity of TH in these cells (60% inhibition with 1mM of THIP). Moreover, baclofen which is $GABA_B$ agonist also decreased the activity of TH in cultured bovine adrenal chromaffin cells (30% inhibition with 1mM of baclofen). GABA and THIP inhibited the activity of TH in high K^+-stimulated chromaffin cells but baclofen does not. We are continuing the investigation of the mechanisms for this inhibitory effect of GABA related drugs on TH activity in cultured bovine adrenal chromaffin cells. (This research is supported by grant from the USPHS NS-09199).

34.14

INSULIN HYPOGLYCEMIA RAPIDLY INCREASES PHENYL-ETHANOLAMINE-N-METHYL TRANSFERASE (PNMT) IN THE BRAIN AND SPINAL CORD. T.D. Gbadebo*, S.A.Wagers*, and J.K. Stewart. Dept. of Biology, Virginia Commonwealth Univ., Richmond, Virginia 23284

Oscillation and immobilization stress, hypertension, and diabetes are associated with increased PNMT activity in the medulla/pons but not in the hypothalamus. In this study, male Sprague Dawley rats were sacrificed 4 hrs after a single injection of saline or insulin (10 U/kg body wt, s.c.). Insulin significantly elevated PNMT activity in the hypothalamus, medulla/pons and spinal cord:

TISSUE	SALINE	INSULIN	p≤
Spinal Cord	0.62±.08(7)	1.11±.16(7)	0.05
Medulla/Pons	2.35±.28(7)	3.71±.33(6)	0.01
Hypothalamus	4.3 ±.41(9)	7.9 ±.64(5)	0.001

Values=mean±S.E.M. (pmol/(60 min x mg protein)
The number of animals is in parentheses.

These findings suggest that insulin hypoglycemia is a potent and rapid stimulator of PNMT activity in the hypothalamus as well as the medulla and spinal cord.
Supported by NIH grant NS 26992

34.15

ALTERATION OF BRAIN NEUROTRANSMITTERS METABOLISM IN RATS CHRONICALLY EXPOSED TO ALCOHOL. M.C. YU and K.W. CHUNG*, Depts. of Anatomy, New Jersey Med. Sch., Newark, NJ 07103 and College of Med. Univ. of Oklahoma Health Sci. Center, Oklahoma City, OK. 73190.

The purpose of this study was to determine the alteration of several neurotransmitters and their metabolites in several brain regions after chronic ingestion of ethanol. Sixty days old male rats were fed a liquid diet for 3 months containing 36% of total calories as ehtanol or the isocaloric equivalent of sucrose. For control, another group of rats of similar age was fed a regular rat chow diet. Tissues were dissected out and homogenized in 10 volumes of IN perchloric acid containing 1 ng/µl DHBA as internal standard, centrifuged and filtered. The supernatant was used for quantitative assay by HPLC while the lower solid phase was used for protein analysis.

The norepinephrine levels in cerebrum, corpus striatum, hypothalamus and hippocampus were significantly higher in alcoholic rats than controls. The dopamine levels in the striatum and hippocampus were higher in alcoholic rats than controls, whereas in the cerebrum it was less than the controls. The 3,4-dihydroxyphenyl-acetic acid levels in the corpus striatum and hypothalamus showed no difference between the control and alcoholic rats, but in the cerebrum it was higher in the controls. The serotonin levels showed no difference between controls and alcoholic animals in the 4 brain regions examined.

34.16

ESTRADIOL MODIFIES THE EFFECT OF LITHIUM ON RAT BRAIN DOPAMINE METABOLISM. M. Morissette and T. Di Paolo. Dept of Molec. Endocrinology, CHUL Res. Centre, Laval Univ. Medical Centre, Québec, G1V 4G2 and Sch. of Pharmacy, Laval Univ., Québec, G1K 7P4, CANADA.

The effect of 17ß-estradiol(E2) on the response of dopamine (DA) to lithium in the brain of ovariectomized (OVX) rats was investigated. An E2 injection (100 ng,sc) left striatal DA levels unchanged while its metabolites levels, DOPAC and HVA, increased 30 min later. Injection of LiCl (10 mEq) decreased striatal DA levels while DOPAC levels as well as DOPAC/DA and HVA/DA ratios increased at 60 and 90 min and HVA levels slightly decreased at 30 and 120 min. A combined injection of LiCl (10 mEq) with E2 gave a similar effect in the striatum than LiCl alone but the response of LiCl increased at 30 min where the levels of DOPAC, HVA and DOPAC/DA and HVA/DA ratios were higher than those observed with LiCl or E2 alone. In the frontal cortex, an E2 injection left DA and HVA levels unchanged while DOPAC levels, DOPAC/DA and HVA/DA ratios increased 30 min later. An injection of LiCl (10 mEq) increased frontal cortex DA levels at 30 min and HVA levels at 90 and 120 min while DOPAC levels remained unchanged. DOPAC/DA and HVA/DA ratios decreased at 30 min but an increase of HVA/DA occured from 60 to 120 min. Injection of LiCl (10 mEq) with E2 enhanced the effect of LiCl in the frontal cortex on DA, DOPAC and HVA levels 60 min later. Our results indicate that E2 can modify the effect of lithium on brain DA metabolism. Supported by the MRC of Canada.

34.17

EFFECT OF REDUCED NICOTINAMIDE ADENINE DINUCLEOTIDE (NADH) ON MONOAMINE METABOLISM IN RAT BRAIN. A.B. Naini*, B.A. Haslund, L.J. Cote*, V. Jackson-Lewis*, J.L. Cadet*. Department of Neurology, College of P &S, Columbia University, New York, NY 10032

NADH given IV significantly ameliorates the symptoms of Parkinson's disease (Birkmayer et al, J Neural Transm 1: 297, 1989). In the present study we investigated the effect of NADH injected i.p. on regional monoamine metabolism.

Sprague-Dawley rats, 5 per group, received NADH (100mg/kg) i.p. and were decapitated at timed intervals after NADH administration. The striatum, nucleus accumbens and hippocampus were quickly dissected out and assayed for monoamines by HPLC-EC.

Effects of NADH on Regional Monoamines in Rat Striatum

	Control	30 min	60 min	120 min
DA	56.2 ±2	60.0 ±3	45.4 ±4*	42.5 ±2*
HVA	4.4 ±0.4	4.4 ±0.3	5.6 ±0.2*	5.6 ±0.3*
NE	2.4 ±0.3	2.7 ±0.2	1.8 ±0.2*	1.9 ±0.1

* Significantly different from control ($p<0.05$)

Results (table) show decreases in dopamine (DA) and norepinephrine (NE) with an increase in homovanillic acid (HVA) in the striatum. No changes were seen in the hippocampus or the nucleus accumbens. Serotonin was not affected in the regions studied. The mechanism by which NADH affects catecholamine metabolism is under investigation.

Supported by the Parkinson Disease Foundation, New York, NY 10032.

34.18

A TYROSINE HYDROXYLASE ASSAY IN MICROWELLS USING COUPLED NONENZYMATIC DECARBOXYLATION OF DOPA. **J.R. Bostwick*, W. Le*, and S.H. Appel. Dept. of Neurology, Baylor College of Medicine. Houston, Texas 77030**

A radiometric assay for tyrosine hydroxylase employing a coupled nonenzymatic decarboxylation of ^{14}C-L-dopa formed from ^{14}C-L-tyrosine has been adapted for performance in a 96 microwell culture plate. The method requires the use of an easily manufactured plate holder to compress blotting paper impregnated with methylbenzethonium hydroxide against the top rim of each well. This forms isolated, air-tight compartments in which $^{14}CO_2$ is evolved and quantitatively absorbed into the blotting paper. The method is sensitive enough to detect less than 0.3 x 10^{-6} units of enzyme activity. Tyrosine hydroxylase activity in homogenates of adult rat striata was linear with time (up to 25 minutes) and protein. The enzyme exhibited an apparent K_m of 155 µM for L-tyrosine. A major advantage of this system is that cells can be grown in tissue culture and subsequently assayed for tyrosine hydroxylase activity in the same well. A 3.8-fold increase in enzyme activity was measured in cultures of dissociated cells from rat ventral mesencephalon grown in a defined medium for six days. The method is more facile than previously devised procedures, allowing for the simultaneous assay of up to 96 samples totally contained in a single, compact, portable unit.

Supported by the American Parkinson's Disease Association

34.19

DETERMINATION OF DOPAMINE AND SEROTONIN IN SINGLE CELL CYTOPLASM BY CAPILLARY ELECTROPHORESIS. A. Ewing, T. Olefirowicz and P. Curry, Jr. Dept. of Chemistry, Eberly College of Science, Penn State Univ., Univ. Park, PA 16802.

Dopamine and serotonin concentrations have been measured in the cytoplasm of single invertebrate nerve cells. An injection scheme capable of acquiring picoliter volumes of cell cytoplasm and subsequently injecting these samples into an electrophoresis capillary has been developed. This injector is constructed at the end of a 5-µm diameter fused silica capillary column. An 8-10 µm tip is formed by removing the polyimide coating and then etching the fused silica in hydrofluoric acid. Electromigration is used to inject analytes into the injector tip and through the separation capillary. Attomole detection limits are obtained in volumes as small as 0.3 picoliters.

34.20

IMRPOVEMENTS IN NEUROBIOLOGICAL ANALYZER (NEUBA®) FOR RAPIDLY DETERMINING MULTIPLE NEUROCHEMICAL SPECIES, **D.J. TURK AND C.L. BLANK,** Dept of Chemistry and Biochemistry, U. of Oklahoma, Norman, OK 73019.

The determination of a wide variety of catecholamine, indoleamine and acetylcholine related neurochemicals and metabolites has been demonstrated using a novel Neurobiological Analyzer previously. This multiple column, multiple electrode system provides substantial amounts of quantitative and qualitative information useful in the analysis of a variety of interesting electroactive neurochemicals during a single 22-30 minute analysis.

The device consists of four individual liquid chromatographs connected to one autoinjector and one multiple potential-controlling, current-monitoring potentiostat. The three primary liquid chromatographs possess multiple amperometric electrochemical detectors to aid in qualitative identification of individual components and overall selectivity of determinations. The fourth primary system is devoted to the determination of acetylcholine, choline, and related species. Improvements in the data acquisition, hardware and software associated with this unit have resulted in a linear dynamic range of 6-7 orders of magnitude and detection limits for favorable species in the low femtomole range (at a S/N ratio of 2).

35.1

PRENATAL COCAINE PRODUCES LASTING CHANGES IN SEROTONIN AND CATECHOLAMINE FIBER ORGANIZATION IN RAT BRAIN.

H.M. Akbari and E.C. Azmitia.
Dept. of Biology, N.Y.U., New York, NY 10003.

Cocaine is neurotoxic to cultured fetal rat serotonin (5-HT) neurons (Azmitia et al., 1989). We examined the effects of prenatal exposure to cocaine. Pregnant rats were injected subcutaneously with either cocaine (40 mg/kg/2cc) or vehicle on days 13-16 of gestation. At 4 and 6 weeks postnatal, male pups were perfused and brains were processed for 5-HT and tyrosine hydroxylase (TH) immunocytochemistry. Decreases in 5-HT and TH immunoreactive (IR) fibers were found in the trigeminal motor nucleus and raphe magnus. 5-HT IR fiber density was decreased in the ventral tegmental area, the nucleus accumbens septi, and layer IV of cortex. An increase in TH IR fibers was observed in the cerebellum. Interestingly, increased 5-HT and decreased TH IR fiber densities were seen in the dorsal striatum. In the septohippocampal system, the density of 5-HT IR fibers was increased in the hippocampus, while TH IR fibers were decreased in the septum. No differences in either 5-HT or TH IR cell number were observed. We are in the process of doing biochemical assays to correlate with our anatomical findings. Our results indicate that cocaine is a potent drug for disrupting the normal balances between the serotonergic and catecholaminergic systems within the brain. The implications of these effects to the functional abnormalities seen in "crack babies" remains to be established.

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35.2

FUNCTIONAL CONSEQUENCES OF THE INTERACTION OF COCAINE WITH THE SEROTONIN TRANSPORTER. W.A. Wolf* and D.M. Kuhn, Lafayette Clinic and Cellular and Clinical Neurobiology Program, Department of Psychiatry, Wayne State University School of Medicine, Detroit, MI 48207 USA

An important role for the 5-HT transporter in mediating the rewarding aspects of cocaine use is suggested by recent results which indicate that fluoxetine, a 5-HT uptake inhibitor, can reduce self-administration of cocaine in rats. We have investigated the mechanism by which cocaine interacts with the 5-HT transporter and whether cocaine modifies 5-HT release through its actions at the 5-HT uptake site. Cocaine displaces ^{3}H-paroxetine binding to rat brain membranes in a competitive manner with a Ki of 150 nM in rat brain membranes. Solubilisation of the 5-HT transporter (0.9% digitonin) did not alter the kinetics of ^{3}H-paroxetine binding or the Ki of cocaine (155 nM), suggesting that cocaine interacts directly with the macromolecular transporter complex. The effects of cocaine on 5-HT release were examined in superfusion studies using rat hippocampal slices. Cocaine enhanced both basal and KCl-induced ^{3}H-5-HT release from slices which were prelabelled with ^{3}H-5-HT. The effects of cocaine on KCl-induced release were attenuated by the 5-HT uptake inhibitor, fluoxetine, as well as by the 5-HT$_1$ agonist, 5-methoxytryptamine (5-MeOT), although release of endogenous 5-HT release from superfused slices was not altered by 5-MeOT. The data suggest complex interactions occur between cocaine, the 5-HT transporter and presynaptic autoreceptors to modify 5-HT release from different intraneuronal pools.

35.3

ACTIONS OF COCAINE AND AMPHETAMINE IN NUCLEUS PREPOSITUS HYPOGLOSSI. D.H. Bobker & J.T. Williams. Vollum Institute, Oregon Health Sciences University, Portland OR 97201

Intracellular recordings were made from guinea-pig prepositus hypoglossi (PH) neurons in vitro. Neurons within 300 μm of the 4th ventricle are innervated by terminals that upon electrical stimulation release 5-HT to mediate an inhibitory postsynaptic potential (IPSP). Cocaine caused a prolongation of that IPSP. The prolongation was dependent on the concentration of cocaine applied and was 11.5 fold at 10 μM. The amplitude of the IPSP was increased by concentrations of cocaine <3 μM and was depressed by higher concentrations. Fluoxetine had similar actions on the IPSP and procaine (10 μM) was without effect. Cocaine (up to 10 μM) had no action on the membrane potential or rate of spontaneous firing. The prolongation of the IPSP can be explained by inhibition of 5-HT reuptake. Inhibition of the IPSP, an action shared with other uptake inhibitors but not local anesthetics is most likely due to a presynaptic action, possibly resulting from local accumulation of 5-HT acting on presynaptic 5-HT$_{1D}$ receptors.

Amphetamine, unlike cocaine, evoked the release of 5-HT from the nerve terminals causing a spiperone-sensitive hyperpolarization. In addition, amphetamine depressed the amplitude and prolonged the duration of the IPSP.

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35.4

CORRELATION BETWEEN [^{3}H]PAROXETINE BINDING AND REGIONAL SEROTONIN CONCENTRATION IN RAT AND RABBIT BRAIN. K.M. Dewar, T.A. Reader, L. Grondin* and L. Descarries. CRSN, Département de physiologie, Université de Montréal, Montréal Québec, Canada H3C 3J7.

Serotonin (5-HT) uptake recognition sites were quantified by measuring the high affinity binding of [^{3}H]paroxetine to membranes (Habert et al., Eur. J. Pharmacol. 118:107, 1985) from different regions of adult rat and rabbit brain: cingulate, frontal, parietal, piriform, entorhinal and visual cortex; dorsal and ventral hippocampus; rostral and caudal neostriatum (rat) or caudate nucleus and putamen (rabbit). From the same brain regions, the levels of 5-HT and its metabolite 5-HIAA were determined by HPLC. [^{3}H]Paroxetine binding showed a considerable regional heterogeneity, with the highest B_{max} values in the entorhinal and piriform cortex and the neostriatum. The concentrations of 5-HT and 5-HIAA were also the highest in these same brain regions. Regions with low 5-HT and 5-HIAA contents, such as the visual cortex and the dorsal hippocampus, showed lower densities of [^{3}H]paroxetine binding. In contrast, the 5-HIAA/5-HT ratio was constant throughout. Interestingly, [^{3}H]paroxetine binding and 5-HT content were high in the rat and not in the rabbit cingulate cortex. Therefore, in both species, there was a close correlation between the B_{max} of [^{3}H]paroxetine binding and the concentrations of 5-HT and 5-HIAA, confirming that the high affinity binding of this 5-HT uptake blocker is a reliable index of 5-HT innervation density.

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35.5

MANIPULATION OF LIGHT/DARK CYCLE ALTERS THE DEVELOPMENT OF THE SUPRACHIASMATIC SEROTONIN INNERVATION.

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The serotonin (5-HT) innervation of the suprachiasmatic nucleus (SCN) is among the densest in the mammalian CNS. 5-HT fibers innervate the SCN on postnatal day 10 following the arrival of optic tract fibers on postnatal day 4. We examined whether altered physiological activity in the SCN mediated by visual inputs could influence the 5-HT innervation of the nucleus. Rat pups were reared from birth in either constant light (L), constant dark (D), or a 12 hour light/dark cycle (L/D). The SCN in male pups was then examined with 5-HT immunocytochemistry at 4 and 8 weeks postnatal. 5-HT fibers in the SCN of the L/D animals emanated from the ventral and medial aspects of the nucleus to cover its full extent. L animals had an abnormal innervation pattern with decreased fiber density along the medial and dorsal aspects, and general flattening and dimunition of the area of innervation. D animals had normal patterns yet significantly sparser 5-HT innervation. The 5-HT innervation of a non-visual hypothalamic region (the pre-optic area) was unchanged by these environmental manipulations. These results indicate that the physiological activity of the target neurons can determine the development of the pattern and density of 5-HT innervation in the SCN.

Supported by NIMH 23433 and NSF 88-12892.

35.6

DOSE-DEPENDENT SEROTONERGIC MODULATION OF CORTICAL EPSPS. H.L. Read and N.J. Dun. Dept. of Pharmacol., Loyola Univ. Med. Center, Maywood, IL 60153.

Induction of synchronous cortical activity appears to be inhibited by brainstem serotonergic input. Accordingly, intracortical EPSPs which are necessary for the entrainment of neighboring pyramidal neurons are inhibited by 5-HT. In contrast, 5-HT can act postsynaptically to increase the NMDA component of cortical EPSPs--which should promote synchronization. Using the *in vitro* slice preparation, we have investigated the physiologic and pharmacologic differences between these opposing effects. Intracellular recordings were restricted to lamina V of the rat dorsal anterior cingulate and precentral motor cortices. Stimulation of the callosum evokes a short-latency EPSP which has an early APV-insensitive and a late APV-sensitive component. Superfusion of 5-HT for 3 minutes reversibly inhibited both components of the EPSP causing little or no change in membrane potential or input resistance. The dose-response relationship for the inhibition was steep such that the threshold concentration was 1 μM and the maximal inhibition was attained with 50 μM. When stimulus intensities were adjusted to attain a 60-90% maximal amplitude EPSP, the maximal inhibition by 5-HT was 70-90%. At lower doses (1 to 10 μM) 5-HT reversibly increased the EPSP amplitude (10-20%) and with repeated brief exposures this augmentation persisted for a longer period (minutes to hours). When the NMDA component of the EPSP was blocked by D-APV (10-20 μM), the dose-response relationship for 5-HT inhibitory effect was shifted to the left. The inhibitory effect is probably presynaptic since the depolarizing response to glutamate (in the presence of APV) was not reduced by 5-HT. Since the opposing effects of 5-HT occur in tandem, the presynaptic inhibition may mask the postsynaptic excitation. Functionally, activation of serotonergic input could have dual modulatory affects on cortical synchronization: i.e. inhibiting the synchronization and at the same time 'priming' the cortex for future synchronization. (NS18710 & NS24226)

35.7

5-$HT_{1C/2}$ MODULATION OF REFLEXES, POTASSIUM RELEASE, AND EXCITATORY AMINO ACID RESPONSES IN THE FROG SPINAL CORD. J.C. Hackman, A.M. Holohean, S.B. Shope and R.A. Davidoff, Dept. Neurology, Univ. Miami Sch. of Med. and Neurophysiology Lab, VAMC , Miami, FL 33101.

The present studies investigated the effects of activating 5-$HT_{2/1C}$ receptors with high concentrations (> 3 μM) of serotonin (5-HT) on reflexes, motoneuron depolarizations, and changes in $[K^+]_o$ evoked by single and tetanic stimulation of dorsal root (DR) fibers and by application of excitatory amino acid (EAA) agonists. Sucrose gap recordings were made from the ventral root (VR) of the isolated, hemisected frog spinal cord superfused with HCO_3^--buffered Ringer's solution. K^+ levels were measured with ion sensitive microelectrodes positioned in the dorsal horn.

Superfusion with 5-HT (100 μM, 20 min) significantly reduced motoneuron depolarizations evoked by supramaximal DR stimulation (73 $\pm$ 9%, n = 11) and the release of K^+ caused by tetanic DR stimulation (1.0 msec pulses, 25 Hz, 10 sec) (71 $\pm$ 18%, n = 3). Motoneuron depolarizations produced by addition of L-glutamate or L-aspartate (1 mM, 10 sec applications) were also reduced by 5-HT (80 $\pm$ 4%, n = 5; 82 $\pm$ 5%, n = 5 respectively). Similar results were obtained by 5-HT following the addition of N-methyl-D-aspartate (NMDA, 100 μM, 10 sec, 81 $\pm$ 3%, n = 3), quisqualate (30 μM, 10 sec, 80 $\pm$ 4%, n = 5) and kainate (10 μM, 10 sec, 80 $\pm$ 3%, n = 3). Addition of ketanserin (10 μM) reversed the 5-HT-induced depression of NMDA responses. Changes in $[K^+]_o$ evoked by NMDA was reduced (68 $\pm$ 12%, n = 2), but quisqualate-induced changes were unaffected (111 $\pm$ 6%, n = 2).

In sum, concentrations of 5-HT (> 3 μM) that activate 5-$HT_{1C/2}$ receptors reduce the amplitude of VR reflexes, EAA-evoked depolarizations, and the release of K^+. These results differ those produced by low concentrations of 5-HT that activate 5-HT_{1A} receptors and facilitate NMDA responses. (Supported by VA MRIS Funds and USPHS grants NS17577 & NS07044.)

35.8

CELLULAR MECHANISM UNDERLYING FAST TRANSIENT INCREASE IN HIPPOCAMPAL PYRAMIDAL CELL EXCITABILITY BY 5-HT. S. G. Beck. Department of Pharmacology, Loyola University Chicago Stritch School of Medicine, Maywood, IL 60153

In the presence of 10 μM spiperone, 5-hydroxytryptamine (5-HT) elicits a rapid and transient increase in the extracellularly-recorded population spike amplitude evoked by stratum radiatum stimulation (Beck et al., EJP, 116, 1985). This response cannot be attributed to the 5-HT-mediated decrease in afterhyperpolarization (AHP) amplitude due to the difference in the time course of the responses. We have been investigating the cellular mechanism(s) underlying the increase in pyramidal cell excitability using intracellular recording techniques in area CA1 of the hippocampal slice preparation. In the presence of 10 μM spiperone, 5-HT (3-15 μM) increased EPSP amplitude in 50% of the cells tested. This response occurred within 1 min of the start of drug perfusion, without affecting EPSP rate of rise, IPSP amplitude, membrane potential, or the amplitude of the AHP. 5-HT increased the rectification in the subthreshold region of the current-voltage plot, requiring less current to elicit an action potential. The selective 5-$HT_{2/1C}$ agonist DOI (0.1-10 μM) increased EPSP amplitude, subthreshold rectification and the amplitude of the AHP. These data support the hypothesis that the receptor mediating the fast transient increase in pyramidal cell excitability is different from the 5-HT_{1A} receptor and the 5-HT receptor mediating the decrease in AHP amplitude. Supported by MH41917.

35.9

CHARACTERIZATION OF 5-HT RESPONSES IN RAT MOTONEURONS. M. Y. Wang*, S. Y. Wu*, N. J. Dun and A. G. Karczmar. Dept. of Pharmacol., Loyola Univ. Med. Ctr., Maywood, IL 60153

Intracellular recordings were obtained from motoneurons (MNs) in transverse spinal cord slices (500 µm) of neonate (12-22 days) rats. 5-HT (10-50 µM) elicited in 70% of MNs a slow depolarization (inward current) and a slow hyperpolarization (outward current) in 10% of MNs. 5-HT responses persisted in a low Ca/high Mg solution or in TTX (0.3 µM), indicating a direct action on MNs. The inward current was associated with decreased membrane conductance and nullified between -80 and -100 mV; the reversal potential was shifted to a positive direction in high K solution. The outward current was associated with increased membrane conductance. The depolarization was antagonized by methysergide, spiperone and ketanserin and mimicked by the 5-HT_2 agonist DOI. The hyperpolarization was mimicked by 8-OH-DPAT, a 5-HT_{1A} agonist. In addition, 5-HT consistently attenuated the EPSPs evoked by dorsal root stimulation. The depression occurred independent of any membrane potential change of the MNs and in the absence of any reduction of glutamate-induced depolarizations. The depressant effect was not antagonized by methysergide, ketanserin and MDL 72222. 8-OH-DPAT diminished the EPSPs in a manner similar to 5-HT. It is concluded that 5-HT depolarizes and hyperpolarizes MNs by decreasing and increasing membrane K conductance via 5-HT_2 and 5-HT_{1A} receptors. Additionally, 5-HT acting on presynaptic 5-HT_{1A}-like receptors reduces the release of transmitter.

35.10

SEROTONIN IN THE CERVICAL SPINAL CORD OF NEONATAL RAT. A.D. Lindsay, N.E. Schwartz* & J.L. Feldman. Systems Neurobiology Laboratory, Dept. of Kinesiology, UCLA, LA, CA, 90024-1568

In the mid-cervical spinal cord of adult rat, axons immunoreactive for serotonin (5-HT) form dense arborizations throughout the gray matter and make numerous presumptive synaptic contacts with phrenic motoneurons (PMNs; Zhan et al, Neurosci. 31:105, '89). In addition, 5-HT mediates some of the raphe-spinal excitation of PMNs in the adult cat (Holtman et al, Brain Res. 417:12, '87). We investigated:

(i) The termination pattern of axons immunoreactive for 5-HT in the mid-cervical spinal cord of neonatal (0-7 day old) rats. PMNs were retrogradely labeled with horseradish peroxidase (HRP), the tissue frozen, sliced in 10 µm horizontal sections and reacted on slides with diaminobenzidine and 5-HT antisera. 5-HT positive fibers formed a dense network throughout the cervical spinal cord of all ages of neonatal rats studied. Labeled fibers formed bands of varying density spanning the ventral horn and perpendicular to the midline. Many presumptive synaptic contacts were made with PMNs.

(ii) The responses of cervical motoneurons to exogenously applied 5-HT agonists in 0-7 day old rats. C4 ventral root, phrenic nerve and vagus nerve activity were recorded in a neonatal rat brainstem-spinal cord preparation maintained *in vitro*. A Vaseline partition separated the spinal cord compartment from the brainstem compartment. 5-HT (≥10µM) applied to the spinal cord caused tonic firing of all cervical motoneurons. The tonic activity was more robust in the C4 ventral root than the phrenic nerve. There was no change in respiratory rate after spinal cord application. When 5-HT was applied to the brainstem, no tonic motoneuron firing was produced, but time and concentration-dependant changes in respiratory rate and pattern were seen. Spinal cord application of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI-HCl; ≥10µM), a potent and selective 5-HT2 agonist, mimicked the tonic firing produced by application of 5-HT to the spinal cord, and produced an increase in respiratory rate when applied to the brainstem. High concentrations (1mM) of phenylbiguanide, a 5-HT3 agonist, had no effect when applied to either the brainstem or spinal cord.

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35.11

SEGMENTAL DISTRIBUTION OF SEROTONINERGIC FIBERS AND CELL BODIES IN CAT SPINAL CORD. H. Zhuo*, S.J. Fung, V.K. Reddy* and C.D. Barnes. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

The segmental distribution of serotoninergic fibers and cell bodies in the spinal cord of the cat were studied, using the immunofluorescent staining technique. Immunoreactive serotoninergic fibers and terminals were seen in the gray matter of all spinal cord segments, with the highest density in the nucleus intermediolateralis pars principalis (IMLp) and pars funicularis (IMLf), with less density in the nucleus intercalatus (IC), the central autonomic areas (CA), dorsal and ventral horns, and with the lowest density in the nucleus thoracicus (Clarke's column). In the white matter of the spinal cord, these immunoreactive serotoninergic fibers were located immediately subjacent to the pia mater in the dorsolateral and ventrolateral funiculi.

Two types of serotoninergic immunoreactive cell bodies, multipolar and bipolar, were found in the areas dorsal and ventral to the central canal and in the medial half of the lamina VII of the cervical, lumbar, sacral and caudal segments. Some of the serotoninergic immunoreactive neurons were in close proximity to blood vessels. (Supported by NIH grant #NS24388).

35.12

ULTRASTRUCTURAL FEATURES OF SEROTONIN (5-HT) AXON TERMINALS IN ADULT RAT HIPPOCAMPUS. S. Oleskevich, K.C. Watkins*, L. Descarries and P. Séguéla. Centre de recherche en sciences neurologiques , Université de Montréal, Montréal (Qué.), Canada H3C 3J7.

PAP-immunocytochemistry with a polyclonal antiserum against 5-HT-glutaraldehyde-protein conjugate (kindly donated by Michel Geffard, Bordeaux) was used to characterize the 5-HT innervation of adult rat hippocampus at the electron microscopic level. Initial fixation of the tissue was by perfusion with 3% glutaraldehyde; 100-µm-thick-vibratome sections were processed free-floating without Triton X (1:1000 dilution of the primary antibody), and serial or single ultrathin sections were respectively cut perpendicular or parallel to the surface of vibratome sections. Several hundred immunostained varicosities from the oriens and the radiatum layer of CA3, or the molecular and the polymorph layer of the dentate gyrus (DG) were examined in single sections (n = 338); those examined in serial sections (n = 57) were all from CA3. The average size, vesicular content and relative frequency of synaptic membrane specialization, synaptic targets and juxtaposed elements were similar in each layer of both regions. A junctional complex (most often asymmetrical) was present on 10% of the single-sectioned profiles, indicating a 24% synaptic incidence when stereologically extrapolated to whole varicosities. A comparable value of 18% was directly observed in serial sections. In CA3, only dendritic shafts were synaptically contacted by 5-HT varicosities, whereas in both layers of DG, a few axo-spinous synapses were also seen. The most frequent neuronal elements in the immediate micro-environment of immunostained profiles were other axonal varicosities (30%), neurites (25%) and dendritic shafts (13%). Dendritic spines were relatively infrequent (4%). Thus, the 5-HT innervation of adult rat hippocampus is predominantly nonjunctional (70-80% of its varicosities) and shows similar internal and relational features in the neuropil layers of CA3 and DG.
(Supported by the FRSQ and MRC grant MT-3544).

35.13

METABOLISM OF ^{14}C-SEROTONIN BY HUMAN AND RAT PINEAL GLANDS. S. Daya and F. Briceland* Department of Biochemistry, Rhodes University, Grahamstown, South Africa, 6140.

Investigations were carried out to determine whether differences exist in the metabolism of exogenous radio-labelled serotonin by the rat and human pineal gland. Human pineals were obtained 6h after death from medico-legal autopsies. Pieces of pineal tissue were weighed and incubated for 24h in BGJb culture medium with 0.4 μCi carbon 14 labelled serotonin creatinine sulfate. Rat pineals were removed immediately after sacrifice of the animals by neck fracture and cultured individually as above. All culture media were analysed for their content of radiolabelled indoles using two dimensional thin layer chromatography as described elsewhere (Daya, S. and Fata, M. IRCS Med. Sci., 14 : 1153, 1986).

The results indicate that human and rat pineals produce relatively similar proportions of indoles from exogenous serotonin. The human pineal removed 6h after death appears to be metabolically active and could be used for a variety of scientific manipulations.

35.14

STIMULATION OF ATROGLIAL 5-HT$_{1A}$ RECEPTORS RELEASES THE SEROTONIN GROWTH FACTOR, S-100, AND ALTERS ASTROGLIAL MORPHOLOGY. P.M. Whitaker-Azmitia and E.C. Azmitia Dept. of Psychiatry, SUNY, Stony Brook, New York and Dept. of Biology, NYU, New York, N.Y.

We have recently shown that stimulation of a subtype of serotonin receptor, the 5-HT$_{1a}$ receptor, on astroglial cells, causes the release of a factor or factors into the culture media, which can regulate the development of serotonergic neurons in primary culture (Brain Res. 497:80, 1989). In order to identify this factor, we preincubated the conditioned media (referred to as GCM) with an antibody raised against S-100, an astroglial specific protein which has been shown by others to be releasable by cAMP and to be a neurite extension factor. Specifically, we tested media produced by stimulating astroglial cultures with the 5-HT$_{1a}$ receptor agonist ipsaperone (the media is thus referred to as GCM-IPS). We found that the growth-promoting properties of GCM-IPS on serotonin neurons is inhibited by pre-incubation with this antibody. Moreover, the morphology of the IPS treated astroglial cultures is changed to a more mature form.

This work is supported by a grant from NINDS to P.M. Whitaker-Azmitia.

35.15

5-HT$_{1A}$ RECEPTORS REGULATE HIPPOCAMPAL S100$_B$, A 5-HT AND CORTICAL GROWTH FACTOR

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5,7-DHT intracerebral injections into fornix or cingulum bundle produces a delayed increase in hippocampal (HIPP) 5-HT homotypic collateral sprouting and increased soluble trophic-extract. S-100$_B$ but not NGF, EGF, Insulin and calmodulin, is a serotonergic growth factor in dissociated mesencephalic cultures (Azmitia et al, Brain Res. 1990). Ipsapirone (IPS), a specific 5-HT$_{1A}$ agonist, releases a 5-HT trophic factor from cultured astrocytes which is sensitive to antibodies raised against S-100$_B$. Desipramine pretreated adult female Sprague-Dawley rats (200 gms) were bilaterally injected with 5,7-DHT (5 ug/0.4ul) into fornix-fimbria/cingulum bundle and after one month intraperitoneally injected with 1 mg/kg ipsapirone at 12:00, 18:00 and 9:00 four hours before decapitation. The S-100$_{beta}$ levels were measured in soluble or detergent-treated homogenates by RIA. Free S-100$_{beta}$ was not increased in control HIPP by IPS injections (0.226 ± .06 vs 0.232 ± .06 ugm/mg protein). One month after HIPP 5-HT deafferentation the S-100$_{beta}$ levels were reduced from control levels (0.201 ± .014) but significantly increased after IPS treatment (0.287 ± .012). Detergent extracted S-100$_{beta}$ levels were not changed after 5,7-DHT or IPS treatment. Our results indicate that 5-HT can regulate its own growth by activation of 5-HT$_{1A}$ astroglial receptors which increase the availability of S-100$_{beta}$. Our results may be applicable to Alzheimer's disease since S-100$_{beta}$ is a cortical trophic factor and this factor and 5-HT$_{1A}$ receptors are changed in post-mortem Alzheimer's brains. Supported by NSF 88-12892 and NINDS.

35.16

SEROTONIN RELEASE FROM HYPOTHALAMIC TISSUE IS MODULATED BY TRYPTOPHAN AVAILABILITY.

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Experiments performed *in vivo* have provided evidence for a physiological coupling between brain tryptophan (trp) levels and serotonin (5HT) release. However, variable results regarding trp effect on 5HT have been reported with *in vitro* studies. We examined the relationship between trp availability and 5HT release under experimental conditions that resemble those occuring physiologically. An *in vitro* perifusion system was established to examine the release 5HT from perifused hypothalami taken from mature pigs under basal conditions and in response to trp and KCl administration. Hypothalami were perifused with Dulbecco's modified eagle's medium continuously at 37°C for 5 hrs at a flow rate of 100 μl/min. The basal release level plus 2 standard deviation was used as reference value to determine a significant response. Tryptophan significantly increased 5HT levels ($P<0.05$). At the end of the experiment, potassium depolarization caused a rapid and significant release of 5HT ($P<0.05$) indicating viability of the neuronal tissue. We conclude that availability of tryptophan modulates the hypothalamic output of serotonin.

35.17

CHANGING SEROTONERGIC INNERVATION OF THE CEREBRAL CORTEX IN THE FIRST TWO POSTNATAL MONTHS OF LIFE.

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The cerebral cortex is densely innervated by serotonergic (5-HT) axons which arise from the dorsal and median raphe nuclei. The axons appear in the cortex already in fetal life but there is a significant change in their disposition and density during early postnatal life. In postnatal cats aged P1 to P60 we have demonstrated the serotonergic axons with immuno-histochemical methods and, using a semiautomatic computerized technique, determined the changing density and innervation patterns of the two types of serotonergic axons (Mulligan and Törk, J. Comp. Neurol. 270: 86-110, 1988) in the cortex. In the prefrontal, primary auditory and primary visual cortical areas we have found that while at birth the 5-HT fiber distribution is relatively even across the cortical layers, later a reorganization of the innervation pattern occurs in each cortical area. Although there is a steady increase in the density of serotonergic innervation in the supragranular layers, the increase is strongest in layer II of the prefrontal and layer I of the visual and auditory cortex. The increase in fibre density seems particularly significant in the case of the small varicose fibers. The large varicose fibers are hardly demonstrable at birth but their density increases evenly across the cortex with increasing age. At P23 the first pericellular basket-like arrays can be observed and their number increases with age. These observations indicate that the development of the serotonergic innervation pattern in the neocortex may be linked to the maturation of the intrinsic cortical elements. Moreover the results indicate that the two independent serotonergic systems develop according to a differential timescale and that the large varicose (basket) fibers innervate their target areas of the cortex later than the fine varicose system. This study was supported by a grant from the National Health and Medical Research Council of Australia.

35.18

INVESTIGATING RELATIONSHIPS BETWEEN 5-HT SYNTHESIS AND EXTRACELLULAR CONCENTRATIONS BY BRAIN DIALYSIS IN INDIVIDUAL, CONSCIOUS RATS.

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We have adapted rat brain dialysis procedures to allow studies of the effects of altered 5-hydroxytryptamine (5-HT) synthesis on extracellular 5-HT concentrations in individual, conscious rats. Concentric dialysis probes (5mm membrane length) were implanted in the frontal cortex of anaesthetised rats. 18-24 hours later, basal and K^+ stimulated changes (100mM in perfusate, 20 min) of dialysate 5-HT concentration were determined. 5-HT synthesis rates were then investigated in the same animals as indicated by the accumulation of 5-hydroxytryptophan (5-HTP) after systemic administration of an aromatic amino acid decarboxylase inhibitor (NSD 1015, 150 mg/Kg i.p.). 5-HT, 5-HTP, 5-HIAA and NSD 1015 were analysed by HPLC with coulometric detection. Pretreatment of rats with the tryptophan hydroxylase inhibitor, p-chlorophenylalanine (150 mg/Kg i.p., 24 hours prior to dialysis) substantially reduced basal and K+ evoked dialysate 5-HT values (approximately 60%) even though the rates of accumulation of dialysate 5HTP and also frontal cortex 5-HT and 5-HIAA values of the subsequently killed rats showed only small changes. The above procedures should be applicable in studies of relationships between the synthesis of 5-HT and its availability to receptors.

35.19

Modulation of [3H]-Paroxetine Binding by Ca^{++} and Mg^{++} but not by GTP-gamma-S.

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We have previously seen that 5-HT_{1b} receptor agonists, RU24969 and TFMPP, and the 5-HT_2 receptor agonists, DOI and and NPP, interact with the binding of [3H]-paroxetine to the serotonergic high-affinity uptake site (transporter) in rat brain membrane preparations (1mg/ml). To examine which regulator or effector systems might be involved in these interactions, we tested the effects of GTP-gamma-S on the binding of [3H]-paroxetine. 1 uM GTP-gamma-S, a non-hydrolyzable GTP analogue, did not affect the Kd nor the Bmax suggesting that the interaction does not occur at the level of the receptor/G-protein complex. Application of 2 mM EGTA, a Ca++ chelator, increased the Bmax (Ctl=134 fmol/mg prot;EGTA=184 fmol/mg prot) without changing the Kd (Ctl=0.09nM;EGTA=0.1nM). Different calcium concentrations (5,10,20mM) were tested. The affinity (Kd=0.18,0.35,0.57nM) and the Bmax values decreased as [Ca++] is increased. Mg++ was tested to control for increasing divalent ion concentrations. We found that the affinity (Kd=0.11,0.15,0.27) and the Bmax values decreased but not as potently as seen in the presence of Ca++. This suggests that Ca++, which is a primary regulator of signal transduction, may play a role in the interaction between the 5-HT1b and 5-HT2 receptors and [3H]-paroxetine binding. We are currently probing the effects on [3H]-paroxetine binding with co-administrations of the 5-HT receptor agonists with EGTA or increasing Ca++ concentrations on rat membrane preparation and tissue culture.

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35.20

PKC Stimulation of 5-HT Release is Sensitive to An L-type Ca^{++} Channel Blocker But Independent of External Ca^{++}

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The intracellular mechanisms regulating evoked neurotransmitter release were studied in synaptosome by adding PMA (phorbol 12-Myristate 13-Acetate)-PKC activator. A release assay was used to study the differential effects of Ca^{++} channel blockers (Nickel, Cobalt and Nimodipine) and TTX (tetrodotoxin) on PMA in different media, (i,e, Ca^{++}-free, and Na^{+}-free Krebs-Ringer media). Synaptosomes were preload the with ^{3}H-5HT ($5x10^{-8}$M) at 37^{0}C for 25 min, incubate it with drugs 10 min before adding PMA at 37^{0}C for 5 min. Our results showed that in both Ca^{++}-containing and Ca^{++}-free medium, PMA stimulated the release of 5-HT at 10^{-4}M to $5x10^{-6}$M and Ionomycin (10^{-5}M)-an Ca^{++} ionophore potentiated the effect of PMA. The stimulatory effects of PMA in Ca^{++}-free medium was not fluoxetine-sensitive. The effects of PMA in Ca^{++}-containing medium was attenuated by Nickel (10^{-5}M), Cobalt (10^{-5}M) and Nimodipine (10^{-8}M). In addition, TTX (10^{-8}M) blocked PMA effects and there is no stimulatory effects of PMA in Na^{++}-free medium. PKC may regulate release of 5-HT from synaptosomes by a voltage-dependent mechanism sensitive to L-type Ca^{++}-channel antagonist but not to external Ca^{++}. Supported by NIDA Contract 271-87-8114.

35.21

REGULATION OF TRYPTOPHAN HYDROXYLASE ACTIVITY BY PROTEIN KINASES. P.A. Johansen and D.M. Kuhn, Lafayette Clinic and Cellular and Clinical Neurobiology Program, Department of Psychiatry, Wayne State University School of Medicine, Detroit, MI 48207 USA

Tryptophan hydroxylase (TPH) is the initial and rate limiting enzyme in the biosynthetic pathway of the neurotransmitter serotonin (5-HT). TPH activity is increased when the enzyme is exposed to in vitro conditions favoring protein phosphorylation. Previous studies established that the activation of TPH by phosphorylating conditions was mediated by a Ca^{2+}-calmodulin-dependent protein kinase. Certain in vitro experiments have suggested that a cAMP-dependent protein kinase can also activate TPH. Our experiments were aimed at determining if TPH, like tyrosine hydroxylase, is regulated by different protein kinases. Exposure of TPH from rat brain to conditions favoring protein kinase A (cAMP-dependent) including cAMP (1-100 μM), the phosphodiesterase inhibitor theophylline, or the catalytic subunit of protein kinase A, did not activate TPH. Furthermore, the stimulation of TPH activity by Ca^{2+}-calmodulin dependent protein kinase was not decreased by the protein kinase inhibitors W-7 or the Walsh inhibitor. TPH was also activated in a calmodulin independent manner by Ca^{2+} and phosphatidylserine. The ability of the protein kinase C inhibitor staurosporine to block this effect indicate that protein kinase C can also activate TPH. As previously reported, the largest stimulation of TPH was mediated by calmodulin dependent conditions. The activation of TPH by protein kinases was manifested as a decrease in the K_m of TPH for BH_4, with no change in the apparent kinetic constants of TPH for tryptophan. Studies are underway to determine which serine residues in TPH are phosphorylated by the different protein kinases in brain.

$GABA_A$ RECEPTORS I

36.1

ANTAGONISTS OF THE NMDA SENSITIVE GLUTAMATE RECEPTOR DOWN REGULATE mRNAS ENCODING VARIOUS $GABA_A$ RECEPTOR SUBUNITS IN CEREBELLAR GRANULE NEURONS. D.R. Grayson, M. Memo, P. Bovolin and E. Costa. FGIN, Georgetown University, Washington, D.C. 20007.

The genes encoding the subunits of the $GABA_A$ receptor constitute a complex and probably tightly regulated multigene family. At least 14 different, evolutionarily related cDNAs have been cloned, each of which encodes a receptor subunit located in both neurons and glial cells. Two allosteric modulatory sites of GABA action located in extracellular and channel domains, have different structural requirements. The modulatory properties of the extracellular domain receptors on GABA operated channels are expressed only when the heteroligomeric complex includes α and γ subunits.

Using quantitative PCR, we have analyzed the levels of $GABA_A$ receptor subunit mRNAs present in primary rat cerebellar granule cells. Isosteric agonists and antagonists of $GABA_A$ receptors fail to change the expression of $GABA_A$ receptor subunits indicating that homologous receptor stimulation is not involved in the regulation of the receptor subunit genes. In contrast, specific antagonists of NMDA-sensitive glutamate receptors mediate a pronounced down regulation of the mRNAs encoding the α-1, β-1, β-2 and γ-2 receptor subunits. The results indicate that the regulation of the $GABA_A$ receptor subunit genes may involve an interplay between cell specific signals and signals generated through heterologous receptor stimulation.

36.2

DIFFERENTIAL EXPRESSION OF $GABA_A$ RECEPTOR SUBUNITS IN PRIMARY NEURONAL AND GLIAL CELL CULTURES. P. Bovolin, M. Memo, E. Costa, and D. R. Grayson. FGIN, Georgetown University, Washington, D.C. 20007.

To determine the pattern of $GABA_A$ receptor subunit mRNAs expressed in defined cell types, we have quantitated the mRNA levels corresponding to various receptor subunits present in primary neuronal and glial cell cultures, prepared from neonatal rat brain, using the polymerase chain reaction. Subunit specific oligonucleotides were used to amplify the reversed transcribed mRNA isolated from each culture.

Cerebellar granule cells maintained in culture for 7 days contained comparable amounts of the α_1, β_1, β_3 and δ subunit mRNAs and smaller amounts of α_4, β_2 and γ_2. We found that the mRNA levels encoding the β_2 subunit decreased continuously from day 5 to day 15 in culture, while changes in the α_1, α_4 and γ_2 subunit mRNAs with time in culture are presently under investigation. In contrast, in primary cortical glial cells, the α_4, β_1 and β_3 were the most abundant with minor amounts of the α_1, β_2 and γ_2 mRNAs. A similar analysis of the $GABA_A$ receptor subunit mRNAs present in cerebellar glial cells, including data for the γ_1 subunit, will be presented. Our results suggest that the $GABA_A$ receptor subunit mRNAs are regulated by both an ontogenetic program, which provides environmental cues related to cell type specificity, and heterologous receptor stimulation, which allows for an additional modulation through neuron to neuron and neuron to glial cell signalling.

36.3

EXPRESSION OF GABA$_A$ RECEPTOR SUBUNIT mRNAs IN CULTURED CEREBELLAR NEURONS: ANALYSIS BY THE POLYMERASE CHAIN REACTION. C.E. Beattie and R. E. Siegel, Dept. of Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

Molecular biological studies have revealed that the GABA$_A$ receptor complex is composed of multiple subunits. For most of these subunits multiple subtypes exist. In earlier studies we have examined the distribution of these subunits in the developing and adult rat brain by in situ hybridization histochemistry. These studies have raised the possibility that cell-cell interactions play a role in subunit gene expression in the rat cerebellum. To begin to address this issue, we have developed a system for detecting subunit mRNA expression in tissue culture. Cerebellar neurons dissociated from embryronic day 19 rats are maintained for several weeks in culture in medium containing 25mM potassium. To analyze mRNA expression, cells are lysed in the culture wells and processed for the reverse transcriptase reaction utilizing random primers. The polymerase chain reaction (PCR) is then performed with primers for neuron specific enolase (NSE) and GABA$_A$ subunit specific primers. Our studies indicate that NSE mRNA can be detected from as few as ten neurons in culture. In addition, the β2 and γ2 subunits, which are relatively abundant in early postnatal development, can readily be detected by PCR in culture wells plated at a density of two thousand cells. This experimental paradigm is being used to examine GABA$_A$ receptor subunit mRNAs and factors that modulate their expression.

36.4

DIFFERENTIAL EXPRESSION OF RAT GABA$_A$ RECEPTOR SUBUNIT mRNAs DURING DEVELOPMENT. C. Gambarana, C. E. Beattie, and R. E. Siegel. Dept. of Pharmacology, Case Western Reserve University, Cleveland, OH 44106.

The GABA$_A$ receptor complex, site of action of the inhibitory neurotransmitter γ-aminobutyric acid (GABA) and the anxiolytic benzodiazepines, is composed of multiple structurally distinct subunits. For most of the subunits, several isoforms have been identified by molecular cloning, and the mRNAs encoding these subunit subtypes exhibit different regional distributions in the adult brain. In addition, our previous studies indicated that the expression of at least one subunit, α1, changes during ontogeny. To further examine the developmental expression of GABA$_A$ receptor subunit mRNAs, we have performed quantitative hybridization histochemistry with probes specific for the α1, β1, β2, β3, and γ2 subunits. Our studies demonstrate that all subunit mRNA levels increase for 2-3 weeks postnatally, although significant regional differences in the onset of expression are apparent. For example, while the β2, β3, and γ2 mRNAs are observed in the cerebellar cortex during the first postnatal week, the α1 mRNA only becomes detectable in the second week. Furthermore, the levels of all subunit mRNAs peak by approximately day 21 and then decline to the adult levels. In the mature brain, the α1, β2, and γ2 mRNAs are widely distributed and exhibit similar regional patterns of expression. In contrast, the β3 subunit is abundant only in the hippocampus and cerebellar granule cell layer. Furthermore, the β1 subunit mRNA, whose expression declines to the greatest extent, is present in relatively low levels in the cerebral cortex and hippocampus. These findings underscore the differential regulation of GABA$_A$ receptor subunit genes.

36.5

COMPARATIVE STUDY OF AGONIST AND ANTAGONIST BINDING TO GABA$_A$ RECEPTORS IN RAT BRAIN. Y.Ito, K.Ishige*, M.Makimura*, H.Fukuda* and Y.Murakoshi*. Dept. of Pharmacology, Col. of Pharmacy, Nihon Univ. Funabashi-shi, Chiba 274, Japan.

The properties of ^{3}H-SR 95531, a synthetic GABA$_A$ antagonist, binding in the frontal cortex (FC), cerebellum(CB) and cultured cerebellar granule cells(CBCUL) were compared with those of ^{3}H-GABA. The treatment of the membranes with Triton X-100 and phospholipase A$_2$ dramatically increased ^{3}H-GABA binding, whereas ^{3}H-SR 95531 binding was significantly decreased in the FC, CB and CBCUL. Scatchard analyses revealed that the Triton X-100 treatment caused a significant decrease in the Bmax values of high- and low-affinity sites for ^{3}H-SR 95531 in the FC, while it increased those for ^{3}H-GABA. The K_D values for both radiolabelled ligands were not affected in this region. On the other hand, in the CB, the treatment increased the K_D values of high- and low-affinity sites for ^{3}H-SR 95531, whereas it decreased the values for ^{3}H-GABA with no change in the Bmax values. These results suggest that there are different conformational states of the GABA$_A$ receptor binding, and that the regulatory mechanism of these states in the FC is different from that in the CB.

36.6

THE RELATIVE EFFICACY OF THE BENZODIAZEPINE RECEPTOR ANTAGONIST (Ro15-1788) IN THE $^{36}Cl^-$ UPTAKE ASSAY DEPENDS ON GABA CONCENTRATION AND THE PRESENCE OF GABA RECEPTOR ANTAGONISTS. E.Malatynska, S.C. Dilsaver, M.L. Giroux*, R.J. Knapp and H.I. Yamamura. Dept. of Pharmacology, The University of Arizona, Tucson, AZ 85724 and Dept. of Psychiatry, The Ohio State Univ., Columbus, OH 43210.

The benzodiazepine antagonist (Ro15-1788) has been shown to have partial agonist or partial inverse-agonist activity in some behavioral and electrophysiological studies. In this study we have shown that in the $^{36}Cl^-$ uptake assay, Ro15-1788 may have agonist, antagonist, and inverse-agonist efficacy at the benzodiazepine receptor depending on the GABA concentration. Ro15-1788 did not alter the effect of 30 μM GABA on $^{36}Cl^-$ uptake. However, it did inhibit $^{36}Cl^-$uptake produced by 100 μM GABA, and enhanced $^{36}Cl^-$ uptake mediated by 10 μM GABA. 1 μM Ro15-1788 shifted the concentration-response curves of the GABA$_A$ receptor antagonists (bicuculline, SR 95531, amoxapine and amitriptyline) to the left. The shift in EC-50 value by Ro15-1788 for these drugs was inversely proportional to their affinity for the GABA$_A$ receptor. We suggest that the activity of Ro15-1788 depends on changes in the conformational state of the GABA$_A$ receptor produced by increasing concentrations of GABA or the GABA receptor antagonists.

36.7

SYNTHESIS OF AN AFFINITY AGAROSE SPECIFIC FOR THE PERIPHERAL-TYPE BENZODIAZEPINE RECEPTOR. **A.L. Parola*, N. R. MacKenzie*, and H. E. Laird II, Dept. of Pharmacology and Toxicology and Dept. of Pharmaceutical Sciences, Univ. of Arizona, College of Pharm., Tucson, AZ 85721**

PK 14105, a photoaffinity ligand specific for the peripheral-type benzodiazepine receptor (PBR) was photochemically coupled to ω-aminobutyl agarose (ABAg) to yield PK 14105 agarose (PKAg). ^{19}F and ^1H nuclear magnetic resonance spectroscopy was consistent with the proposed site of coupling at the 2'-fluorine of PK 14105 by the primary amine moiety of ABAg. Quantitation of the affinity gel using two different colorimetric assays for primary amines suggests approximately 50% of the available primary amine groups of ABAg were bound by PK 14105. The estimated concentration of PK 14105 bound to ABAg was 2.3 μmol/ml of settled gel (2.3 mM immobilized ligand concentration). PKAg specifically binds the bovine PBR solubilized by digitonin. The affinity of PKAg for the soluble PBR was estimated by varying the concentration of PKAg. PBR binding to PKAg was saturable and the apparent affinity of PKAg for the bovine receptor was estimated from the saturation data. A PKAg affinity column bound 85% of the solubilized PBR from cow adrenal or from rat adrenals partially purified by anion exchange chromatography. These results indicate PKAg is a receptor specific affinity media which may be useful in the purification of the native PBR from various species. (Supported by Ariz. Dis. Control Res. Comm. #82-9290 and Coll. of Pharm., BRSG Fund).

36.8

EFFECT OF THIOL REAGENTS ON THE GABA/BENZODIAZEPINE CHLORIDE CHANNEL COMPLEX A.M. Allan and G.G. Mayes*
Department of Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

Agents which modify thiol groups have been shown to alter ligand binding at a variety of receptor sites; in addition, alkylation of sulfhydryls have been shown to block ion channel conductance. We studied the effects of thiol reagents on: GABA activated chloride flux ($^{36}Cl^-$) (JPET 247:1012, 1988),[^{3}H]-muscimol (Life Sci. 39:2005, 1986) and [^{3}H]-diazepam (Brain Res. 452:118, 1988) binding in mouse brain membrane preparation (microsacs). Incubation of microsacs in the presence of: mercuric chloride ($HgCl_2$), p-chloromercuriphenylsulfonic acid (pCMBS) or hydroxymercuribenzoate (HMB) attenuated GABA-stimulated Cl uptake (IC_{50}s 130-150 uM). Iodoacteic acid (IAA) also attenuated Cl uptake, but was not as potent as the mercurial compounds. The thiol reagents reduced both maximal stimulation and the potency of GABA to induce Cl uptake. Supernatant prepared from pCMBS (200 uM) treated microsacs stimulated Cl uptake (3 sec exposure) in the absence of GABA agonist in microsacs unexposed to thiol reagents. The supernatant was still effective after boiling or the addition of 1mM dithiothreitol (DTT). pCMBS treated supernatant lowered specific binding of [^{3}H]-muscimol but stimulated [^{3}H]-diazepam binding. Stimulation of diazepam binding by supernatant was blocked by 50 uM bicuculline. From the present findings it appears that these thiol reagents may act either to release or prevent the reuptake of GABA, although other explanations have not been excluded. Supported by USPHS grants AA08219 and DA06106.

36.9

FUNCTION OF GABA BINDING SITES ON GABA$_A$ RECEPTORS
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Experiments with never-frozen membranes from rat cerebral cortex incubated at 37°C for 10 min in the presence of 100 or 400 mM NaCl and (-)-nipecotate (50 μM) indicate that GABA binds to three sites on GABA$_A$ receptors, the K_d values of which are ~30 nM, >0.3 but <3 μM, and >3 μM. Under these conditions GABA increases ^{3}H-flunitrazepam binding by 2.5-fold (EC_{50} ~1 μM), and exerts a biphasic effect on ^{35}S-TBPS binding; increasing the amount bound at concentrations <1 μM, but decreasing the amount bound at higher concentrations. For 10 min incubation periods the GABA-induced increase in ^{35}S-TBPS binding was enhanced by increasing NaCl from 100 to 400 mM. Concentration-response curves for the GABA-induced increase in ^{35}S-TBPS and ^{3}H-flunitrazepam binding were coincident up to 1 μM GABA, but at higher GABA concentrations the curves diverged due to the GABA-induced inhibition of TBPS binding. The EC_{50} for the inhibition of TBPS binding was ~10 μM, similar to the EC_{50} for GABA-induced Cl^- fluxes in neurosomes. Our results suggest that only the low-affinity site is associated with receptor activation, whereas the middle-affinity site serves a negative modulatory function, and contributes to the apparent positive cooperativity of receptor activation. The high-affinity site may exist only on immature or desensitized receptors.

36.10

ALLOSTERIC INTERACTIONS AT THE GABA$_A$ RECEPTOR BINDING DOMAINS IN RAT BRAIN SYNAPTONEUROSOMES. T. M. DeLorey, G. B. Brown, and I. Kissin.* Behavioral Neurobiology, Dept. of Psychiatry, and the Dept. of Anesthesiology, Univ. of Alabama at Birmingham, Birmingham, AL 35294.

The rat brain cerebrocortical synaptoneurosomal preparation has in the last few years become a useful model for the study of GABA$_A$ receptor pharmacology in the brain. Studies from several laboratories have demonstrated the suitability of this vesicular model for measurement of GABA-mediated $^{36}Cl^-$ flux. Since the $^{36}Cl^-$ flux measurements are performed under more physiological conditions and the data appear to reflect a more physiologically relevant description of the GABA$_A$ receptor complex than the standard binding data, we initiated a series of studies to adapt the synaptoneruosomal model to direct radioligand binding measurements. We have previously reported results with GABA agonists and antagonists in this system. Estimates of kinetic parameters describing the dose-response curves of these ligands in this system revealed significant differences from those determined by the standard radioligand binding measurements using frozen/thawed brain membrane fragments (Soc. Neurosci.(abstracts) 14:977, 89).We have extended these studies and report initial results for the binding of benzodiazepines, barbiturates, and their allosteric interactions, at the GABA$_A$ receptor complex in rat brain synaptoneurosomes. ^{3}H-diazepam binding experiments revealed a K_d comparable to that using standard assay techniques. Muscimol potentiates ^{3}H-diazepam binding with an EC_{50} value of 1 μM and a maximal enhancement of 65%. Although binding of pentobarbital could not be directly measured, we did observe a dose-dependent increase in ^{3}H-muscimol produced by this barbiturate (EC_{50}=40 μM with max. enhance.=40%). A similar result has been reported by Willow and Johnston (Neurosci. Lett. 18:323-7, 80) with crude synaptosomal membranes. The standard assay fails to display this pentobarbital-mediated enhancement of muscimol binding. Other allosteric interactions between the GABA, benzodiazepine and barbiturate sites were studied using this new protocol, and were found to be qualitatively different. (Support: NIH grant GM 39344 to I. Kissin)

36.11

ENHANCEMENT OR INHIBITION OF ^{35}S-TBPS BINDING BY BICUCULLINE IS REGION SPECIFIC IN RAT BRAIN. J. Peris. Dept. of Pharmacodynamics, University of Florida Health Science Center, Gainesville FL, 32610.

The GABA$_A$ receptor/chloride ionophore complex includes at least three additional binding sites that can allosterically affect GABA receptor binding: the benzodiazepine receptor, the barbiturate receptor and the picrotoxin/convulsant receptor. Occupation of the benzodiazepine and barbiturate sites by agonists increases GABA binding while occupation of the picrotoxin site inhibits GABA receptor binding and vice versa. Recent measurements of the regional expression of a variety of mRNAs for the different subunits of the GABA$_A$ receptor complex using in situ hybridization revealed markedly different profiles of the subtypes for the α, β and γ subunits in different brain regions, particularly substantia nigra (SN), cerebellum (CBL) and inferior colliculus (IC; Richards et al., Soc. Neurosci. Abstr. 15:642, 1989). It is thus possible that allosteric interactions of the binding sites would differ across brain regions. Specific binding of ^{35}S-TBPS to the picrotoxin site was studied in homogenates of cortex (CTX), superior colliculus (SC), CBL, IC and SN and the allosteric regulation of this binding was measured. Bicuculline methiodide, a GABA$_A$ antagonist, enhanced binding by 50% in CTX and SN (EC_{50} = 300 nM) and enhanced binding by 2-fold in CBL (EC_{50} = 10 μM). However, bicuculline inhibited binding in IC by 30% (IC_{50} = 4 μM) and did not affect binding in SC. This regional difference was also found using quantitative autoradiographic analysis of binding in thin tissue sections. These findings provide a functional basis in support of the heterogeneity of GABA$_A$ receptor subunit composition in different brain regions. This work was supported by PHS grant AA 08262.

36.12

DIFFERENT TEMPERATURE SENSITIVITY OF TYPE I AND TYPE II BENZODIAZEPINE RECEPTORS. P.A. Maguire*, M.F. Davies, H.O. Villar*, and G.H. Loew*, Molecular Research Institute, Palo Alto, CA 94304.

The temperature dependence of the binding kinetics of BDZ receptors was studied in two putative single receptor tissues, rat cerebellum (Type I) and spinal cord (Type II). Displacement of [^{3}H]Ro15-1788 (1nM) by flunitrazepam and zolpidem (agonists), β-CCE (inverse agonist), Ro15-1788 (antagonist), and AHR11797 (selective muscle relaxant agonist) was carried out at 0°C and 37°C. Our results indicate that each tissue contains only one BDZ receptor. Using LIGAND, a one-site model in both cerebellum and spinal cord provided the only reliable set of K_ds for each ligand and B_{max}s for each tissue at each temperature. The results at 0°C predict significant Type I selectivity only for zolpidem and β-CCE (47 and 7.5 respectively). Increasing the temperature to 37°C produced a shift in the K_d to a lower affinity in both tissues. This shift was 8-10 fold in the cerebellum for all ligands except AHR11797 (3.5 fold). In the spinal cord, the temperature-induced shift in K_d was ~1/2 that in cerebellum for all ligands except flunitrazepam, which was equally sensitive to temperature in each tissue. The net result of the disparate temperature dependence of binding at the two receptor subtypes, is the loss of Type I selectivity at elevated temperatures, with only zolpidem retaining significant (19-fold) Type I selectivity, but with modest affinity (136 nM). Thus at 37°C there does not appear to be a high affinity selective ligand for either receptor type.

36.13

THE NMDA RECEPTOR ANTAGONIST MK-801 PREVENTS KINDLING INDUCED BY PENTYLENETETRAZOL (PTZ) IN RATS. M.G. Corda, O. Giorgi, M. Orlandi*, D. Lecca*, G. Biggio. Dept. of Exp. Biology, Chair of Pharmacology, Univ. of Cagliari, ITALY.

The present study was designed to investigate the role of GABA and excitatory aminoacid (EAA) neurotransmissions in the chemical kindling produced by PTZ, a selective blocker of the Cl^- channel coupled to the GABA receptor. Chemical kindling was observed in 90% of the rats treated with 30 mg/kg of PTZ daily or every second day for 8 to 10 weeks. This effect was remarkably enduring since rats were still sensitized to PTZ up to one year after drug discontinuation. The administration of the non-competitive NMDA receptor antagonist MK-801, at a dose of 1 mg/kg, i.p., 30 min before each injection of PTZ completely prevented the development of kindling. In fact, no convulsions were observed in rats receiving PTZ plus MK-801 during the chronic treatment or following a challenge dose of PTZ administered 5 days after the last injection of PTZ plus MK-801. Biochemical studies revealed a decrease in both 35S-TBPS binding and GABA-stimulated 36Cl uptake in the cerebral cortex of rats chronically treated with PTZ up to 24 days after the completion of the chronic treatment, suggesting an involvement of the GABAergic transmission in PTZ-induced kindling. We are at present investigating whether MK-801 is able to prevent the neurochemical changes in the GABA receptor complex induced by PTZ-kindling.

36.14

FAILURE OF EXCITATORY AMINO ACID TO ALTER THE FUNCTION OF GABA$_A$ RECEPTOR COMPLEX
M. Serra*, A. Concas, E. Sanna*, C. Foddi*, G. Santoro* and G. Biggio, Dept. of Experimental Biology, University of Cagliari, Italy

Recently, it has been proposed that excitatory amino acids may affect the strenght of synaptic inhibition by GABA (Nature 337: 170, 1989). Thus, we sought to determine whether kainic acid was able to modify the function of GABA$_A$ receptor. Kainic acid (8 mg/kg, i.p.) markedly potentiated the convulsant activity of isoniazid, an inhibitor of GABAergic transmission; in fact, it shortened the latency of the appearance of convulsion and augmented the expression of each seizures induced by isoniazid. On the other hand, [^{35}S]TBPS binding, which is very sensitive to the modulatory action of drugs that affect GABAergic transmission, was not significantly changed after "in vivo" administration of kainic acid. Moreover, kainic acid does not modify the isoniazid-induced increase of [^{35}S]TBPS binding. Consistently, [^{35}S]TBPS binding in the cerebellum was not modified following either the activation of the climbing fibers by harmaline or the degeneration of this pathway induced by 3-acetylpyridine. The data suggest that the activation of excitatory amino acid system does not alter the function of GABA$_A$ receptor complex.

36.15

IS GABA THE ONLY ENDOGENOUS LIGAND FOR GABA-RECEPTOR? M. Yarom, X. W. Tang*, J. Bao*, Y. H. Lee* & J.-Y. Wu. Department. of Physiology and Cell Biology, University of Kansas, Lawrence, KS 66045.

γ-Aminobutyric acid (GABA) is a major inhibitory neurotransmitter in the mammalian CNS. The complexity and heterogeneity of GABA receptors led us to search for possible endogenous compounds which may interact with GABA receptors and modulate GABA binding sites. Pig brain P_2 membranes were used as a source for those modulators. Successive extractions of the membranes were filtered through a PM 10 membrane (exclusion limit: 10,000-dalton) and concentrated by lyophilization. Samples of the concentrated filtrates were tested for their ability to affect [^{3}H] muscimol (GABA$_A$-receptor agonist) binding, using the extensively washed pig brain membranes as the receptor source. The extracts that contained inhibitory activity were purified by a series of column chromatographies; gel filtration, ion exchange, and C_{18} HPLC columns. Three peaks of inhibition were obtained by Bio-Gel P-2 column. The major peak co-migrated with [^{3}H] GABA and couldn't be distinguished from GABA by further chromatographies. The two minor peaks which were separated from GABA, were not retained on anion exchange column, AG-1-X8, suggesting that they are probably neutral or positively charged compounds. It is most likely that they represent a new group of substances that can modulate GABA receptor activity. These two inhibitors are now subjected to further characterization and purification. (Supported by NIH Grant NS20978 and BNS-8820581 from NSF).

36.16

THE GENERAL ANAESTHETIC PROPOFOL ENHANCES THE FUNCTION OF THE GABA$_A$ IONOPHORE RECEPTOR COMPLEX. A. Concas, G. Santoro*, M.P. Mascia*, M. Serra*, E. Sanna* and G. Biggio, Dept. of Experimental Biology, Chair of Pharmacology, University of Cagliari, Italy

The effect of the general anaesthetic propofol (PF) has been evaluated "in vitro" on the function of the GABA$_A$-ionophore receptor complex and compared to that of pentobarbital (PB) and alphaxalone (AF) drugs known to enhance the GABAergic transmission. PF, mimicking the action of PB and AF, inhibited 35S-TBPS binding to rat cortical membranes in a concentration dependent-manner. However, while the efficacy of these drugs was similar (maximal inhibition 100%), they differed markedly in potency being AF > PF > PB. Consistently with its action on 35S-TBPS binding, PF, like AF and PB, enhanced muscimol-stimulated 36Cl$^-$ uptake in membrane vesicles from rat cerebral cortex (maximal enhancement +50% at 10 μM) but failed to affect basal 36Cl$^-$ uptake in the absence of GABA agonist. Although the effect of PF was similar to that of steroids and barbiturates, different sites of action were demonstrated by the observation that these drugs, when included together with PF, produced additive effects on 35S-TBPS binding as well as on 36Cl$^-$ uptake. The effect of PF on the function of the GABA-coupled chloride channel is dependent on the interaction of GABA with its recognition site. In fact, bicuculline reversed completely the effect of this drug on 35S-TBPS binding and muscimol-stimulated 36Cl$^-$ uptake. However the effect of these compounds is not due to a competitive interaction with GABA$_A$ receptors. In fact PF, like AF and PB enhanced 3H-GABA binding in the rat cerebral cortex. These data suggest that PF exerts its pharmacological effect by enhancing the function of the GABA$_A$ ionophore receptor complex.

36.17

EFFECTS OF GABA, PROPOFOL AND PENTOBARBITONE ON CHLORIDE CONDUCTANCE IN CHICK EMBRYO SENSORY NEURONS. M. Nobile*, V. Magnelli*, E. Maestrone*+, and C. Usai* (SPON: European Neuroscience Association) Istituto di Cibernetica e Biofisica, Genova, Italy, and +Ospedale Civile di Sondrio, Sondrio, Italy.

The effects of gamma-aminobutyric acid (GABA) and of general anaesthetics Propofol (PR) and racemic Pentobarbitone (PB) on Chloride channels in neurons of the chick embryo dorsal root ganglion, were investigated using the patch-clamp whole-cell recording technique. External and internal solutions blocking Na^+, K^+ and Ca^{2+} ionic currents were employed. GABA and PR increased PR and GABA induced I_{Cl}, respectively. Similar results were observed using either GABA and PB, or PR and PB. This potentiating effect occurred pretreating the preparation with one of the three drugs. Every drug elicited I_{Cl} in cells insensitive to both the other ones, and, in some cases, this activity induced a subsequent response to the other drugs. External perfusion with bicuculline or picrotoxin inhibited cell responses to GABA, PR and PB. Further investigations are in progress in order to assess the antagonistic effects of blockers on drugs induced I_{Cl}. Our results might display the response of the developing GABA$_A$ receptor complex with different binding sites for the three agents described or drugs' different receptors.

36.18

EFFECT OF CYPROHEPTADINE, BICUCULLINE, PICROTOXIN, AND PCPA ON NEGATIVE CONTRAST. P.S. Grigson and C.F. Flaherty. Rutgers University, New Brunswick, NJ 08903.

Rats shifted from a 32% to a 4% sucrose solution drink less 4% sucrose than controls having only experienced the 4% sucrose. This contrast effect was eliminated by the non-specific serotonin antagonist cyproheptadine (3 or 6 mg/kg), but was not reduced by the ip administration of PCPA (150 or 300 mg/kg). The effect of cyproheptadine was not altered by PCPA pretreatment. Additional studies with bicuculline, picrotoxin, buspirone, gepirone, ritanserin, ketanserin, and methysergide suggest that the effects of cyproheptadine are not mediated by a serotonergic system, but may partly involve the BDZ/GABA complex. In addition, an independent contrast reducing action of bicuculline (2 mg/kg) and picrotoxin (1 and 2 mg/kg) raises the possibility than an endogenous ligand may be responsible for the initiation of contrast and that this ligand may act via the BDZ/GABA complex.

36.19

BENZODIAZEPINES INHIBIT TRIIODOTHYRONINE TRANSPORT INTO HUMAN LIVER CELL LINE

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L-triiodothyronine (T3) accumulates stereospecifically, with energy-dependency and pM affinity, into HepG2 cells. The postulated iodothyronine carrier, though not yet isolated, is characterized by analogue competition and enzyme kinetic studies (Movius et al Endocrinology 124: 1988). CNS benzodiazepine (BZ) receptors are modulated by thyroid hormones in vitro (Nagy & Lajtha J Neurochem 40:414) and in vivo (Go et al J Neurochem 51:1497). Peripheral BZ sites are not linked to GABA receptor /Cl channel complexes and bind with micromolar affinity. Specific ligands bind CNS (RO15788) and peripheral (RO54864) sites. We report here, for the first time, that diazepam and its peripheral site analogue inhibit high affinity T3 accumulation into Hep G2 cells.

Confluent HepG2 cells in multiwell plates were incubated prior to assay with serum free media. Wells +/- drug and 60 pM 125I-T3 incubated x 1 hr, then cells were placed on ice, media was aspirated and cells washed. NaOH 0.5 M was added to wells and the hydrolysate was gamma counted. Nonspecific proportion equalled uptake with 1 uM unlabelled T3 or on ice.

MEAN % CONTROL (N):		1 uM unlab T3	61 (5)	4 C x 1 hr	34 (7)
1 uM Diazepam	78 (3)	RO54864	68 (2)	RO15788	97 (2)
10 uM	65 (5)		59 (2)		93 (2)

No effects of BZ's were seen under low temperature and uM T3 conditions. We conclude that peripheral BZ ligands interfere with high affinity T3 transport into liver cells and may be interacting directly with the iodothyronine carrier. Study supported by NIH grant 1K11DK0145601A2 to Dr. Kragie. Dr. DJ Triggle generously provided H. LaRoche BZ analogues and advice.

36.20

EFFECTS OF GABA ON EXTRACELLULAR pH IN TURTLE CEREBELLUM. JCT Chen, M Chesler & ME Rice. Dept. Physiology & Biophysics, Dept. Neurosurgery, NYU Med. Ctr., 550 1st. Ave., N.Y., NY 10016.

The GABA-gated anion channel is permeant to HCO_3^- in CNS neurons (Bormann, J. et al., J. Physiol. 385:243, 1987) and crayfish muscle (Kaila & Voipio, Nature 330:163, 1987). To determine if such channels can therefore modulate brain pH, we studied the effect of GABA on pH_o in turtle cerebellum *in vitro*. Microelectrodes were used to measure pH_o, $[K^+]_o$, and field potentials in the molecular layer in 35mM HCO_3^- (5% CO_2) or 35mM HEPES Ringer.

Superfusion of 1mM GABA caused an alkaline shift (AS) of 0.05±0.02 pH units (±SD, n=29 animals, range 0.02-0.09). A similar AS was evoked in the granular layer. The AS was blocked by picrotoxin and was evoked by the GABA-A agonists muscimol and isoguvacine (10^{-5}-10^{-3}M), but not by the GABA-B agonist baclofen (10^{-3}M). Since GABA and isoguvacine also caused a 1-3 mM rise in $[K^+]_o$, we tested for a synaptic origin of the AS: solutions containing zero Ca^{2+} or 4mM kynurenate did not block the AS. Replacement of HCO_3^- with HEPES reversibly abolished the AS, suggesting that the response was caused by the efflux of HCO_3^- through GABA-gated anion channels. Indeed, formate (which passes through these channels), when added to HEPES Ringer, restored the GABA-evoked AS.

Since excitatory synaptic activity is known to evoke an extracellular alkalinization (Kraig R.P. et al. J. Neurophysiol. 49:831, 1983) we tested whether GABA plays a role in such responses. In contrast to the GABA-evoked AS, the AS evoked by parallel fiber stimulation (5-10 Hz) was blocked by 1 mM GABA, was insensitive to picrotoxin (10^{-3}M), and was enhanced in HCO_3^--free solution. These data indicate that the GABA and parallel-fiber-evoked alkalinizations arise through different mechanisms. Our results suggest that local brain pH may be modulated independently by excitatory and inhibitory synaptic transmission. Supported by NINDS: NS27011 & NS13742.

37.1

ELECTROPHYSIOLOGICAL EVIDENCE DEMONSTRATING THAT BOTH D1 AND D2 RECEPTORS MEDIATE THE INHIBITORY EFFECT OF DOPAMINE IN THE MEDIAL ZONA INCERTA. M.J. Eaton and R.L. Moss. Dept. Physiol., Univ. of Texas Southwestern Med. Ctr., Dallas, TX 75235.

In vitro electrophysiological experiments have demonstrated that dopamine (DA), which is contained within many medial zona incerta (ZI) neurons, inhibits the spontaneous activity of one third of these neurons. D1 receptors in the medial ZI have been implicated in facilitation of luteinizing hormone secretion, whereas D2 receptors are believed to be the autoreceptor subtype. The purpose of the present study was to determine which DA receptor subtype is mediating the inhibitory response of medial ZI neurons to DA applied via micropressure. This was investigated in two ways: by attempting 1) to mimic DA inhibition with the selective D1 and D2 agonists, SKF82526 and LY163502; 2) to block the inhibition with the selective D1 and D2 antagonists, SCH23390 and sulpiride.

Out of 33 neurons tested, the inhibition noted during DA application was mimicked in 14 cases by only the D2 agonist. Only the D1 agonist mimicked the DA inhibition in 6 of the cells tested. In 5 cases, both agonists effectively inhibited the neuronal firing of the medial ZI neuron under test. In the antagonist portion of this study, 20 neurons which were inhibited by DA were tested with the selective DA receptor antagonists. In 10 of 19 cases tested with the D2 antagonist, there was at least 30% attenuation of the DA inhibition. In 7 of 11 neurons tested, the D1 antagonist attenuated the DA inhibition. In one instance, both selective antagonists attenuated the inhibition caused by exogenous DA application. The results of this study suggest that both D1 and D2 receptor subtypes may mediate the inhibitory effect of DA on medial ZI neurons. In some cases, only one receptor subtype appeared to be involved in the inhibitory response, whereas in other neurons, activation of either DA receptor subtype caused inhibition of cellular firing in the medial ZI. Supported by NIH grants HD09988 & HD07062.

37.2

EFFECT OF SKF 77434 ON A9 DOPAMINE NEURONS FIRING RATE IN IMMOBILIZED RATS.
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Previous studies (Diana et al 1989 Soc Neurosci Abstr, vol 15 part 2 p 1001, 1989) have shown that the new selective dopaminergic D1 agonist SKF 77434, administered intravenously, dose-dependently decreases the firing rate of nigro-striatal dopaminergic neurons through a mechanism which appears to be located posterior to the globus pallidus since SKF 77434-induced inhibition of firing persisted after hemitransection of the brain. These experiments were performed in urethane-anesthetized rats. In order to avoid possible interactions with urethane we repeated the experiments in immobilized, d-tubocurarine paralized and artificially respired rats. Male Sprague-Dawley (200-350 gr) rats were temporarily ahesthetized with alothane then incannulated with a tracheal catheter for artificial respiration and in the femoral vein for intravenous administration of drugs. D-tubocurarine (4mg/kg iv) was administered and once muscular paralysis was obtained the rats were placed on a stereotaxic frame (Kopf). SKF 77434 was administered at exponentially increasing doses (12.5-1600 ug/kg). When dopamine neurons were recorded from anterior substantia nigra pars compacta (coordinates from lambda: AP 2.0; L 1.9 nose bar -2.5) SKF 77434 decreased the firing rate of the majority of dopamine neurons (65-70%). However, when dopamine neurons were recorded from a different subregion (coord. AP 1.8; L 1.8) only a small number of neurons (10-15%) were found to be sensitive to SKF 77434. Experiments are under way to characterize the differences between the two populations.

37.3

DIHYDREXIDINE ACCUMULATES RAPIDLY AND SPECIFICALLY IN RAT BRAIN AFTER SYSTEMIC ADMINISTRATION. Q. David Walker, Cindy P. Lawler, Barbara Faggin, Stan Southerland, Julie Bennett, Mark H. Lewis, D.E. Nichols*, and Richard B. Mailman. Curriculum in Toxicology, Brain and Development Research Center, Departments of Psychiatry and Pharmacology, University of North Carolina at Chapel Hill, NC 27599 and School of Pharmacy, Purdue University*, West Lafayette, IN 47907

Dihydrexidine (trans–10,11–dihydroxy–5,6,6a,7,8,12b–hexahydrobenzo[a]phenanthridine) is a high potency full efficacy D_1 dopamine receptor agonist which we hypothesize will have specific utility as both a neuropharmacological probe and a potential therapeutic agent for parkinsonism. An HPLC method was developed to permit the quantification of dihydrexidine in discrete brain nuclei following systemic administration. Dihydrexidine was separated from endogenous monoamines, their metabolites and precursors on a reverse phase 10 cm C18 3 μm column. Monoamines were selectively retained by 18–crown–6–ether, enabling a total analysis time of 15 minutes. Dihydrexidine was detected electrochemically (750 mV), with a detection limit of about 10 pg. Striata and cerebelli were dissected on ice, and homogenized in the mobile phase. After centrifugation, an aliquot of the supernatant was alumina–extracted. Recovery of dihydrexidine was ca. 55%.

Adult Sprague–Dawley rats were treated with 3 mg/kg dihydrexidine (s.c.) and sacrificed 0, 10, 20, 40, or 120 minutes after dosing. This dose has been shown to induce selectively and rapidly behaviors commonly thought to be mediated by D_1 dopamine receptors. Peak concentrations of dihydrexidine were found in both striatum and cerebellum 20 minutes post–dosing. Dihydrexidine was almost undetectable at 120 minutes after treatment. Concentrations of dihydrexidine in striatum (ca. 18 pg/mg tissue) was about twice as great as that in cerebellum 10 and 20 minutes post dosing. The dihydrexidine concentrations in striatum reflect drug bound to dopamine receptors, as well as drug non–specifically bound to tissue or blood. The time course of peak brain concentrations of dihydrexidine correlates well with the time course of peak behavioral effects induced by the drug. The described technique should be useful in characterizing further the in vivo pharmacology of this new drug.

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37.4

DIHYDREXIDINE, A NOVEL AGONIST TO STUDY THE PHARMACOLOGY AND STRUCTURAL REQUIREMENTS OF D_1 DOPAMINE RECEPTORS. D.M. Mottola[1], W.K. Brewster[2*], D.E. Nichols[2] and R.B. Mailman[1]. University of North Carolina[1], Chapel Hill, N.C. 27599. Purdue University[2], West Lafayette, IN. 47907

Dihydrexidine is a member of a new class of D_1 dopamine receptor ligands (trans–hexahydrobenzo[a]phenanthridines). This agent possesses high affinity for the D_1 receptor, is a full efficacy agonist, and is a structurally rigid compound. These features make dihydrexidine an important tool to study the pharmacology and structural requirements of D_1 dopamine receptors. Pharmacological studies have shown that dihydrexidine competes stereoselectively for D_1 (i.e. ^{3}H–SCH23390) binding sites in rat striatal membranes with an IC50 of ca. 12 nM and for D_2 sites (i.e. ^{3}H–spiperone) of ca. 120 nM. Adenylate cyclase assays show that dihydrexidine is able to activate the D_1 receptor to the same degree as dopamine itself. The N–alkyl derivatives of dihydrexidine show higher IC50 values for D_1 sites, but lower IC50's for D_2 sites.

This study also reports preliminary data regarding the functional groups required for activation of the D_1 receptor. The D_1 receptor pharmacophore was assessed by computerized molecular modeling of dihydrexidine, the thienopyridine compound THP (a less potent, full efficacy D_1 agonist), and (R)–SKF38393 (partial agonist). These three agonists were overlaid and minimized by the Maximin program in SYBYL. Comparisons of these ligands allowed various hypotheses to be generated. First, based on the degree of overlap with (R)–SKF38393, it was predicted that 6a(R),12b(S)–dihydrexidine and (R)–THP are the active enantiomers. Secondly, the orientation of the phenyl ring of (R)–SKF38393 was perpendicular to the catechol moiety, while the accessory rings of the two full agonists were essentially planar. Perhaps this ring orientation is responsible for the fact that (R)–SKF38393 is a partial agonist. In addition, all three agonists were able to conform to a D_1 receptor pharmacophore consistent with that proposed by Nichols (*Dopamine Receptors, pg. 201, 1983*). A union of each ligands volume map generated the excluded volume map of the D_1 receptor. The less active structural analogs of dihydrexidine (IC50 > 500 nM) were subsequently minimized and their volume maps compared to the excluded volume map to derive the receptor essential volume of the D_1 receptor. These molecular modeling studies using the Active Analog Approach permit our working model of the D_1 receptor to be refined further, and can lead to the design of novel, more selective D_1 ligands.

37.5

SELECTION FOR HALOPERIDOL-RESPONSIVE(HR) AND HALOPERIDOL NON-RESPONSIVE(HNR) LINES OF MICE.
R.Hitzemann, K.Dains*, Y.Qian, B.Hitzemann* and N.Zahniser[1]. Departments of Psychiatry and Neurobiology, SUNY at Stony Brook, Stony Brook, NY 11794 and [1] Department of Pharmacology, U. Colorado, Denver, CO. 80262.

Using HS/Ibg mice as the parent strain, we have selected mice over nine generations for response/ non-response to haloperidol-induced catalepsy. The selection has been asymmetric, with a greater selection divergence for the HNR line. The haloperidol ED_{50s} at S_6 for the HR and HNR line were 0.4 $\pm$ 0.1 and 4.3 $\pm$ 0.4 mg/kg, respectively. Fluphenazine, trifluoperazine, spiroperidol and raclopride showed a 7-fold or greater discrimination between lines. Chlorpromazine, (+) butaclamol, cis-flupenthixol and thiothixene showed a 3-4 fold discrimination. SCH-23390, was a potent cataleptogenic agent (ED_{50} = 0.1 mg/kg), which did not discriminate between the lines. D_2 receptor density in either the whole caudate or nucleus accumbens was similar in both lines. However, D_2 somatodendritic receptor density in the substantia nigra, was 80% higher in the HNR line. Evidence that this difference in receptor density may be involved in the differential response to haloperidol will be presented.

37.6

THE DENSITY AND TURNOVER OF POST-SYNAPTIC D_2 DOPAMINE RECEPTOR ON THE HALOPERIDOL-RESPONSIVE (HR) AND HALOPERIDOL NON-RESPONSIVE (HNR) LINES OF MICE.
Y. Qian, R. Hitzemann and B. Hitzemann*. Dept.'s of Psychiatry, and Neurobiology, SUNY at Stony Brook, NY 11794.

The HR and HNR lines of mice differ >10 fold in their sensitivity to haloperidol-induced catalepsy. We now report on D_2 density in discrete regions of the caudate-putamen. Significant caudal to rostral differences were found between the HR and HNR lines in D_2 density. The HR line showed the typical caudal-to-rostral decrease in D_2 receptor density whereas the HNR line showed the highest density in the medial caudate. In the rostral caudate, the receptor density was significantly higher in the HR line. In the caudal caudate, density was higher in the HNR line. Receptor turnover, assessed by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ), was significantly faster in both lines in the caudal aspects of the caudate as compared to the more medial and rostral areas. In the rostral caudate, receptor turnover was faster in the HNR line. In contrast, in the caudal part of caudate, it was faster in the HR line. These data illustrate that there are discrete differences in post-synaptic D_2 receptor density between lines.

37.7

DUAL ACTION OF FCE 23884, A NEW ERGOLINE DERIVATIVE, ON DAergic SYSTEM. N. Carfagna*, C. Caccia*, S. Cavanus*, M.G. Fornaretto*, M. Buonamici* and R.G. Fariello

R&D., Farmitalia Carlo Erba - Erbamont Group, CNS Dept, 20014 Nerviano, Italy

FCE 23884 has been shown to possess an unusual pharmacological spectrum. Behaviorally, the compound exhibited dopamine (DA) antagonistic profile in normal animals and a strong DA agonist action in models of denervation, such as 6-OHDA lesioned rats, reserpinized rodents and MPTP-treated monkeys.

We studied the interaction of FCE 23884 with central DAergic system in normal and reserpine-treated rats.

In vitro binding assays showed that FCE 23884 bound to D-2, α-2 and 5-HT1A sites in the nM range; the affinity for D-1 and S-2 receptor was moderate (sub μM). FCE 23884 potently stimulated the basal activity of adenylate cyclase in vitro (E_{50}=0.6μM). The compound dose dependently accelerated the turnover of DA in rat brain as demostrated by the increased levels of HVA, and DOPAC (ED_{50}=0.1 mg/Kg s.c.) and by the enhancement of DA synthesis in both striatum and n. accumbens of normal rats. FCE 23884 was also able to reverse apomorphine inhibitory action on γ-butyrolactone activity indicating a presynaptic DA antagonistic action. These profiles are consistent with the antidopaminergic effect of FCE 23884 in normal animals. In reserpine pretreated rats (18hr), FCE 23884 (1 mg/kg s.c.) significantly antagonized reserpine induced striatal HVA and DOPAC elevation (±40%), an effect notably displayed by DA agonists. Taken as a whole our neurochemical data support the earlier assumption based on behavioral studies that FCE 23884 possesses both DA antagonist and agonist actions depending on the substrate.

As judged from this unique pharmacologic profile FCE 23884 is expected to be of therapeutic value in the neuropsychiatric disorders associated with DA dysfunction.

37.8

THE EFFECT OF SCH 23390 AGAINST TOXIC DOSES OF COCAINE, d-AMPHETAMINE AND METHAMPHETAMINE. R.W. Derlet, T.E. Albertson and P. Rice*. Dept. Internal Med. & Pharmacology, U.C.D. School of Med., Davis, CA 95616.

The effect of SCH 23390, a dopamine-one (D_1) antagonist, in preventing acute toxicity induced by lethal doses of cocaine, d-amphetamine, and methamphetamine was studied in the rat. Animals were first pretreated with SCH 23390 (0.0, 0.5, 1.0, and 2.5 mg/kg, i.p.) and then were challenged with cocaine (70 mg/kg, i.p., an LD_{85}), d-amphetamine (75 mg/kg, i.p., an LD_{95}), and methamphetamine (100 mg/kg, i.p., an LD_{90}). SCH 23390 did not significantly alter the incidence of seizures compared to the vehicle controls for each of the three stimulants tested. Significant protection against cocaine-induced death was afforded only by the lowest dose of SCH 23390 tested, with death occurring in 50% of the animals ($p \leq 0.05$). Significant protection against d-amphetamine-induced death was provided by all doses, with a dose dependent effect noted so that the death incidence decreased from 95% for vehicle to 30% ($p \leq 0.01$) with 2.5 mg/kg SCH 23390 pretreatment. No statistically significant reduction in the incidence of methamphetamine-induced death was seen with SCH 23390 pretreatment. The ability of SCH 23390 to protect against d-amphetamine, but not methamphetamine-induced death, suggests differing mechanisms of toxicity may exist between these drugs at high doses. The antagonistic effect of SCH 23390 against cocaine at a low dose is consistent with other reports.

37.9

TWO NEW RADIOIODINATED LIGANDS WITH HIGH AFFINITY AND SELECTIVITY FOR D-1 AND D-2 DOPAMINE RECEPTORS. M.P. Kung, J. Billings, S. Chumpradit, R. Murphy, Y. Yang and H. Kung, Univ. of Pennsylvania, Philadelphia, PA 19104.

Radioodinated ligands with high specific activity and selective affinity are useful probes for various neurotransmitter systems. Several selective iodinated benzamides for D-2 receptor and benzazepines for D-1 receptor have been developed.

One of the benzamides,5-iodo-7-N-[1-ethyl-2-pyrrolidinyl)methyl] - carboxamido-2,3-dihydrobenzofuran(IBF) displays in vitro binding specificity to D-2 dopamine receptors with high affinity and low nonspecific binding (Kd=0.106nM, rat striatum).The competition data with various neurotransmitter ligands indicate that IBF is very selective and the results are consistent with the pharmacological profile of a D-2 receptor antagonist. In vivo biodistribution studies and ex vivo autoradiography results in rats with IBF further confirmed the specific localization of this agent in the basal ganglia region where the D-2 receptor is concentrated. 7-chloro-8-hydroxy-1-(3'-iodophenyl)-3-methyl-2,3,4,5-tetrahydro-1H-3-benzazepine(TISCH) is the best D-1 ligand for in vitro and in vivo characterization. The binding constant is comparable to that of SCH-23390 (0.2nM vs 0.32nM, rat striatum). The displacement studies with various ligands indicated that TISCH binds specifically to D-1 receptor with high selectivity and the binding is stereoselective with the preference for the R(+)isomer. Furthermore, the in vivo organ distribution and the ex vivo autoradiography studies with R(+) TISCH displayed high uptake in striatum and substania nigra, region known to have a high concentration of D-1 receptors, while the S(-) isomer(inactive) displayed no specific uptake.

Radioiodinated IBF and TISCH are valuable pharmacological tools for probing D-1 and D-2 dopamine receptors under in vitro and in vivo conditions.

37.10

BEHAVIOURAL, BIOCHEMICAL & ELECTROPHYSIOLOGICAL STUDIES ON THE MOTOR DEPRESSANT & STIMULANT EFFECTS OF BROMOCRIPTINE D.M. Jackson✚ ■ L.P. Martin● L.-G. Larsson *✚, R.F. Cox●, B.L. Waszczak● & S.B. Ross*✚ ✚ Astra Research Centre, Södertälje, Sweden, ■ Pharmacology Department, Sydney University, NSW, Australia, ● Pharmacology Section, Pharmacy & Allied Health Professions College, Northeastern University, MA 02115, USA.

Bromocriptine (BRC) produced a biphasic motor effect in mice: early depression which lasted for about 1 h & later stimulation which lasted from about 1 to 5 h & which was blocked with SCH23390. Both phases were accompanied by reductions in dihydroxyphenylacetic acid (DOPAC) & homovanillic acid (HVA) levels & a reduction in DOPA accumulation (after inhibition of nigro-striatal dopamine (DA) nerve firing & of DOPA decarboxylase). However, while the autoreceptor (AR) effects were still evident during the stimulant phase, there was a gradual rise in DOPAC & HVA from 1 to 4 h after injection, indicating an increase in DA turnover, due perhaps to acute desensitization of the synthesis regulating DA ARs. Using behavioural & biochemical paradigms, no change in DA AR sensitivity occurred after one dose of BRC & challenge with a second dose. In electrophysiological studies, it was found that prior exposure of rats to 1 dose of BRC rendered them subsensitive to the rate-inhibiting effects of a 2nd dose of BRC, as measured in anaesthetized animals using extracellular recordings of DA neurons in the substantia nigra pars compacta. In conclusion (1) the stimulant phase of BRC in mice occurs despite continued occupation of the DA ARs by BRC because adequate DA is available to provide the required D1 receptor stimulation; (2) the terminal ARs in the striatum may be regulated differently from the somatodendritic ARs.

37.11

QUANTITATIVE AUTORADIOGRAPHY OF DOPAMINE RECEPTORS IN THE SPONTANEOUSLY HYPERTENSIVE RAT. K. Kujirai*, S. Przedborski, V. Kostic*, V. Jackson-Lewis, S. Fahn and J. L. Cadet. Columbia University, New York, New York 10032.

The spontaneously hypertensive rat (SHR) has been used to evaluate the role of catecholaminergic systems in the development of hypertension. Some differences in the dopamine system have been reported between SHR and normotensive rats. The present study assessed possible differences in dopamine (DA) D1 and D2 receptors between SHR and normotensive rats. The status of DA uptake sites was also evaluated. Quantitative receptor autoradiography revealed significantly higher binding of D1 and D2 receptors in the caudate-putamen (CPu), the nucleus accumbens (NAc) and olfactory tubercle (OT) of SHR in comparison to Sprague-Dawley (SD) rats. High-to-low lateromedial gradients were observed in D2 receptors in both strains. There were no differences in DA uptake sites between the two strains. Unilateral injection of 6-hydroxydopamine (6-OHDA) into the rat striatum resulted in greater than 90% depletion of dopamine uptake sites in the striatum of both strains of animals. These changes were associated with significant increases in striatal D2 receptors which were of similar magnitude in both strains of rats. Striatal D1 receptors were not affected by the 6-OHDA-induced lesions. These results demonstrate that, although the SHR have higher concentration of both D1 and D2 receptors in the basal ganglia, these receptors are influenced in a similar fashion by striatal DA depletion.

37.12

COMPARISON OF THE SELECTIVITY AND SPECIFICITY OF ASCORBIC ACID ANTAGONISM OF DOPAMINERGIC AGONIST BINDING. L.C. Tolbert, J.J. Spollen*, P.E. Morris*, Dept. of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL 35294.

Ascorbic acid (AA) is a water-soluble vitamin with multiple known functions in the body. In recent years a number of investigators using both in vivo and in vitro techniques have raised the possibility that AA may function as a neuromodulator in CNS dopaminergic function. For example, it has been reported to modulate dopamine agonist binding and cyclic AMP stimulation as well as antagonize the behavioral and physiological effects of amphetamine and potentiate the effects of haloperidol. The purpose of the present study was to evaluate the selectivity and specificity of the AA effect on dopaminergic function. In vitro binding studies were utilized as a system uncomplicated by questions of transport, etc. [^{3}H]N-0437,, was chosen as the ligand to define D_2 agonist binding and H] SKF 38393, was chosen as the ligand to define D_1 agonist binding for reasons of stability and selectivity. The effect of AA, d-ascorbic acid (iso) and D-glucoascorbic acid (gluco) on the binding of these two ligands to striatal membranes from rats was evaluated. The rationale for the choice of these three compounds was that they have relatively similar redox properties but if the AA effect was a specific receptor-mediated phenomena the compounds should have differences in potency. Using 2.5 nM ^{3}H-N-0437 (~ K_D) and increasing concentrations of the 3 compounds we confirmed significant differences in their inhibition curves. The IC_{50} were: AA 93.3, iso 199.5, and gluco 9,549.9 micromolar. Using a similar paradigm for SKF 38393 a similar rank order of potency of the three compounds was obtained but all were significantly less potent at inhibiting the D_1 agonist binding (IC_{50}'s): AA = 1,897 and ISO = 2,828 μM). Possible differences in mechanisms of antagonism will be discussed.

37.13

PRAMIPEXOLE (SND 919), A NEW DOPAMINE D-2 RECEPTOR AGONIST, DECREASES THE EXTRACELLULAR CONCENTRATIONS OF DOPAMINE AND ITS METABOLITES IN THE STRIATUM OF FREELY MOVING RATS. A. J. Carter and R. E. Müller (SPON: R. Anderson), Department of Pharmacology, Boehringer Ingelheim KG, 6507 Ingelheim, West Germany.

Pramipexole is a new D-2 autoreceptor agonist which is structurally related to the potential antipsychotic agent, B-HT 920. We have studied the effects of pramipexole on the extracellular concentrations of dopamine (DA), 3,4-dihydrophenylacetic acid (DOPAC) and homovanillic acid (HVA) in the striatum of freely moving rats by means of microdialysis and HPLC with electrochemical detection. Pramipexole (0.1 mg/kg s.c.) caused long-lasting (≥ 2 h) decreases in DA, DOPAC and HVA. Haloperidol (0.3 mg/kg s.c.), a DA antagonist, caused a transient (ca. 20 min) increase in DA and long-lasting (≥ 2 h) increases in DOPAC and HVA. Haloperidol also reversed the effects of pramipexole. These results indicate that the D_2 autoreceptor agonist, pramipexole, reduces the extracellular concentrations of DA and its metabolites in vivo by a reversible interaction with the DA receptor. Pramipexole could therefore be used as an alternative treatment for schizophrenia.

37.14

PHARMACOLOGICAL CHARACTERIZATION OF THE INHIBITORY RESPONSES INDUCED BY VTA STIMULATION IN THE RAT PREFRONTAL CORTEX. R. Godbout, J. Mantz*, S. Pirot*, A.M. Thierry*, J. Glowinski* Chaire de Neuropharmacologie, Collège de France, 75231 Paris Cedex 05, France.

The medial prefrontal cortex (PFC) receives converging projections from DA neurons located in the VTA and NA fibers running through the dorsal VTA. Electrical stimulation (1Hz) of the VTA induces an inhibition (Dur.=110ms) of spontaneous firing in 80% of the PFC cells recorded in layers III to VI. To determine the type of receptor mediating this response, we analyzed the effect of microiontophoretic applications of NA and DA receptor antagonists on VTA-induced inhibitions in ketamine-anesthetized rats. Firing inhibition following VTA stimulation was suppressed by application of the selective D_2/D_4 antagonists sulpiride (61% of the 48 cells tested), RIV 2093 (88% of 16 cells), and LUR 2366 (88% of 8 cells). Inhibitions were still present upon the application of the D_1 antagonist SCH-23390 (n=11) as well as that of the ß-(propranolol; n=11), α_1-(prazozin; n=7) and α_2- (yohimbine; n=13) adrenoreceptor antagonists. In conclusion, the receptor mediating the inhibition of PFC spontaneous firing induced by VTA stimulation has the pharmacological profile of a D_2/D_4 recognition site.

Supported by INSERM and FRSQ.

37.15

REGULATION OF BASAL GANGLIA GLUCOSE UTILIZATION BY THE D1 DOPAMINE RECEPTOR. J.M. Trugman, C.L. James and G.F. Wooten. Dept. of Neurology, Univ. of Virginia, Charlottesville, VA 22908.

In rats with unilateral dopamine depletion, D1 agonists increase regional cerebral glucose utilization (RCGU) in the subthalamic nucleus, entopeduncular nucleus, and substantia nigra pars reticulata (SNr) (J. Neurosci. 7:2927, 1987). In contrast, it has been reported that RCGU is not altered by D1 stimulation or antagonism in rats with intact dopamine innervation (Brain Res. 327:390, 1985). To re-examine this question, we studied the effects of a selective D1 agonist (SKF 38393, 30 mg/kg) and antagonist (SCH 23390, 0.5 mg/kg) on RCGU in naive, male Sprague-Dawley rats using quantitative [14C]-2-deoxyglucose autoradiography. Administration of SKF 38393 increased RCGU in the SNr (+26%), but did not alter RCGU in other basal ganglia regions. Administration of SCH 23390 decreased RCGU in the caudate-putamen (-15%), globus pallidus (-29%), entopeduncular nucleus (-30%), subthalamic nucleus (-26%), and SNr (-28%); RCGU was increased in the lateral habenula (+17%). We conclude that stimulation of the D1 receptor by endogenous dopamine contributes substantially to RCGU in multiple basal ganglia nuclei of naive, awake, behaving rats.

37.16

EFFECTS OF DOPAMINERGIC AGONISTS ON CULTURED SUBSTANTIA NIGRA NEURONS. K-M. Kim*, Y. Nakajima and S. Nakajima. Dept. of Pharmacology and Dept. of Anatomy and Cell Biology, Univ. of Illinois, Chicago, IL 60612.

We have cultured neurons separately from the pars compacta and the pars reticulata of the substantia nigra of newborn rats and investigated dopamine effects with the whole-cell patch clamp technique. When the cell was clamped at -74mV, dopamine (0.1-30μM) or (-)-quinpirole (D_2 agonist, 0.1-10μM) produced a conductance increase concomitant with an outward current, suggesting that the agonists increased K-conductance. These effects were found mainly on neurons from the pars compacta, and were blocked by 1-3 μM of S(-)-sulpiride (D_2 antagonist). Pretreatment of cultures with pertussis toxin (500 ng/ml) overnight abolished the conductance increase induced by the agonists. But in some cells an outward current, not accompanied by conductance increase, remained after the pertussis toxin treatment, suggesting that two different effects are produced by the agonists. Intracellular application of GTP-γS (500 μM) through the patch pipette spontaneously increased conductance concomitant with an outward current in the absence of agonist. In some cells, especially neurons from the pars reticulata cultured for a long time (> 3 weeks), dopamine (10 - 20 μM) or R(+)-SKF38393 (D_1 agonist, 25 μM) decreased conductance concomitant with an inward current. Supported by NIH grant, NS24711.

37.17

STRIATAL INJECTION OF RECEPTOR INACTIVATOR EEDQ REVEALS NO ROLE FOR STRIATAL DOPAMINE (DA) RECEPTORS IN INHIBITION OF NIGRAL DA CELL FIRING BY I.V. R(-)NPA. R.F. Cox, L.P. Martin and B.L. Waszczak. Pharmacol. Sect., Northeastern Univ., Boston, MA 02115.

We previously reported that intranigral injection of EEDQ reduced the ability of i.v. R(-)N-n-propylnorapomorphine (NPA) to inhibit substantia nigra (SN) DA cell firing (Soc. Neurosci. Abstr. 15:427,1989). This effect was revealed as a 4-fold increase in the ED50 for NPA and a 20% decline in maximum response. We have now carried out complementary studies to assess the contribution of striatal DA receptors toward the same response. Rats were anesthetized and pretreated with i.p. prazosin (5 mg/kg), idazoxan (1.3 mg/kg), and ketanserin (5 mg/kg) 30 minutes before striatal injections. EEDQ (200 nmol, 2 ul total) or hydroxypropyl-B-cyclodextrin vehicle (VEH) were injected stereotaxically at 3 sites in the left striatum. Extracellular single unit recording studies were performed on the next day. Unlike results after intranigral injections of EEDQ, responses of SN DA cells to i.v. NPA were not significantly affected by striatal DA receptor inactivation. The mean ED50 was 0.38 ± 0.20 ug/kg after intrastriatal EEDQ (n=9) versus 0.37 ± 0.12 ug/kg after VEH injections (n=6). In both groups full inhibition of DA cell firing occurred at the same dose of NPA (5 ug/kg). Autoradiographic analysis of the degree of DA receptor loss after similar intrastriatal EEDQ injections showed >90% losses of D-1 (3H-SCH 23390) and D-2 (3H-YM-09151-2) binding sites in the injected striata. These studies confirm previous assertions that nigral DA receptors (most likely somatodendritic autoreceptors) play a predominant role in mediating the inhibition of SN DA cell firing by systemically administered direct-acting agonists like NPA. Conversely, striatal DA receptors appear to be unnecessary for this response. (Supported by NS 23541.)

37.18

RECEPTOR-MEDIATED PRESYNAPTIC REGULATION OF DOPAMINE SYNTHESIS IN RAT STRIATAL TISSUE. R.G. Booth* and R.J. Baldessarini, Harvard Medical School (Psychiatry & Neuroscience), and Mailman Research Center-McLean Hospital, Belmont, MA 02178

Modulation of striatal dopamine (DA) synthesis by presynaptic receptors in minced rat corpus striatum was probed by measuring the ability of selective agents to inhibit or stimulate tyrosine hydroxylase (TH) activity monitored with [1-^{14}C]-L-tyrosine. S(+)N-propylnorapomorphine (NPA), a relatively potent D_2 *autoreceptor* agonist, inhibited TH (IC_{50}=1.0 µM) as did the D_2 agonists R(–)-NPA (0.3 µM) and quinpirole (5.0 µM); these effects were blocked selectively by D_2 antagonists and reversed by c-AMP-increasing agents (eg, forskolin). The D_1 agonist CY-208-243, as well as α_1, α_2, β_1, and β_2 adrenergic agonists and 5HT agonist quipazine, had no effect on TH (up to 10 µM). The adenosine A_1 agonist, N^6-cyclopentyladenosine, weakly inhibited TH (IC_{30}=100 µM) but the A_1-A_2 agonist 2-Cl-adenosine stimulated TH activity (EC_{50}=60 µM); this stimulation was blocked by theophylline (A_1-A_2 antagonist) but not 8-cyclopentyltheophylline (A_1 antagonist), suggesting A_2 mediation, and was attenuated by D_2 agonists but not additive with excess forskolin, suggesting involvement of c-AMP. (+)N-allylnormetazocine (σ opiate agonist) also potently stimulated TH (EC_{30}=0.1 µM); this effect was attenuated by S-NPA and not additive with forskolin. The results suggest that D_2 autoreceptors negatively modulate striatal DA synthesis while presynaptic A_2, and perhaps σ receptors, act via opposing positive neuroregulatory mechanisms that may be cAMP-mediated. [Support: NIMH grants 34006, 47370, 14275, & BJ Anderson Foundation]

37.19

THE D1/D2 AGONIST PERGOLIDE AND THE D2 AGONIST BROMOCRIPTINE MEDIATE BODY TEMPERATURE DIFFERENTIALLY IN RESERPINIZED MICE THROUGH DISTINCT RECEPTOR SUBTYPES. D. R. Helton, D. O. Calligaro, and D. L. Modlin. Lilly Research Laboratories, Eli Lilly and Company, Greenfield, IN 46140.

The nonselective potent D1/D2 agonist, pergolide, and the selective D2 agonist, bromocriptine, both produce hypothermia in control mice. However, in male CD-1 mice made hypothermic by prior administration of reserpine (2.5 mg/kg IP), these compounds produced differential effects. Administration of pergolide (0.3 to 30 mg/kg, PO) or the selective D1 agonist, SKF 38393 (1 to 10 mg/kg, IP), produced significant dose-dependent increases in body temperature. Bromocriptine (0.3 to 30 mg/kg, PO) and the D1 antagonist SCH 23390 (1 to 10 mg/kg, IP) further decreased body temperature in reserpinized mice. Increasing doses of SKF 38393 in combination with bromocriptine (3 to 10 mg/kg) produced a temperature increase in hypothermic mice equal to those seen with SKF 38393 alone. In addition to the hypothermia, the change in D1/D2 receptor density in reserpinized mice was determined using radiolabeled SCH 23390 and spiroperidol. These results indicate that the hypothermic and hyperthermic effects of dopamine agonists are mediated via distinct receptor subtypes. Activation of the D1 receptor is responsible for the increase in body temperature in reserpinized mice, while activation of the D2 decreases temperature.

37.20

D_1 AND D_2 DOPAMINE RECEPTORS IN THE INFANT HUMAN NEOSTRIATUM. C.E. Adams and S.J. Boyson. Department of Neurology, University of Colorado Health Sciences Center, Denver, CO 80262.

D_1 and D_2 receptors were examined in the infant human neostriatum using quantitative autoradiographic techniques. D_1 receptors were labeled with ^{3}H-SCH-23390 while D_2 receptors were labeled with ^{3}H-spiperone. ^{3}H-diprenorphine binding was used as a marker for the striosomal compartment.

D_1 and D_2 dopamine receptors were heterogeneously distributed within the neostriatum at both ages examined (32 weeks and 4 days postnatal), although this heterogeneity differs from that reported in adults. Islands of intense receptor binding were embedded in a background of moderate intensity. Occasionally, the islands were bordered by narrow regions of little to no binding. The D_1 and D_2 islands were only partially coextensive and bore no relation to the striosomes as defined by the ^{3}H-diprenorphine binding.

D_2 receptor density was greater than D_1 receptor density within each age group, the reverse of adult data. Total D_1 receptor density was greater at 32 weeks than at 4 days, possibly due to a decrease in neostriatal cellular density with increasing age. Subdivisions exhibiting greater D_1 receptor densities at 32 weeks included the ventromedial quadrant of the caudate as well as the dorsomedial, dorsolateral and ventrolateral quadrants of the putamen.

PEPTIDES—RECEPTORS: CCK, SOMATOSTATIN

38.1

MOLECULAR CLONING OF A CCK/GASTRIN-RESPONSIVE G PROTEIN-COUPLED RECEPTOR. L.D. McVittie, F.J. Monsma, Jr. C.R. Gerfen, R.M. Burch*, D.R. Sibley & L.C. Mahan*. ETB, NINDS & LCB, NIMH, NIH, Bethesda, MD 20892 & Nova Pharmaceutical Corp., Baltimore, MD 21224.

We have used the polymerase chain reaction (PCR) method to selectively amplify G protein-coupled receptor cDNA sequences from rat striatal mRNA. Poly (A)$^+$ RNA was first sized on sucrose gradients followed by reverse transcription of the 2-3 kb mRNA fractions. The resulting cDNAs were amplified using sets of highly degenerate primers derived from the transmembrane sequences of previously cloned G protein-coupled receptors. A novel cDNA fragment was identified which exhibits considerable homology to various members of the G protein-coupled receptor family. This PCR fragment was used isolate a full-length cDNA from a rat striatal library. A 2.2 kb clone was isolated encoding a 326 amino acid protein which contains 7 transmembrane domains as predicted by hydropathy analysis. The extracellular NH_2 terminus consists of 10 amino acids and lacks N-linked glycosylation sites while the 3rd cytoplasmic loop is 35 residues long and contains 1 phosphorylation site for cAMP-dependent protein kinase. Northern blot analysis in brain tissues reveals 2 transcripts of 5.6 and 3.1 kb. Both mRNAs are highly abundant in cortex, hippocampus, cerebellum, and striatum with lower levels in the olfactory bulb, mesencephalon, pituitary and retina. *In situ* hybridization analysis also indicates a high abundance of mRNA in the cortex, hippocampus and thalamus with lesser amounts in the striatum. To establish the identity of this receptor, we have transcribed RNA from the cDNA clone for expression in *Xenopus* oocytes. Out of numerous agonists tested for their ability to induce $^{45}Ca^{2+}$ efflux activity, both CCK and gastrin exhibited the greatest stimulation of efflux (2-4 fold) in RNA- but not H_2O-injected oocytes. Additional pharmacological experiments to confirm the identity of this receptor clone are presently being performed.

38.2

NT AND CCK RECEPTOR cDNAs: ENRICHMENT BY OOCYTE EXPRESSION AND cDNA LIBRARY SIB SELECTION. B.F. O'Hara, J.M. DiGiorgianni,* S. Shimada, E.M. Landau, G.R. Uhl and H. Meiri. Lab. of Mol. Neurobiol., NIDA/ARC, and Depts. of Neurol. & Nsci., Johns Hopkins Sch. of Med., Bx 5180, Baltimore, MD 21224. Mt. Sinai Sch. of Med. & Bronx VAMC, Dept. of Psych., Bronx, NY 10468.

Receptors for the neuropeptides cholecystokinin (CCK) and neurotensin (NT) participate in many CNS and peripheral functions. Injection of appropriate RNAs into Xenopus oocytes can lead to the expression of either receptor. This expression can be monitored by electrophysiological recording following the application of ligand. Oocytes injected with RNA from rat cerebral cortex gave positive signals for both receptors. In vitro RNA transcripts from a L-Zap cDNA library constructed from this RNA also produced responses to CCK and NT, in addition to 5-HT, bombesin and bradykinin. Fractionation of this library by sib selection has produced small pools of clones, one of which appears to contain a cDNA for the CCK receptor and another for the NT receptor. Under voltage clamp conditions (-70mV), inward currents have ranged from 20-3000nA upon application of ligand. In general, signal size has increased at each fractionation level consistent with an increased purification of receptor clones. Isolation of individual cDNAs encoding the CCK and NT receptors should be possible using this approach.

38.3

EVIDENCE THAT GASTRIN RECEPTORS ON AR4-2J CELLS ARE DISTINCT FROM CCK-B RECEPTORS, USING NOVEL PYRAZOLIDINONE CCK ANTAGONISTS. J.J. Howbert, K.L. Lobb*, R.F. Brown*, J.K. Reel*, L.G. Mendelsohn, N.R. Mason, D.F. Mahoney*, and R.F. Bruns. Lilly Research Labs., Eli Lilly & Co., Indianapolis, IN 46285.

The CCK/G receptor family is thought to contain three receptor subtypes: CCK-A (pancreas) [A], CCK-B (brain) [B], and gastrin (stomach fundus) [G]. B and G subtypes are difficult to distinguish with existing tools, suggesting they are very similar or identical receptors. Several studies have demonstrated that pancreatic acinar tissues and cell lines contain a B or G-like receptor, as well as an A receptor. This study reports characterization of the former on the rat acinar cell line AR4-2J, using a novel series of non-peptide, pyrazolidinone CCK antagonists.

Four compounds (including LY262684 and LY262691) were evaluated. They inhibited binding of ^{125}I-CCK-8S to mouse brain (IC_{50}'s 6-31 nM) and ^{125}I-gastrin I to guinea pig stomach (IC_{50}'s 25-490 nM). IC_{50}'s vs. ^{3}H-MK-329 in rat pancreas were $\geq$ 6 μM, showing their high selectivity for B and G over A receptors. Phosphoinositide turnover elicited in AR4-2J cells by gastrin was clearly mediated by a B or G-type receptor, since the A-selective antagonist MK-329 blocked it only at higher concentrations (Ki > 100 nM). The LY compounds reversed the gastrin response with K_i's of 16-270 nM, with the same rank order of potency as in B and G binding. Absolute potencies in PI turnover, however, correlated much more closely with G binding than B binding. This provides evidence that the second PI-linked CCK/G receptor on AR4-2J cells is a true gastrin, rather than a CCK-B, receptor. While G to B ratios for the compounds were modest (4-18), use of several with differing potencies greatly strengthened comparisons among assays, and illustrated the utility of this series for studying CCK-dependent systems.

38.4

LY262684, A NOVEL PYRAZOLIDINONE CCK ANTAGONIST: STUDIES OF CONTRACTION IN GUINEA PIG ILEUM. V.L. Lucaites*, L.G. Mendelsohn, N.R. Mason, M.L. Cohen*, K.L. Lobb*, R.F. Brown*, J.K. Reel*, and J.J. Howbert. Lilly Research Labs., Eli Lilly & Co., Indianapolis, IN 46285.

Studies of contraction induced by CCK peptides in longitudinal muscle of guinea pig ileum suggest mediation by at least two CCK receptors, corresponding to CCK-A (pancreatic) and CCK-B (brain) receptors. This tissue has now been further studied with LY262684, a new CCK antagonist with a novel, non-peptide, pyrazolidinone structure.

LY262684 displaced ^{125}I-CCK-8S from mouse brain with an IC_{50} of 6 nM, and ^{3}H-MK-329 from rat pancreas with an IC_{50} of 7900 nM, documenting its potent and highly selective interaction with CCK-B receptors. This was very similar to the binding profile of L-365,260, a known CCK-B-selective antagonist (IC_{50} of 8 nM in brain and > 1000 nM in pancreas). In ileum, L-365,260 showed greater potency as an antagonist of contractions to CCK-4 (pK_b = 9.24) than contractions to CCK-8S (pK_b < 7), consistent with the selectivity of CCK-4 as an agonist for CCK-B receptors. LY262684, however, while showing B receptor affinity similar to L-365,260 in brain binding, was less potent in blocking CCK-4 contractions (pK_b = 8.45), and more potent against CCK-8S contractions (pK_b = 7.64) than L-365,260, thus showing less selectivity for the putative CCK-B receptor in the ileum. Similar results were found for several analogs of LY262684. The differential effects of LY262684 and L-365,260 underscore the heterogeneity of CCK-induced contractions in the ileum, and raise the possibility that they are mediated, in part, by a novel non-A, non-B-type CCK receptor for which these antagonists have differential affinity. This finding helps establish LY262684 as an important new tool for probing CCK-dependent mechanisms in smooth muscle and other systems.

38.5

VISUALIZATION AND CHARACTERIZATION OF CHOLECYSTOKININ BINDING SITES IN THE PITUITARY INTERMEDIATE LOBE.

P. Gaudreau, M. Migneault* and G.L. Lavigne. Notre-Dame Hosp. Res. Center and Faculty of Dental Medicine, Univ. of Montreal, Montreal, CANADA, H2L 4K8.

Peripheral administration of cholecystokinin octapeptide (CCK-26-33) elevates plasma immunoreactive concentrations of ß-endorphin in rats and the effect can be antagonized by L-364,718. However, the locus of this action has not yet been established. We therefore searched, by in vitro autoradiography, for CCK binding sites in rat and guinea pig pituitary. Twenty um-thick slide-mounted sections of pituitaries were pre-incubated at 23°C for 35 min in 50 mM Tris.HCl buffer containing 0.2% BSA, pH 7.4. They were incubated at 23°C for 120 min in the pre-incubation buffer containing 5 mM $MgCl_2$, 0.35 mM bacitracin and 35 pM N [^{125} I-desaminotyrosyl] CCK-26-33, pH 7.4 in the absence or presence of CCK-A and CCK-B receptor agonists and antagonists and submitted to two 14-min washes in 4°C pre-incubation buffer. Slide-mounted sections of rat brain were used as control. These sections were dried and apposed to tritrium-sensitive film for 30 days. The autoradiograms revealed a high level of CCK specific binding confined to the intermediate lobe of guinea pig pituitary. This binding was totally inhibited by 1 nM of CCK-26-33, CCK-28-33, L-364,718 and L-365,260. A low and homogeneous level of binding was observed in the rat gland. Altogether, these results suggest that only in guinea pig, the CCK-induced ß-endorphin secretion could be mediated by pituitary CCK binding sites.Supported by MRCC.

38.6

LESION OF THE PARABRANCHIAL NUCLEI INCREASES CCK-RECEPTOR BINDING IN THE VENTROMEDIAL NUCLEUS OF THE HYPOTHALAMUS.

M. Schumacher, H. Coirini, L. Zaborszky and B.S. McEwen. The Rockefeller University and University of Virginia.

The ventromedial nuclei (VMN) of the hypothalamus contain high levels of CCK receptors (Neuroendocrinology, 45: 257) and are innervated by CCK-immunoreactive fibers which originate from the nuclei parabrachialis (PDB) (Brain Res., 303: 225; J.Neurosci.,4: 1289). Within the VMN, estrogen decrease the binding of sulfated [^{125}I]CCK (Neuroendocrinology, 45: 257) but do not affect the binding of sulfated [^{3}H]CCK . Ovariectomized females were injected with vehicle (controls) or with estradiol benzoate (EB; 50 ug/100 g body weight) and brain were sampled 48 hrs later for the quantitative autoradiography of [^{3}H]CCK receptor binding. The estrogen treatment did not affect the binding of [^{3}H]CCK within the VMN. In a second experiment, either the right or the left PDB of ovariectomized females was lesioned. Two week later, females were injected with vehicle or with EB like in the previous experiment. Unilateral lesion of the PDB increased [^{3}H]CCK receptor binding by about 30% within the ipsilateral VMN, but not in the area surrounding the VMN or in other brain nuclei. There was no effect of the EB-treatment on [^{3}H]CCK binding in the VMN ipsilateral or contralateral to the lesion site.

We conclude that CCK fibers originating from the PDB selectively innervate the ipsilateral VMN where CCK interacts with postsynaptic receptors.

38.7

BINDING OF [^{125}I]-D-TYR^{0}(NLE^{28,31})-CCK 26-33 TO CCK RECEPTORS IN CORTICAL MEMBRANES AND BRAIN SECTIONS.

M. Carlberg*, B. Jarrott*, B.G. Livett† and P.M. Beart. University of Melbourne, Clinical Pharmacology and Therapeutics Unit and Department of Biochemistry†, Parkville 3052, Australia.

High affinity binding to cholecystokinin (CCK) receptors is dependent on the tetra C-terminal sequence of CCK-8S. We have characterized the binding of a new commercially available, non-oxidizable, peptidase resistant and iodinable CCK analogue, D-Tyr^{0}(Nle^{28,31})-CCK 26-33 in cortical membranes and sections of rat brain. The peptide was reacted with Na^{125}I in the presence of Chloramine-T and purified by reverse phase liquid chromatography. Binding to cortical membranes was of high affinity (K_d ± s.e.m 0.14 ± 0.03 nM, n=3), saturable (B_{max} 60 ± 31 fmol/mg protein, n=3), reversible ($t_{1/2}$ association 20 min, k_{+1} 8.9 $\cdot 10^{-5}$ $min^{-1}pM^{-1}$, $t_{1/2}$ dissociation 27 min, k_{-1} 0.025 min^{-1}) and to a single population of sites. Specific binding represented 50-70% of the total binding. Binding was dependent on divalent cations and inhibited by the non-hydrolyzable guanine nucleotide Gpp(NH)p, indicating binding to a receptor coupled to a G protein. Binding could be completely displaced by CCK analogues and inhibitors with the following K_i values (n=3): CCK-8S 3.3 ± 2.3 nM, unsulfated CCK-8 23 ± 9 nM, CCK-4 27 ± 18 nM, L-365,260 6 ± 3 nM, L-364,718 130 ± 28 nM. These competition data indicate receptors of the CCK_B type. Binding to 10 µm slide-mounted sections showed 80-90% specificity and the same pharmacological characteristics as those found in cortical membranes. Several parts of the brain were discretely labeled, including anterior nucleus accumbens, anterior cingulate, frontoparietal and primary olfactory cortex. The high specific activity of the iodinated peptide, its stability and low non-specific binding make it an excellent ligand for the autoradiographic localization of CCK receptors. Supported by NH&MRC (Australia) and the Swedish Natural Science Research Council.

38.8

CHARACTERIZATION AND AUTORADIOGRAPHIC LOCALIZATION OF CHOLECYSTOKININ (CCK) RECEPTORS IN THE BOVINE BRAIN.

M.A. Morency, L.K. Srivastava and R.K. Mishra. Neuropharmacology Laboratory, Departments of Psychiatry and Biomedical Sciences, Health Sciences Centre 4N52, McMaster University, Hamilton, Ontario, Canada L8N 3Z5.

Cholecystokinin (CCK) binding sites have been characterized in the rodent, primate and human brain. In the present communication, we report the characterization and autoradiographic localization of CCK receptors in the bovine brain.

Bovine cortical membrane homogenates were incubated with [^{3}H]pCCK$_8$ for 120 min at 25°C. Nonspecific binding was determined in the presence of 1 µM unlabelled CCK_8. Scatchard analysis revealed saturable binding to a single class of high affinity sites; K_d = 0.922 ± 0.047 and B_{max} = 51.87 ± 8.22. Hill coefficients did not differ significantly from unity; n_H = 1.02 ± 0.05.

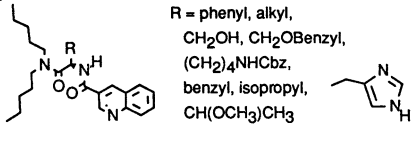
Quantitative autoradiography revealed that [^{125}I]BH-CCK_8 and [3H]pCCK8 binding sites were heterogeneously distributed in bovine brain. Moderate to high densities were present in the cortex, olfactory bulb, nucleus accumbens, caudate, claustrum, amygdala, hippocampus, superior colliculus, and substantia gelatinosa of the cervical spinal cord. Very low to low binding densities were observed in the globus pallidus, internal capsule, cerebellum, and most thalamic and hypothalamic nuclei. Thus, the autoradiographic distribution of CCK binding sites in bovine brain is fairly similar to those reported in rodent, primate, and human brain. Supported by the Natural Sciences and Engineering Research Council of Canada.

38.9

CHOLECYSTOKININ ANTAGONISTS: NOVEL COMPOUNDS WITH A DEFINED PHARMACOPHORE LINKING A NUMBER OF TYPE A CCK ANTAGONIST SERIES.

James F. Kerwin, Jr.*, Frank Wagenaar*, Hana Kopecka*, Chun Wei Lin*, Thomas Miller*, David Witte*, Alex M. Nadzan. Neuroscience Research Division, Pharmaceutical Discovery D-47H, Abbott Laboratories, Abbott Park, Illinois 60064.

We have reported on the structural similarities of glutamic acid based CCK antagonists (A-64718, A-65186) and the benzodiazepine CCK antagonist L-364,718 and also recently presented a new series of antagonists that attempts to relate benzotript with proglumide and with the benzodiazepine class of CCK antagonists. Here we report an array of new classes of CCK antagonists that still maintain potency and selectivity for the CCK type A receptor. Capitalizing on our previous design concepts, a minimium pharmacophore for all these classes was found along with a corresponding variable region of the CCK antagonist construct. This variable position was extensively examined by structural substitution for changes in potency and selectivity and was found to permit great latitude in substitution as evidenced by the number and types of charged groups as well as hydrophobic residues that were accommodated. With a larger variety of functional groups at this site sub-differentiation of the CCK type A receptor may be more feasible. We will report on the discovery of these compounds and provide preliminary *in vitro* chacterization of the binding and functional activity (amylase release) for these derivatives. Examples of these classes (IC_{50}'s < 100 nM) are depicted in Figure 1.



38.10

PHYSICAL PROPERTIES OF THE SOLUBILIZED RAT BRAIN SOMATOSTATIN RECEPTOR.

S. Rens-Domiano and T. Reisine. Dept. of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104

Somatostatin (SRIF) is a neurotransmitter in the brain, whose physiological actions are mediated by cell-surface receptors. Previous studies have suggested that these receptors are glycoproteins, but little is known about the composition of the oligosaccharide component(s), or its functional role. We have begun to address these issues using lectin affinity chromatography and exoglycosidases. High affinity SRIF receptors were solubilized from rat brain using the detergent CHAPS, and detected using the SRIF analogue, [125I] MK-678. Lectin affinity chromatography revealed that the solubilized receptor bound specifically to WGA (NAcGlc, Sialic acid), *Ricinus Communis* I (ß-Gal) and II (ß-Gal/NAcGal) and *Sambucus nigra L.* (Neur5Acα(2,6)Gal/NAcGal), but not to Con A (Mannose), succinylated WGA (NAcGlc), DBA (NAcGalα(1,3)), *Ulex Europaeus* (L-Fucose) nor Jacalin (O-linked). These results suggest that the carbohydrate moities are either an N-linked complex or hybrid structure that contains terminal sialic acid groups. In order to assess the functional significance of the oligosaccharide component of the SRIF receptor, solubilized membranes were treated with neuraminidase from *Vibrio Cholerae*, which cleaves all terminal sialic acid residues. After a 24 hr incubation, no detectable agonist binding was present. In contrast, treating the solubilized membranes with Newcastle Disease Virus, which removes only α(2,3)-linked sialic acids, did not result in any loss in agonist binding. This would suggest that sialic acid, in an α(2,6)-linkage plays a functional role in agonist binding to the solubilized SRIF receptor. Supported by NIMH 45533, MH 14654 and the Juvenile Diabetes Foundation.

38.11

IDENTIFICATION OF G PROTEINS COUPLED TO SOMATOSTATIN RECEPTORS S.F. Law, S. Rens-Domiano, D. Manning*, and T. Reisine Dept. of Pharmacology, Univ. of Pennsylvania, School of Medicine, Philadelphia, PA 19104.

Somatostatin (SRIF) is a neurotransmitter in brain that modulates various cellular effector systems by interacting with membrane bound receptors. Many receptors are coupled to second messengers via a family of G proteins. The ability of neurotransmitter receptors to modulate effector systems may be influenced by the specificity of receptor/G protein/second messenger interactions. In order to study these interactions, SRIF receptors from the cell line AtT-20 (which expresses a high density of SRIF receptors) were solubilized and their coupling to G proteins studied using peptide directed antisera against different alpha subunits of the G proteins. The AtT-20 cell SRIF receptor couples to its cellular effector systems via pertussis toxin sensitive G proteins (either G_i and/or G_o). Immunoprecipitation studies using antisera 8730, directed against G_{ia}, removes 60% of SRIF receptor binding from the supernatant, as measured using the high affinity SRIF agonist [125I] MK 678,. This effect is specific since it is blocked by the peptide to which the antisera was generated. Furthermore an antisera directed against the beta36 subunit of G proteins did not precipitate the SRIF receptor. Western blotting on solubilized AtT-20 cell membranes revealed that all the subtypes of G_i and G_o are expressed in these cells. Further studies are in progress to characterize which G_{ia} subunits are coupled to the SRIF receptor. Supported by NIMH grant 45533 and the NIH Cardiovascular Training Grant 5T32HL07502.

38.12

SUBTYPES OF SOMATOSTATIN RECEPTORS CAN BE DIFFERENTIALLY REGULATED AND CAN COUPLE TO DISTINCT CELLULAR EFFECTOR SYSTEMS. Terry Reisine, Karen Raynor, Hung-Li Wang, and Marc Dichter Depts. of Pharmacol. and Neurol., Univ. of Pennsylvania and Graduate Hospital, Philadelphia, PA 19104.

Subtypes of somatostatin (SRIF) receptors can be distinguished by the peptides MK 678 and CGP 23996. Each peptide binds to pharmacologically distinct SRIF receptors in brain as well as other tissues. Two model cell systems, GH_3 cells and rat neocortical neurons in culture, were used to further explore the properties of the SRIF receptor subtypes. GH_3 cells express SRIF receptor subtypes with similiar characteristics as those in rat brain. The SRIF receptor subtypes in GH_3 cells can be differentially regulated. Continous exposure of GH_3 cells to 1 uM MK 678 abolished [125I] MK 678 binding to GH_3 cell membranes but did not significantly affect [125I] CGP 23996 binding. Differential regulation of [125I] MK 678 and [125I] CGP 23996 binding sites further indicates that these peptides bind to distinct receptors. To further examine the cellular effector systems coupled to each SRIF receptor subtype, the actions of MK 678 and CGP 23996 on K^+ currents in rat neocortical neurons in culture were measured using whole cell patch clamp techniques. We previously showed that somatostatin-14 stimulated a delayed rectifier K^+ current in these cells. MK 678 also stimulated this current ($26 \pm 2\%$ increase in 8 of 14 neurons tested) but 1 uM CGP 23996 did not. These studies indicate that CGP 23996 and MK 678 sensitive SRIF receptors can couple to different cellular effector systems, possibly to mediate distinct biological actions of SRIF. Supported by NIMH grant 45533, the Office of Naval Research and the Juvenile Diabetes Foundation.

38.13

DIFFERENTIAL REGULATION OF SOMATOSTATIN RECEPTOR SUBTYPES IN ADULT RAT BRAIN. Melanie Tallent, Karen Raynor, and Terry Reisine. Depts. of Pharmacol. and Inst. of Neurol. Sci., Univ. of PA, Philadelphia, PA 19104.

Pharmacologically and functionally distinct subtypes of somatostatin (SRIF) receptors are expressed in the rat brain. These SRIF receptor subtypes are distinguished by the SRIF analogues MK 678 and CGP 23996 and are found to be differentially localized by autoradiographic analysis. We now show that these receptor subtypes can be differentially regulated in the rat striatum. Rats were treated with 0.9% NaCl or cysteamine (300 mg/kg, s.c.), a compound previously shown to deplete SRIF-like immunoreactivity in the rat brain. The specific binding of [125I] CGP 23996 in the striatum was found to be increased by 13% at 30 min and by 58% at 4 hrs after cysteamine treatment relative to saline-injected animals. Neither the cortex nor the hippocampus demonstrated corresponding increases in specific [125I] CGP 23996 binding. Likewise a 33% increase was found in the specific binding of [125I] MK 678 binding at 4 hrs with no change in the other regions of the brain. Whether these increases reflect changes in affinity or binding capacity will be discussed. We have shown that the striatum expresses a high density of MK-sensitive SRIF receptors, but that this subpopulation of receptors does not appear to be efficiently coupled to the inhibition of forskolin-stimulated adenylyl cyclase in the striatum. However, MK 678 can inhibit forskolin-stimulated adenylyl cyclase activity in both the hippocampus and the cortex to the same extent as SRIF. The differential effects of SRIF depletion on receptor subpopulations in the striatum vs. other brain regions further substantiates heterogeneity of MK-sensitive SRIF receptor subpopulations in the brain. Supported by NIMH Grants 45533 and MH17168.

38.14

THE SOMATOSTATIN ANALOGUE MK 678 PRODUCES INCREASES IN LOCOMOTOR ACTIVITY WHEN INJECTED INTO THE NUCLEUS ACCUMBENS OF THE RAT. Karen Raynor, Irwin Lucki, and Terry Reisine. Depts. of Pharmacology and Psychiatry, Univ. of PA, Philadelphia, PA 19104.

The tetradecapeptide somatostatin (SRIF) is found in higher concentration in the nucleus accumbens than in any other region of the striatum. However, the function of SRIF in the nucleus accumbens has not been elucidated. Subtypes of SRIF receptors are distinguished in the rat brain by the stable SRIF analogues, MK 678 and CGP 23996. Autoradiographic analysis reveals a moderate density of MK-sensitive sites (94 fmol/tissue slice) in the nucleus accumbens with fewer corresponding CGP 23996 sites (15 fmol/tissue slice). SRIF administered icv has been shown to increase locomotor activity in rats. Here we report that MK 678, a stable and specific analogue of SRIF, produces increases in locomotor activity similar to that seen with amphetamine when microinjected directly into the anterior nucleus accumbens of the rat.

Rats were surgically implanted with indwelling cannulae aimed at the anterior nucleus accumbens. After recovery, animals received bilateral injections of amphetamine, MK 678, or saline and locomotor activity was measured in automated activity cages. Amphetamine (10 ug/ul/side) produced a 4-fold increase in activity. The metabolically stable SRIF analogue MK 678 (3-100 ng/ul/side) produced an effect similar in magnitude to amphetamine. The effects of CGP 23996 on locomotor activity and the possible mediation of the effects of SRIF analogues by dopaminergic systems will be discussed. Supported by NIMH Grants MH455 3 and MH17168.

38.15

DIFFERENTIAL DISTRIBUTION OF SOMATOSTATIN RECEPTOR SUBTYPES IN RAT BRAIN REVEALED BY NEWLY DEVELOPED SOMATOSTATIN ANALOGS. J-L. Martin*, M-F. Chesselet* and T. Reisine*. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, PA 19104 .

Somatostatin (SRIF) receptor subtypes were labeled with the SRIF analogs [125I] CGP 23996 and [125I] MK 678 and the distribution of these receptors in rat brain was investigated using quantitative autoradiography. [125I] CGP 23996 and [125I] MK 678 specifically label different populations of SRIF receptors in rat brain. In a number of brain regions striking differences in the distribution of the SRIF receptor subtypes labeled by each peptide were observed. High levels of binding sites for both [125I] CGP 23996 and [125I] MK 678 were present in the cerebral cortex, CA1 region and subiculum of the hippocampus. In contrast, high levels of [125I] MK 678 binding were found in the dentate gyrus of the hippocampus while few [125I] CGP 23996 binding sites were observed in this brain region. [125I] CGP 23996 binding was detected in the central region of the interpeduncular nucleus whereas the dorsal and lateral subnuclei of this brain area expressed mainly SRIF receptors with high affinity for MK 678. The locus coeruleus as well as regions of the superior colliculus and hypothalamus selectively express [125I] MK 678 sensitive SRIF receptors. Furthermore, limbic structures such as the lateral septum, the nucleus accumbens and ventromedial striatum had much higher levels of [125I] MK 678 binding sites than [125I] CGP 23996 binding sites. Differences in the expression of the SRIF receptor subtypes were also detected in the substantia nigra. [125I] CGP 23996 binding was present in the pars reticulata but not the pars compacta whereas the reverse distribution for [125I] MK 678 binding sites was observed. The differential distribution of [125I] CGP 23996 and [125I] MK 678 binding sites in rat brain supports the hypothesis that these peptides selectively label different SRIF receptor subtypes in the central nervous system.

38.16

LACK OF CROSS-DESENSITIZATION OF SOMATOSTATIN-14 AND SOMATOSTATIN-28 RECEPTORS COUPLED TO POTASSIUM CHANNELS IN RAT NEOCORTICAL NEURONS. Hung-Li Wang , Marc Dichter and Terry Reisine . Departments of Pharmacology and Neurology, University of Pennsylvania School of Medicine and Graduate Hospital, Philadelphia PA 19104.

The effects of somatostatin-14 (SOM-14) and somatostatin-28 (SOM-28) on the delayed rectifier K^+ current (I_K) in rat neocortical neurons in culture were measured using whole cell patch clamp techniques. SOM-14 stimulated I_K in a reversible manner. Continuous application of SOM-14 to the neocortical neurons led to a gradual desensitization of the SOM-14 response. Many cells completely desensitized to SOM-14. SOM-28 also modulated I_K in neocortical cells. However, SOM-28 reduced I_K. This response was also reversible. Continuous application of SOM-28 to neocortical neurons led to a desensitization of the SOM-28 inhibition of I_K. Many of the neurons that responded to SOM-28 became completely refractory to the peptide following prolonged SOM-28 pretreatment. While most neocortical neurons responded either to SOM-14 or SOM-28, a population of neurons responded to both peptides. Chronic application of SOM-14 to these neurons completely desensitized the SOM-14 stimulation of I_K, but did not affect SOM-28 inhibition of this potassium current. Similarly, complete desensitization of SOM-28 responses in these cells was not associated with a modification of SOM-14 stimulation of I_K. The lack of cross-desensitization between SOM-14 and SOM-28 induced responses suggests that these peptides act through different receptors to regulate I_K. This work was supported by NIMH grant 45533, the Office of Naval Research and Juvenile Diabetes Foundation (TR) and NIH grant NS24927 (MD).

38.17

MECHANISM OF ACTION OF A LONG-ACTING ANALOG OF SOMATOSTATIN G. Hermann, M.S. O'Dorisio*, T.M. O'Dorisio*, and W.B. Malarkey*, Depts Physiology, Pediatrics and Medicine, OSU, Columbus, OH

These experiments were designed to compare the mechanism of action of somatostatin (SOM) and octreotide acetate, a long-acting analog of somatostatin being used to treat several neuroendocrine diseases. The GH_3 rat pituitary tumor cell line, an in vitro model of prolactin secretion, was utilized for these studies. Prolactin secretion can be stimulated by VIP while SOM inhibits VIP-stimulated release. These studies examined the mechanism of SOM inhibition of VIP-mediated prolactin secretion and tested whether the synthetic analog of SOM, octreotide, acts by a similar mechanism.

Octreotide competitively inhibited binding of ^{125}I-(tyr^{11})SOM to GH_3 cells; the apparent affinity of octreotide was 100-fold less than SOM. Similar to SOM, octreotide was an effective antagonist of VIP-mediated stimulation of adenylate cyclase but did not inhibit forskolin-mediated stimulation of adenylate cyclase. Octreotide treatment did not affect either the number or affinity of VIP receptors expressed on GH_3 cells. Taken together, these results suggest that SOM and octreotide block VIP-mediated prolactin secretion by interfering with VIP-receptor G-protein coupling to the adenylate cyclase complex. The ability of octreotide to mimic native somatostatin actions and its resistance to degradation make it a valuable research tool for further delineation of the multiple physiological functions of SOM as well as being useful in the clinical treatment of selected neuroendocrine disorders.

EXCITATORY AMINO ACIDS: RECEPTORS I

39.1

RESPONSES MEDIATED BY N-METHYL-D-ASPARTATE RECEPTORS IN CULTURED HIPPOCAMPAL NEURONS FROM MOUSE TRISOMY 16, A MODEL OF DOWN SYNDROME. E.J.Coan, B.Ault & S.I. Rapoport. LNS, NIA, NIH, Bethesda, MD 20892.

The trisomy 16 (Ts16) mouse is considered to be a model of human trisomy 21 (Down syndrome) because of genetic homology between human chromosome 21 and mouse chromosome 16. This laboratory previously has reported that cultured dorsal root ganglion neurons from the Ts16 embryo have several voltage dependent electrical properties that differ from those of neurons from littermate controls. Down syndrome subjects show developmental retardation and, after the third decade of life, develop Alzheimer-like dementia and neuropathology. As the N-methyl-D-aspartate (NMDA) receptor may play a significant role in development and may also be involved in Alzheimer's disease experiments were performed to investigate responses to NMDA in cultured hippocampal neurons from Ts16 mice. Whole cell patch clamp recordings were made from 2-4 week old primary cultures of hippocampal neurons prepared from Ts16 embryos and littermate controls at gestation day 14-16 using standard techniques. Tetrodotoxin (0.2 uM) picrotoxin (50 uM) and glycine (1 uM) were added to a standard extracellular medium. Intracellular medium included KMeSO4 + TEA or CsCl, and ATP. NMDA was applied either via a puffer pipette (200 uM) or via a fast perfusion system (20 uM) while the cell was voltage clamped at membrane potentials between -80 mV and + 20 mV. I-V plots were then constructed. In 7/7 cells from Ts16 mice, NMDA produced an inward current which was voltage-dependent, and had a reversal potential of approximately 0 mV. The differences between NMDA responses produced in cultured hippocampal neurons from Ts16 mice and controls are being investigated.

39.2

MULTIPLE EFFECTS OF TETRAETHYLAMMONIUM (TEA) ON N-METHYL-D-ASPARTATE RECEPTOR-CHANNELS IN CULTURED MAMMALIAN NEURONS. Jerry M. Wright* and Linda M. Nowak, Dep't of Pharmacology, NYSCVM Cornell University, Ithaca, NY 14853.

The effects of TEA on NMDA receptor-channels were studied in outside-out patches. The extracellular solution contained 300 nM TTX and (in mM): 150 NaCl, 2.8 KCl, 1.0 $CaCl_2$, 10 HEPES-Na. The patch pipette solution was (in mM): 140 CsCl, 10 EGTA-K/1.0 $CaCl_2$, and 10 HEPES-K. Different effects were seen depending upon which membrane surface was exposed to TEA. Extracellular TEA produced two inhibitory effects: 1) channel conductance (γ) decreased, and 2) the frequency of channel events decreased. The γ decrease was voltage-dependent and the data were fit by the Woodhull model with the block site sensing 60% (δ = 0.6) of the transmembrane potential. The apparent dissociation constant (K_d) of channel block was 11 mM at –60 mV and 45 mM at 0 mV. There was no change in mean open time and no new fast closed time was observed. The greater inhibitory effect was the reduction in frequency of opening with an IC_{50} of 5 mM TEA; this effect was apparently voltage independent. The reduction in frequency of opening was not due to competitive inhibition at either the NMDA or glycine sites. Partial (70 mM) or total substitution of TEA for Cs in patch pipetts revealed that NMDA channels are TEA permeable with P_{TEA}/P_{Cs} = 0.45. Supported by NS24467

39.3

EQUILIBRIUM AND KINETIC MEASUREMENTS SUGGEST NMDA RECEPTOR ACTIVATION REQUIRES BINDING OF TWO MOLECULES EACH OF NMDA AND GLYCINE. M.L. Mayer, M. Benveniste and D.K. Patneau. Unit of Neurophysiology and Biophysics, NICHD, Bethesda, MD 20892.

The activation of ligand gated ion channels usually requires binding of more than one molecule of agonist (e.g., nicotinic ACh: 2; $GABA_a$: 2; neuronal ATP: 3). The NMDA receptor is unusual in that both glutamate and glycine must bind to activate the receptor channel complex. We have used whole cell recording to study the stoichiometry of activation of NMDA receptors by measuring the concentration-dependence and kinetics of activation of NMDA receptors evoked by rapid application of glutamate- and glycine-site agonists and antagonists.

With 3 μM glycine present, a log-log plot of the NMDA dose-response curve had a limiting slope of 2 at low concentrations of agonist, suggesting two binding sites for NMDA. Consistent with this, at low concentrations of NMDA, activation kinetics were non-exponential, but could be well fit by a model which assumed two equivalent excitatory amino acid binding sites (for NMDA we obtained k_{on} = 2.08 μM^{-1} sec^{-1}, k_{off} = 22.87 sec^{-1}). The microscopic dissociation constant calculated from these values is 11 μM, close to that obtained when a 2 equivalent binding site model was used to fit the NMDA equilibrium dose response curve (16 μM).

Interpretation of the limiting slope of the glycine dose-response curve, recorded with 100 μM NMDA, is complex due to contamination by an unknown concentration of endogenous glycine. However, the activation kinetics for both glycine and for the lower affinity analogue L-alanine were also sigmoidal, and could be fit by a model which assumed two equivalent binding sites for glycine-like amino acids. Experiments with 7Cl-Kynurenic acid, a competitive antagonist for the glycine site, confirmed that a single binding site model would not fit the onset of and recovery from antagonism in the presence of 100 μM NMDA and 100 μM L-alanine, but a model assuming two equivalent sites for L-alanine gave good fits (7Cl-Kynurenic acid k_{on} = 40.77 μM^{-1} sec^{-1}, k_{off} = 10.68 sec^{-1}; K_i = 262 nM).

39.4

INDEPENDENT STRUCTURAL FEATURES INFLUENCE BINDING AND DISSOCIATION KINETICS FOR NMDA COMPETITIVE ANTAGONISTS. M. Benveniste and M. L. Mayer. Unit of Neurophysiology and Biophysics, LDN, NICHD, NIH, Bethesda, MD 20892.

Antagonism of NMDA activated currents was studied using whole cell voltage clamp of mouse dissociated hippocampal neurons cultured for 10 to 15 days. The kinetics of NMDA receptor antagonism were measured by applying drugs with a multibarrel flow pipe, allowing rapid solution changes around the cell in less than 10 msec. Mathematical solutions for both one and two equivalent site models for antagonism were determined according to the differential equations outlined by D. Colquhoun and A. G. Hawkes (Proc. R. Soc. Lond. B. 199:231, 1977). The kinetics for the onset of (k_{on}) and recovery from (k_{off}) antagonism gave better fits for the two-equivalent site model, and also yielded k_{off} / k_{on} values which well approximated equilibrium K_i's. Six competitive antagonists were compared to determine how changes in structure influence ligand binding and dissociation. The results indicate that single chain non-constrained compounds associate faster than cyclic constrained antagonists; and that compounds in which the α–carbon and phosphorous are 5 atoms apart associate faster than their 7-atom analogs. However, the number of atoms separating the α–carbon and phosphorous atoms does not influence k_{off}. Our results suggest that sterically restricted antagonists are more tightly bound when compared to non-constrained antagonists.

Compound	k_{on} ($\mu M^{-1} sec^{-1}$)	k_{off} (sec^{-1})
d-AP5	22.23	19.45
d-AP7	14.08	20.34
CGP 37849	12.95	1.12
d-CMP	10.79	7.53
CGS 19755	6.96	1.69
d-CPP	3.56	1.10

M.B. is supported by a National Research Council Fellowship.

39.5

EFFECTS OF SIGMA LIGANDS ON NMDA-INDUCED SINGLE CHANNEL CURRENTS. E. Siqueira-Rocha, E.X. Albuquerque, and A.S. Ramos. Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, RJ, 21944, Brazil and Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. of Med., Baltimore, MD 21201.

The antipsychotic agent haloperidol, a high affinity ligand for sigma (σ) receptors, modulates the NMDA response of hippocampal neurons (Rocha *et al.*, Soc. Neurosci. Abst., 1989). Here, we have examined whether σ-haloperidol receptors mediate this effect. Using outside-out patches from rat hippocampal neurons in culture, we compared the effects of haloperidol and high affinity σ ligands 1,3-di(2tolyl)guanidine (DTG) and 3-(3-hydroxyphenyl)-N-(propyl)piperidine (3-PPP) on single channel ion currents induced by application of NMDA (0.5-20 μM). Each patch was studied at several concentrations of each σ ligand. At relatively low concentrations (0.1-1 μM), haloperidol potentiated the NMDA response in 20 out of 30 patches by increasing the frequency of NMDA-induced openings. In contrast, potentiation was not observed after application of DTG (1-50 nM) or 3-PPP (0.1-1 μM) at concentrations that, according to previous binding studies, assured a high level of specificity for σ receptors. At higher concentrations, DTG (0.1-100 μM) and 3-PPP (1-100 μM) shared with haloperidol (1-30 μM) the ability to reduce the NMDA response by markedly decreasing frequency of openings and channel lifetime. These blocking effects may result from low affinity binding at a site within the NMDA receptor ion channel. Although mechanisms underlying the potentiating effects of haloperidol remain unknown, the results observed with DTG and 3-PPP suggest that σ receptors are not directly involved with modulation of NMDA receptor function. Support: CNPq, FINEP Brazil, FINEP Maryland, and NIH #P50-MH44211.

39.6

PYRAZOLE ACTIVATES N-METHYL-d-ASPARTATE (NMDA)-LIKE CURRENTS. E.F.R. Pereira, Y. Aracava and E.X. Albuquerque. Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, RJ, 21944, Brazil and Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. of Med., Baltimore, MD, 21201.

Pyrazole prevents alcohol toxicosis and some experimental data show that this compound potentiates other general depressants (*J. Pharm. Pharmacol.*, 39:658, 1987). In this work we have examined the effects of pyrazole on hippocampal pyramidal cells in culture using the outside-out patch clamp technique. We used a nominally Mg^{2+}-free external solution for our records. Pyrazole (0.5-10 μM) did not block significantly the NMDA-activated currents. Surprisingly, however, pyrazole alone activated currents that appeared as bursts similar to those evoked by NMDA itself. They had a conductance of about 54 pS at 28°C. The frequency and characteristics of channel bursts were both concentration- and voltage-dependent. The closures within the burst could be fit to a double exponential function whereas the openings disclosed a single exponential distribution. In addition, hyperpolarization decreased the open time, increased the number of intraburst events and prolonged the burst duration. Potentials more negative than -100 mV decreased the channel open probability such that long bursts were followed by silent periods that lasted several seconds. Furthermore, APV (50 μM), a competitive antagonist of the NMDA receptor, blocked the pyrazole-activated currents. Though some subtle differences were noticed, these data altogether suggest that pyrazole may activate NMDA receptors. Support: CNPq/ FINEP/ UFRJ-UMAB Training Program.

39.7

COMPUTER SIMULATIONS OF QUANTAL EVENTS MEDIATED BY NMDA RECEPTORS. J.C. Wathey. Computational Neurobiology Laboratory, Salk Institute, POB 85800 San Diego, CA 92138.

Postsynaptic currents mediated by N-methyl-D-aspartate (NMDA) receptors typically decay to baseline with a time constant on the order of 100 ms. Single-channel recordings indicate, however, that the mean open time of these channels (~10 ms) is too short to account for this slow decay. One possible explanation is that the diffusion of neurotransmitter molecules from the synaptic cleft is retarded by repeated binding to receptors, thus limiting the decay phase of the postsynaptic current. To test the plausiblity of this hypothesis, quantal events mediated by NMDA receptors were simulated using a computer program originally developed for modeling the quantal event at nicotinic synapses (Wathey et al. 1979, Biophys J 27:145-164). The program simulates the diffusion of transmitter within the cleft and the reaction of transmitter with receptor molecules and with an uptake system. The NMDA receptor was modeled using a 3-state reaction scheme, in which two independent binding sites bind transmitter, and the doubly-bound receptor opens via a rate-limiting conformational change. The closing rate was set to 100/s, the reciprocal of the mean open time. The other rate constants were chosen so as to be consistent with the dose-response data of Patneau & Mayer (J Neurosci, in press). With 70 receptor molecules covering a postsynaptic area of 0.07 μm^2, in a synaptic cleft 0.6 μm^2 in area, a quantum of 1000 glutamate molecules opened 11 NMDA receptor channels, a value similar to experimentally observed peak amplitudes (Bekkars & Stevens 1989 Nature 341:230-233). The decay phase was exponential with a time constant of 13 ms. Diffusion of transmitter became the rate-limiting process in the decay only with unrealistically small diffusion constants or unrealistically large values of receptor density or cleft size. These manipulations produced peak amplitudes much larger than experimentally measured values. The results indicate that buffered diffusion of neurotransmitter contributes little to the slow decay of the NMDA response. The prolonged decay may instead result from intrinsic properties of the receptors, such as their tendency to open in bursts or clusters of bursts.

39.8

EVIDENCE THAT ZINC INHIBITS NMDA RECEPTOR-GATED ION CHANNEL ACTIVATION BY NON-COMPETITIVE ANTAGONISM OF GLYCINE BINDING. G.C. Yeh, D.W. Bonhaus, J.O. McNamara. Duke & V.A. Medical Center. Durham, N.C. 27705

Zinc noncompetitively inhibits NMDA receptor-mediated responses. To investigate the mechanism of this inhibition, we measured the effects of zinc on ligand binding to three distinct sites (phencyclidine, glycine and NMDA binding sites) on the receptor-channel complex. Zinc dose-dependently decreased the association and dissociation of [^{3}H]TCP but had no effect on steady-state levels of binding. This suggests that zinc inhibits the receptor-gated access of [^{3}H]TCP to (and from) its binding site. Zinc inhibition of [^{3}H]TCP binding was not mediated by an action on NMDA recognition sites since zinc had no effect on NMDA-sensitive [^{3}H]glutamate binding. However, zinc dose-dependently inhibited [^{3}H]glycine binding by a non-competitive mechanism. Stoichiometric analysis of equilibrium binding data disclosed the presence of two [^{3}H]glycine binding sites per [^{3}H]TCP binding site. Comparison of the potencies of zinc in inhibiting glycine-dependent [^{3}H]TCP association and [^{3}H]glycine binding suggests that blockade of only one of the two glycine sites is sufficient to prevent [^{3}H]TCP association. We hypothesize that synaptically released zinc inhibits NMDA receptor mediated responses by binding to a distinct site on the receptor-channel complex, reducing glycine binding, and thereby decreasing what would otherwise be a tonically present action of endogenous extracellular glycine.

39.9

CONCENTRATION-DEPENDENT SINGLE CHANNEL KINETIC PROPERTIES OF NMDA RECEPTORS OF MOUSE SPINAL CORD NEURONS IN CULTURE. D.M. Rock@, T. Ryan-Jastrow+, R.E. Twyman+ and R.L. Macdonald+#. Neuroscience Program@ and Depts. of Neurology+ and Physiology#, Univ. of Michigan, and Parke-Davis Pharm. Res.@, Ann Arbor, MI 48104.

To investigate the gating kinetic properties of the NMDA receptor, single channel currents evoked by NMDA (1-50 μM) and glycine (2 μM) were studied using the outside-out patch clamp technique. Patches were obtained from cultured mouse spinal cord neurons at room temperature in a 1 mM Ca^{++} and nominal Mg^{+2} recording solution.

Currents with multiple amplitudes were evoked by NMDA. However, openings of channels with 52 and 37 pS conductances were predominant. Kinetics of these conductance states were analyzed. Percent open and opening frequency of both conductance states increased with NMDA concentration. Mean open duration increased at low concentrations but remained relatively constant at high concentrations. Analysis of open duration frequency histograms for both conductance states revealed the presence of multiple open states that had concentration-dependent properties. Closed and burst duration frequency histograms analyzed in a similar fashion also revealed the presence of multiple closed and burst states. Microscopic kinetic gating schemes for the two dominant conductance states are presented. Low μM concentrations of CPP, a competitive NMDA antagonist, reduced opening frequency.

The results suggest that the gating properties of the NMDA receptor were similar to those described for the nicotinic acetylcholine, glycine and $GABA_A$ receptors.

39.10

CHANNEL BLOCK BY Mg^{2+} OF THE NMDA RECEPTOR LARGE CONDUCTANCE STATES OF MOUSE NEURONS IN CULTURE. R.L. Macdonald+#, N.M. Porter+, R.M. Green+, and R.E. Twyman+. Depts. of Neurology+ and Physiology#, Univ. of Michigan, Ann Arbor, MI 48104.

The actions of magnesium (Mg^{2+}) on NMDA receptor currents were characterized by studying the kinetic properties of the large conductance states (50 and 38 pS) of the NMDA receptor. Outside-out patches from cultured mouse spinal, cortical, and striatal neurons were obtained in 1 mM Ca^{+2} and nominal Mg^{2+} solutions. Single channel data were digitized at 20 kHz with a 2 kHz Bessel filter interposed. Currents evoked by NMDA (50 μM) and glycine (2 μM) were reduced by Mg^{2+} and were voltage-dependent. At -75 mV, Mg^{2+} (1 to 200 μM) produced a concentration-dependent decrease in mean channel open time and opening frequency. Frequency distributions of channel open, closed and burst durations of the conductance states were analyzed by curve fitting to sums of exponential functions.

Open duration time constants decreased and closed time constants were altered with increasing Mg^{2+} concentration. Mean burst durations increased slightly and then decreased as Mg^{2+} concentration was increased.

These results suggest that Mg^{2+} reduced NMDA-activated current by a mechanism that cannot be explained purely by simple open channel blockade. Channel block appeared to occur by a complex open channel block mechanism and/or by allosteric regulation of channel gating. A microscopic reaction scheme consistent with the results is presented.

39.11

NOREPINEPHRINE MODULATION OF THE NMDA RESPONSE IS AFFECTED BY GLYCINE. M.S. Rocha and A.S. Ramoa. Lab. Mol. Pharmacol. II. UFRJ, RJ 21944, Brazil and Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. of Med., Baltimore, MD 21201.

Norepinephrine (NE) has been suggested to modulate the NMDA receptor function in hippocampal neurons (Stanton *et al.*, Exp. Brain Res., 77:517, 1989). Here, we have examined whether NE directly interacts with the NMDA receptor macromolecular complex. Single channel ion currents were recorded from cultured rat hippocampal neurons using the outside-out patch-clamp configuration. Application of NE (0.1-100 μM) frequently led, within a few seconds, to an increased frequency of NMDA induced channel openings and longer burst durations (40% of 41 patches). These effects are similar to those previously reported for glycine, suggesting a common mechanism of action. Consistent with this possibility, kynurenic acid (10 μM), an antagonist of glycine at its strychnine-insensitive site, blocked the NE potentiation. In addition, the NMDA response was not potentiated or even depressed by NE, when glycine was previously added at a high concentration (10 μM). Thus, effects of NE are dependent on extracellular glycine concentration. These findings indicate that complex interactions are involved in the regulation of NMDA response by NE. Direct interaction with the NMDA receptor could contribute to the role of NE in modulation of synaptic transmission and plasticity. Support: CNPq, FINEP (Brazil & US).

39.12

EXCITATORY AMINO ACID-EVOKED INTRACELLULAR CALCIUM TRANSIENTS IN SPINAL CORD NEURONS MEASURED WITH INDO-1. D.B. Reichling and A.B. MacDermott. Dept. Physiology and Cellular Biophysics, and Center for Neurobiology and Behavior, Columbia Univ., New York, NY 10032.

The calcium indicator dye, indo-1, was used to measure changes in intracellular free calcium ion concentration ($[Ca^{2+}]_i$) evoked in spinal cord dorsal horn neurons by excitatory amino acid agonists. Cultured neurons from the embryonic rat dorsal horn were loaded with indo-1-AM. The flowing bath contained $5x10^{-7}$ M TTX and 5 μM glycine. Drugs were applied rapidly via a large Y-tube to the entire neuron. Fluorescence was monitored in single cell bodies using dual photometers. Concentrations of NMDA from 5 to 50 μM stimulated peak $[Ca^{2+}]_i$ increases of 50 nM to >1 μM. $[Ca^{2+}]_i$ transients evoked by a non-saturating dose of NMDA (20 μM) were maximally antagonized by 1 mM magnesium (Mg^{2+}); while at a saturating dose of NMDA (100 μM), 10 mM Mg^{2+} was required to completely block the $[Ca^{2+}]_i$ transients. Thus, physiological $[Mg^{2+}]$ can modulate excitatory amino acid-evoked $[Ca^{2+}]_i$ transients. The dose-response relationship for kainate-evoked $[Ca^{2+}]_i$ transients between 10 and 50 μM kainate was steeper than that for NMDA. 100 μM lanthanum (La^{3+}), which blocks voltage-gated calcium channels as well as NMDA-gated channels, almost completely inhibited $[Ca^{2+}]_i$ transients evoked by saturating doses of NMDA. This $[La^{3+}]$ enhances kainate-gated currents by about 50%, yet it blocks nearly all of the $[Ca^{2+}]_i$ increase evoked by kainate, implying that under these conditions $[Ca^{2+}]_i$ elevation results mainly from entry of extracellular Ca^{2+}.

39.13

SPONTANEOUS AND EVOKED OSCILLATIONS OF INTRACELLULAR CALCIUM IN CULTURED RAT CEREBELLAR GRANULE CELLS. L.D Artman* and E.F. Nemeth. Natural Product Sciences Inc., Salt Lake City, UT 84108.

The concentration of intracellular free calcium ($[Ca]_i$) was measured in cultured rat cerebellar granule cells using fura-2. In 6-8 day old cultures incubated in nominally magnesium-free buffer supplemented with 1 μM glycine, we occasionally observed rapid and transient increases (oscillations) in $[Ca]_i$ that peaked within 30 sec and then plummeted to baseline levels. Oscillations could be elicited almost at will simply by adding 1 mM dithiothreitol. The magnitude of the oscillations varied from 200-2000 nM calcium (basal = 80-150 nM). Oscillations occurred with a frequency of 1-3/min and were dependent on the presence of extracellular calcium. Stepwise increases in the concentration of N-methyl-d-aspartate (NMDA) produced increases in $[Ca]_i$ and progressively damped oscillations of cytosolic Ca^{2+}. Competitive (CPP) and non-competitive (MK-801) inhibitors of the NMDA-receptor/channel complex were found to totally block the oscillations. Tetrodotoxin (TTX) at 1 μM inhibited oscillatory activity. Low concentrations (10 nM) of ω-conotoxin GVIA decreased the frequency of oscillations without changing the amplitude, whereas concentrations above 1 μM were capable of reducing both amplitude and frequency. GABA (1 mM) and the GABA-B agonist baclofen (100 μM) blocked the oscillations. Picrotoxin (100 μM) increased the frequency of the oscillations without changing the magnitude. The competitive antagonist of glycine, 7-chlorokynurenic acid, blocked the glycine-induced oscillations. The subsequent addition of glycine (100 μM) reversed the inhibitory effects of 7-chlorokynurenic acid. Nifedipine (5 μM) reduced the amplitude and frequency of oscillations but did not produce a complete inhibition. The effects of ω-conotoxin, together with those of the NMDA antagonists, suggests that excitatory amino acid synaptic transmission contributes significantly to these oscillatory changes in $[Ca]_i$.

39.14

REDOX STATE ALTERS NMDA RECEPTOR-MEDIATED RISE IN $[Ca^{2+}]_i$ IN RETINAL GANGLION CELLS (RGCs). Linda A. Wong, Nikolaus J. Sucher & Stuart A. Lipton. Dept. of Neurol, Children's Hospital & Harvard Med Sch, Boston, MA.

In patch-clamp recordings, the reducing agent dithiothreitol (DTT) selectively increases NMDA responses in RGCs and other central neurons; the oxidizing agent DTNB reverses this effect (Aizenman, Lipton & Loring, *Neuron* 1989;2:1257). Glutamate or NMDA kills RGCs in a Ca^{2+}-dependent manner (Hahn, Aizenman & Lipton, *PNAS* 1988;85:6556). Furthermore, we have shown that NMDA receptor-mediated neurotoxicity in RGCs is increased with DTT treatment and that this effect is blocked by DTNB (Levy, Sucher & Lipton, *Neurosci. Lett.* 1990;110:291). These previous findings suggest that the redox state of the NMDA receptor is crucial for the survival of neurons facing glutamate-related injury and predicts that excessive calcium entry may be associated with cell death. Therefore, the present study was undertaken to investigate the effect of NMDA receptor activation on $[Ca^{2+}]_i$ and its modulation by DTT. Cultured RGCs from postnatal rat (P2-11) were exposed to the fluorescent dye fura-2/AM (20 μM, 1 h). Fluorescence (excitation: 350 and 380 nm, emission: 495 nm) was recorded with a SIT camera and fed to a Quantex image-processing system. $[Ca^{2+}]_i$ was calculated from intensity ratios (350/380 nm). Baseline $[Ca^{2+}]_i$ was 56 ± 7 nM (n = 14). In low $[Mg^{2+}]_o$, 200 μM NMDA reversibly increased $[Ca^{2+}]_i$ to 421 ± 70 nM in ~80% of the cells tested (n = 11). This effect was completely blocked by 200 μM APV or 1 mM $[Mg^{2+}]_o$, but only slightly (<20%) by 10 μM CNQX. Exposure to 2 mM DTT had no effect on resting levels of $[Ca^{2+}]_i$. The NMDA-induced rise in $[Ca^{2+}]_i$, however, was increased up to two fold after DTT treatment.

39.15

REDUCTION OF NMDA-INDUCED RISES IN $[Ca^{++}]_i$ BY PHORBOL ESTERS IN CULTURED RAT HIPPOCAMPAL NEURONS. K.G. Baimbridge and G.A. Joy* Dept. Physiology, University of British Columbia, Vancouver, B.C., Canada, V6T 1T7

The potential intracellular regulation of NMDA receptors by protein kinase C (PKC) was studied by determining the effect of the phorbol ester, PMA, on the [Ca++]i response to transient applications of NMDA to cultured rat hippocampal pyramidal neurons The cultures were loaded with Fura-2-AM and $[Ca^{++}]_i$ was monitored from single neurons perifused with a salt solution containing no added Mg++, 1µM glycine and 0.5 µM TTX.

PMA perifused at 500 nM for 10 min caused a partial blockade of NMDA responses in 9 of 14 cells tested. The effect was highly dependent upon the age of the cultures in that three week old cultures more consistently responded to PMA whereas no effect was seen after one week in culture. These results are consistent with the reported development of PKC in vivo, and may also reflect a differential response of the pyramidal cell types present in the cultures. (Supported by the MRC of Canada)

39.16

NMDA STIMULATED CALCIUM UPTAKE INTO PRISMS FROM CHICKEN FOREBRAIN: CHANGES DURING MATURATION J.A.P. Rostas, J.M.Kavanagh* and D.A.Powis* The Neuroscience Group, Faculty of Medicine, University of Newcastle, NSW Australia

In chicken forebrain the maturation phase of synapse development, which has been proposed as a model for plasticity in the adult, is protracted and occurs well after synapse formation. We have investigated whether, during maturation, there is a change in functional response (Ca^{2+} uptake) to stimulation of the NMDA receptor since this receptor is known to be associated with postsynaptic densities (PSDs) which themselves change markedly during maturation. At 10-12 weeks (mature brain) and 8-14 days (when most synapses are immature but synapse formation is virtually complete) $^{45}Ca^{2+}$ uptake was measured into 250um prisms at 30°C under conditions which were linear for both time and protein. NMDA (1mM) stimulated $^{45}Ca^{2+}$ uptake was maximal by 4 min (37%, $p<0.05$) in mature brain and was maintained for at least 10 min. In immature brain NMDA stimulated $^{45}Ca^{2+}$ uptake was much slower and did not reach statistical significance until 10 min (29.5%, $p<0.05$). The stimulation was totally inhibited by 1mM CPP. At both ages glutamate (2.5mM) stimulated $^{45}Ca^{2+}$ uptake was more rapid, reaching similar maximal levels at 2.5 min (31% and 38%, respectively) and was only partially blocked by CPP. This change in the NMDA receptor response appears to occur slowly over the same period as the changes in PSD suggesting that PSDs may modulate receptor-mediated events.

39.17

GLUTAMATE AND QUISQUALATE EVOKE AN OUTWARDLY-RECTIFYING CURRENT IN *APLYSIA* NEURONS. P.S. Katz and I.B. Levitan. Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254.

Neurons of the sea hare, *Aplysia californica*, have been previously shown by other researchers to have a variety of different responses to glutamate and glutamatergic agonists. We are currently performing voltage clamp recordings in neurons isolated in primary cell culture and have found a previously undescribed voltage-sensitive response to pressure-applied glutamate. This response is a sustained outward current due to a conductance increase. The current is outwardly rectifying; it goes to zero at holding potentials more negative than -75mV. Occasionally, this current was observed to reverse to a small inward current at this potential, which is the equilibrium potential for potassium in *Aplysia* neurons. Quisqualate also evokes this outwardly rectifying current. Furthermore, saturating concentrations of quisqualate prevent glutamate from activating this current, but do not antagonize another transiently activated, non-rectifying response to glutamate. Thus, it is likely that quisqualate is a specific agonist for the glutamate-evoked outwardly-rectifying current. This response to glutamate is not present in all *Aplysia* neurons, but has been seen repeatedly in the large identified buccal neurons, B1 and B2.

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EXCITATORY AMINO ACIDS: RECEPTORS II

40.1

B_6B_{21}, A MONOCLONAL ANTIBODY THAT BINDS TO NMDA RECEPTORS, MODULATES NOREPINEPHRINE RELEASE FROM HIPPOCAMPAL SLICES IN A GLYCINE-LIKE MANNER. P.E. Potter[1], J.R. Moskal[2]. Departments of Anesthesiology[1], Neurosurgery[2] and Neuroscience[2], Albert Einstein College of Medicine, Montefore Medical Center, Bronx, N. Y. 10467.

We have previously shown that a monoclonal antibody, B_6B_{21}, binds to the N-methyl-D-aspartate receptor-ionophore complex (NMDAR) in a glycine-like fashion. To further characterize the interaction of B_6B_{21} with NMDAR, we have examined NMDAR-mediated release of ^{3}H-norepinephrine (NE) from hippocampal slices.

Hippocampal slices were labelled with ^{3}H-NE in normal Krebs for 30 min, then perfused at 0.4 ml/min with Mg^{2+}-free Krebs. NE release was induced by perfusion with 60 µM NMDA for 5 min. Two stimulation periods were used. Drugs to be tested were added between the first (S_1) and second (S_2) stimulation periods. Results are expressed as the ratio between the amount of NE release in the two periods (S_2/S_1). A decrease in this ratio indicates inhibition of release.

Kynurenic acid (100 µM) decreased the S_2/S_1 ratio by almost 50% from a control value of 0.80 ± 0.11 to 0.44 ± 0.09. Addition of 100 µM glycine reversed this effect and brought the S_2/S_1 ratio back up to 0.99 ± 0.22. The antibody B_6B_{21}, like glycine, also increased the S_2/S_1 ratio in the presence of kynurenic acid to 0.81 ± 0.12 . These results further support the hypothesis that B_6B_{21} binds to the glycine site of the NMDAR, and that glycine-like modulation of NMDAR regulates release of NE *in vivo*.

40.2

A MONOCLONAL ANTIBODY WHICH MIMICKS GLYCINE ACTIONS ON N-METHYL-D-ASPARTATE RECEPTORS HAS COMPLEX EFFECTS ON CHANNEL ACTIVATION AND NEURONAL SENSITIVITY TO HYPOXIA. P.K. Stanton[1,3], R. Sircar[3,4] and J.R. Moskal[1,2]. Dept. Neuroscience[1], Neurosurgery[2], Neurology[3], Psychiatry[4], Albert Einstein Coll. Med., Bronx, N. Y.

We have previously demonstrated that monoclonal antibody B_6B_{21} binds to the N-Methyl-D-Aspartate (NMDA) receptor and acts like glycine in its ability to stimulate [^{3}H]TCP binding to extensively washed hippocampal membrane preparations. In electrophysiological recordings from hippocampal slices, B_6B_{21} application prior to a hypoxic insult led to long-term potentiation of synaptic transmission, but did not exacerbate the loss of synaptic function. In order to explain this apparent paradox, we have measured the actions of B_6B_{21} and glycine on [^{3}H]MK-801 binding to rat cortical and hippocampal slices.

B_6B_{21} at 1, 5, and 10 µg/ 100 µl in 5 mM Tris-acetate buffer (pH 7.4) increased [^{3}H]MK-801 binding by 120%, 140% and 210%, respectively. In contrast, 50 µg/ 100 µl B_6B_{21} reduced binding to 87% of control. Glycine also showed a biphasic effect on [^{3}H]MK-801 binding. At 0.1 µM, glycine stimulated binding to 130% of control, but was not significantly increased when the glycine concentration was increased to 1 or 10 µM. Glycine at 0.1 µM also potentiated glutamate-stimulated [^{3}H]MK-801 binding (130% of control) but at 1 µM or 10 µM, glycine reduced this binding to 110% and 105% of control, respectively. With 1, 5 and 10 µg/ 100 µl B_6B_{21} in the presence of glutamate, binding was 160, 130 and 105% of control. Thus, modulation of the glycine site of the NMDA receptor appears to be biphasic, with potentially important implications in regulation of the neurotoxic effects of ischemia.

40.3

INHIBITION OF NMDA-EVOKED DOPAMINE RELEASE FROM MESENCEPHALIC CELL CULTURES BY NEUROPEPTIDE Y (NPY) AND PEPTIDE YY (PYY). H. Mount, I. Chaudieu, Y. Dumont, P. Boksa, & R. Quirion. Douglas Hospital Research Ctr., Depts. Pharmacol. & Therap. and Psychiat., McGill University, Verdun, PQ, Canada.

NPY and PYY have recently been reported to possess high affinity for the PCP receptor (within the NMDA receptor channel)(Roman et al., *Eur. J. Pharmacol.* 174:301-302, 1990). To test for a functional interaction with the PCP receptor, we examined effects of NPY-related peptides on NMDA-stimulated [^{3}H]dopamine ([^{3}H]DA) release from fetal rat dissociated mesencephalic cell cultures. We have previously shown NMDA-evoked [^{3}H]DA release to be potently and selectively inhibited by PCP (Boksa et al., *Soc. Neurosci.* 15:946, 1989).

NMDA-stimulated [^{3}H]DA release was significantly blocked by high concentrations (10 uM) of PYY or NPY. Spontaneous and kainate-evoked [^{3}H]DA release were only marginally affected by either peptide. NPY[13-36] ($\leq$ 10 uM), an NPY fragment that selectively activates Y_2 NPY receptors, had no effect on NMDA-evoked [^{3}H]DA release. Calcitonin gene-related peptide (hCGRP), another neuropeptide for which binding sites are localized in dopaminergic regions of the brain (T. Dennis et al., this meeting) failed to affect spontaneous or NMDA-stimulated [^{3}H]DA release. Thus, NPY and PYY may affect the NMDA receptor complex. However, the physiological relevance of this action remains to be established, since relatively high concentrations of the peptides were required to produce the effect. *Supported by the FRSQ.*

40.4

HIGH AFFINITY [^{3}H]-DEXTRORPHAN RECOGNITION SITES IN RAT BRAIN ARE ASSOCIATED WITH THE NMDA-OPERATED CATION CHANNEL. P.H. Franklin and T.F. Murray
College of Pharmacy, Oregon State University, Corvallis, OR 97331.

Although essentially devoid of µ opioid receptor activity, electrophysiological, behavioral and ligand binding data indicate that dextrorphan(DX), the dextrorotatory analog of morphine in the morphinan series, is a non-competitive antagonist of NMDA receptor activation(Church, et al. Eur. J. Pharmacol., 111:185, 1985; Murray and Leid, Life Sci., 34:1899, 1984) Based on electrophysiological data DX and other PCP-like drugs have been proposed to act as open-channel blockers of NMDA gated conductances. We report evidence of a high affinity DX recognition site at or near the NMDA-operated cation channel. Employing assay conditions which promote the binding of non-competitive NMDA antagonists(NCNA), equilibrium saturation analysis of [^{3}H]DX binding indicates that DX labels a site in rat forebrain with an apparent K_D of 60.2 ± 3.5 nM and B_{max} of 2.62 ± 0.06 pmol/mg protein. Regional analysis of [^{3}H]DX binding demonstrates a differential distribution in rat brain: hippocampus ≥ cortex > striatum ≥ thalamus > cerebellum > hypothalamus. As with other NCNA, specific [^{3}H]DX binding is increased by glycine and glutamate in a concentration-dependent manner, and is completely blocked by the specific NMDA antagonist AP-5. [^{3}H]DX binding is stimulated by the polyamines spermidine and spermine but not by putrescine. Competition analysis reveals the following rank order of potency against [^{3}H]DX: MK-801 > phencyclidine ≥ DX > dextromethorphan >> (+)-3-(Hydroxyphenyl)-N-propylpiperidine. Nifedipine, naloxone, haloperidol, and dynorphin A(1-13), were essentially inactive against [^{3}H]DX binding. In summary the pharamacological signature of [^{3}H]DX binding and the concordant responses of [^{3}H]DX and other NCNA to the influence of modulators of NMDA receptor activity suggest that DX acts as a NCNA at what may represent a unique site in the domain of the NMDA-operated cation channel.

40.5

CPP MORE POTENTLY ANTAGONIZES HIGH AFFINITY BINDING SITE FOR Mg^{2+} THAN L-GLUTAMATE RECOGNITION SITE.
K.Hatta*, T.Yamamoto, T.Hori*, K.Yoshikawa and T.Moroji*. Dept. of Psychopharmacology, Psychiat. Res. Inst. of Tokyo, Tokyo 156, JAPAN.

3-(2-Carboxypiperazine-4-yl)propyl-1-phosphonate (CPP) has been demonstrated to be highly selective and competitive NMDA antagonist. Recently, we have shown that low concentrations of Mg^{2+} markedly stimulate [^{3}H]TCP binding to rat cortical membranes, indicating the presence of a novel Mg^{2+} recognition site with high affinity on the NMDA/PCP receptor ion channel complex. In this study, we report the effects of CPP on [^{3}H]TCP binding in rat cerebral cortex.

CPP markedly inhibited [^{3}H]TCP binding stimulated by 300 uM Mg^{2+} in a concentration-dependent manner with an IC_{50} of 0.427±0.043 uM compared to those stimulated by 1 uM L-glutamate (IC_{50}=36.0±3.8 uM). CPP did not affect the basal level of [^{3}H]TCP binding. The IC_{50} value of CPP for Mg^{2+}-stimulated [^{3}H]TCP binding was one eighty-fourth of that for L-glutamate-stimulated [^{3}H]TCP binding. The present results show that CPP more potently antagonized [^{3}H]TCP binding stimulated by Mg^{2+} than L-glutamate.

40.6

ETHYLENEDIAMINETETRAACETIC ACID (EDTA) POTENTIATES A GLYCINE-STIMULATED [^{3}H]TCP BINDING IN RAT CORTICAL MEMBRANES.
T.Yamamoto, T.Hori*, K.Hatta* and T.Moroji*. Dept. of Psychopharmacology, Psychiatric Research Institute of Tokyo, Tokyo 156, JAPAN.

The binding of [^{3}H]TCP to the well washed rat cortical membranes was stimulated by glutamate and glycine. The prior reports indicate that glycine requires for the stimulation of [^{3}H]TCP binding by glutamate, and vice versa.

However, it remains unclear whether or not these two amino acids stimulate [^{3}H]TCP binding in an additive manner. . The present study was conducted to examine the effects of glutamate and glycine on binding properties of [^{3}H]TCP in the presence of EDTA.

EDTA markedly potentiated [^{3}H]TCP binding stimulated by glycine. The basal and glutamate-stimulated binding were only slightly stimulated in a concentration dependent manner (max. at 1 mM EDTA). In the absence of EDTA, glycine had an addtive effect on glutamate-stimulated [^{3}H]TCP binding. In the presence of 1 mM EDTA, however, no additive effect was observed. Similar results were obtained with EGTA. These results suggest that EDTA modulates an inter-relationship between glutamate- and glycine-sites on the NMDA/PCP receptor ion channel complex via unknown membrane factors.

40.7

INHIBITION OF [^{3}H]CPP BINDING TO BRAIN SYNAPTIC MEMBRANES BY ORGANOPHOSPHORUS AGENTS.
P.S. Johnson and E.K. Michaelis Dept. of Pharmacology and Toxicology, University of Kansas, Lawrence, KS 66045

Several competitive antagonists of the N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptors are phosphonate analogs of L-glutamic acid. The position of the phosphonate has been shown to be important in the structure-activity relationships of these analogs. To investigate whether other phosphorous-containing compounds had activity at the NMDA receptor, several neurotoxic organophosphorous compounds were tested for the ability to inhibit the specific binding of 3-(±)-2-carboxypiperazin-4-yl)-[1,2-^{3}H]propyl-1-phosphonic acid ([^{3}H]CPP), a selective antagonist of NMDA receptors, to brain synaptic membranes. Diisopropylfluorophosphate (DFP) was found to be the most potent of the organophosphates tested (K_i = 6 uM) and this inhibition was irreversible. Also, preincubation with excess NMDA receptor agonists and antagonists prior to exposure to DFP prevented inhibition by DFP of [^{3}H]CPP binding. These observations are suggestive of an interaction between organophosphates and the NMDA receptors. Current studies are focusing on determining which binding site the organophosphates may be interacting with.
(Supported by grants AA04732 and DAAL 03-88-K0017).

40.8

TCP BINDING TO CEREBRAL CORTEX MEMBRANES FROM DEVELOPING AND AGEING MICE.
P. Saransaari and S.S. Oja. Tampere Brain Research Center, Department of Biomedical Sciences, University of Tampere, Box 607, SF-33101 Tampere, Finland.

TCP (N-[1-(2-thienyl)cyclohexyl] piperidine) is an analogue of phencyclidine which is bound to sites associated with the NMDA subclass of excitatory amino acid receptors in the brain. We characterized the binding of tritiated TCP to cerebral cortex membranes isolated from developing and ageing mice. TCP binding was saturable in the studied concentration range of 2 to 200 nM, exhibiting one binding component in the cortex of 7-day-old mice as well as during the whole lifespan up to 24 months of age. The maximal binding capacity calculated per protein content decreased during postnatal development until 3 months of age, remaining thereafter constant in ageing mice. The binding constant did not change during development and adulthood but increased during the second year of life. Glycine potentiated the binding concentration-dependently in the adult cerebral cortex, whereas only a small stimulation was discernible in 7-day-old mice. L- and D-glutamate also enhanced TCP binding; the effects being again somewhat smaller in the developing brain. The differences in the properties of cortical PCP binding sites in the developing and ageing brain are likely to be of great importance in the function of NMDA receptors.
Supported by the Emil Aaltonen Foundation, Finland.

40.9

EX VIVO [^{3}H]MK-801 BINDING TO MOUSE CEREBRAL MEMBRANES: EFFECT OF COMPETITIVE AND NON-COMPETITIVE NMDA RECEPTOR ANTAGONISTS.
C.E. Stidsen, Novo Nordisk A/S, Dept. of Biochemical Pharmacology, CNS Division, DK-2880 Bagsvaerd, Denmark.

[^{3}H]MK-801 binds specifically and with high affinity to freshly dissected and homogenized mouse forebrain. Non-competitive NMDA receptor antagonists (MK-801, PCP, Ketamine) administered i.p. to NMRi mice 15-30 mins before decapitation displaced [^{3}H]MK-801 in a dose-dependent manner. The ED_{50} values obtained for the individual compounds correlated well with IC_{50} values found by in vitro binding to the same homogenate and was comparable to ED_{50} values reported from inhibition of pentylenetetrazole induced seizures in mice On the other hand competitive NMDA receptor antagonists (CPP, CGP 37849, CGP 39551) as well as glycine antagonists (HA-966 and 7-chlorokynurenic acid) up to 100 mg/kg had no effect on ex vivo [^{3}H]MK-801 binding.

These results suggest that this method could be predictable for behavioral effects of non-competitive NMDA antagonists. On the other hand high levels of endogenous glutamate and glycine might abolish the antagonistic effects of competitive NMDA antagonists or glycine antagonists.

40.10

ANALYSIS OF THE INTERACTIONS OF N-ACETYL-ASPARTYLGLUTAMATE (NAAG) WITH NMDA, QUISQUALATE AND KAINATE BINDING SITES IN COMPETITIVE EQUILIBRIUM BINDING ASSAYS.
H. M. Valivullah, J. Lancaster*, P. Sweetnam and J.H. Neale, Dept. Biology, Georgetown University, Wash, D.C. 20057 and NOVA Pharmaceutical Corp., Balt, MD 21224.

The structure of the neuropeptide, NAAG, suggests that it may act through acidic amino acid receptors. However, attempts to test this concept with physiological and ligand binding assays often have provided equivocal results. One reason for this is the presence in neuronal membranes of peptidase activity which hydrolyzes NAAG to glutamate and N-acetylaspartate. In this study, we have obtained equilibrium binding conditions which substantially reduce or abolish the peptidase activity while permitting assay of ^{3}H-CGS 19755 (NMDA-receptor antagonist), ^{3}H-AMPA (quisqualate agonist), and ^{3}H-Kainic acid (kainic acid agonist) binding. At the NMDA receptor, N-acetylaspartate (NAA) showed no inhibition, but NAAG had an IC_{50} of >3 μM. Under ^{3}H-CGS 19755 binding conditions, peptidase activity was not totally inhibited. Therefore, this very modest apparent competition for the NMDA site may have been due to glutamate (IC_{50}=350 nM) released from NAAG in the course of the assay.

40.11

N-METHYL-D-ASPARTATE RECEPTORS IN BATS AND SHREWS. D. S. Higgins, R.L. Albin, J.B. Penney, A.B.Young. Department of Neurology, University of Michigan, Ann Arbor, MI, 48104-1687.

Using an autoradiographic assay we describe the regional distribution of N-methyl-D-aspartate (NMDA) receptors in the brain of megachiroptera and microchiroptera, insectivora and rodentia. Twenty three regions in the forebrain, brainstem and cerebellum were examined. NMDA receptor binding was greatest in forebrain structures, the highest levels being found in the hippocampal formation and outer cortical laminae. Within the hippocampus, the stratum radiatum of the CA-1 subfield uniformly evidenced the highest number of NMDA receptors. Certain thalamic nuclei and the caudate-putamen contained 30% of the binding seen in the stratum radiatum of the hippocampus. The external plexiform layer of the olfactory bulb contained at least 50% greater binding than other olfactory laminae. Binding minimally above background was found in most brainstem structures, the reticular nucleus of thalamus and the granular layer of the cerebellum. NMDA receptor distribution was similar amongst these eutherian mammals though there was some inter-species variation in the rank order of receptor density found in the medial geniculate nucleus and the superficial layers of the superior colliculus. Supported by USPHS Grants NS 01300, NS 19613, and NS 07222.

40.12

[^{3}H]MK-801 AND NMDA-SENSITIVE [^{3}H]GLUTAMATE BINDING IN TURTLE BRAIN. A.B. Young, S.Y. Sakurai, and R.L. Albin. Dept. of Neurology and Neuroscience Program, University of Michigan, Ann Arbor, MI, 48109.

Turtle neurons are resistant to glutamate neurotoxicity (Wilson and Kriegstein, Soc. Neurosci., p. 763, 1989). Since NMDA receptors have been implicated in the pathogenesis of glutamate neurotoxicity, we examined NMDA-sensitive [^{3}H]glutamate binding and [^{3}H]MK-801 binding in the red-eared turtle (P. Scripta).

NMDA-sensitive [^{3}H]glutamate binding was present in turtle forebrain. The competitive antagonists, CPP (100 μM) and AP7 (100 μM) completely inhibited NMDA-sensitive [^{3}H]glutamate binding. Specific [^{3}H]MK-801 binding was also present in the turtle forebrain. The non-competitive NMDA antagonists, PCP, TCP and ketamine (each 100 μM) displaced total binding by 80%. CPP (1mM) inhibited only 20% of specific [^{3}H]MK-801 binding. CNQX (100 μM) did not inhibit binding while 7-chlorokynurenic acid (100 μM) inhibited specific binding by 40%. Glutamate (1mM) enhanced binding by 200% whereas glycine (1mM) had no effect. These data suggest that the pharmacology of the NMDA-receptor complex in turtle may differ from mammalian brain. Supported by USPHS Grant NS 19613 and NS 01300.

40.13

[^{3}H]MK-801 BINDING IN PIGEON FOREBRAIN. S.Y. Sakurai, R.L. Albin, A. Reiner, and A.B. Young. Dept. of Neurology and Neuroscience Program, University of Michigan, Ann Arbor, MI, 48109; Dept. of Anatomy, University of Tenn. Health Science Center, Memphis, TN 38163.

Pigeons have been used extensively in excitatory amino acid (EAA) behavioral pharmacology. We have examined the distribution and pharmacology of NMDA receptors in White Carneaux pigeon brains using [^{3}H]MK-801 and NMDA-sensitive [^{3}H]glutamate quantitative autoradiography.

Specific [^{3}H]MK-801 binding was present in pigeon forebrain regions homologous to mammalian cortex and striatum. In the pigeon neostriatum, TCP (100 μM) and ketamine (100 μM) displaced [^{3}H]MK-801 binding by 94% and 98%, respectively. The competitive NMDA antagonist, CPP (100 μM), inhibited specifc [^{3}H]MK-801 binding by 93%. Zinc ions (100 μM) inhibited specific binding by 81%. In addition, the quinoxalinedione compound, CNQX (100 μM), reduced [^{3}H]MK-801 binding by 40%. The distribution of NMDA-sensitive-[^{3}H]glutamate binding sites in pigeon forebrain was similar to the distribution of [^{3}H]MK-801 binding sites. NMDA-sensitive [^{3}H]glutamate binding was inhibited by CPP but not by CNQX. These data suggest that the pharmacology of the NMDA receptor in pigeon brain is similiar to the mammalian brain. Supported by USPHS Grant NS 19613.

40.14

AGE-RELATED CHANGES OF THE NMDA RECEPTOR COMPLEX IN THE RAT BRAIN STUDIED BY IN VITRO AUTORADIOGRAPHY. R. Miyoshi, S. Kito and T. Nomoto*. Dept. of Pharmacology, Tokyo Women's Medical College, Tokyo 162 and Division of Health Sci., Univ. of the Air, Chiba 260, Japan.

Receptors for excitatory amino acid, L-glutamate have been divided into three subtypes referred to N-methyl-D-aspartate (NMDA), quisqualate and kainate receptors. Among them, the NMDA subtype has been supposed to play an important role in long term potentiation and neuronal cell death due to brain ischemia and Alzheimer's disease. This type consists of the receptor complex containing an agonist recognition site, a cation channel and a strychnine-insensitive glycine modulatory site. For observing age-related changes of these binding sites, quantitative in vitro autoradiography with ^{3}H-CPP, ^{3}H-MK 801 and ^{3}H-glycine was done with use of the rat brain. ^{3}H-glycine binding sites were severely decreased in telencephalic regions including the hippocampus and cerebral cortex of aged animals. ^{3}H-CPP binding sites were well preserved in the aged brain. ^{3}H-MK 801 binding itself was at very low levels throughout the brain and did not significantly changed in aged animals. However, ^{3}H-MK 801 binding in the presence of both L-glutamate and glycine was potentiated and this potentiation was reduced in telencephalic regions of aged rats. These results indicate that glycine binding sites are primarily affected in the normal aging process.

40.15

THE DISTRIBUTION OF N-METHYL-D-ASPARTIC ACID RECEPTOR BINDING IN NEONATAL AND ADULT MOUSE LUMBAR SPINAL CORD D.L. Gonzalez, J.L. Fuchs, M.H. Droge. Dept. of Biol., Texas Woman's Univ., Denton, TX. 76204; (JLF) Dept. of Biol. Sci., Univ. of North Texas, Denton, TX. 76203.

The objective of this study was to characterize the distribution of N-Methyl-D-Aspartic Acid (NMDA) receptors in the lumbar spinal tissue of young (aged birth to 10 days) and adult Balb/C mice. Transverse, 20 μm frozen sections were mounted and dried. Sections were incubated in 200 nM [^{3}H]-glutamate in the presence of 1 uM quisqualic acid to inhibit non-NMDA glutamate binding. To determine nonspecific binding, adjacent sections were treated as above but with excess NMDA present. Sections were apposed to tritium sensitive Hyperfilm for 3 weeks. Densitometric analyses were carried out using a video-based image analysis system. In neonates, binding was slightly higher in the ventral horn than dorsal horn. This order was reveresed in adults. While the neonatal gray matter was fairly uniformly labeled, the adult showed the highest levels in the substantia gelatinosa, along the medial border of the dorsal horn, and in lamina X. Supported by NIH-MBRS Grant 41424

41.1

AGE-RELATED DECREASES IN CORTICOTROPIN-RELEASING FACTOR RECEPTORS IN THE BRAIN AND ANTERIOR PITUITARY GLAND OF THE RAT. J.A. Heroux, D.E. Grigoriadis, and E.B. De Souza. Neurobiology Laboratory, Addiction Research Center, National Institute on Drug Abuse, Baltimore, MD 21224

Age-related changes in basal and stress-induced hypothalamic-pituitary-adrenocortical (HPA) hormone secretion have been previously reported. In the present study, corticotropin-releasing factor (CRF) receptors were measured in homogenates of discrete areas of brain and in anterior pituitary of 4, 12, 18, and 24 month old male Fisher rats using ^{125}I-Tyr^{0}-ovine CRF (^{125}I-CRF). At a single saturating concentration of CRF (1nM), significant decreases were observed in ^{125}I-CRF binding in the anterior pituitary gland and hypothalamus with respect to increasing age. Maximal reductions in CRF binding were observed in the anterior pituitary (60% decrease) and hypothalamus (27% decrease) of 24 month old rats when compared to binding in 4 month old rats. No significant age-related changes in CRF binding were observed in midbrain, cerebellum, olfactory bulb, pons medulla, hippocampus, striatum, frontal cortex, parietal cortex, or spinal cord. Saturation experiments of ^{125}I-CRF binding in the anterior pituitary of 4 and 24 month old rats revealed about a 60% reduction in the density of CRF receptors (B_{max}) in 24 versus 4 month old rats with no age-related differences in the affinity (K_D) of CRF for it's receptor. The mechanisms involved in the age-related decreases in CRF receptors in the anterior pituitary and hypothalamus are currently being investigated. The age-related decline of CRF receptors in the hypothalamus and anterior pituitary may play an important role in the changes in basal and stress-induced HPA hormone secretion seen in aged rats.

41.2

EFFECT OF SHORT AND LONG DURATION HYPOTHYROIDISM AND HYPERTHYROIDISM ON HYPOTHALAMIC CORTICOTROPIN-RELEASING HORMONE mRNA RESPONSES TO STRESS. T.C Kamilaris, J. Redwine, M. Smith, E.O. Johnson, P.W. Gold, and G.P. Chrousos. CNB/NIMH & DEB/NICHD, Bethesda, MD, 20892.

We used *in situ* hybridization histochemistry on rat hypothalamus to examine the effect of the duration of hypo- and hyperthyroidism on the levels of mRNA encoding corticotropin-releasing hormone (CRH mRNA). We studied parvocellular neurons of the rat paraventricular nucleus under basal and prolonged stress conditions in male SD rats with short- (7 days) or long-standing (60 days) hypothyroidism (thyroidectomy + placebo), euthyroidism (sham thyroidectomy + placebo), or hyperthyroidism (thyroidectomy + thyroxine 50 ug/day). Prolonged stress was induced by daily ip injections of 1.5 M hypertonic saline at 09:00 for 5 consecutive days (Lightman & Young, PNAS 86:43-6, 1989). Control animals received an equivalent volume (1.8 ml/Kg BW) of 0.15 M saline or no saline. Hypo- and hyperthyroidism of short duration had no significant effect on basal or stress-stimulated CRH mRNA levels. In contrast, long-lasting hypothyroidism was associated with reduced stress-stimulated CRH mRNA levels ($p<0.001$). No changes in basal or stress-stimulated CRH mRNA levels were found in long-standing hyperthyroid animals. These findings suggest that stress-stimulated synthesis of hypothalamic CRH mRNA is significantly affected in severe, long-standing hypothyroidism which may compromise their responsiveness to and coping with stress. The data also suggest a central effect of thyroid hormone on CRH system independently from the known effect on corticosteroid secretion and metabolism.

41.3

IN VITRO REGULATION OF PITUITARY ACTH SECRETION IN INFLAMMATORY DISEASE SUSCEPTIBLE LEWIS (LEW/N) AND INFLAMMATORY DISEASE RESISTANT FISCHER (F344/N) RATS. P. Zelazowski*, M.A. Smith, P.W. Gold*, G.P. Chrousos, R.L.Wilder* and E.M. Sternberg NIMH, NIAMS, NICHD, NIH, Bethesda, MD 20892

We have previously shown that susceptibility to inflammatory disease in LEW/N rats is related to deficient glucocorticoid counter-regulation of the immune response resulting from deficient hypothalamic corticotropin releasing hormone (CRH) responsiveness to inflammatory and other stress mediators. LEW/N rats also show deficient *in vivo* ACTH responses to CRH stimulation. In order to determine whether this blunted ACTH response is related to secondary changes in the functional responsivensss of the pituitary or to enhanced sensitivity to glucocorticoid negative feedback, we studied basal and CRH stimulated ACTH secretion and content in primary pituitary cells and pituitary organ explants, and POMC mRNA expression by *in situ* hybridization in LEW/N and histocompatible F344/N pituitaries. LEW/N rats contain 50% fewer POMC expressing pituitary cells and 50% less ACTH compared to F344N rats. Basal ACTH secretion and the peak ACTH response to CRH is also 50% less in LEW/N compared to F344/N rats. The percent secretion of ACTH stimulated by all doses of CRH, when corrected for the lower baseline, is the same in LEW/N and F344/N rats. At low doses of CRH stimulation, LEW/N pituitary cells show a dose-dependent greater sensitivity of both basal and CRH-stimulated ACTH secretion to dexamethasone suppression compared to F344/N cells, suggesting enhanced sensitivity of LEW/N pituitary cells to glucocorticoid negative feedback. We speculate that the lower LEW/N ACTH responses and lower percent of POMC-expressing cells reflect the long-standing CRH hyporesponsiveness and/or the sustained effects of enhanced glucocorticoid negative feedback on the pituitary. This could reflect either a global defect in glucocorticoid receptor function that restrains POMC message expression or be secondary to the CRH hyporesponsiveness that we have previously described *in vivo* and *in vitro*. These changes in pituitary corticotroph cell function could render LEW/N rats further susceptible to inflammatory diseases.

41.4

INCREASE IN CORTICOTROPIN RELEASING HORMONE (CRH) CELL SIZE IN MALE AND FEMALE FISCHER 344 RATS IN RESPONSE TO CHRONIC BEHAVIORAL STRESS. D.D. Kelly and A.J. Silverman. NYS Psychiatric Inst. & Dept. Anat. & Cell Biol., Columbia Univ., NY, NY. 10032

The neurohormone CRH is synthesized in the hypothalamic paraventricular nucleus (PVN). In previous studies, we observed a significant increase in detectability, staining intensity and size of PVN CRH cells in male Wistar rats exposed for 10d to a chronic behavioral stress paradigm. We since serendipitously observed that in Fischer 344 rats, both staining intensity and CRH cell number were high in non-stressed individuals. We have now examined whether males (n=8) and females (n=8) of this strain respond similarly to stress with an increase in CRH cell size. Experimental animals were placed in a sound proof chamber, food and water ad lib. and exposed to alternating 30-min sessions of white noise (90db) and conditioned emotional response training for 10 d. Controls remained in their home cage. Animals were perfused and tissue prepared for CRH immunocytochemistry. Cell size measurements were made without knowledge of the animal's condition. CRH cells of non-stressed females were larger than those in age matched males (117.8 ± 40.3 vs 99.8 ± 54.0 μm^2, respectively). In both sexes, there was a robust response to stress with mean cell size increasing to 142.6 ± 49.3 and 151.2 ± 64.1 μm^2. These results suggest that alterations in CRH cell size are not attributable to the amount of reaction product per cell, but to a real increase in plasma membrane and intracellular organelles. These data also indicate that the PVN of both sexes responds to chronic stress in a similar, morphologically plastic manner, but not to the same degreee. NS 23858

41.5

CHARACTERIZATION OF THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS IN THE ZUCKER OBESE RAT. P.M. Plotsky, S. Otto*, C. Rivier and S.W. Sutton*. The Clayton Foundation Labs for Peptide Biol, The Salk Institute, La Jolla, CA 92037.

Genetically obese rats of the Zucker strain have numerous endocrine, metabolic and behavioral abnormalities. Compared to non-obese littermates (**Fa**/-), Zucker obese (**fa/fa**) rats have larger adrenals (45 ± 2 vs 37 ± 1 mg; $p<0.01$), elevated morning corticosterone (24 ± 4 vs 49 ± 10 ng/ml; $p<0.01$), and lack a robust pituitary-adrenocortical circadian rhythm. Although both ACTH and corticosterone responses to exogenous rCRF-41 challenge (1 µg, iv) in the morning were equivalent, the duration of the corticosterone response was prolonged in the **fa/fa** rats. Possibly underlying these changes, initial secretion of hypothalamic corticotropin releasing factor (CRF) was significantly lower in **fa/fa** vs **Fa**/- rats (65 ± 8 vs 237 ± 34 pg/ml; $p<0.001$) and no increased secretion in response to hypotensive challenge was evident. Despite these differences in CRF secretion into the hypophysial-portal circulation, neither hypothalamic CRF content (762 ± 45 vs 836 ± 49 pg/median eminence; $p>0.5$) nor mRNA levels were significantly different in **fa/fa** and **Fa**/- rats. These characteristics are similar to those of the chronically stressed rat model.

41.6

MODULATION OF HYPOTHALAMIC CRF-41 BY ACTIVIN-A. S.W. Sutton*, A. Kjaer*, W. Vale and P.M. Plotsky. The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037.

A diverse array of inputs modulates corticotropin releasing factor (CRF-41) secretion and gene expression in the hypothalamic parvicellular paraventricular nucleus (PVN). Among these inputs is an inhibin βA subunit immunopositive pathway arising from the caudal nucleus of the solitary tract. Central (icv) administration of recombinant activin-A, an inhibin βA/βA homodimer with growth factor activity, is associated with stimulation of adenohypophysial ACTH secretion. At concentrations of 0.6-3.0 nmole, activin-A facilitates hypothalamic secretion of CRF-41 (maximal 4.1-fold elevation from mean initial levels of 228 ± 48 pg/ml), but is without effect on vasopressin secretion. This response is attenuated by bilateral PVN infusion of inhibin βA-antiserum into the PVN prior to challenge. In vitro, activin-A (24 h, 10 ng/ml) enhances basal and norepinephrine-stimulated CRF-41 release from primary cultures of neonatal rat hypothalamic cells in an additive fashion, but is without effect on acetylcholine-stimulated CRF-41 secretion. Interactions between activin-A and other putative transmitters on CRF-41 secretion and gene expression are being characterized.

41.7

NEONATAL ISOLATION INCREASES HYPOTHALAMIC CORTICOTROPIN RELEASING FACTOR mRNA. B.L. Firestein and P.M. Plotsky. The Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA 92037.

Neonatal stress can lead to long-term changes in hypothalamic-pituitary-adrenal (HPA) axis function, including hyper- or hyporesponsiveness of the axis to stressors and glucocorticoid feedback. We have examined the effects of neonatal handling or isolation on hypothalamic CRF mRNA levels in adult rats. Litters of 8 pups each (n=3/treatment) were subjected to one of 3 treatments: UNDISTURBED (no human contact until weaning), HANDLED (5 min handling every morning), and ISOLATED (6 hr separation from dam removed every morning to incubator) during the second neonatal week. Total hypothalamic RNA was prepared when rats were 40 days old for sequential Northern blot analysis of rCRF mRNA (riboprobe α^{32}P-UTP labeled; $1x10^6$ cpm/ml) and human β-actin mRNA (40-mer oligoprobe 5'-end labeled with γ^{32}P-ATP; $1x10^6$cpm/ml). RNA was loaded onto a formaldehyde gel (20 µg/lane). After transfer of the blots to HyBond membrane, hybridization was performed (65 C) and films exposed for 3 days. Films were semiquantified by scanning densitometry. Rat hypothalamic CRF mRNA (1.3 kb band) from the ISOLATED group was significantly elevated ($p<0.05$) in comparison to the UNDISTURBED or HANDLED groups. No differences were apparent between these latter two groups, nor was a sex difference observed for any treatment These studies provide a central molecular basis for long-term changes in HPA axis function following neonatal stress.

41.8

TIME-COURSE OF NEUROPEPTIDE AND PROTO-ONCOGENE UP-REGULATION IN NEURONS OF THE PARVOCELLULAR PARAVENTRICULAR NUCLEUS. S. S. Kollack, J. P. Herman, and S. J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48198-0720.

We have looked at the inductive effects of immobilization stress on parvocellular neurons of the rat paraventricular nucleus (PVN). Male rats were sacrificed at various time points following the onset of immobilization stress, including: 15 min., 30 min., 45 min., 1 hr., 1.5 hr., 2 hr., 4 hr., 6 hr., and 1 day. Animals that received no stress served as controls. cRNA probes complementary to corticotropin releasing hormone (CRH) mRNA and c-fos mRNA were used to assay levels of message via *in situ* hybridization histochemistry. Results demonstrated a different time-course of induction of these two messages. Expression of c-fos mRNA was undectectable in the PVN of unstressed rats. In response to stress, c-fos mRNA was induced in PVN neurons, achieving peak levels 30 minutes after onset of stress and returning to undetectable levels by 90 minutes. In contrast, induction of CRH mRNA peaked 90 minutes after onset of stress and declined more slowly. These data demonstrate that increases in cytoplasmic proto-oncogene and CRH mRNAs occur in different time domains, perhaps commensurate with different post-stress functions.

These observations indicate that immobilization stress activates the hypothalamo-pituitary-adrenocortical axis and results in transient increases in levels of message for both CRF and c-fos in parvocellular neurons of PVN. We are currently assessing the relationship between stress-induced changes in CRH and c-fos mRNA with that of other ACTH secretagogues (e.g., vasopressin) and proto-oncogenes (e.g., c-jun) in the parvocellular PVN.

Supported by NS 08267 and MH 422251.

41.9

ALTERATIONS IN C-FOS EXPRESSION IN THE RAT SUPRAOPTIC AND PARAVENTRICULAR NUCLEI AFTER ADRENALECTOMY, DEXAMETHASONE, AND CLONIDINE. M. Jarnot and L. Jennes, Dept. of Anatomy, Wright State Univ. Sch. of Med., Dayton, OH 45435.

The presence of the protooncogene c-fos was used to examine the effects of adrenalectomy, dexamethasone treatment, and the noradrenergic agonist clonidine on cells of the supraoptic (SON) and paraventricular (PVN) nuclei of the rat. In untreated control as well as in adrenalectomized animals, c-fos immunoreactivity was absent in cell nuclei of the SON. 1 hr after dexamethasone (100 ug/kg BW, iv) and 1 hr after clonidine (100 ug/kg BW, iv), certain vasopressin (VP), but not oxytocin containing neurons showed c-fos in their nuclei. In the control PVN, c-fos was consistently present in many parvocellular neurons, but absent from the magnocellular compartment. After adrenalectomy, the number of c-fos containing neurons in the parvocellular PVN was slightly reduced. However, iv injections of clonidine as well as dexamethasone caused a strong transient increase in the number of c-fos immunoreactive neurons in both the parvo- and magnocellular PVN. This increase in c-fos immunoreactivity was restricted to VP and corticotropin releasing factor (CRF) containing neurons. The results suggest that both clonidine and dexamethasone affect selectively the VP-CRF neuronal system through different mechanisms which result in an increased expression of c-fos. Supported by NIH HD-24697.

41.10

MODULATION OF CORTICOTROPIN-RELEASING FACTOR (CRF) OR GLUCOCORTICOID RECEPTOR (GR) GENE EXPRESSION IN TRANSGENIC MICE: MODELS FOR NEUROENDOCRINE CHANGES SEEN IN DEPRESSION Nicholas Barden, Francine Morin*, François Pothier*, James Lee† and Marie-Claude Pepin. Molecular Psychogenetics, Laval Univ. Hospital Research Ctr, Ste Foy, Québec, Canada, G1V 4G2. † Psychiatry Dept., Duke University Medical Ctr, Durham, N.C.

Increased activity of the HPA axis, as indicated by hypersecretion of CRF and cortisol, and premature escape from the cortisol suppressant action of dexamethasone is often associated with mood disorders. The resistance of cortisol secretion to suppression by dexamethasone is suggestive of GR malfunction at the limbic-hypothalamic system level and this hypothesis is supported by our finding of tricyclic antidepressant drug modulation of GR mRNA concentrations in hypothalamic neurons. We have attempted to reproduce in an animal model, by manipulation of genes involved in the control of the HPA axis, two of the changes which can accompany depression, namely, increased CRF secretion and a reduced neuronal sensitivity to glucocorticoids. In order to produce animals hypersecreting CRF, the cloned human CRF gene was introduced into the mouse genome by microinjection of vector-free DNA into a pronucleus of a fertilized mouse egg. When this gene was under control of its homologous promoter, insertion into mouse DNA was generally lethal. However, construction of a chimeric gene consisting of the metallothionein promoter/human CRF gene coding region permitted the establishment of a stable line of transgenic animals with characteristic phenotype. To reduce neuronal GR levels in transgenic mice, cellular GR production was modified by introduction of a complementary GR anti-sense RNA strand. To produce anti-sense RNA, an 1800 bp GR cDNA fragment was inverted downstream from a human neurofilament gene promoter This construction lead to transcription of the anti-sense strand of the GR cDNA fragment instead of the sense strand and, when transfected in cell cultures, caused a functional reduction in GR activity. When incorporated into the mouse genome, a line of animals which pass the transgene from generation to generation and show modified HPA axis parameters was established. These transgenic mice colonies could be invaluable for assessment of new antidepressant therapies.

41.11

CORTICOTROPIN-RELEASING HORMONE STIMULATION TEST IN PATIENTS WITH DEPRESSION SECONDARY TO OTHER PSYCHIATRIC ILLNESSES, PRIMARY DEPRESSION, SCHIZOPHRENIA AND NORMAL CONTROLS. SD Samuelson, RG Kathol, TL Gehris*, BT Carroll, AF Pitts, WH Meller*, and J Carter*. The University of Iowa College of Medicine, Iowa City, IA 52242.

Disturbances in the hypothalamic-pituitary-adrenal (HPA) axis have been well documented in depressed patients. In this study, we investigated HPA axis functioning in patients with other psychiatric illnesses.

The ovine corticotropin-releasing hormone (oCRH) stimulation test (0.1 µg/kg) was performed at 1600 hours on twenty-five patients with primary major depressive disorder (MDD), twelve patients with major depressive episodes secondary to concurrent or previous diagnoses of other psychiatric illnesses (SDD), eight patients with schizophrenia, and twenty-seven healthy controls. No statistically significant group differences in cortisol or ACTH response to oCRH could be demonstrated between MDD, schizophrenic, or control subjects. However, SDD patients had significantly ($p<0.05$) higher baseline and peak stimulated cortisol levels, and greater mean area under the curve for the cortisol response compared with the controls. Our SDD group, however, was atypical in that most of these patients had depressions secondary to psychotic illnesses, or to drug or alcohol abuse. Although no differences in ACTH or cortisol secretion could be attributed to the presence of psychosis, there was a significant association between previous alcohol or drug history and a blunted ACTH response ($t=2.8$; $p=0.01$) as well as increased cortisol secretion ($t=3.4$; $p=0.005$) following oCRH administration when compared to the SDD without a history of alcohol or drug abuse.

41.12

TIME-COURSE OF ACTION OF THE TRIAZOLOBENZODIAZEPINE, ALPRAZOLAM, ON HPA AXIS FUNCTION IN RATS. M.A. Vargas*, M.J. Owens, J.C. Ritchie and C.B. Nemeroff. Depts. Psychiatry & Pharmacology, Duke Univ., Durham, NC 27710.

Corticotropin-releasing factor (CRF) functions to integrate not only the endocrine, but autonomic and behavioral responses to stress. We have previously shown that 60 minutes following a single injection of alprazolam or adinazolam, biochemical indices of HPA axis function are altered in a pattern opposite to that observed after exposure to stress. To further scrutinize the effects of triazolobenzodiazepines on HPA axis function, rats received a single sc injection of alprazolam (1 mg/kg) and were killed at various times (15-240 min) post injection. In contrast to our previous data in which alprazolam decreased plasma ACTH and corticosterone concentrations in non habituated rats, alprazolam did not alter the already low basal concentrations of the pituitary adrenal hormones at any time point studied. Similarly, median eminence CRF content was unchanged. However, in agreement with our previous work, CRF concentrations in the locus coeruleus were decreased between 30-180 minutes post-injection. These results further suggest that the anxiolytic and/or purported antidepressant properties of alprazolam may be related to their direct or indirect actions on CRF neurons innervating the locus coeruleus. These results will be discussed in relation to the pharmacokinetics of alprazolam in plasma and brain. Supported by NIMH MH-42088.

41.13

EFFECT OF RESERPINE AND COLCHICINE ON NEUROPEPTIDES mRNA LEVELS IN THE RAT HYPOTHALAMIC PARAVENTRICULAR NUCLEUS. S. Ceccatelli, R. Cortés and T. Hökfelt. Dept. of Histology and Neurobiology, Karolinska Institutet, P.O. Box 60400, S-104 01 Stockholm, Sweden.

In the present study we have analysed peptides in the hypothalamic paraventricular nucleus (PVN), using in situ hybridization and immunohistochemistry. We have studied possible changes in mRNA and peptides levels in the PVN 24 hr after a single large dose of reserpine (10 mg/kg) and 24 hr after colchicine (120 µg/20 µl NaCl) intraventricularly (i. c. v.) injected. Sections of the PVN were hybridized using synthetic oligonucleotide probes complementary to CRH, NT, ENK, VIP and TRH mRNAs. For immunohistochemistry rabbit antisera to CRH, NT, ENK, VIP and TRH were used. In situ hybridization showed a clear increase in CRF mRNA as compared to control rats after both treatments. Also NT and VIP mRNA could be seen in parvocellular neurons in reserpine and in colchicine treated rats, whereas we so far have not been able to demonstrated these mRNAs in untreated rats. No changes in TRH mRNA could be detected after reserpine. With immunohistochemistry, after reserpine, many CRH-, but not NT- or VIP- positive neurons could be observed in the parvocellular part of the PVN. This immunoreactivity could only be seen in a few cells in normal rats with the present sensitivity of our immunohistochemical technique and increase dramatically after i. c. v. colchicine.

The present results demonstrate that treatment with two drugs, the monoamine depleting drug reserpine and the mitosis inhibitor colchicine, causes increased levels of mRNA for several peptides in neurons of the PVN, located almost exclusively in its parvocellar part and confined to the hypothalamo-pituitary adrenal axis.

41.14

EFFECTS OF INTRAHIPPOCAMPAL COLCHICINE ON HYPOTHALAMIC CORTICOTROPIN-RELEASING HORMONE mRNA LEVELS IN RATS. L. S. Brady, A. B. Lynn, H. J. Whitfield and M. Herkenham. Section on Functional Neuroanatomy, Clinical Neuroendocrinology Branch, NIMH, Bethesda, MD 20892.

Evidence suggests that the hippocampus exerts an inhibitory influence on corticotropin-releasing hormone (CRH) gene expression in the paraventricular nucleus (PVN) of the hypothalamus. We examined mRNA expression of CRH in rats after selectively lesioning the dentate gyrus of the hippocampus with colchicine. Rats were sacrificed at varying time intervals after bilateral infusion of colchicine (15 µg/rat) into the hilus of the dentate gyrus. Cryostat-cut sections through the PVN were hybridized with an ^{35}S-labeled probe for CRH and exposed to film for quantitative autoradiography. CRH mRNA was reduced in a time-dependent manner:

CRH mRNA (% of unoperated control)

Time (days)	1	2	3	7	14
Lesion	71±7†	64±3*	39±12*	45±12*	59±22*
Sham	85±15	84±7	132±17†	142±12†	95±12

† $p < 0.003$; * $p < 0.0001$ relative to control

Our findings show that selective lesion of the dentate gyrus decreases CRH mRNA in the PVN in contrast to hippocampectomy which increases CRH mRNA levels (*J. Neurosci.* 9: 3072, 1989). These data suggest that discrete subfields of the hippocampus differentially modulate CRH gene expression in the hypothalamus.

NEUROENDOCRINE REGULATION: OTHER I

42.1

6-HYDROXYDOPAMINE (6-OHDA) EFFECTS ON PITUITARY INNERVATION: DEGENERATIVE AND REGENERATIVE CHANGES. L.C. Saland, J.A. Carr, A. Samora*, S. Benavidez* and T. Romero*. Dept. of Anatomy, Univ. of New Mexico Sch. Med., Albuquerque, NM 87131.

The catecholamine neurotoxin, 6-OHDA, has been shown to induce degeneration, and to alter serotonin (5-HT) immunostaining in pituitary neurointermediate lobe (NIL) nerve fibers. Here, pituitaries from 6-OHDA treated rats were studied for possible regenerative changes after initial degeneration of terminals. Adult male Sprague-Dawley rats received two 6-OHDA injections by tail vein (150 mg/kg in 1mg/ml ascorbic acid in saline) on days 1 and 3, then were ether anesthetized and perfused with buffered aldehydes for light or electron microscopy (EM) at 1, 2, 3 or 4 weeks after day 1 of injection. Initial fiber degeneration in the NIL was confirmed by EM after 1 week, with gradual reappearance of normal appearing terminals over the remaining time. Tyrosine hydroxylase (TH) immunostaining was intensified, as compared to vehicle-treated controls, with "swollen" terminals seen at 1 week. Fiber staining gradually returned to more normal patterns over 2-4 weeks. TH-containing cell bodies in the hypothalamus appeared normal at 1 and 2 weeks. Chemical denervation of the NIL, followed by apparent regeneration, can be used to examine plasticity of neural regulation of the endocrine cells of the intermediate lobe. Supported by NIH NS 21256, GM 08139, BRSG 400257 (LCS); F32 NS 08447 (JAC).

42.2

IN-VIVO EFFECTS OF SEROTONERGIC AGENTS ON PEPTIDE RELEASE FROM RAT INTERMEDIATE PITUITARY. J.A. Carr, L.C. Saland, A. Samora*, S. Benavidez*, T. Romero*. Dept. of Anatomy, Univ. of New Mexico Med. Sch., Albuquerque, NM 87131.

We used an in-vivo pharmacological approach to investigate the potential influence of serotonin (5-HT) on pro-opiomelanocortin-derived peptide release from the intermediate lobe (IL) of the pituitary in adult male Sprague-Dawley rats. After drug treatment, rats were rapidly decapitated and plasma collected for RIA of α-melanocyte-stimulating hormone (α-MSH) and β-endorphin. Pituitaries were collected for the ultrastructural analysis of IL cells. Administration of 5-hydroxy-L-tryptophan (30 mg/kg ip), the synthetic precursor to 5-HT, or the 5-HT reuptake blocker fluoxetine (10 mg/kg ip) elevated plasma levels of β-endorphin but not α-MSH. The non-selective agonist MK-212 produced an elevation in plasma β-endorphin at both doses tested (2.5, 5.0 mg/kg ip) while only the larger dose elevated plasma α-MSH. Rats treated with three successive daily ip injections of the 5-HT synthesis inhibitor parachlorophenyl-alanine (300,100,100 mg/kg) and then sacrificed one week after the first injection showed an elevation in plasma β-endorphin but no change in plasma α-MSH. Qualitative ultrastructural analysis of IL cells revealed changes consistent with drug effects on plasma α-MSH. Drugs which influence 5-HT synthesis/reuptake alter release of β-endorphin but not α-MSH, suggesting an action on anterior lobe (AL) but not IL POMC cells. MK-212 appears to stimulate POMC peptide release from both the AL and the IL. Supported by F32 NS08447 (JAC); NIH NS21256, GM 08139, BRSG 400257(LCS).

42.3

TEMPORAL CHARACTERISTICS OF DOPAMINERGIC REGULATION OF THE RAT INTERMEDIATE PITUITARY: SECRETION, POMC AND D2 RECEPTOR GENE EXPRESSIONS AND CELL PROLIFERATION. Bibie M. Chronwall[a], John M. Farah[b], Stephen J. Morris[a], David R. Sibley[c] and William R. Millington[a]. a. UMKC School of Basic Life Sciences, Kansas City MO, b. G.D. Searle & Co, St. Louis, MO, c. NIH, Bethesda, MD.

This study demonstrates the time sequence of changes in pro-opiomelanocortin (POMC) mRNA, rough endoplasmic reticulum, mitochondria, secretion, D2 dopamine receptor mRNA and proliferation induced in melanotropes after treatment with the D2 dopamine receptor antagonist haloperidol. A detailed time course study (10 min, 30 min, 1 h, 2h, 4h, 6 h, 12 h and 24 h) after a single injection of haloperidol (5mg/kg) showed that peptide secretion increased rapidly, peaking at 30 min and depleting the secretory vesicles. Within 2 h of treatment POMC mRNA levels were significantly elevated; POMC mRNA was maximally elevated at 6 h and remained at these levels throughout the 24 h period. An increase in the number of biosynthetically active organelles was first observed at 24 h and after two days there was an increase in melanotrope proliferation. Subchronic treatment (2.5 d) with haloperidol (2 mg/kg), stimulated peptide secretion from the IL and elevated POMC mRNA levels to 180% of control values. Chronic treatment (12 d), increased the amount of biosynthetically active organelles in melanotropes and further increased POMC mRNA to 300% and cell proliferation to 200%. D2 receptor mRNA increased to 150% of control values. These studies show that the dopaminergic regulation of the IL is multifaceted, incorporating a triad of temporally related events and that the IL functions as an integrated multicellular unit that uses both intra- and intercellular mechanisms to coordinate its total production of peptide hormones.

42.4

POMC GENE EXPRESSION IN RAT PITUITARY INTERMEDIATE LOBE: A COMPARISON OF RADIOACTIVE AND NON-RADIOACTIVE *IN SITU* HYBRIDIZATION HISTOCHEMISTRY. B. Pratt, S.J. Morris and B.M. Chronwall, School of Basic Life Sciences, University of Missouri-Kansas City, Kansas City, MO 64108.

Individual melanotropes of the intermediate lobe (IL) differ in their content of biosynthetically active organelles as well as pro-opiomelanocortin (POMC) mRNA. POMC gene expression is mainly regulated by dopaminergic agents; haloperidol increases and bromocriptine decreases POMC mRNA. The focus of this study is to use quantitative *in situ* hybridization histochemistry to further demonstrate melanotrope biosynthetic heterogeneity and to quantitate relative changes in gene expression in the IL after drug treatment. A comparison of radioactive and non-radioactive detection of the hybrids was made to develop a method that detects cellular biosynthetic heterogeneity in a histologically homogenous tissue. The results are as follows. Radioactive detection of hybrids: Highly sensitive, can be used on 15µm frozen and 3µm paraffin sections as well as on floating lobules fixed in 4% paraformaldehyde and 0.1% glutaraldehyde and postfixed in OsO_4, which are then embedded in plastic and semithin sectioned. The tissue preservation in paraffin sections allows assignment of grains to individual cells. In the plastic sections silver grains can be quantitated over cells with retained cytology. Relative quantitation over whole sections after drug treatments is easy to perform using automated counting of silver grains. It is possible but time consuming to count grains over individual cells in paraffin and plastic sections. Refocussing is sometimes necessary to see cell borders. The long exposure time (2 weeks using ^{35}S) is a draw back. Non-radioactive detection of hybrids: Less sensitive, does not readily detect hybrids to mRNA in paraffin embedded tissue. Better cellular resolution than radioactive labeling on 15µm frozen sections. Possible but not easy to quantitate by densitometry; less accurate than grain counting. Results can be obtained in 2-3 days. In summary, radioactive detection gives a more exact quantitation at a higher level of resolution.

42.5

CHARACTERIZATION OF PROLACTIN RECEPTORS IN DOVE BRAIN BY AUTORADIOGRAPHY AND EFFECTS OF PROLACTIN INJECTIONS ON BINDING ACTIVITY. J.D. Buntin and E. Ruzycki*, Dept. of Biol. Sciences, Univ. of Wisconsin, Milwaukee, WI 53201.

Prolactin (PRL) receptors have been detected and characterized in ring dove brain membranes (Gen. Comp. Endocr. 65:243,1987). In addition, in vitro autoradiographic data indicates that ^{125}I-ovine PRL (oPRL) binds specifically to several dove forebrain and midbrain areas, with highest levels in the preoptic region and medial-basal hypothalamus (Br. Res. 487:245,1989). In this study, we examined the affinity, specificity, and in vivo saturability of ^{125}I-oPRL binding in several of these regions using film autoradiography and densitometry on slide-mounted sections (20μm). In specificity tests, 44, 440, or 4400 pM unlabelled oPRL significantly reduced the in vitro specific binding of ^{125}I-oPRL (30pM) in preoptic area (POA) and three hypothalamic regions. In contrast, turkey GH, ovine GH, and ovine LH were ineffective competitors at these concentrations. Binding affinity was similar across the brain regions examined (average K_d = .17nM). Unlabelled oPRL (37nmoles) reduced the uptake of ^{125}I-oPRL (37 pmoles) in POA and tuberal hypothalamus by 30-45% at 2 hours after intravenous injection. Moreover, chronic oPRL treatment (590nmoles/day x 7 days, s.c.) reduced the specific binding of ^{125}I-oPRL to these areas in vitro by over 65%. These results suggest the existence of specific, high affinity PRL receptors in dove brain that are accessible to blood-borne PRL. Supported by MH 41447.

42.6

CO-CULTURE OF ANTERIOR AND POSTERIOR PITUITARY CELLS INCREASES PROLACTIN CELL CONTENT AND RESPONSIVENESS TO TRH. J. Dymshitz* and N. Ben-Jonathan. Dep. Physiology, Indiana Univ. Med. Sch., Indianapolis, IN 46202.

The synthesis and release of PRL are regulated by multiple hypothalamic and posterior pituitary (PP) factors. We have shown that cells of the intermediate lobe produce PRL-releasing factor. The objective of this study was to determine whether co-culturing of anterior pituitary (AP) and PP cells influences the production and release of PRL. Pituitaries from adult male rats were separated into anterior and posterior (intermediate+neural) lobes and the cells were cultured in serum-free medium; AP cells were plated either alone (control) or with PP cells (AP+PP). PRL and GH in cells and media were measured by RIA. After 4 days, the PRL cell content (ng/10,000 cells) in AP+PP was 157±5 vs 80±3 in controls; GH cell content was the same in both groups. The levels of either hormone in media were not affected by PP cells. A similar effect of PP cells on PRL cell content was observed after 8 days. Moreover, the PRL levels in medium from AP+PP were 40% higher than those in controls. Incubation of cells cultured for 4 days with TRH (25 nM for 20 min) increased PRL release from controls and AP+PP by 350% and 700%, respectively. In contrast, dopamine (50 nM) caused a 50-60% inhibition of PRL secretion in either group.

Conclusions: 1) PP cells selectively stimulate the accumulation of PRL by lactotrophs, 2) the rise in intracellular PRL levels reflects, at least in part, an increase in the releasable pool of this hormone. Supported by NIH grants DK 39551 and NS 13234.

42.7

ASSESSMENT OF HYPOTHALAMIC DOPAMINERGIC AND SEROTONERGIC ACTIVITY DURING PREGNANCY-INDUCED PROLACTIN SURGES USING PUSH-PULL PERFUSION. A. M. MISTRY* AND J. L. VOOGT Dept of Physiology, Univ Kansas Med Ctr, Kansas City, KS 66103

Mating results in two daily surges of prolactin (PRL) in the rat. A nocturnal (N) surge occurs between 0200h and 0600h. This study evaluated dopaminergic and serotonergic activity in the hypothalamus during the entire time period of the N surge on day 8 of pregnancy and on day 16, at which time the surge is no longer present. Female Sprague-Dawley rats were implanted with push-pull cannulae so that the cannula tip was in the arcuate-median eminence region. On day 8 or 16 artificial cerebrospinal fluid was perfused from midnight to 0600h using two identically precalibrated pumps. Perfusates were collected over ice every 15 min and injected immediately onto a HPLC column. Blood samples were withdrawn hourly from previously implanted carotid catheters and plasma PRL assayed by RIA. Histological sections determined correct placement of the cannula. Dopamine (DA) and serotonin (5-HT) were detected in a limited number of samples. However, dihydroxyphenylaceticacid (DOPAC) which reflects neuronal dopaminergic activity, and 5-hydroxyindoleaceticacid (5-HIAA) the metabolite of 5-HT were consistently detected. Approximately half the rats failed to show a N PRL surge on day 8. These rats had significantly ($p \leq .001$) lower 5-HIAA levels compared to rats which displayed a surge of PRL. There was no difference in DOPAC levels between these two groups. On day 16 when PRL surges were absent, 5-HIAA levels were significantly ($p \leq .001$) lower than in rats that surged on day 8. Release of DOPAC was also significantly ($p \leq .01$) lower compared to day 8 rats, suggesting that low PRL at this time is not dependent on increased dopamine secretion. In conclusion a greater overall release of 5-HIAA is seen *in vivo* during early pregnancy in freely moving conscious rats which display a surge of PRL. During the second half of pregnancy, when PRL release was low there was a remarkable reduction in 5-HIAA suggesting that a positive serotonergic input is important for generating the PRL surge. This is supported by studies showing that 5-HT antagonists blocked the PRL surge.(Supported by grant HD 24190)

42.8

SPONTANEOUS ELECTRICAL AND SECRETORY ACTIVITY OF RAT LACTOTROPES ARE INDEPENDENT OF SODIUM CURRENTS. K.A. Gregerson, Depts. Pediatrics & Physiology, Univ. MD, Baltimore, MD 21201.

Sodium currents (I_{Na}) play a critical role in the function of nerve and muscle cells, but their importance in secretory mechanisms of endocrine cells has not been determined. We have investigated the contribution of I_{Na} to electrical properties and secretory function of rat lactotropes. Cells were derived from female rats and identified for patch clamp studies using the hemolytic plaque assay. Whole-cell voltage clamp recordings demonstrated no inward current that was sensitive to either TTX (4uM) block or substitution of external Na^+. These prolactin (PRL)-secreting cells can exhibit spontaneous depolarizing action current which may correlate with their secretory activity. Again, TTX had no effect on this spontaneous spiking activity as monitored during whole-cell current clamp. Moreover, PRL release from acutely dissociated or primary cultures of lactotropes was unchanged by TTX as determined by both quantitative plaque assay of individual cells and static release studies of pituitary cell populations. GH_3 cells, clonal cells derived from a rat pituitary tumor which secreted both PRL and growth hormone, did exhibit a large, rapidly-inactivating I_{Na} which could be blocked by TTX. However, TTX did not alter PRL release from these cells either. A TTX-sensitive I_{Na} does not appear to be important for spontaneous PRL release. Further studies are required to determine if I_{Na} plays a role in regulated PRL secretion. Supported by NIH grant DK-40336.

42.9

DIFFERENTIAL PROLACTIN RESPONSE TO DOMPERIDONE DURING THE NOCTURNAL PROLACTIN SURGE IN PREGNANT RATS. J. R. Mathiasen and J. L. Voogt. Department of Physiology, University of Kansas Medical Center, Kansas City, KS 66103.

Pregnant rats exhibit 2 daily surges of prolactin (PRL) during the first half of pregnancy. The nocturnal surge peaks at 0400h and the diurnal at 1800h. PRL is under the inhibitory influence of dopamine (DA). Domperidone (DOM), a DA D2 antagonist causes release of PRL by inhibiting the action of DA at the pituitary. DOM was used to measure the degree of endogenous DA inhibition of pituitary PRL at various times prior to and during the nocturnal PRL surge on Day 8 of pregnancy. A large PRL response to DOM suggests substantial DA interaction at the lactotroph, whereas a smaller PRL response to DOM indicates less DA acting at the lactotroph. Carotid cannulas were placed in pregnant rats on Day 6 and blood samples were taken hourly from 2400h through 0500h on Day 7. On Day 8, DOM (100 ug/kg i.v.) was given at either 2400h, 0200h, 0400h or 1200h, and blood samples taken 0, 5, 15, 30 and 60' after DOM. Whole blood was assayed for PRL by double antibody RIA. PRL levels in response to DOM averaged 630 ng/ml above preinjection levels of 15 ng/ml at 2400h and 1200h, two times when a PRL surge is not normally present. PRL response to DOM given at 0400h was moderate, increasing 442 ng/ml above the preinjection surge level of 146 ng/ml. PRL response to DOM at 0200h, however, was significantly lower, increasing 330 ng/ml above a preinjection level if 52 ng/ml, suggesting that at the beginning of the nocturnal PRL surge there was a transient fall in DA interaction at the pituitary lactotroph. This transient fall in DA could serve to initiate the PRL surge, while PRL-releasing factors may maintain the surge. Supported by NIH grant HD 24190.

42.10

CENTRAL CATECHOLAMINES AND THE PROLACTIN RESPONSE TO NICOTINE. SG Matta and BM Sharp*. Minneapolis Medical Research Fndn. and Depts. of Med., Hennepin County Medical Center and Univ. Minn., Minneapolis, MN, 55404.

The mechanism(s) whereby i.v. nicotine stimulates prolactin (PRL) release has not yet been described. The current studies showed that a fourth ventricular (IV) injection of nicotine (N; 0.125 - 2.5 ug) produced a dose-dependent PRL response [Buffer < N 0.125 = N 0.25 ($p<.05$) < N 0.5 = N 2.5 ($p<.01$)]. Injection of mecamylamine [nicotinic (NAch) antagonist] into IV (20 or 40 ug) or i.v.(0.5 mg/kg b.wt.) prior to nicotine given by the alternate route showed nicotine's effect was due to NAch receptors accessible from the IV and that sites adjacent to IV mediate the PRL response to i.v. nicotine. Since brainstem catecholaminergic neurons project to hypothalamic structures modulating PRL release, the role of these neurons in nicotine-stimulated PRL release was studied. 6-Hydroxydopamine i.c.v. abolished the PRL response to nicotine injected into the IV (1 or 2.5 ug) or i.v.(0.03 or 0.05 mg/kg b.wt.). To determine the role of epinephrine,SKF 64139 or DCMB (synthesis inhibitors) were given prior to nicotine (0.05 mg/kg b.wt); both reduced the PRL response ($p<.01$). These compounds may also act at alpha2 adrenoreceptors; yohimbine (1 ug), injected into the third ventricle prior to nicotine (1 ug) into IV blocked the peak PRL response ($p<0.05$). Further evaluation showed that both alpha1 or ß antagonists were effective ($p<.05$). Thus, i.v. nicotine acts centrally via catecholaminergic neurons to stimulate the release of PRL. (Supported by DA03977).

42.11

CHRONIC, BUT NOT ACUTE, EXPOSURE TO PHENCYCLIDINE FACILITATES THE 5-HYDROXYTRYPTOPHAN (5HTP)-INDUCED RELEASE OF PROLACTIN James F. Hyde, Dept. of Anatomy and Neurobiology, Univ. Kentucky, Coll. of Med., Lexington, KY 40536.

Phencyclidine (PCP) is known to interact with dopaminergic and serotonergic neurons which inhibit and stimulate prolactin secretion, respectively. Acute exposure to PCP inhibits prolactin release, presumably by increasing hypothalamic dopamine release. The affects of PCP on hypothalamic serotonin neurons are not clear. To determine if PCP alters the serotonergic control of prolactin release, female Sprague-Dawley rats (n=5-7/group) were injected with PCP (10 mg/kg, ip) or vehicle (saline) for 14 days. Jugular cannulae were implanted one day prior to experiments. Serial blood samples were collected before and 10, 20, 30, 60 and 90 min after 5HTP (20 mg/kg, iv) injection. In control rats, plasma prolactin levels peaked (199.5 ± 29.2 ng/ml) 20 min after 5HTP injection and returned to basal levels (12.9 ± 3.4 ng/ml) by 90 min. Chronic PCP treatment did not alter basal prolactin levels (13.9 ± 3.3 ng/ml), however, 5HTP increased prolactin levels to (682.3 ± 170.9 ng/ml) at 20 min ($p<0.05$). Prolactin levels remained elevated at 90 min (44.0 ± 19.2 ng/ml). A single injection of PCP, 90 min prior to 5HTP administration, did not alter the profile of prolactin release. Although basal prolactin levels (5.9 + 1.6 ng/ml) were lowered by acute exposure to PCP, peak prolactin values (224.0 ± 23.7 ng/ml) were similar to vehicle controls. These data indicate that chronic, but not acute, exposure to PCP alters the function of the hypothalamic serotonergic system involved in the regulation of prolactin secretion. (Supported by NIH RR-05374 and UKMC Research Funds from the University of Kentucky.)

42.12

MECHANISM FOR DECREASED RESPONSIVENESS TO ß-ENDORPHIN DURING THE NOCTURNAL PROLACTIN SURGE IN PREGNANT RATS. C.A. Sagrillo and J.L. Voogt, Dept. of Physiology, University of Kansas Medical Center, Kansas City, KS 66103.

Exogenous opioids stimulate prolactin (PRL) release in the pregnant rat. We reported (Endocrine Society Meeting, 1990) that Day 8 pregnant rats showed dramatic PRL responses to Beta-endorphin (ß-end) when given at midnight (presurge) or 1200h (intersurge), but greatly attenuated responses at 0200h (early surge) and 0400h (peak surge). Animals treated at 0600h (postsurge) showed recovered responsiveness to ß-end. The following experiments were designed to determine what accounts for these significantly lower PRL increases to ß-end during the surge. To determine what dose to use, Day 8 pregnant rats received 2.5, 10, 25 or 100 ng/µl/min ß-end at 1200h, a nonsurge time, for 15 minutes via a right lateral ventricular brain cannula. The dose used in subsequent experiments was chosen to ensure maximal opioid receptor stimulation. Day 8 pregnant rats received ventricular infusions of 100ng/µl/min ß-end at 1000h and then again at 1200h. All animals showed PRL increases greater than 1100ng/ml at 1000h, and those that responded again at 1200h (4 of 7) had 50% lower PRL levels. The other 3 rats were unresponsive. In another experiment, ß-end was infused at midnight and the animals were monitored for a subsequent endogenous nocturnal PRL surge. All animals showed a rapid increase in PRL after ß-end, followed by a decrease to near pretreatment levels. Subsequently, 5 of 8 rats did not have a nocturnal PRL surge. This suggests that the initial ß-end may down regulate its receptor or deplete significant amounts of pituitary PRL. To determine whether pituitary PRL reserves may be a factor for the low response to ß-end during the surge, hemipituitary content was measured and found to be significantly lower ($p<0.01$) at 0200h compared to 1200h, whereas serum PRL was 456ng/ml (0200h) and 26ng/ml (1200h). Based on these results, lower pituitary PRL reserves during the nocturnal surge may partially account for the attenuated PRL response to ß-end. Continuous PRL secretion throughout surge periods may lower releasable PRL which is then restored during postsurge periods for subsequent surges. Supported by HD24190.

42.13

REGULATION OF PROLACTIN SECRETION IN THE DIABETIC MALE RAT. S.G. Kienast, R.N. Hails and R.W. Steger, Dept of Physiology, Southern Illinois University School of Medicine, Carbondale, IL 62901.

We have previously demonstrated that diabetes in the male rat is associated with abnormalities in gonadotropin secretion secondary to changes in hypothalamic neurotransmitter metabolism (*Endocrinology* 124:1737, 1989). In the present study we looked at the effect of streptozotocin-induced (STZ) diabetes on prolactin (Prl) secretion. Adult male rats made diabetic with STZ(50mg/kg) showed markedly reduced plasma levels of Prl within 1 wk of treatment and levels remained low for at least 2 months. Insulin replacement restored normal Prl levels in diabetic rats. Pituitary Prl content, in vitro Prl secretion and the in vitro response to the Prl inhibitory effects of 10^{-8} M DA did not differ between diabetic and control animals. The diabetes-related reduction of in vivo Prl secretion did not appear to be due to changes in tuberoinfundibular dopaminergic activity since DA turnover (as estimated by measuring the rate of DA depletion after tyrosine hydroxylase inhibition) in the median eminence did not differ between control and STZ-treated rats. The ability of exogenous Prl (1 ug ovinePrl/day for two days)to increase DA synthesis (as estimated by measuring DOPA accumulation after decarboxylase inhibition) in the median eminence was similar in control and STZ-treated rats. However, STZ-treatment was shown to reverse hyperprolactinemia resulting from the grafting of donor pituitaries under the kidney of male rats while insulin replacement restored the ability of ectopic pituitary grafts to secrete Prl. In conclusion, diabetes related changes in Prl secretion are not due to changes in DA metabolism or pituitary DA response but may be due to the direct effects of hypoinsulinemia on pituitary Prl secretion. (Supported by a grant from the Juvenile Diabetes Foundation International.

42.14

ANGIOTENSIN II (AII) AND PROLACTIN (PRL) SECRETION IN THE MALE RAT. M.K.Steele and L.S.Myers, Dept. of Physiology, University of California, San Francisco, CA 94143 and Dept. of Psychology, CSU, Stanislaus, Turlock, CA 95380

We have previously shown that intracerebroventricular (ICV) injections of AII lower plasma PRL levels in female rats; however endogenous brain AII actively inhibits PRL only in the presence of ovarian steroids (Myers and Steele, J. Neuroendo, 1:299, 1989). The present studies investigated the role of the brain renin-angiotensin system in the regulation of PRL secretion in the intact male rat. Third ventricle cannulae were implanted at least one week prior to and jugular cannulae were inserted 48 hours before use in experiments. Blood samples were taken from conscious rats prior to and following administration of test substances. In the first series of experiments, we determined the sensitivity of the PRL response to ICV-AII. In the second series, we investigated the role of endogenous AII by ICV infusion of saralasin, an AII receptor blocker, (15 ug/25 ul/hr) or ICV injection of enalaprilat, a converting enzyme inhibitor, (100 ug/2 ul). PRL levels were significantly suppressed, in a dose-related manner, by AII (500 to 10 ng). A dose of 1 ng did not affect PRL, compared to values from vehicle-injected animals. Neither saralasin nor enalaprilat administration resulted in any significant changes in PRL levels. These results suggest that, while exogenous AII can suppress basal PRL levels and this response is quite sensitive, endogenous AII in the brain does not play a role in the tonic regulation of basal PRL levels in the intact male rat. (Supported by USPHS Grants HD18020, HL29714 and DK07265)

42.15

ANALYSIS OF PULSATILE TSH SECRETION IN THE RAT. T.O. Bruhn, M.B. McFarlane* and I.M.D. Jackson. Division of Endocrinology, Rhode Island Hospital, Brown University, Providence, RI 02903.

To determine the pattern of TSH secretion, adult male rats were fitted with indwelling venous and arterial catheters exteriorized through a spring tether and connected to a swivel. Blood was constantly withdrawn under low stress conditions with a roller pump which also served to reinfuse blood for maintainance of volume and hematocrit. Blood samples were fractionated at 2.5 min intervals. TSH data was subjected to "Pulsar" analysis.

TSH secretion was pulsatile in both eu- and hypothyroid rats. Mean TSH plasma concentrations and peak amplitude were 18.2 and 25.8 fold elevated in hypothyroid rats in comparison to euthyroid controls. Pulse frequency and peak lengths were similar in both groups (1-1.5 pulses/h and 10-12 min). Passive immunization with TRH antiserum caused a significant 55% decrease of TSH levels and the disapperance of TSH pulses in hypothyroid rats. We conclude that 1. TSH secretion is pulsatile in the rat, 2. TRH appears to control both basal TSH secretion and TSH pulsatility and 3. hypothyroidism upregulates both basal and peak TSH levels without influencing the pattern of TSH secretion.

42.16

THYROIDECTOMY INDUCES FOS-LIKE IMMUNOREACTIVITY IN THE PARVOCELLULAR PARAVENTRICULAR HYPOTHALAMIC NUCLEUS OF THE RAT. N. Koibuchi[1], R.B. Gibbs[1], M. Suzuki[2], and D.W. Pfaff[1]. [1]Lab. of Neurobiology and Behavior, Rockefeller University, N.Y., N.Y. 10021, [2]Dept. of Physiology, Inst. of Endocrinology, Gunma University, Maebashi 371, Japan.

The expression of fos-like immunoreactivity (IR) was examined in the adult rat brain as a function of thyroidectomy (TX). Thirty-two male, Sprague Dawley rats (250-300g) were used. All animals were handled for at least 1 week prior to surgery. Of the 32 animals, 20 had their thyroid gland surgically removed while remaining animals received sham surgery. One (n=10), 3 (n=10), and 6 (n=12) days after surgery, rats were sacrificed by transcardiac perfusion with 4% paraformaldehyde in 50 mM PBS. Blood was collected and plasma levels of T_4, T_3, and TSH were measured by radioimmunoassay. Brains were removed and 30 µm frozen sections were cut and processed for immunocytochemistry using a rat monoclonal antibody (IgG) which binds to immunoprecipitated fos protein (Microbiological Associates, Inc., Bethesda, Md.). Numbers of fos-like IR cells in the magnocellular and parvocellular regions of the paraventricular nucleus of the hypothalamus (mPVN & pPVN), the anterior hypothalamus, the lateral hypothalamus, and the pyriform cortex were subsequently counted and compared.

Within 6 days after TX, a specific increase (212%) in the number of fos-like IR cells in the pPVN was observed. In contrast, no specific change in the number of fos-like IR cells was observed, independent of environmental variables, within any of the other brain areas examined 1-6 days after TX. The time course for the effect of TX in the pPVN correlated with changes in plasma levels of TSH ($r=0.749$, $p<0.001$). Since levels of TSH are, in part, a reflection of TRH release, we are currently examining whether TX induces fos-like IR specifically within TRH-containing neurons located in the pPVN.

42.17

EFFECT OF INDUCED HYPOTHYROIDISM ON ADULT RAT BRAIN MEMBRANE FLUIDITY. LIPID CONTENT AND COMPOSITION. Maria Teresa Tacconi, Giovanni Cizza°, Giovanni Fumagalli*, Pierluigi Sarzi Sartori* and Mario Salmona*. Istituto di Ricerche Farmacologiche "Mario Negri", Milano, Italy.

This study was designed to establish whether fluidity and lipid composition of brain membranes were affected by hypothyroidism, in the hope of clarifying the mechanisms through which hypothyroidism might influence nerve cell functions. Rats were made hypothyroid by injections of PTU (dissolved in 0.005 M NaOH, 50 mg/kg, ip, daily for 28 days). Membrane fluidity, chol and PL content, and PL and their fatty acid composition were measured in plasma, erythrocyte plasma membranes, liver microsomes and brain subcellular fractions. P_2 pellets from brains of hypothyroid rats were less fluid than those of euthyroid ones; subcellular fractionation showed that mitochondrial membranes were responsible for the rigidity observed. Similar changes were found in erythrocyte "ghosts". The reduced fluidity seemed to be related more to alterations in the ratios between PC, Sph and PE, than to those of chol/PL, protein/PL or FA unsaturation index. The well known alteration in hypothyroidism-induced desaturase activity, which in peripheral tissue leads to a reduction of 20:4n-6 and to an increase in its precursors (18:2n-6 and 20:3n-6)is barely detectable in brain membranes. Only in PC of synaptosomes and myelin did slight changes in percentages of the n-6 family fatty acids result in a significant alteration of 20:4n-6/20:3n-6 ratios.

PAIN MODULATION: ANATOMY AND PHYSIOLOGY I

43.1

RAPHE-SPINAL AND SUBCOERULEO-SPINAL MODULATION OF TEMPERATURE SIGNAL TRANSMISSION IN RATS. H. Sato* and H. Nishino. Dept. of Physiology, School of Medicine, Nagoya City University, Mizuho-cho, Mizuho-ku, Nagoya 467, Japan.

To verify the possibility that the nucleus raphe magnus (NRM) and subcoeruleus (SC) modulate the spinal transmission of temperature signals from the periphery, extracellular recordings of dorsal horn neuron activity responsive to unnoxious thermal stimulation (10-40°C) of the scrotal skin were made in urethane-anesthetized Wistar rats, and effect of electrical stimulation in the NRM and SC on these activities was investigated. NRM stimulation suppressed excitatory responses of the neurons induced by skin warming (92%) whereas facilitated those of the neurons induced by skin cooling (57%). SC stimulation suppressed excitatory responses of both types of neuron (62%, 80%). Data suggest that the spinal transmission of temperature signals contributing to thermoregulation is descendingly controlled by both the raphe-spinal and subcoeruleo-spinal pathways in such the manner that warm signals are inhibited by both pathways whereas cold signals are facilitated by the former and inhibited by the latter.

43.2

PROJECTIONS FROM THE VENTROMEDIAL MEDULLA TO PONTINE CATECHOLAMINE NUCLEI. F.M.Clark and H.K. Proudfit, Dept of Pharmacol., Univ. of Il at Chicago, Chicago Il. 60680.

Stimulation of neurons in the ventromedial medulla (VMM) produces antinociception which is partially mediated by bulbospinal noradrenergic (NE) neurons. Since no NE neurons are located in the VMM, it is likely that neurons located in the VMM have axonal connections with the spinally-projecting NE neurons located in the A5, A6 (locus coeruleus), or A7 NE nuclei. To provide evidence for such connections, the anterograde tracer, phaseolus vulgaris-leucoagglutinin (PHA-L), was injected into the VMM and labeled axons were identified near NE neurons labeled with dopamine-ß-hydroxylase-immunoreactivity (DßH-ir). A dense field of PHA-L-positive terminals was seen within the A7 nucleus. Many PHA-L-positive terminals were closely apposed to DßH-ir A7 perikarya or proximal dendrites. A modest number of terminals was seen within the A5 and LC nuclei. In the second experiment, a unilateral injection of the retrograde tracer, Fluoro-Gold (FG), was made into the A7 nucleus and brainstem sections were processed for serotonin (5HT) immunocytochemistry. Many neurons retrogradely labeled with FG were seen in the VMM but less than 5% of these FG-labeled cells contained 5HT-immunoreactivity. The results of these experiments indicate that the VMM has a substantial population of non-5HT neurons which project to the A7 NE nucleus. (This was supported by USPHS Grant DA 03980.)

43.3

DIFFERENCES IN COERULEOSPINAL PROJECTIONS IN RAT SUBSTRAINS. H.K. Proudfit, D.C. Yeomans and F.M. Clark, University of Illinois at Chicago, Chicago, IL 60680.

Noradrenergic neurons in the locus coeruleus (LC) are known to have prominent projections to the spinal cord. However, reports that describe the location of terminal fields arising from these neurons in the rat are conflicting. For example, some studies indicate that coeruleospinal neurons project primarily to the dorsal horn while others have demonstrated major projections to the ventral horn. Since some of these conflicting results were obtained using nearly identical anatomical methods, it is difficult to reconcile these divergent results on the basis of methodological differences. However, these conflicting results may be due to genetic differences in rats obtained from different vendors. To test this hypothesis unilateral injections of the retrograde tracer Fluoro-Gold (FG) were made into the ventral horn of rats obtained from Harlan or Sasco. Many retrogradely-labeled neurons were seen in the ipsilateral LC in Sasco rats, but very few LC neurons were labeled in Harlan animals. These results are consistent with previous observations using anterograde tracers which indicate that LC neurons in Sasco rats project primarily to the ventral horn. These results are also consistent with similar experiments done using Harlan rats which demonstrated that LC neurons have sparse projections to the ventral horn. These observations indicate that different substrains of rats exhibit fundamental differences in the projections of coeruleospinal neurons. (This work was supported by USPHS Grant DA 03980.)

43.4

DESCENDING FACILITATION AND INHIBITION OF SPINAL NOCICEPTIVE TRANSMISSION FROM THE NUCLEI RETICULARIS GIGANTOCELLULARIS (NGC) AND GIGANTOCELLULARIS PARS ALPHA (NGCα) IN THE RAT. M.Zhuo and G.F.Gebhart. Dept. Pharmacology, Univ. of Iowa, Iowa City, Iowa 52242.

The effect of activation of the NGC/NGCα on responses of spinal dorsal horn neurons to noxious heating of the skin was studied. At 30 of 51 sites in the NGC/NGCα, electrical stimulation facilitated responses of dorsal horn neurons to noxious heating at low intensities (12.7 ± 1.3 µA) and inhibited the responses of the same neurons at greater intensities (46.2 ± 3.0 µA) of stimulation. Stimulation produced only inhibition at the other 21 sites of stimulation in the NGC/NGCα. Stimulation in the NGC/NGCα decreased the slope of the stimulus response function (SRF) at intensities inhibiting unit responses to noxious heating; stimulation at facilitation-producing intensities produced a leftward parallel shift of the SRF. Glutamate microinjection into the NGC/NGCα also facilitated responses of dorsal horn neurons to noxious heating at a low concentration (1.7 nM) and inhibited responses at a greater concentration (50 nM). Inhibition produced by electrical stimulation was attenuated by transection of the dorsolateral funiculi (DLF), but facilitation was unaffected or enhanced. The results suggest that (1) activation of the NGC/NGCα can facilitate and inhibit spinal nociceptive transmission and (2) different descending pathways are involved in mediating descending facilitation and inhibition.

43.5

RVM STIMULATION REDUCES NOXIOUS STIMULUS-EVOKED FOS EXPRESSION AND IS ANTAGONIZED BY THE ALPHA$_1$ ANTAGONIST PRAZOSIN. A.Z. Murphy, F.P. Zemlan, R.M. Murphy and M.M. Behbehani. Depts. of Psychiatry and Physiology, Univ. of Cincinnati College of Medicine, Cinti., OH 45267-0559.

Microinjection of glutamate into the rostral ventral medulla (RVM) inhibits nociceptive dorsal horn neurons. The present study determined whether the selective alpha$_1$ antagonist prazosin could attenuate the antinociceptive actions produced by microinjection of glutamate into the RVM using immunocytochemistry to examine the pattern of noxious-stimulus evoked expression of the proto-oncogene c-fos in the spinal cord of the rat. Prior to microinjection of glutamate into the RVM, animals received bilateral injection of 4% formalin into the hind paws; animals were then post-treated i.p. with either prazosin (5 mg/kg) or its vehicle. Two and one half hours after formalin injection, animals were perfused intracardially with 4% paraformaldehyde and 60 micron sections of the lumbar spinal cord were immunostained for Fos protein. Quantitative analysis revealed that formalin injection alone produced intense bilateral Fos-like immunoreactivity (FLI) in laminae I, II, V and VI. Less intense labelling was noted in lamina VII, VIII, and X. RVM stimulation produced a unilateral suppression of FLI in all laminae, most notably in the deeper laminae, V, VII, VIII, and X, and a substantial reduction in FLI occurred in lamina I and II. This inhibition of FLI was partially reversed by administration of prazosin. It is concluded that RVM induced analgesia involves activation of alpha$_1$ adrenergic receptors.

43.6

THE ONTOGENY OF ANALGESIA INDUCED BY INTRASPINAL INJECTION OF SEROTONERGIC DRUGS. M. N. Schwarz and G.A. Barr. Biopsychology Doctoral Program, Dept. of Psychology, Hunter College, CUNY, New York, N.Y. 10021

Recent evidence has shown that descending bulbospinal monoamine pathways are involved in pain modulation. Intrathecal application of noradrenergic compounds in infant rats attenuates the withdrawal response to a painful mechanical stimulus to a greater extent than to a noxious thermal stimulus. Therefore, norepinephrine receptors in the spinal cord are functional in the modulation of specific types of pain in neonatal rat pups. The present study examines the ability of serotonergic drugs to produce analgesia in young infant rat pups, with attention paid to stimulus modality and to somatotopic localization of the analgesia. Intraspinal injection of serotonin in 4 and 10 day old rat pups was tested using a thermal or a mechanical stimulus applied to the forepaws, hindpaws or tail. Serotonin produced analgesia at both ages that was more pronounced in the hindpaws and tail than the forepaws and against the thermal stimulus more than the mechanical stimulus. These results demonstrate the presence of functional spinal cord 5-HT receptors in infant rats and provide evidence for the differential modulation of thermal and mechanical pain in the developing animal.

43.7

AUTORADIOGRAPHIC MAPPING OF SEROTONIN$_1$ RECEPTORS IN HUMAN SPINAL CORD. R.M. Murphy and F.P. Zemlan. Dept. of Psychiatry, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0559.

In vitro autoradiography was used to study the distribution and types of serotonin$_1$ (5-HT$_1$) receptors in human spinal cord. Tissue sections (10 uM) from cervical and lumbar spinal cord were cut at -15^{o}C with a cryostat and thaw-mounted onto gelatin-coated slides. Following preincubation, sections were incubated for one hour with 2 nM ^{3}H-5-HT or ^{3}H-8-OH-DPAT, and for 2 hours with 5 nM ^{3}H-mesulergine in a 0.17M Tris-HCl buffer (pH 7.6), in the presence and absence of 10 uM cold 5-HT, washed twice with ice-cold (4^{o}C) buffer, and dried with cool air. Slides were opposed against ^{3}H-Ultrofilm, exposed for 1-2 months, and analyzed by micro-densitometry.

Across both spinal cord levels, the total number of 5-HT$_1$ receptors was 2-3 times higher in the dorsal horn than in the ventral horn, with the highest densities in the substantia gelatinosa and dorsal funicular gray. The highest densities of 5-HT$_1$ receptors in the ventral horn were found within the motor nuclei. Approximately 30-40% of the 5-HT$_1$ receptors in human spinal cord were labelled by ^{3}H-8-OH-DPAT (5-HT$_{1A}$ subtype) and 4-15% were labelled by ^{3}H-mesulergine (5-HT$_{1C}$ subtype). Presently, experiments are being conducted to determine if the 35-65% 5-HT$_1$ receptors remaining in the cord are similar to the 5-HT$_{1S}$ receptor subtype recently identified in rat spinal cord.

43.8

SELECTIVE 5HT$_3$ AGONIST INHIBITS EXCITATORY AMINO ACID (EAA)-EVOKED EXCITATION IN RAT SPINAL PROJECTION NEURONS. Sizheng Lei and George Wilcox. Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455, U.S.A.

The function of serotonin in mammalian spinal cord has been controversial. At least part of the effect mediated by spinal 5HT receptors is believed to be involved in spinal antinociception, possibly through only certain subtypes of 5-HT receptors including the recently identified 5-HT$_3$ receptor. The action of the selective 5TH$_3$ agonist, 2-methyl serotonin (2-me-5-HT), on EAA-induced excitation of lumbar dorsal horn projection neurons in adult rats was examined.

Extracellular recordings were conducted *in situ* in the lumbar spinal cord of urethane-anesthetized male rats. Tungsten microelectrodes (1MΩ) glued to seven barreled borosilicate micropipettes were used to record from single spinal neurons and to apply various drugs iontophoretically. All neurons tested were dorsal horn projection neurons and the cutaneous receptive field for each neuron was characterized by mechanical stimuli. NMDA was used as a selective NMDA receptor agonist and α-amino-3-hydroxy-5-methyl-4-methyl-4-isoxasole-proprionic acid HBr (AMPA) was used as an AMPA receptor agonist. One or both of these selective excitatory amino acid agonists excited the cells studied here when applied iontophoretically.

Iontophoretic application of the 5-HT$_3$ agonist produced profound current-related inhibition of the neuronal excitation produced by NMDA in all twenty spinal projection neurons tested. In addition, NMDA-induced excitation was also blocked by the selective GABA$_A$ receptor agonist, musicmol. Both the 5-HT$_3$ antagonist, zacopride, and the GABA$_A$ antagonist, bicuculline, reversed the inhibition of 2-me-5-HT in a dose–dependent manner. A similar but less consistent inhibitory effect was seen when AMPA was used as the excitant. In conclusion, this study has demonstrated that spinal 5-HT$_3$ receptors produce inhibition of nociceptive neurotransmission in spinal cord dorsal horn, perhaps by causing release of the inhibitory neurotransmitter, GABA. (Supported by USPHS grants DA-01933 and DA-04274).

43.9

NUCLEUS RAPHE MAGNUS (NRM) STIMULATION INHIBITS NOCICEPTIVE DORSAL HORN CELL ACTIVITY AND INCREASES DORSAL HORN AMINO ACIDS LEVELS WITHOUT DETECTABLE CHANGES IN SEROTONIN (5HT) LEVELS. **L.S. Sorkin, D.J. McAdoo and W.D. Willis. Univ. of TX Medical Branch, MBI, Galveston, Texas 77550.**

We collected excitatory (EAA) and inhibitory amino acids and 5HT from the dorsal horn during NRM stimulation, while recording from nearby cells. The NRM was stimulated at several intensities to determine the relationship between NRM-induced inhibition and neurotransmitter release. Dialysis tubes were positioned in the dorsal horn of anesthetized (α-chloralose) adult cats and perfused (5μl/min) with artificial CSF; continuous samples were collected in 15 min aliquots. Microelectrodes placed rostral to the tube recorded activity from cells with nociceptive input. Stimulating electrodes were placed in the NRM. Chemical analysis was done with HPLC; amino acids with fluorescence detection, 5HT with electrochemical detection. Detection limits were approximately 10^{-8} M for 5HT and 10^{-7} M for the amino acids. NRM stimulation at 1-4 times the intensity necessary to inhibit 50% of peripherally evoked activity increased levels of aspartate, glutamate, glycine, serine and/or GABA. 5HT increases were observed only with higher stimulus intensities. These results suggest that there is an EAA mediated raphe-spinal pathway that inhibits nociceptive activity via an inhibitory interneuron. **Supported by grants: NS 11255 and the John Sealy Foundation.**

43.10

"5HT-ONLY" CELLS DO NOT CONSTITUTE A SPECIFICALLY ANTINOCICEPTIVE CATEGORY OF SEROTONERGIC NEURON. W. Wu and M. Wessendorf. Dept. Cell Biology and Neuroanatomy, Univ. Minnesota, Minneapolis, MN 55455

Although fibers containing serotonin (5HT) are common in the superficial dorsal horn, few of these also stain for substance P (SP) or thyrotropin-releasing hormone (TRH). This suggests that 5HT cells containing neither SP nor TRH ("5HT-only cells") might constitute a specifically antinociceptive subpopulation of 5HT cells. The present study examined whether coexistence of SP or TRH was less common among the 5HT fibers apposing a nociceptive group of cells (spinothalamic tract neurons: STT cells) than among a non-nociceptive group of cells (post-synaptic dorsal column cells: PSDC cells).

Injections of Fluoro-Gold were made into either the thalamus or the dorsal column nuclei of rats. After perfusion, tissue was immunofluorescently stained either for 5HT and SP, or for 5HT and pre-pro-TRH 160-169 (ppT: a marker peptide for TRH). Sections were examined for immunoreactive fibers apposing retrogradely labeled cell profiles. A minimum of 50 cell profiles were examined in each case.

Among 5HT-stained fibers apposing PSDC cell profiles, only about 1% were also stained for SP and none was stained for ppT. Among 5HT-stained fibers apposing STT cell profiles, only 1% were also stained for SP but 15% were stained for ppT. This difference was statistically significant ($p<.005$; chi-square test).

The apposition of numerous "5HT-only" fibers to PSDC neurons suggests that "5HT-only" neurons are not specifically antinociceptive. In addition, the apposition of "5HT+ppT" fibers to STT neurons suggests some "5HT+ppT" cells might have antinociceptive actions.

The assistance of Stephen Schnell in these studies is gratefully acknowledged. These studies were supported by DA 05446 to M.W.

43.11

RE-EXAMINATION OF THE SEROTONERGIC RAPHESPINAL PROJECTION IN THE RAT: A RETROGRADE IMMUNOHISTOCHEMICAL STUDY. S.L. Jones and A.R. Light. Dept. of Pharmacology, Univ. of Oklahoma, Oklahoma City, OK 73190 and Dept. of Physiology, Univ. of North Carolina, Chapel Hill, NC 27599.

Until recently, the NRM has been considered to be composed primarily of serotonin-containing neurons. However, it is now recognized that the NRM is a heterogeneous population of cells. The objective of this study was to re-examine the serotonergic raphespinal projection to the lumbar spinal cord. The retrograde tracer apo-horseradish peroxidase-wheatgerm agglutinin conjugated to colloidal gold (apo-HRP-WGA*gold) was used in combination with serotonin immunohistochemistry. Visualization of the apo-HRP-WGA*gold and serotonin immunoreactivity was achieved using silver precipitation and avidin biotin techniques, respectively. Following microinjections (8-10µl) of apo-HRP-WGA*gold into the lumbar spinal cord of adult, male Sprague Dawley rats, large numbers of retrogradely labeled neurons were identified in the pons and medulla. To date (n=4), regional distributions of retrogradely labeled neurons also demonstrating serotonin-like immunoreactivity have been noted throughout the rostral-caudal and medial-lateral extent of the NRM. Few serotonergic raphespinal neurons have been identified in the rostral NRM; in the caudal NRM, double-labeled neurons extend into the lateral aspects of the NRM. Supported by DA05341 and NS16433.

43.12

INCREASED RESPONSIVENESS OF DORSAL HORN NEURONS DURING LIDOCAINE BLOCKADE OF THE DORSOLATERAL FUNICULUS. N.A. Bernau, D.A. Simone, J.A. Atkinson*, and L.M. Pubols. R.S. Dow Neurological Sciences Institute, Good Samaritan Hospital & Medical Center, Portland, OR 97209.

The responses of cat lumbar dorsal horn neurons to mechanical stimulation of skin and electrical stimulation of the tibial nerve were examined before and after lidocaine injection into the ipsilateral T^{12} dorsolateral funiculus (DLF). Cells that responded maximally to innocuous mechanical stimuli (LT) were unaffected by lidocaine blockade. However, cells that were activated by noxious, but not by innocuous, mechanical stimuli (HT), and those that responded in a graded fashion to both types of stimuli (MR) showed enhancement of responding to stimulation of tibial nerve A-delta and C fibers during lidocaine blockade of the DLF. A majority of these cells also showed increased sensitivity to natural stimuli during blockade, and 2 of 4 HT studied acquired responsiveness to innocuous stimuli. The white matter region common to all lidocaine injections causing a C-fiber response greater than twice the pre-injection level was located ventromedially in the DLF, adjacent to the grey matter. A lesion of this region also caused a large increase in C-fiber responding.

These experiments have confirmed the existence of a powerful, tonic, descending inhibitory projection in the ipsilateral DLF, which acts selectively on cells with nociceptive inputs. (Support: NIH NS19523)

43.13

AN ANALYSIS OF A REAL ANTINOCICEPTIVE NEURONAL SYSTEM IN THE ROSTRAL VENTROMEDIAL MEDULLA OF ANAESTETIZED RATS. S. McGaraughty* and S. Reinis. Department of Psychology, University of Waterloo, Waterloo, ON., Canada.

The functional interactions of cells in the rostral ventromedial medulla (RVM) were investigated using a computer-assisted method developed in our laboratory. This technique allows the simultaneous recording of activity of small groups of neurons with the calculation of their interactions. The effects of noxious heat applied to the end of a rat's tail on the activity of individual neurons and groups of neurons were analyzed with rats under Ketamine-Rompun anesthesia. Auto-, mass- and cross-correlograms were calculated, and showed existence of three types of systems which were active both in control and stimulated condition. All systems initially responded to a common input. Then, in some cellular systems, there was an abundance of short interspike intervals, less than 10 ms long. The second type of systems inhibited themselves, after the initial input, for about 30 - 40 ms, and then resumed firing, while yet others showed neither an inhibition nor preference for any duration of interspike intervals. We assume that of the three classes of RVM cells, it is the inhibited type that is involved in the control of analgesia; the higher is the number of inhibited cell systems in response to pain the more analgesia is produced.

43.14

MORPHINE APPLIED IONTOPHORETICALLY DEPRESSES ACTIVITY OF ON-CELLS IN ROSTRAL VENTROMEDIAL MEDULLA OF LIGHTLY-ANESTHETIZED RATS. M.M. Heinricher, M.M. Morgan and H.L. Fields. Depts. of Neurology and Physiology, Univ. Calif., San Francisco, CA 94143

The rostral ventromedial medulla (RVM) is involved in opioid-sensitive mechanisms that modulate nociceptive transmission. Two classes of putative nociceptive modulatory neurons have been identified in the RVM, and morphine, when given in doses sufficient to block the tail-flick response (TF), invariably has the same effect on all neurons of a given class: **off-cells** (which are likely to exert a net suppressive effect on nociceptive transmission) show an increase in spontaneous activity and an elimination of the TF-related pause, whereas the activity of **on-cells** (which appear to have a facilitating effect on nociception) is suppressed. These effects are seen whether morphine is given systemically or by microinjection into the periaqueductal gray. The present experiments use single unit recording and iontophoretic techniques to determine the **direct** effects of opiates on activity of **on-** and **off-cells** in RVM.

Rats are maintained in a lightly-anesthetized state by a continuous infusion of methohexital. **On-cell** firing can be depressed by iontophoretic application of morphine: spontaneous, TF-related, and glutamate-evoked activity are substantially reduced. These effects can be reversed by naloxone (given systemically or by iontophoresis). In contrast, we have not to date observed an effect of iontophoretically-applied morphine on the firing of **off-cells**.

These findings indicate that morphine can have a **direct** action upon the activity of **on-cells**, and underscore the importance of the **on-cell** as a site of access for opiate action in the RVM.

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43.15

LACK OF INHIBITION OF A NOCICEPTIVE REFLEX BY HETERO-SEGMENTAL NOXIOUS INPUT: BEHAVIOR CORRELATES WITH ACTIVITY IN RVM NEURONS. M.M. Morgan, M.M. Heinricher and H.L. Fields. Depts. of Neurology and Physiology, Univ. of California, San Francisco, CA 94143.

Two neuron populations in the rostral ventromedial medulla (RVM) show abrupt changes in activity immediately preceding nocifensive reflexes. These changes consist of a burst (**on-cell**) or a pause (**off-cell**), and appear to play an important role in modulation of nociception. Since sustained noxious tail heat has been shown to inhibit nociception, the effect of such a stimulus on RVM neurons was assessed in lightly-anesthetized rats.

Male Sprague-Dawley rats were anesthetized with pentobarbital for surgical preparation and then maintained on a continuous infusion of methohexital for extracellular unit recording in RVM. The latency to elicit a paw-withdrawal reflex to noxious heat was determined before, during, and after submersion of the distal half of the tail in 40 or 50 °C water for 5 min. Such stimulation produced a prolonged **on-cell** burst and **off-cell** pause. The occasional change in cell activity during stimulation appeared to constitute part of the cycling typical of these neurons (such changes were more common with 40 °C water). At no time was the paw-withdrawal reflex inhibited by tail heating.

These findings demonstrate that RVM neurons respond in a consistent manner to **sustained** as well as brief noxious stimuli. In addition, the pattern of cell activity is consistent with the observed preservation of the paw-withdrawal in the presence of a continuing heterosegmental noxious stimulus.

Supported by PHS grants DA01949, NS07265, and a Pain Research Grant from the Bristol-Myers Squibb Company.

43.16

DISTRIBUTION OF GABA-IMMUNOREACTIVE SYNAPSES ONTO NOCICEPTION-MODULATING NEURONS IN THE ROSTRAL VENTROMEDIAL MEDULLA. K. Skinner, P. Mason, A.I. Basbaum and H.L. Fields. Depts. of Neurology, Anatomy and Physiology, UCSF (94143) and the Institute on Aging, Mount Zion Hospital, San Francisco, CA. (94115).

The rostral ventromedial medulla (**RVM**) makes an important contribution to opioid antinociception. One class of RVM cells, the off-cells, is inhibited by noxious stimulation and excited by opiate administration. Off-cells are hypothesized to inhibit nociceptive transmission in the dorsal horn. To examine the anatomical basis for the hypothesis that opioid excitation of off-cells results from removal of a GABA-mediated inhibition, off-cells in the nucleus raphe magnus were intracellularly characterized and filled with horseradish peroxidase in the lightly anesthetized cat. The labeled cells were reconstructed from vibratome sections and prepared for electron microscopy. The soma and fourteen regions of the dendritic arbor, at defined distances from the soma center, were then immunolabeled for GABA using a postembedding immunogold method. Although off-cell soma and proximal dendrites receive an extensive synaptic input, few GABA-labeled boutons are found within 50um of the soma. A GABAergic synaptic input onto distal dendrites is, however, more common. Both the number of boutons and the percentage of synaptic membrane contacted by the labeled boutons increase distally. The GABA synapses onto labeled off-cells are all symmetrical and contain predominantly round vesicles and often dense core vesicles. These results demonstrate an anatomical substrate for the GABAergic control of off-cells, consistent with the hypothesis that the opioid excitation of off-cells is mediated by an opioid inhibition of this GABAergic inhibitory regulation.

44.1

TAIL-FLICK PARAMETERS CAN DETERMINE WHETHER NALTREXONE DECREASES OR INCREASES FLICK LATENCIES FOLLOWING FOOTSHOCK STRESS. J.T. Cannon, E.W. Walsh*, R.F. Henry* and E.A. Bolan*. Depts. of Psychology and Chemistry, University of Scranton, Scranton, PA 18510-2192.

D'Amour & Smith's (1941) seminal work on the tail-flick response to heating involved average baseline latencies in the 4.5-5.0 sec range. A survey of recent investigations found that test stimuli differed sufficiently across laboratories to produce average baseline latencies ranging from approximately 1.5 to 10.0 sec. Such variations in test parameters may alter the tail-flick, potentially changing its neural substrates and/or its sensitivity to antinociceptive mechanisms (e.g., Jensen & Yaksh, 1986; Ness & Gebhart, 1986). We examined whether tail-flick test parameters could alter the effects of naltrexone on elevations in latencies produced by footshock stress.

Forty-eight male albino rats (Holtzman) were individually housed with food and water freely available on a 12/12 hour light/dark cycle. Testing occurred during the dark phase of this cycle. Animals (8/group) were assigned to either footshock stress or no stress conditions that were paired with one of 3 sets of tail-flick test parameters: 1) 3.5 sec baseline, 7.0 sec cutoff; 2) 4.5 sec baseline, 7.0 sec cutoff; or 3) 4.5 sec baseline 10.0 sec cutoff. Footshock consisted of constant current 3.0 mA pulses (1 sec pulse every 5 sec) delivered for 3 min. Tail-flick latencies were measured at 1 min intervals: 5 pre- and 12 post-footshock. Ten min prior to pre-shock testing the animals were injected with either naltrexone (5 mg/kg/ml) or an equal volume of saline.

Naltrexone significantly reduced post-shock tail-flick latencies in the 3.5 sec baseline condition; no significant Trial x Drug interaction was observed. In both 4.5 sec baselines conditions, naltrexone significantly elevated tail-flick latencies. For only the 10.0 sec cut-off condition, there was a significant Trial x Drug interaction.

These data demonstrate that tail-flick test parameters can modify the direction and time-course of naltrexone's effect on post-shock tail-flick latencies. Such dynamics may be a source of some conflicting observations across laboratories. These and other findings (see above) also suggest that systematic consideration of test parameters may expand the utility of the tail-flick, allowing it to assess multiple facets of nociception.

44.2

EFFECT OF IT NALTREXONE ON INESCAPABLE TAIL SHOCK ANALGESIA. J. Grisel, L.Watkins, L. Yuva, S. Ryan, & S. Maier. Dept. of Psychology, University of Colorado, Boulder, CO 80309.

Some forms of stress induced analgesia are mediated by endogenous opiates. Increasing numbers of inescapable tail shocks produce 3 successive analgesic peaks as measured by the tailflick test. IP naltrexone (7mg/kg) blocks the first & third analgesias, induced by 1-5 & 40-80 1.6 mA shocks respectively. Also, rats given 100 inescapable tail shocks demonstrate naltrexone-blockable (7mg/kg) analgesia when later exposed to 5 mild grid shocks (.8 mA).

Experiment I tested intrathecal (IT) naltrexone on analgesia induced by inescapable tail shock. Male Holtzman rats (400-500 g), implanted with lumbar IT catheters, were randomly divided into 2 groups receiving either naltrexone (2.0 µg in 0.5 µl; n=7) or 0.5 ml saline (n=8). All rats received 80 0.5 sec 1.6 mA tail shocks; tail flick latencies were recorded prior to (baseline), & after 1, 5, 10, 20, 40, 60, & 80 shocks. IT naltrexone or vehicle was delivered before shock, & after 10 & 40 shocks. IT naltrexone blocked analgesia induced by less than 5 & greater than 40 shocks, implicating spinal opiate mediation.

Experiment II used parallel methods to investigate IT naltrexone on reinstatement analgesia. Rats, implanted with lumbar IT catheters, received restraint (n=8) or 80 5 sec 1.O mA tail shocks (n=24). Twenty-four hr later, previously shocked rats were given either no injection, IT saline (0.5 µl) or IT naltrexone (2.0 µg in 0.5 µl; n=8 in all groups), & all rats were exposed to 5 0.8 mA grid shocks, in a plexiglass chamber. Naltrexone significantly blocked analgesia compared to saline-injected & non-injected shock groups, and produced latencies equal to the previously non-shocked group. These data indicate a spinal opiate component in reinstatement analgesia.

Since spinal opiates clearly play a part in the analgesias induced by inescapable tail shock, continuing studies attempt to test the effect of κ, δ & µ specific opiate antagonists on the 3 peaks of analgesia produced during acute tail shock, as well as the analgesia observed during reinstatement. BNS-8808840.

44.3

DEVELOPMENT AND REVERSAL OF HYPOALGESIC RESPONSES IN RHESUS MONKEYS. A. L. Beggs. Department of Psychology, University of Southwestern LA, Lafayette, LA 70504.

Two experiments were conducted to examine the effects of inescapable shock on foot withdrawal responses to radiant heat. In the first experiment, latency measures of left foot withdrawal to radiant heat indicated that inescapable shock to the right leg increased reaction time compared to no-shock control subjects ($p < .05$). Additionally, reaction times for all subjects decreased over stimulus presentations ($p < .01$). Assuming that the group differences observed in the first experiment were mediated by endogenous opiates, a second experiment was conducted to compare foot withdrawal responses of naloxone treated subjects to saline controls which had also received right leg shocks. Analysis of the latency data revealed group differences with the naloxone treated subjects demonstrating faster reaction times ($p < .01$). Again, reaction times decreased over trials for both groups ($p < .01$). The results of this study indicate that shock activated an opioid system sensitive to naloxone antagonism and suggest neural mediation of reaction to radiant heat over trials.

44.4

ADRENALECTOMY AND DEXAMETHASONE ATTENUATE MILD SHOCK-INDUCED ANALGESIA. M.K. Biles, J.M. Barter* and J.W. Grau. Department of Psychology, Texas A&M University, College Station, TX 77843.

Prior work suggests that the opioid analgesia observed in rats after an extended exposure (e.g. 20 min or more) to intermittent shock is "hormonal" in nature because it is blocked by adrenalectomy and dexamethasone. The opioid analgesia observed after less severe shock schedules is thought to be "neural" in nature since it is not affected by manipulations that disrupt the pituitary-adrenal axis (Terman, G.W. et al., Science, 226, 1270, 1984). We have previously shown that exposure to very mild shock (3, 0.75-s, 1.0 mA tail-shocks) can elicit both a transient nonopioid and long-lasting opioid analgesia on the tail-flick test (Grau, J.W., Beh. Neurosci., 101, 272, 1987). It is currently unclear whether the pituitary-adrenal axis plays a role in this analgesia. We addressed this issue by testing the impact of adrenalectomy and dexamethasone (1 mg/kg administered 2 hrs before testing) on mild shock-induced analgesia. We found that both manipulations attenuated, but did not eliminate, the analgesia. The results suggest that the pituitary-adrenal axis also plays a role in the production of mild shock-induced analgesia. Supported by DA-05846-01 to J.W.G.

44.5

LATENT INHIBITION AND OVERSHADOWING OF A CONDITIONED ANTINOCICEPTIVE RESPONSE IN THE SPINALIZED RAT. J.A. Salinas† P.A. Illich and J.W. Grau. Dept. of Psychology, Texas A&M University, College Station, TX 77843.

We have previously shown that a conditioned antinociception can be established and extinguished in the spinalized rat (Grau et al., Beh. Neurosci., 104, 489, 1990). The present study explored whether this conditioned response would exhibit latent inhibition and overshadowing. In both experiments testing was conducted 24 hr after subjects received a spinal transection at T2. Mild shock to the left or right paw served as the conditioned stimulus (CS). The unconditioned stimulus (US) was an intense tail-shock. Antinociception during the CS was assessed with the tail-flick test 1 hr after training. To show latent inhibition subjects were preexposed to either the CS+, CS- or nothing (n=10). All of the subjects then received differential conditioning in which one CS (the CS+) was paired with the US while the other was presented alone (the CS-). We found that preexposure to either the CS+ or CS- attenuated classical conditioning. To test for overshadowing subjects received either: 1) a mild CS paired with the US; 2) the mild CS plus a strong CS paired with the US; or 3) the mild CS and US unpaired. Only subjects in the first training condition exhibited conditioned antinociception. Supported by BNS 881981 to J.W.G.

44.6

THE IMPACT OF SCOPOLAMINE ON CONDITIONED AND SHOCK-INDUCED ANALGESIA. P.S. Chen*, P.A. Illich, M.W. Meagher and J.W. Grau. Department of Psychology, Texas A&M University, College Station, TX 77843.

Rats exposed to long shocks exhibit a nonopioid analgesia on the tail-flick test which is eliminated by spinal transection but not decerebration. It is currently unclear whether cholinergic sytems play a role in this analgesia. We addressed this issue by testing the impact of the cholinergic antagonist scopolamine (1 mg/kg) on the analgesia observed after three 25 s, 1.0 mA, tail-shocks. Prior to shock, scopolamine treated subjects exhibited a significant hyperalgesia. The drug did not attenuate the analgesia observed after shock. In fact, when we controlled for the impact of scopolamine on baseline levels of pain reactivity, it actually potentiated shock-induced analgesia. Methylscopolamine, which does not cross the blood-brain barrier, had no effect. We also tested whether cholinergic systems play a role in conditioned analgesia. One stimulus (the CS+) was paired with shock while the other was presented alone (CS-). Each subject received 6 presentations of each stimulus spaced over 2 hr. Testing was conducted 24 hr later. We found that saline treated subjects were analgesic during the CS+ relative to the CS-. This conditioned analgesia was blocked by scopolamine but not methylscopolamine. Supported by BNS 881981 to J.W.G.

44.7

VAGAL STIMULATION ALTERS THE ACTIVITY OF NEURONS IN THE ROSTROVENTRAL MEDULLA (RVM) WHICH RESPOND TO NOXIOUS STIMULI. C. L. Thurston and A. Randich, Department of Psychology, The University of Iowa, Iowa City, IA 52242.

Electrical stimulation of the vagus produces antinociception which is dependent on the RVM. The present studies examined the effects of vagal stimulation on RVM neurons which respond to noxious stimuli. Rats were anesthetized with pentobarbital sodium and prepared for electrophysiological recordings of RVM neurons and for electrical stimulation of the left cervical vagus. Rats were maintained in a light plane of anesthesia with methohexital to enable classification of neurons as ON, OFF, or NEUTRAL cells based on their response to tail-flick trials. Vagal stimulation appears to have no effect on NEUTRAL cells. However, vagal stimulation excited 14/19 ON cells, inhibited 1, had variable effects on 1, and had no effect on 3. In contrast, vagal stimulation inhibited 4/7 OFF cells, excited 1, and had no effect on 2. These data are contradictory to present knowledge of vagal stimulation produced antinociception and the proposed role of ON and OFF cells in antinociception derived from the NRM, since excitation of OFF cells and inhibition of ON cells would be predicted to support antinociception. Supported by NIH grants NS24958 and HL07121.

44.8

SPINAL NEUROTRANSMITTERS INVOLVED IN FACILITATION OF THE NOCICEPTIVE TAIL FLICK REFLEX BY VAGAL AFFERENT STIMULATION. K. Ren, A. Randich and G.F. Gebhart. Departments of Pharmacology and Psychology, The University of Iowa, Iowa City, IA 52242.

We have previously demonstrated that the nociceptive tail flick (TF) reflex in the rat is facilitated at low and inhibited at high intensities of vagal afferent stimulation (VAS) (Brain Res. 446:285,1988). This experiment was conducted to characterize the spinal neurotransmitter(s) involved in the facilitation of the TF reflex produced by VAS. Rats were lightly anesthetized with pentobarbital (5-10 mg/kg/hr iv). The central end of the cut vagus nerve was electrically stimulated (2.0 ms, 20 Hz and 2-15 μA). VAS at a mean intensity of 7 ± 1 μA (n=39) facilitated the TF reflex independent of the baseline TF latency. Intrathecal administration of pharmacologic receptor antagonists into the subarachnoid space of the lumbar enlargement indicated that the facilitation of the TF reflex by VAS was antagonized by methysergide, naloxone or nor-BNI, but not by the 5-HT_2 and 5-HT_3 receptor antagonists LY53857 and ICS 205-930, the delta opioid receptor antagonist naltrindole, or the adrenoceptor antagonists yohimbine or prazosin. These results suggest that subtypes of spinal 5-HT and opioid, but not noradrenergic receptors are activated by a group of low threshold cervical vagal afferents to facilitate spinal nociceptive processing.

44.9

CHARACTERIZATION OF THE VISCEROMOTOR RESPONSE TO COLORECTAL DISTENSION IN THE HALOTHANE LIGHTLY-ANESTHETIZED RAT. A. Burnett, T.J. Ness and G.F. Gebhart. Departments of Pharmacology and Anesthesia, University of Iowa, Iowa City, Iowa, 52242.

Responses to colorectal distension have been demonstrated to be useful in studying visceral pain when performed in awake, unrestrained rats. The present study was undertaken to validate the use of this model in the halothane lightly-anesthetized rat to facilitate parametric evaluations of visceral and cutaneous pain. Male Sprague Dawley rats were initially deeply anesthetized with 5% halothane and 95% O_2; a tracheotomy was performed and animals were maintained in the lightly-anesthetized state with constant inhalation of 0.5-1.5% halothane and O_2. Vigorous flexion reflexes were present in this state. A reliable visceromotor response was obtained with repeated colorectal distension (80 mmHg; 10s) at 2 min intervals for 2 hrs. Abdominal constriction was assessed using EMG recordings. Graded EMG responses to graded intensities of distension were also demonstrated. There was an obvious threshold for the response using a phasic stimulus. Electrical stimulation in the midbrain produced a reliable and reproducible inhibition of the response at intensities as low as 40μA. The use of this model in the lightly-anesthetized animal will allow comparative studies of descending modulation of visceral and cutaneous pain.

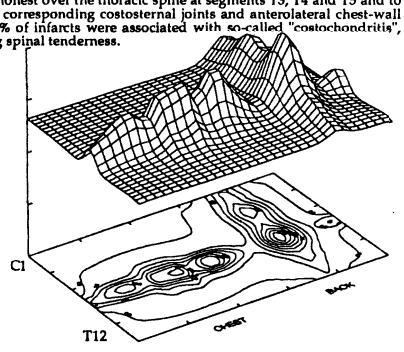
44.10

EFFECT OF LASER ACUPUNCTURE ON ANIMAL PAIN THRESHOLD AND STUDY OF ITS MECHANISMS. C. Yu, K. Zhang*, G. Lu* and Q. Wang*, College of Veterinary Medicine, Beijing Agricultural University, Beijing, P. R. China.

In order to investigated the changes of the animal pain threshold and the action of endorphin, serotonin etc., in the process of irradiating the acupoints of animals with laser, from 1977-1985, the animals of different kinds-50 rats, 51 dogs, 463 rabbits, 15 pigs, 68 goats and 73 sheep-were divided into experimental groups and control groups. Experimental groups were irradiated and then injected the drugs D-phenylalanine, D-leucine etc. or irradiated and at the same time injected the drugs L-tryptophan, insulin, acetylcholine etc. The control groups were only irradiated or only injected the drugs aforementioned or normal saline. The changes of animal pain threshold were observed by using *WQ-9D SURVEYING INSTRUMENT OF PAIN THRESHOLD* with the penetration of potassium ion (K^+) to be measured. The result in the present study was the average of three measured values of the pain threshold. The summary of the results are as follows: (1). The pain threshold in rabbits, dogs, pigs, sheep and goats significantly increased ($p<0.01$) before and after irradiation of laser. The pain threshold significantly increased ($p<0.05$) in irradiation group of laser than in traditional acupuncture group in rabbits, and in irradiation group, the effect of CO_2 laser stronger than that of He-Ne laser in dogs. (2). The increase of pain threshold were related to endorphin, serotonin and neurotransmitter releasing from cholinergic neurons, which potentiated each other in analgesic process. (3). The increase of pain threshold were enhanced by L-tryptophan, D-phenylalanine, D-leucine, acetylcholine and insulin.

44.11

THE HYPERALGESIA OF MYOCARDIAL INFARCTION: A PROSPECTIVE STUDY. G. A. C. Jones* and R. J. Milne. Dept. of Physiol., Univ. of Auckland, New Zealand. Hyperalgesia (musculoskeletal tenderness) of cardiac origin, potentially dangerously misleading in the evaluation of chest pain, is known to occur but has not been systematically documented. As well as an important clinical question viscerosomatic hyperalgesia paralleling somatosomatic hyperalgesia is an important neurophysiological question. Hence this study. All cases admitted to the 5 bed coronary care unit of a general hospital over a 12 week period with 20 minutes or more of cardiac-like chest-pain were reviewed on the morning following admission. A single examiner blind to the diagnosis graded tenderness to palpation over 151 points of the cervical spine and thorax. Of the 56 cases subsequently confirmed to have infarction 52% showed hyperalgesia. The graph below shows probability of hyperalgesia on the z axis, C1-T12 levels along the y axis and location around the chest wall on the x axis. Hyperalgesia was commonest over the thoracic spine at segments T3, T4 and T5 and to a lesser extent over the corresponding costosternal joints and anterolateral chest-wall maximal on the left. 40% of infarcts were associated with so-called "costochondritis", 95% of these also having spinal tenderness.



44.12

RESPONSES TO TOOTHPULP STIMULATION IN CONSCIOUS CATS: DIGASTRIC REFLEX AND PROPERTIES OF NEURONES IN THE TRIGEMINAL BRAINSTEM. D.Banks* and B.Matthews. (SPON: Brain Research Association). Dept. of Physiology, University of Bristol, England BS8 1TD.

We have investigated the digastric response to toothpulp stimulation and recorded from trigeminal brainstem neurones in lightly anaesthetised and awake, unrestrained animals. The animals were prepared under general anaesthesia (alphaxalone/ alphadolone, 18mg/kg I.M. and 5-8mg/kg/h I.V.). A connector block with a miniature socket and venous injection port was fixed to the skull over the frontal sinus. Stainless-steel wires were passed subcutaneously from the block to Ag/AgCl fillings in the right canine teeth for electrical stimulation and to the right digastric and masseter muscles for recording EMGs. A cannula from the injection port was inserted into the anterior facial vein. A small plastic block was positioned stereotaxically over a craniotomy which was sealed with silicone elastomer. The block held a miniature micromanipulator which remained in place between recording sessions. Recordings were made with Teflon-coated platinum/ iridium microwires.

As shown previously, the conscious animals showed no aversive behaviour to toothpulp stimulation over the range tested (up to 1mA, 0.1ms and 0.1Hz). The digastric reflex stimulus/ response curve in the awake and lightly anaesthetised (alphaxalone/ alphadolone, 4mg/kg, I.V.) animal had one and sometimes two notches in the range 25-100μA. Under similar conditions the responses to toothpulp stimuli recorded from neurones in the trigeminal brainstem nuclei (spinal, pars oralis) also showed a decrease in excitability corresponding to the notches in the digastric stimulus/ response curve. The inhibition of neuronal activity applied equally to the short (~3ms) and long (~25ms) latency discharges. The results indicate that toothpulp afferents produce inhibitory as well as excitatory effects on neurones in the trigeminal brainstem and on digastric motoneurones.

44.13

TAIL FLICK LATENCY IN LACTATING RATS. P.Pacheco, R.Chirino*, M.Martínez-Gómez and F.Mena*.Instituto Investigaciones biomédicas-UNAM,México 04510,DF.;CIRA-UAT-CINVESTAV, Panotla,Tlax.México; CIB-U.Veracruzana,Xalapa,Ver.México.

During suckling the lactating mother rat exhibits immobility and relaxated behavior, synchronous EEG, increased autonomic activity, and changes in peripheral cathecolamines levels. This suggests that sensitivity to nociceptive stimuli may be altered during lactation. We analyzed the tail flick latency (TFL) evoked by noxious heating of the tail before, during and after suckling of lactating rats. Before each test the pups were separated from the mother 6 or 12 h. RESULTS: a) During lactation TFLs progressively decreased as compared to those measured in the same rats before pregnancy. At weaning the TFLs values were increased. b) Mothers separated 12 h from their pups, showed higher TFLs than mothers separated 6 h. c) TFLs were significantly decreased during a 15-30 min suckling period. Following suckling TFLs gradually returned to previous values. d) The reduction in TFLs during suckling was significantly higher at mid lactation. We conclude that, lactating rats as compared to virgin rats show hyperalgesia, which may be due to suckling by the pups. Such effect of suckling may blocks central inhibitory influences on nociceptive input.

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44.14

CAROTID SINUS NERVE STIMULATION INHIBITS SPINOTHALAMIC AND SPINORETICULAR TRACT NEURONS. W. S. Ammons. Dept. of Physiology, Thomas Jefferson Univ. Philadelphia, PA 19107.

This study was designed to test the hypothesis that stimulation of the carotid sinus nerve inhibits activity of ascending pain pathways. Extracellular recordings were obtained from lumbosacral spinothalamic tract (STT) neurons in 5 monkeys and spinoreticular tract (SRT) neurons in 10 cats under chloralose anesthesia. A bipolar electrode was placed on the left carotid sinus nerve. Stimulation of the carotid sinus nerve (20 Hz, 0.1 ms, 7-12 V) inhibited spontaneous activity of 9/16 STT and 12/20 SRT cells. Two response patterns were observed: rapid (< 1 s) inhibition that was maintained during the 10 s stimulus period; rapid inhibition that decreased (escaped) during the stimulus period. Vagotomy attenuated or abolished inhibitory escape. Mean activity of STT neurons decreased from 14 ± 3 to a minimum level of 2 ± 1 spikes/s 3 s into the stimulus period. Activity of SRT cells was reduced from 6 ± 2 to 2 ± 1 spikes/s. Responses to stimulation of A delta and C-fibers in the left sural nerve were inhibited by conditioning the carotid sinus nerve (50 - 200 ms trains). On a percentage basis carotid sinus nerve stimulation reduced responses to C-fiber input significantly more than responses to A delta input. Responses to pinch of skin or skin and muscle of the hindlimb were inhibited by an average of 89%. The results indicate that afferents in the carotid sinus nerve, perhaps baroreceptors, exert antinociceptive effects at the spinal level.

44.15

AGING-RELATED DECREMENTS IN THE ANALGESIC EFFECT OF VAGINOCERVICAL STIMULATION (VS) IN RATS. J.L. Steinman, S. Chinapen*, K. Repola* and B.R. Komisaruk. Institute of Animal Behavior, Rutgers-The State University, Newark, NJ 07102.

The present study compared the magnitude of VS-produced analgesia in sexually mature rats (age 8-9 mos.) with that in substantially older rats (age >16 mos).

After determination of pre-VS measurements of tail flick latency (TFL) to radiant heat and vocalization threshold (VOCT) to electrical shock of the tail, VS was applied as a continuous force against the cervix with a calibrated syringe assembly (5-mm probe); pain thresholds were then determined during VS. All rats were ovariectomized at least one week prior to testing.

TFL and VOCT were significantly elevated during VS (100g) in the 8-9 mo. group but not in the >16 mo. group. At 300g VS, TFL was significantly elevated during VS in both age groups, but VOCT was significantly elevated only in the younger group. These findings indicate that while VS is capable of producing analgesia in both age groups, the magnitude of the analgesia declines significantly with age. A more marked age-related decrement was found on the VOCT than the TFL test. (Support: NIH grants NS 22948, RR 08223 and the Charles and Johanna Busch Foundation [BRK])

	Tail Flick Latency (TFL)[a]		Vocalization Threshold (VOCT)[b]	
Age (mos.)	8-9	> 16	8-9	> 16
(n=)	(8)	(11)	(8)	(11)
pre-VS	3.8±0.2	3.4±0.2	.27±.05	.32±.05
VS (100g)	9.4±0.5[§]	4.5±0.8	.31±.04*	.33±.04
VS (300g)	10.0±0.0[§]	9.5±0.4[§]	.38±.05[§]	.34±.03

paired t tests: * $p<0.05$; [§] $p<0.006$-0.0001; compared to corresponding pre-VS values; [a] mean (sec) ± sem; [b] mean (mA) ± sem

CHEMICAL SENSES: CENTRAL PATHWAYS I

45.1

LOCALIZATION OF DOPAMINE RECEPTOR SUBTYPES IN THE OLFACTORY BULB: [^{3}H]SPIPERONE (D2) BINDING IN THE GLOMERULAR AND NERVE LAYERS. W.T. Nickell, A.B. Norman, L.M. Wyatt, and M.T. Shipley, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

There is a substantial population of dopaminergic neurons located in the glomerular layer of the olfactory bulb. Although the structure and development of these neurons has been extensively studied, their cellular targets and physiological function have not been determined. The localization of DA receptor subtypes might provide clues to the function of the dopaminergic neurons; hence, we determined the quantity and localization of D1 and D2 dopamine receptor subtypes using [^{3}H]SCH23390 and [^{3}H]spiperone, respectively.

Saturation studies in homogenates of olfactory bulb demonstrated barely detectable levels of D1 binding and a relatively high level of [^{3}H]spiperone binding (B_{max} = 5.9±0.6 pmol/g tissue). All assays included ketanserin (40 nM) to preclude binding to 5-HT_2 receptors. The location of [^{3}H]spiperone binding in the bulb was determined by autoradiography. As expected there was significant [^{3}H]spiperone binding in the glomerular layer. Unexpectedly, however, there was also a high level of specific binding of [^{3}H]spiperone in the olfactory nerve layer.

Spiperone binding in the nerve layer suggests that olfactory nerve terminals in the glomeruli may be postsynaptic to DA neurons. This hypothesis would provide an explanation for the loss of DA from the glomerular layer following lesion of the olfactory nerve. Lesion of the nerve would then result in loss of the synaptic targets of the DA neurons, thus explaining the loss of DA from the glomerular layer following lesion of the olfactory nerve. Experiments to test this hypothesis are in progress.

Supported by NIDCD DC00347 and DAMD17-86-C-6005.

45.2

EXPRESSION OF CALCIUM BINDING PROTEINS IN THE MAIN OLFACTORY BULB DIFFERS AMONG SPECIES. M.R. Diño*, and E. Mugnaini. Lab. of Neuromorphology, Univ. of Connecticut, Storrs, CT. 06269-4154.

Calcium binding proteins have been used as cell population markers in numerous studies of the CNS. In this investigation we have compared neuron immunostaining with antisera to CaBP 28K and PEP-19 in the main olfactory bulb of rat, guinea pig and cat. Antisera (gift of S. Christakos and J.I. Morgan) were characterized previously . The CaBP 28k antiserum did not cross-react with mammalian CaBP 29k in Western blots. In rat and cat, PEP-19 immunostaining was similar and very dense . It revealed periglomerular cells, granule cells, and superficial and deep short axon cells. In guinea pig, the same types of cells were immunopositive, but only granule cells in the superficial half of the internal granular layer and deep short axon cells were densely stained; the overall staining was moderate . In rat and guinea pig, CaBP immunostaining was similarIt revealed periglomerular cells and a few deep short axon cells. In cat, all types of neurons except the mitral and tufted cells were stained, but the most densely immunoreactive cells were superficial and deep short axon cells, and, particularly , numerous short axon cells in EPL. Thus, the distribution of both calcium binding proteins vary in these species, with CaBP being more variable than PEP-19. This indicates that neurospecific gene expression is not regulated in a conserved manner . These differences reflect species variations in intracellular signal transduction pathways that may ultimately shed light on the biochemical functions of these proteins. (Supported by PHS Grant NS 09904).

45.3

CONVERGENCE OF PRIMARY OLFACTORY AXONS IN RAINBOW TROUT. D.R. Riddle and B. Oakley. Dept. Biology, Univ. of Michigan, Ann Arbor, MI 48104.

To evaluate convergence in the primary olfactory projection of rainbow trout, we labeled the olfactory receptor neurons terminating in small regions of the olfactory bulb by injecting fluorescently tagged latex beads into the glomerular layer of each bulb in 9 trout. In 7 of the fish each bulb was injected at one site with rhodamine beads and at another site with FITC beads. The smallest injections labeled fewer than 100 receptor cells and the largest more than 20,000. Shifting the injection site in the bulb failed to systematically alter which of 12-16 lamellae of the olfactory rosette were best labeled. On each lamella, retrogradely labeled receptor neurons were present and widely distributed, regardless of the size or site of the bulbar injection. Labeled axons were also widely dispersed in the olfactory nerve. Even with maximal separation, double injections in the bulb produced extensively overlapping distributions of labeled receptor neurons in the mucosa. These results provide direct evidence that the olfactory axons that terminate at a given site in the bulb arise from widely dispersed olfactory receptor neurons. This evidence for a convergent projection agrees with our observation that the lectin pokeweed agglutinin (PWA) labels a defined subpopulation of olfactory receptor neurons that is widely distributed in the olfactory mucosa but makes convergent projections to restricted and predictable regions of the glomerular layer. The distribution of labeled axons in the olfactory nerve after discrete injections of fluorescent tracers in the olfactory bulb is consistent with the dispersion of PWA positive axons in the olfactory nerve. Both experiments indicate that there is significant resorting of axons at the interface between the olfactory nerve and bulb, where axons that terminate together first appear to aggregate.

45.4

LACK OF EFFECT OF VALPROIC ACID ON FOOD FINDING IN THE MOUSE T.J. Hoeppner and T. Krug*. Dept. Neurol. Sci., Rush Medical College, Chicago, IL 60612.

We have previously shown that systemically administered valproic acid (VPA) concentrates selectively in the olfactory bulb (OB). Since the primary function of the OB is olfaction we hypothesized that systemically administered VPA would alter olfactory function. Olfactory function was assessed by measuring the time to find a food pellet buried under wood shavings.

Twenty mice (Swiss derived, male, 8-10 weeks of age, approximately 30 g body weight) were deprived of food overnight for eleven nights. On the morning after the first overnight fast each mouse received an i.p. injection of 0.3 ml of saline (.09%) and then 30 min later the time required to find a buried food pellet was determined. On the next 10 mornings 10 animals received an anticonvulsant dose of VPA (300 mg/kg, i.p., as the sodium salt, in 0.3 ml of .09% saline) 30 minutes prior to behavioral testing. Ten control animals received saline injections without VPA prior to behavioral testing. All animals in both groups found the buried pellet each day within 5 minutes. The time required to find the food pellet did not differ significantly for the two groups over the entire period of testing.

These findings indicate that an anticonvulsant dose of VPA does not disturb the ability to find buried food under the conditions of this experiment.

45.5

DEVELOPMENTAL CHANGES IN THE OLFACTORY BULBS OF RAT PUPS FOLLOWING EARLY OLFACTORY LEARNING. C.C. Woo and M. Leon. Department of Psychobiology, University of California, Irvine, CA 92717.

Rat pups will learn to approach an artificial odor following exposure to the odor with concurrent tactile stimulation. Accompanying this behavioral preference is an increase in ^{14}C 2-deoxyglucose (2DG) uptake within the glomerular layer of the olfactory bulb in response to the trained odor on postnatal day (PND) 19. There also is a sensitive period during the first postnatal week for acquisition of this neurobehavioral response (Woo, C.C. and Leon, M., Dev. Br. Res., 36:309, 1987).

In this study we determined the point in development when the behavioral and neural changes emerge. Pups were exposed to peppermint odor and given reinforcing tactile stimulation from PND 1 to PND 8, 11, or 14, and then tested on the following day, rather than on PND 19, to assess their 2DG uptake and behavioral preference for that odor. Both an increase in 2DG uptake and a behavioral preference were observed beginning on PND 9. The increased 2DG uptake persists despite a subsequent increase in 2DG uptake on PND 12 followed by a continuing decrease through PND 19 by both groups.

This research was supported by grants HD-24236 from NICHD to M.L.and MH-09719 from NIMH to C.W.

45.6

ODOR AND TACTILE STIMULATION EACH INCREASE OLFACTORY BULB CATECHOLAMINE RELEASE IN RAT PUPS. R. Coopersmith, F.B. Weihmuller, J.F. Marshall and M. Leon. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717.

Pairing an odor with tactile stimulation induces an odor preference in rat pups which is associated with an increase in the number of olfactory bulb juxtaglomerular neurons in focal glomerular regions. Drug studies raised the possibility that the reinforcing properties of the tactile stimulation are mediated by noradrenergic pathways. We therefore used *in vivo* microdialysis in awake 3-day-old rat pups, to determine whether extracellular olfactory bulb noradrenaline (NA) increases in response to tactile or odor stimulation. Stroking rapidly increased bulb NA levels, followed by a fall to baseline 20 min later. Odor exposure increased NA, but only after a delay of 30 min; this peak was followed by a rapid fall to baseline levels. Dopamine (DA) is present in some juxtaglomerular neurons and we found that DA gradually increased during stroking, with a peak response (> 2-fold) 1 hr post-stimulation. DA also increased slightly (10%) during odor exposure and continued to rise to 200% of baseline 1 hr later. This dual activation of bulb juxtaglomerular neurons by both primary sensory and centrifugal stimulation may play a role in mediating learning-associated changes in these neurons.

Supported by grants ONR N00014-89-J-1960 to M.L. and NSF BNS 8819132 to R.C.

45.7

IMMUNOHISTOCHEMICAL ORGANIZATION OF THE HUMAN OLFACTORY BULB. Robin L. Smith*[1], Harriet Baker[2], Kaare Kolstad*[1], Dennis D. Spencer[1] and Charles A. Greer[1]. Sec. Neurosurgery and Neuroanatomy[1], Yale Univ. Sch. Med., New Haven, CT and Laboratory of Molecular Neurobiology[2], Cornell Univ. Med. Coll., White Plains, NY.

Comparatively little is known of the cytochemical organization of the human olfactory system, despite a growing awareness of its potential involvement in disease states such as Alzheimer's. Consequently, we initiated a series of studies to characterize the organization of the human olfactory bulb (OB) using immunohistochemical probes as phenotypic markers. Thus far we have examined immunoreactivity using antibodies to olfactory marker protein (OMP), tyrosine hydroxylase (TH), 5HT, CCK, GABA, substance P, somatostatin (SST) and synaptophysin (SYP). OMP localized to the olfactory nerve and glomerular layers (GL). TH staining was predominant in the somata and processes of juxtaglomerular neurons. 5HT positive processes were evident as varicose axons in the subependymal core of the OB and in the interglomerular neuropil. CCK axons were evident in the granule cell layer (GCL) and in the interglomerular neuropil. GABA positive neurons occurred in both the GCL and GL layers. Substance P immunoreactive axons localized to the external plexiform layer (EPL) while labeled somata were present in the deep GCL. SST positive somata were seen in the anterior olfactory nucleus, deep to the GCL. Occasional SST positive axons were also found in the EPL. SYP immunoreactivity was strongest in the intraglomerular neuropil and the EPL, consistent with the notion that synaptic density is highest in these areas. Thus far, the results are concordant with prior reports delineating the localization of these antibodies in other mammals.

45.8

SOMATOSTATIN-28-LIKE IMMUNOREACTIVITY IN THE RAT ACCESSORY OLFACTORY BULB. S. Takami*, G.D. Fernandez*, M.H. EL-Hawary and P.P.C. Graziadei. Dept. Biol. Sci. Florida State Univ., Tallahassee, FL 32306-3050. Dept. Zool., Fac. Sci., Assiut Univ., Egypt (M.H.E.-H.).

To characterize the vomeronasal (VN) system morphologically and neurochemically, we have recently investigated its primary CNS center, the accessory olfactory bulb (AOB). We report here that somatostatin (SS)-28-like immunoreactive (LIR) neurons are present in the AOB, and describe their location and morphology.

Brain sections from adult Sprague-Dawley rats were processed following the conventional avidin-biotin-peroxidase complex method. Two kinds of commercially available antibodies (*Incstar*) were used at the dilution of 1,000 to 10,000 times.

In the glomerular layer (GL), a small population of neurons with small somata is SS-28-LIR. The antibodies stain some profusely branched dendrites, but in some cases, they stain only stem dendrites and very few dendritic branchings. In the external plexiform layer (EPL), a subpopulation of the mitral/tufted cells (Takami & Graziadei, *Soc. Neurosci. Abstr.*, 15: 928 '89) is IR. IR dendrites directing to the GL can be sometimes tra ced into the inside of the glomeruli. A very few interneurons located in the EPL are also SS-28-LIR. In the granule cell layer (GRL), the antibodies stain a scattered population of multipolar neurons whose somata are larger than those of the granule cells. The IR dendrites spread in the GRL, but they cannot be tranced into the EPL. The GRL also contains abundant IR fibers some of which are originating from the somata located in the region below the GRL. The above reactivity was completely inhibited when SS-28 was added into the diluted antibody solution, but not weakened when SS-14 was added.

Since the mitral and tufted cells of the MOB are negative to SS-28 antibodies, it can be concluded that the presence of the SS-28-LIR output neurons in the AOB is one of the chemical characteristics of the VN system. Supported by a grant from the NIH (NS 20699) to P.P.C.G.

45.9

LECTINS LABEL SUBPOPULATIONS OF OLFACTORY RECEPTOR CELLS W.B. Stewart and C.E. Touloukian*. Sect. of Gross Anatomy, Dept. of Surgery, Yale Univ. Sch. Med., New Haven, CT. 06510

Several lines of evidence suggest there are subclasses of olfactory receptor cells. Our experiments tested the hypothesis that subpopulations of receptor cells could be distinguished based on lectin binding. A number of lectins were screened on sections of decalcified rat skull that included the olfactory epithelium (OE) and olfactory bulb (OB). Bandeiraea simplifolicia and Vicia villosa stained the vomeronasal organ, nerve and the glomerular layer of the accessory OB. Tetragonolobus pupureas (Lotus) and Glycine max, on the other hand, were of considerable interest because they stained a subgroup of receptor cell bodies, dendrites and axons in the OE as well as a subgroup of OB glomeruli. A detailed map was constructed of the distribution of Lotus staining in the OE and OB. The results were consistent with previous studies on the topography of olfactory nerve projection. There was also close correspondence to maps made by Schwob and Gottlieb (J. Neurosci 6:3393-3404, 1986) for the staining by a monoclonal antibody that recognized a glycoprotein. In conclusion, these studies suggest that there are distinct groups of olfactory receptor cells that differ in membrane glycoprotein specificity and that project to specific regions in the OB. Support by NS-16993 to WBS.

45.10

PROJECTIONS TO OROMOTOR NUCLEI USING TRANS-NEURONAL TRANSFER OF HERPES SIMPLEX VIRUS. J.B. Travers, L.M. Jackson*, T. Copelin*, I. Plaza* and J. Sheridan. Oral Biology, Ohio State Univ. Coll. Dent., Columbus, OH 43210

Decerebration experiments suggest that both gustatory and intraoral tactile stimuli influence ingestive responses through local brainstem substrates but the exact anatomical pathways are still unknown. In the present experiments, pre-motor neurons to the lingual and masticatory muscles were mapped using the retrograde transneuronal transfer of Herpes simplex virus. Either the tongue or anterior digastric muscle (AD) of the hamster was injected with 50 ul of HSV-1 McIntyre (4 x 10^9 pfu/ml) followed by a variable survival time. The distribution of the virus was detected using avidin - biotin immunohistochemistry with a primary anti-HSV glycoprotein antibody. As previously reported (Ugolini et al, 1987), immunoreactivity was detected in motoneurons within 48 hours and Golgi-like labelling was observed in neurons outside the motor nuclei within 72 hours. The first cells to be labelled outside the hypoglossal nucleus following lingual injections included cells in caudal subdivisions of the solitary nucleus (NST) at the IXth nerve level, and the subjacent reticular formation (RF). Following HSV injection into the AD, neurons were also labeled in the caudal NST and the subjacent RF. Only at longer survival times following either muscle injection were cells in the anterior NST labelled. This suggests that the VIIth nerve gustatory zone of the NST may influence lingual and masticatory muscles indirectly via interneurons in the caudal NST and subjacent RF but that IXth nerve influences on oral motorneurons are more direct.
Supported by NIH DC00417 to JBT and NIH GM12264 to IP

45.11

THE EFFERENT PROJECTIONS OF THE BED NUCLEUS OF THE STRIA TERMINALIS IN THE SYRIAN HAMSTER. M. Damlama and J. M. Swann, Institute of Animal Behavior, Department of Biological Sciences, Rutgers University, Newark, NJ. 07102.

The Bed Nucleus of the Stria Terminalis (BNST) is an important center in the neural regulation of reproductive behavior in rodents. In the male Syrian hamster, which relies heavily on chemosensory input for the initiation of mating behavior, lesions of this nucleus disrupt sexual behavior. While the inputs to this nucleus have been identified, the efferents remain to be characterized in this species. This study identifies the efferent projections of the BNST to further elucidate the central pathways controlling mating behavior.

Adult male Syrian hamsters were anesthetized with pentobarbital and iontophoretically injected with PHA-L. Following a one week survival period, animals were perfused with 4% paraformaldehyde, brains removed and sectioned at 40 µm. Free floating sections were immunolabelled using biotinylated antisera to PHA-L and the Vectastain ABC Kit.

Injections of PHA-L confined to the preoptic portion of the BNST resulted in intense terminal labeling in the septohypothalamic nucleus, the anteroventral and medial preoptic nuclei, the rostral extent of the paraventricular hypothalamic nucleus, the retrochiasmatic area, the tuberal area surrounding the ventromedial nucleus of the hypothalamus, the dorsal and ventral mammillary nuclei, and the medial nucleus of the amygdala. Moderate varicose fiber labelling was also seen in the nucleus accumbens, the nucleus of the horizontal limb of the diagonal band, the anterior and lateral hypothalamic areas, the arcuate nucleus, and the dorsal hypothalamic area. Finally, scattered fibers were observed throughout the septum, the ventromedial nucleus of the hypothalamus, the supramammillary nuclei, the zona incerta, the basal and central amygdaloid nuclei, the substantia nigra, and the ventral tegmental area.

The preliminary results indicate extensive BNST connections with limbic areas strongly implicated in the control of sexual behavior and endocrine reproductive functions. In addition, projections to the nucleus accumbens and the substantia nigra suggests that BNST may relay motivational information to areas involved in the execution of motor behavior. Supported by NSF RII 88-17677 to J. M. S.

45.12

INTRAMEDULLARY CONNECTIONS OF THE ROSTRAL NUCLEUS OF THE SOLITARY TRACT IN HAMSTER. M. Beckman* and M. Whitehead. Ohio State Univ. College of Dentistry, Columbus, OH 43210.

The rostral nucleus of the solitary tract (NST) is the major medullary center that receives input from all of the gustatory cranial nerves. Pathways ascending from the rostral NST to the forebrain are better documented than are pathways through the caudal brain stem. To study the latter connections HRP was injected into the rostral NST or into the subjacent reticular formation (RF). NST injection labelled axons extending ventrally into the reticular formation and the facial motor nucleus. Labelled axons also projected caudally within the NST. Cells in the RF below the injected NST were labelled by retrograde transport. Injection of this RF region labelled axons in the NST and dense collections of axons that terminated in the facial, hypoglossal (XII nuc.) and trigeminal motor nuclei and the supratrigeminal nucleus. Injecting the RF ventral to the caudal NST labelled a XII nuc.projection but projections to the other motor nuclei were sparse. Injections of each RF site labelled cells in the rostral NST; injection of the XII nuc. labelled cells in both RF sites. These results indicate that in hamster the rostral NST projects only lightly to one oromotor nucleus but the NST projects more heavily to premotor sites below the NST. These RF sites connect reciprocally with the NST and, as in rat (Tavers and Norgren, '83), project heavily and differentially to oromotor nuclei. The brain stem pathways defined in the present study provide a substrate for gustatory-motor interactions. Support: NIH grant DC00452 and OSU College of Dentistry.

45.13

WITHDRAWN

45.14

DESCENDING PROJECTION FROM THE INSULAR CORTEX TO THE GUSTATORY NTS IN THE HAMSTER. J.A. London, M.A. Barry, and T.S. Donta*. (Dept. BioStructure and Function, Center for Neurological Sciences, UConn Health Center, Farmington, CT 06032)

The descending projection from the insular cortex to the rostral pole of the nucleus tractus solitarius (NTS) was examined in detail in the golden syrian hamster, Mesocricetus auratus. Multiunit activity was recorded in the rostral NTS during the application of taste stimuli to the anterior tongue in order to delimit the boundaries of a taste responsive region. Wheat germ agglutinin horseradish peroxidase was then iontophoretically injected into the center of the taste responsive region. Retrograde label was found bilaterally in the dysgranular and dorsal part of the agranular insular cortex, with the heaviest label being contralateral to the injection site. Neurons were found deeper in Layer 5 ipsilaterally than contralaterally. Neurons that project to non-gustatory NTS were found deep in Layer 5 within middle to ventral parts of the agranular insular cortex. This study has shown the specific location of forebrain neurons which directly project to a gustatory region in the NTS. Further studies on these projections will include double-labelling experiments.

Supported by NIH Grant DC00168 and a UConn Health Center Foundation Grant.

45.15

ANATOMICAL EVIDENCE FOR DIFFERENTIAL OLFACTORY PROCESSING IN THE PRIMATE PREFRONTAL CORTEX. S. T. Carmichael, J. L. Price & M-C. Clugnet. Dept. Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110

Past investigations have revealed several areas of primate orbital cortex responsive to olfactory stimulation. Using electrophysiological recording and retrograde and anterograde tracers we have investigated the olfactory inputs to the monkey (Macaca fascicularis) prefrontal cortex (PFC). Electrical stimulation of the olfactory bulb in halothane-anesthetized monkeys evoked short latency responses in primary olfactory cortex (PC--piriform cortex and AON--anterior olfactory nucleus) as well as posterior and ventral agranular insula (lap and lav) and the posteromedial part of area 13 (13a--located just rostral to AON). Electrode penetrations rostral and lateral to these areas did not reveal any responses. Axonal tracer experiments largely confirmed these results. ^{3}H-Leu injections into the PC produced anterograde label in lap, lav and 13a. Retrograde tracer injections into lav and 13a labelled many cells in the PC.

Retrograde tracer injections into other parts of orbital PFC indicate that the projections of these three olfactory-recipient areas may form the basis for two distinct projection patterns. lap and lav project to lateral 13. Lateral 13 also receives a projection from the primary gustatory area. Lateral 13 in turn projects rostrally to part of area 11. 13a, on the other hand, projects directly to part of area 11. Thus, orbital PFC contains two different olfactory streams beginning in primary olfactory cortex and proceeding to rostral orbital areas: one directly through a secondary olfactory area, 13a, and a second, more indirect, projection through an area which also receives gustatory input, lateral 13. Supported by NIH grant DC00093.

SENSORY SYSTEMS—VISUAL PSYCHOPHYSICS AND BEHAVIOR I

46.1

TONIC SUPPRESSIVE INFLUENCE FROM THE DARK ADAPTED EYE. Thor Eysteinsson*, N. Denny*, and T. E. Frumkes, Dept. Physiol., Univ. Iceland Reykjavik; Dept. Psychol., Queens Col., Flushing, NY 11367

Monocular spatial sensitivity to foveally centered stimuli was examined in humans as a function of the adapted state of the contralateral eye. Visual Occipital Potentials (VOPs) evoked by alternating checkerboards with a Fourier fundamental of 12 cycles per degree (cpd) approximately double in amplitude if the contralateral eye is light adapted and decrease again if the rods in the "adapting eye" are dark adapted. Similarly, psychophysical (PP) modulation sensitivity to sinusoidal gratings with spatial frequencies of 2-20 cpd increases if the contralateral eye is light adapted, and gradually decreases as rods in the "adapting eye" dark adapt. For psychophysical observers, the effect of interocular light adaptation can be duplicated by pressure blinding the "adapting eye." Both our PP and VOP data show that sensitivity with binocular viewing is very similar to that obtained with interocular light adaptation and monocular viewing. Collectively, our results suggest that the increase in visual sensitivity resulting from binocular viewing should be attributed to removal of interocular suppression, and not to binocular convergent summation.

46.2

HUMAN SPEED PERCEPTION IS CONTRAST DEPENDENT. L. S. Stone, P. Thompson, and A. B. Watson. Vision Group, NASA Ames Research Center, Moffett Field, CA 94035-1000.

A moving grating is judged slower than an otherwise identical grating of higher contrast moving at the same speed. However, the uncertainty in this type of speed judgment is largely independent of the contrast ratio. We quantified this phenomenon using a 2AFC staircase paradigm in which both a reference and a test grating patch were presented simultaneously for 500ms above and below a fixation point. Subjects were asked which patch moved faster. For a 1.5 c/d reference grating moving at 2°/s, the percent bias in perceived speed is a linear function of log contrast ratio (mean slope: 1.6 % bias/dB; N = 2). Furthermore, this effect appears robust to changes in spatial frequency, temporal frequency, and even absolute contrast. This latter result suggests that the contrast effect does not saturate, e.g. a 30% contrast grating appeared, on average, 10% slower than one of 70% contrast. Our results shed light on visual motion processing within human cortex.

46.3

PERCEIVED SPEED VARIES INVERSELY WITH LENGTH OF THE MOTION PATH: A MULTI-SENSORY ILLUSION. E. Katz, M. S. Gizzi, E. P. Gardner, R. Malach. Dept. of Neurol., Mt. Sinai Sch. of Med., New York, 10029. Dept. of Physiol. and Biophys., NYU Sch. of Med., New York, 10016. Dept. of Neurobiol., Weizmann Inst. of Sci. Rehovot, Israel.

The dependence of motion perception on spatial frame of reference in the visual and tactile sensory systems of humans was studied using a two-alternative forced-choice paradigm.

Visual targets (0.4x2.0 deg) moving linearly at constant velocities ranging from 11-20 deg/s over distances ranging from 5-25 deg were viewed on a CRT screen placed at a distance of 114 cm. Targets moving at identical velocities but over a shorter distance were perceived as moving faster ($p<.01$) for both periodic motion and for single sweeps across the motion path. The visual illusion of increased speed at shorter path lengths was equally powerful when subjects were free to track the target or fixated a point at the center of the motion path. Decreasing the visible portion of the target path by masking also increased the perceived speed. Matching acceleration profiles at the limits of periodic excursions diminished, but did not eliminate the illusion suggesting that accelerations at the reversal points are important in judging object speed.

Tactile studies were done on the index finger using an OPTACON stimulator containing a 6x24 matrix of probes spaced 1.2 mm apart. Sequential pulses applied to adjacent rows at 17-100 Hz produced sensations of a bar moving along the finger over distances of 9.6-24 mm. Subjects judged stimuli displaced periodically over a shorter distance as moving faster when rows were pulsed at 100 and 50 Hz ($p<.05$). These rapidly applied stimuli were perceived as smooth motion along the finger. However, performance was random at lower frequencies where individual pulses were perceived as discrete events.

Thus, in both sensory systems, path length affects speed perception in similar ways, suggesting that motion perception in both modalities is context sensitive.

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46.4

PATTERN MOTION PROCESSING IN BINOCULAR FUSION OF TWO 2-D PLAIDS. W.A.Ho* and M.A.Berkley. Dept. of Psychology, Florida State Univ., Tallahassee, Fl, 32306.

When two low contrast and low spatial frequency orthogonal drifting square-wave gratings are presented dichoptically to human observers, a fused, coherent drifting 2-D plaid is perceived without the subjects experiencing binocular rivalry (Ho & Berkley, 1989). Our ability to see a single coherent moving pattern in such a display suggests that the underlying motion mechanism is able to generate a unique pattern of image motion by combining separate motion components. However, the combination scheme of Adelson and Movshon (1982) does not predict how the pattern motion mechanism would respond to complex scenes that contain multiple motion components. To study how various motion components are combined in the visual system, we presented two drifting monocular square-wave plaids (of changeable physical parameters) dichoptically to human subjects (so that they could be recombine in six different ways) and timed various perceptual phases during 60 secs long test trials. Our observations indicated that the pattern motion mechanism actively reorganized the 1-D grating components to generate new 2-D drifting plaids when binocular fusion occurred. For example, when a subject was shown two 135 deg monocular plaids (each made up of two 1-D components of .2 c/deg and 10% contrast) that were drifting at right angles to each other, the subject saw mostly (over 50 secs) two transparent 90 deg plaids sliding at an angle of 135 deg to each other. Yet, another possible pair of oppositely drifting 2-D plaids which had different speeds were not seen. Further experiments showed that the pattern motion mechanism preferred to generate pairs of coherent 2-D plaids that belong to one family (i.e. those containing same angle and speed) and avoided yielding pairs that were too different. It is suggested that the pattern motion mechanism functions to minimize the difference in velocity between the two plaids. (Supported by NEI 00953)

46.5

SEGREGATION OF HEXAGONAL MESH TEXTURES AND FOURIER ANALYSIS. P.E. Hallett* and M.I. Hofmann†. *Department of Physiology, Institute of Biomedical Engineering and †Department of Zoology, University of Toronto, Toronto, Ontario, Canada.

Beginning with a hexagonal mesh, a family of equiluminuous geometric textures can be formed by rotating each line segment by a fixed amount. Such textures vary both in line orientation and in the sizes of the intervening spaces. In the Fourier domain the textures have the same discrete components but the amplitudes vary. The segregation of texture pairs as figures in grounds has been assessed for free viewing from the ratings of ten subjects. Line segment orientation information alone cannot explain the results. Linear regression techniques show a significant correlation between the subjects' ratings and the amplitudes of the low-frequency Fourier harmonics of the textures. Patterns composed of mirror-image pairs are very poorly discriminated.

46.6

DISCRIMINATION OF PHASE DIFFERENCES IN PATTERNS COMPOSED OF ROTATED SINE-WAVE GRATINGS. M.I. Hofmann* and P.E. Hallett†. *Department of Zoology and †Department of Physiology, University of Toronto, Toronto, Ontario, Canada.

We have performed experiments in order to examine the visual processing of phase relations between different orientation components of a stimulus. Each pattern is composed of a superposition of three sine-wave gratings rotated 60° relative to one another. The patterns varied in global orientation and relative phase. Subjects were asked to rate the difference between pairs of patterns presented in a figure/ground configuration at 10° retinal eccentricity. The results indicate that subjects used both orientation and phase differences in making their ratings. However it was found that it was not simply the difference in relative phase but rather the difference in the absolute values of the relative phase that was the major phase related cue. This means that mirror image patterns (which may have a large phase difference) were generally poorly discriminated. These results suggest that the visual system does not simply perform local Fourier analysis, but must use some kind of feature detection.

46.7

IS SPATIAL INFORMATION FOR THE RED-GREEN INPHASE AND ANTIPHASE STIMULI PROCESSED BY THE SAME SPATIAL FREQUENCY-TUNED MECHANISMS?

Ram L.P. Vimal and Rita Pandey, Eye Research Institute, 20 Staniford St., Boston, MA 02114.

The bandwidth and peak spatial frequency (SF) of most striate neurons were reported to be the same for both luminance (red-green inphase) and color-varying (antiphase) gratings. However, for antiphase gratings, some cells had lower peak SF and broader bandwidth, and others were spatial low-pass (Thorell et al Vis Res 24:751, 1984). Some of the cells responded more to chromatic or achromatic grating depending on spatiotemporal characteristics of the stimuli (Lennie et al J Neurosci 10:649, 1990). For achromatic patterns, a minimum of 6 SF-tuned mechanisms were necessary to obtain the best fit for the photopic masking data (Wilson et al Vis Res 23:873, 1983). We investigated whether spatial information for red-green inphase and antiphase stimuli was processed by the same SF-tuned mechanisms, and whether they shifted to a lower SF range for the latter. The contrast sensitivity functions (CSF) for the inphase and antiphase, spatially localized stimuli (sixth derivative of spatial Gaussian: 4^0 or 8^0 on dark surround) were measured at about 29 cd/m^2 by the method of constant stimuli. The minimum flicker isoluminant criterion was used to determine the relative intensities of red vs. green stimuli as a function of SF. The SF range was 0.25-11 cpd in 0.5-octave steps. The pattern was presented for 500 msec on a color raster display system (Adage 3006). Powell's achromatizing lens was used to minimize the chromatic aberration. Two observers with normal color vision initiated a trial and reported whether the pattern was seen or not. We found that the CSF for the antiphase stimuli was spatial low-pass (if a sufficient number of cycles were included) and that for the inphase stimuli was band-pass. The six SF-tuned mechanisms were sufficient to predict the inphase CSF but were unable to explain the low-pass antiphase CSF. At least one low-pass mechanism is needed to explain the lower frequency characteristics of the antiphase CSF.

(Supported partly by NSERC A0295 to PK Kaiser).

46.8

A NEW TECHNIQUE FOR DETERMINING EQUILUMINANCE.

A. Chaudhuri. The Salk Institute, La Jolla, CA 92037.

It is generally believed that motion processing in the human visual system is greatly attenuated if the foreground and background components of the stimulus are made to be equiluminant. This has served as psychophysical evidence for the proposed separate neural processing of color and motion. We have now developed a display that produces a vivid and coherent motion percept only at isoluminance. The stimulus is a random-dot pattern composed of red and green elements within which some luminance noise has been added in the form of randomly placed black pixels. If this pattern is displaced in one direction and accompanied by a contrast-reversal of the colored elements, motion of the black pixels is seen only when the red component luminance matches that of the green. This is a very robust effect since it can be observed over a large range of displacements (2.6 - 26.0 min arc), duration of stimulus between displacements (50 - 400 ms), and viewing time (200 - 1000 ms). When the colored components are non-isoluminant, the movement of the black elements is masked by an overriding flicker that is caused by the contrast reversals. The red and green component intensities at which motion is perceived is taken to be equiluminant because the same intensities produce minimum flicker with a 2 deg arc field in a heterochromatic flicker photometry test. We have also found that the moving pattern is capable of generating optokinetic nystagmus (OKN) in both humans and monkeys (*Macaca mulatta*), as determined by magnetic search coil oculography. If used in this manner, the technique provides an independent assessment of isoluminance in chromatic patterns that can be used in addition to an observer's perceptual judgement. (Supported by NIH EY07605).

46.9

PERCEIVED BRIGHTNESS IS DEPENDENT UPON PERCEPTUAL TRANSPARENCY AND STEREO DISPARITY. G.R. Stoner, K.R. Dobkins and T.D. Albright. Vision Center Lab., The Salk Institute, San Diego, CA 92138.

The factors known to effect changes in perceived brightness constrain the types of mechanisms which might underlie brightness perception. For example, if adjacency in the retinal image were the only determinant and depth relationships between objects were irrelevant, then simple lateral inhibitory mechanisms might suffice as an explanation of brightness constancy. However, factors such as perceived depth plane (Gilchrist, Science, 195:185, 1977) have been shown to play a part in apparent brightness. Such findings demonstrate the need for more sophisticated models. We've investigated the conjoint effects of perceptual transparency and stereo disparity on perceived brightness. It was found that perceived brightness depended upon transparency relationships implied by stereo disparity. Human subjects adjusted the brightness of a test disc having a dark "surround" so that it matched the brightness of a reference disc having a bright "surround". This was done under three different stereo disparity conditions: 1) discs in same stereo plane as surrounds 2) discs behind surrounds 3) discs in front of surrounds. As expected from simultaneous contrast induction, subjects consistently adjusted the brightness of the test disc to a much lower luminance than the reference disc in all three conditions. The adjusted luminance values were smallest, however, when the discs were placed **behind** the surrounds. This attenuation of the matched luminance value is consistent with viewing an object placed behind a dark transparent surface. These findings suggest that knowledge about the depth ordering of surfaces and transparency contribute to perceptual brightness. They provide further demonstration of the insufficiency of lateral inhibitory mechanisms as a full explanation of brightness constancy.

46.10

VISUAL SEARCH IN THE PRESENCE OF CONFLICTING DEPTH CUES. V.S. Ramachandran, D.J. Plummer, and H. Pashler. Psychology Department, UCSD, La Jolla, CA 92093.

How are different "depth cues" such as perspective, stereopsis, shading, and occlusion combined in the brain?

We constructed a stereogram composed of 8 pairs of squares. One member of each pair partially occluded the other. All the squares were at zero disparity except for one member of a pair that was stereoscopically nearer than the others. This square was easier to detect when the two cues (occlusion and disparity) were consistent with each other than when the two cues were in conflict. When the cues were in conflict, the occlusion vetoes the stereopsis and makes the target difficult to locate.

Next, we had an identical stereogram which had eight square-pairs but no occlusion cues. In this situation the stereoscopically nearer target square was even easier to detect than when both stereo and occlusion were present (and consistent). To explain these findings we postulate that different depth cues converge on to a common "depth-cue" invariant feature map in which cells respond to relative depth but are indifferent to the actual cue that is used. Such a map would report that each member of a square-pair is in front as a result of occlusion. If the stereo-cue is "redundant" (i.e. consistent) the target does not pop out as easily as when the stereo cue alone is present.

Lastly, we created a stereogram composed of 8 squares. The target square was either smaller or larger than the distractors and was also either stereoscopically nearer or further. It was easier to detect when smaller and nearer (or larger and further). We conclude that "pop-out" occurs after size-constancy correction; it is not based on retinal size alone.

We conclude that visual search and "pop-out" occur in a common "depth-cue invariant" feature map and not at an earlier stage where different cues are represented separately. We thank AFOSR for support.

46.11

AUDITORY-VISUAL INTERSENSORY TEMPORAL RESOLUTION IN HUMAN SUBJECTS. L. J. Achor. Dept. of Psychology, Baylor Univ., Waco, TX 76798.

Auditory-visual intersensory temporal resolution (i.e., the ability to discriminate whether a mixed pair of auditory and visual stimuli is simultaneous as opposed to successive) was investigated in human subjects by determining the intersensory fusion threshold (i.e., the point at which subjects judge successively presented stimuli to be simultaneous 50% of the time).

A double staircase variation of the method of limits was used to determine the intersensory fusion threshold, with 20 stimulus pairs presented from each of the two staircases. 120 young adults were presented stimuli in two conditions: (1) flash followed by a tone and (2) tone followed by a flash. Half of the subjects received the tone-delayed condition first and half received the flash-delayed condition first. Thresholds were obtained for stimulus trials 21-30 (Block 3) and 31-40 (Block 4). Blocks in which a subject made a judgment error on catch trials of 0 or 500 msec temporal separation were excluded from the data analysis.

There was no significant difference in the intersensory fusion thresholds between Blocks 3 and 4 for either the tone-delayed or flash-delayed condition. There was a significant order effect for tone-delayed vs flash-delayed and a significant difference between tone-delayed and flash-delayed conditions.

The intersensory fusion thresholds determined in this study provide an indication of intersensory processing ability. The thresholds will provide normative data for assessing developmental changes and they may also be useful in the evaluation of individuals with perceptual disorders.

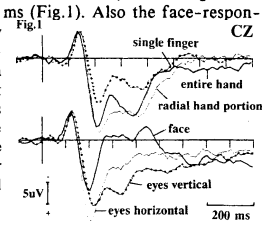
46.12

FACES, HANDS AND OTHER BODY PARTS EVOKE DIFFERENT CATEGORY-RELATED VISUAL RESPONSES IN THE ELECTRO-ENCEPHALOGRAM, Margitta Seeck*, O.-J. Grüsser, Elke Heusser*, W. Seidler*. Dept. Physiol. Freie Univ. 1 Berlin 33, Germany (SPON: European Brain and Behaviour Society)

In the present study we investigated how evoked potential (EP) components related to hands and faces as described in earlier reports change when photographs of parts of hands or faces are used as stimuli. In the first paradigm, slides of black and white photographs of the whole hand, its radial or ulnar half, as well as single fingers were used as stimuli (EEG recordings from T3, T4, T5, T6, Cz, Oz, reference linked mastoids, 8 female, 8 male subjects). In the second paradigm EPs to b/w photographs of the whole face, the face without eyes and both eyes only, positioned horizontally or vertically, were studied (EEG recordings from F3, F4, T5, T6, Cz, Oz, same reference, 9 female, 7 male subjects).

A hand-responsive component was confirmed, consisting of two positive peaks at about 200 and 370 ms (Fig.1). Also the face-responsive component (distinct positivity at 200 ms and rapid consecutive negativity) was found. All EPs have a pronounced positive peak at about 200 ms when different body parts are used as stimuli. The more the hand or face was "mutilated", the less pronounced was the hand- or face-responsive component beyond 200 ms. (DFG Gr 161, S520).

Fig.1

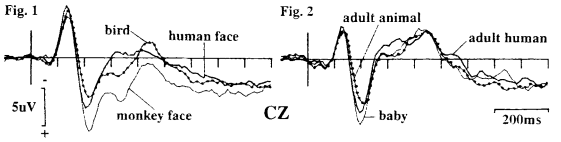


46.13

CATEGORY-SPECIFIC EVOKED POTENTIALS RELATED TO PICTURES OF FACES OF MAN AND ANIMALS. O.-J. Grüsser, L. Fuhry*, M. Seeck*, W. Seidler*. Dept. Physiol. Freie Univ. Berlin (FRG).

In primate studies particular components in epidural monkey-EEGs (EPs) related to pictures of monkey and human faces were found. With the present studies the reverse test was performed: b/w-photographs of monkey, other animal faces and human faces to humans. Differences in the EPs could also be found when pictures of adult humans, babies, adult animals and "baby-animals" of various species were shown (electrodes F3, F4, T5, T6, Cz, Oz, intern. 10/20-recording system, linked mastoids, 16 subjects).

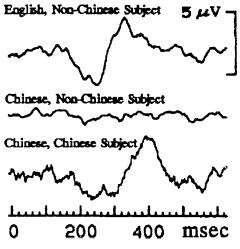
Category-specific differences were most pronounced in the frontal electrodes and Cz. Faces of animals and human babies led to a prominent negative peak at 400-450 ms. The decreasing similarity between the animal and human species was reflected in the averaged EP between 200 and 400 ms. Comparing adult/baby faces, only differences between *human* adult and baby faces could be found (larger positive peak at 200 ms in the category "human baby"). (DFG Gr 161, S520)



46.14

THE RECOGNITION POTENTIAL: A VISUAL RESPONSE EVOKED BY RECOGNIZABLE IMAGES. A. P. Rudell. Dept. of Physiology, SUNY, Health Science Center at Brooklyn, Brooklyn, NY 11203.

The recognition potential (RP) occurs when a subject views a recognizable image. Words, cartoon faces, simple pictures, and geometric patterns evoked RP. Controls (mozaics of small pieces of recognizable images) or words in languages unfamiliar to a subject (Arabic or Chinese) did not. Chinese ideographs did evoke RP for subjects whose native language was Chinese. Fig. 1 shows responses to English or Chinese minus responses to control stimuli. RP had shorter latency than P300 and was less sensitive than P300 to novelty. P300, but not RP, could be recorded from a lead on the vertex, referenced to the forehead. RP was largest for occipital leads when the images were displayed in the opposite visual field. RP for steadily presented recognizable images was equivalent to RP when they were intermixed with controls. Reaction time (RT) and RP latency increased in equal amounts with increasing degradation of the images. RP latency was about 150 msec less than RT. The results imply that RP is not caused by differences in the physical attributes of the stimuli or an emotional, P300-like response. It does depend on a subject's perception and learning experience.



46.15

OPTOKINETIC NYSTAGMUS (OKN), ACCOMMODATION, AND BINOCULAR ALIGNMENT IN NATURALLY STRABISMIC MONKEYS. M. W. Quick*, J. Newbern*, L. Place* and R. G. Boothe. Dept. of Psychology, Ophthalmology, and Yerkes Regional Primate Research Center, Emory University, Atlanta GA 30322.

A photographic corneal light reflex method was used to assess eye alignment in 10 monkeys with naturally occurring strabismus. Assessments were made at 35 target locations throughout the monkey's visual field. Two major findings were: (1) Most monkeys showed nasally directed alignment errors (esotropia); (2) 1/2 of the monkeys showed deficits associated with an accommodative component.

To further examine (1), contrast thresholds for eliciting OKN were measured for nasally and temporally directed drifting stripes. Asymmetric OKN was present in all the strabismic monkeys. This asymmetry was due to abnormally high thresholds for temporal motion. The nasal OKN preference correlates with the nasal alignment deficits and supports the theory that motion asymmetry may play a role in esotropia (Tychsen & Lisberger, J. Neurosci., 1986).

To further examine (2), infrared photorefraction was used to assess accommodative errors at four viewing distances. Two major types of errors relating to the eye alignment deficits were noted during binocular viewing. First, several monkeys exhibited direct accommodative errors in one eye for some fixation targets. Second, some eye alignment errors were consistent with abnormally high AC/A ratios. Supported by NIH RR-00165.

46.16

LONG TERM MD CAT'S DEPRIVED EYE SHOWS A RAPID VISUAL SENSITIVITY INCREASE AFTER EYE LID OPENING. **Z. He* and M. S. Loop Physiological Optics, Sch. of Optometry, Univ. Alabama at B'ham., Birmingham, AL 35294**

It is well known that, within classic critical period, visual experience plays a very important role for the development of mammalian visual system. Here, we ask if there is any effect of visual stimulation on the long term (beyond critical period) monocularly deprived (MD) eye. To answer this question, we measured, for three MD cats', increment thresholds (16° yellow test light on 30 cd/m^2 white background) after opening the eye lid which had been closed for more than 12 months since birth.

Before deprived eye opening, cat Hwa had already learned the two choice task, cats Li and Shou the reaction time task. After Hwa's deprived eye saw normal light for 6 days, sensitivity increased rapidly till day 16 (about 2 log units) followed by a small amount of increase later on. The sensitivities of cats Li and Shou could be obtained after days 9 and 11 of deprived eye open, respectively, and showed a similar sensitivity improvement. However, the reaction time vs subjective intensity (threshold defined) curves of deprived eye showed little change. Compared to the fellow normal eye, the reaction time in deprived eye is not faster at same subjective intensity, which indicates that the abnormally rapid signal transmission by MD LGN Y-cells (Sestokas & Lehmkuhle, 1986) does not translate simply into faster perceptual latencies. This visual sensitivity improvement in long term deprived eye immediately after the eye lid opening may suggest an uncovered neural synaptic mechanism for neurobiologists to explore. (Supported by NIH grant EY 07324)

46.17

BLINDSIGHT: SPATIAL SUMMATION IN VISUAL FIELD DEFECTS. Petra Stoerig, (SPON: European Neuroscience Association) Institute of Medical Psychology, Ludwig-Maximilians-University, Goethestr.32, 8000 Munich 2, FRGermany

The residual visual functions that may be observed in dense visual field defects caused by striate cortical damage (Blindsight) can be used to study the functional properties of the remaining visual pathways. As pathways can be classified by their spatial summation for wavelength and intensity information, increment thresholds were measured as a function of stimulus size in the circumscribed visual field defects of five patients with post-geniculate damage. Circular red and white stimuli, from 7-116' in diameter, were presented on a white photopic background. At a Tübinger perimeter, the patients fixated a central spot and guessed whether or not a target (200ms) that appeared in random alternation with blank trials had been presented. Target luminance was increased in steps of 0.1 log units until the detection became statistically significant. Spatial summation curves measured with the white targets show a marked dip whose position depends on retinal eccentricity, and indicates a change in summation mode. In contrast, curves for the red targets show a smooth increase in sensitivity with stimulus size. Colour-opponent Type I ganglion and LGN cells show a combination of antagonistic summation for intensity and synergistic summation for wavelength information that could explain this result. (Supported by a grant from the DFG (Po121-13/4))

46.18

VISUO MOTOR DISTURBANCES IN DYSLEXIC PATIENTS. RE HABILITATION BY ANIMATED COMPUTER PROGRAMS. I.Zarco de Coronado, A. Gutiérrez López*. Departamento de Fisiología, Facultad de Medicina. AP. P. 70250 U.N.A.M. and Insto. Nal. de la Comunicación Humana. C.P. 01480 México.

Altered visuo motor coordination in dyslexic patients make them disable readers. In order to correct learning problems their training programs must help the patients to mecanizate the procedures involved in the focusing of visual attention and spatial control. The velocity and diversity of stimuli generated by the computer make it a useful instrument for this kind of programs.

It was developed a animated computer program which is going to be used with mexican dyslexic children at the National Institute of the Human Comunication. This program is fully automated and it is based on standard rehabilitation methods and also evaluate and store the results of each session. The difficulty of the next exercise depends of the every day qualification.

46.19

VISUAL PERSISTENCE IS IMPAIRED IN PATIENTS WITH COMPRESSION OF THE OPTIC CHIASMA C.A. Marzi[*1], M.Girelli[*1], G.Tassinari[*1], L. Cristofori[*2], A. Talacchi[*2], G. Marchini[3], M. Gentilin[3] (Spon.: European Neuroscience Association). [1] Inst. of Physiol. [2] Dept. of Neurosurg. [3] Inst. of Ophthalmol., Univ. of Verona, Italy.

Optic fibers originating from retinal ganglion cells of different classes may be selectively affected as a result of compression and stretching due to tumors of the chiasmatic region. To verify this possibility we have studied 8 patients suffering from pituitary adenomas as assessed by NMR. We tested both speed and accuracy in naming digits monocularly presented at 5° of eccentricity in either hemifield. Each of the four digits (2,5,6,9) was randomly split in two portions and each half was flashed for 4 msec, with an interstimulus interval (ISI) of 15,30,45 or 60 msec. Recognition depended on the visual persistence of the first portion and its integration with the second portion. 12 naive subjects of similar age as the patients served as controls. The vocal reaction time of patients was significantly slowed at the longest ISI in the nasal field of both eyes. This result suggests an impairment of beta cell axons, whose sustained pattern of response should allow temporal integration at long ISIs. Recent anatomical data on a monkey with a similar tumoral compression are in agreement with this interpretation (Reese and Cowey, Clinical Vision Science, 4: 341-356, 1989).

46.20

DETECTION AND RECOGNITION THRESHOLDS FOR SPATIALLY DEGRADED IMAGES BASED ON LASER RETINAL INJURY. Zwick, H.*, Robbins, D.O., Mastrianni, G.*, and Monroe, D.* Division of Ocular Hazards, Letterman Army Institute of Research, Presidio of San Francisco, CA. 94129

In previous work, we showed that the rhesus contrast sensitivity function following acute small spot laser exposure was suppressed over a broad range of spatial frequencies. We observed such effects over a wide range of exposure intensities ranging from levels that produced only transient effects to levels that also produced permanent morphological changes in the retina. Long term changes in contrast sensitivity following punctate foveal damage revealed a steepening of the slope of these contrast sensitivity functions suggesting parafoveal compensation for foveal damage. Changes in contrast sensitivity have often been suggested to reflect changes in suprathreshold spatial vision. To evaluate this notion, we have measured human stationary and temporally modulated detection and recognition thresholds for suprathreshold images that have been spatially filtered to reflect the type of contrast sensitivity deficits measured in our exposed animals. A microcomputer system from Delta Technologies was used to fourier filter computerized images and to visually display such images under increasing contrast conditions. Image detection and recognition thresholds for these filtered images were consistent with changes in contrast sensitivity measured in our animal experiments. Differences between image conditions reflect suppressive as well as enhancement effects of exposure, suggesting that spatial contrast threshold mechanisms are relevant to suprathreshold detection and recognition mechanisms.

46.21

ATTENTION: ACETYLCHOLINE, NOREPINEPHRINE AND THE FRONTAL CORTEX. K. Pang, D.S. Olton and H. Egeth. Dept. of Psychology, Johns Hopkins Univ., Baltimore, MD 21218.

Attention is the mechanism that allows expected stimuli to be processed more effectively than unexpected stimuli. A two-choice reaction time task was used to assess attention in rats. Attention was manipulated by varying the relative probability of two stimuli, light and tone; each stimulus required a different response. On each trial, a single stimulus was presented. The rat responded by releasing the appropriate lever. As the percentage of visual stimuli increased, the reaction time to the light decreased and the bias to respond to the lever indicating light increased. As the percentage of auditory stimuli increased, the reaction time to the tone remained constant and the bias to respond to the lever indicating tone increased. Stimulus detectability (d') remained constant for all stimulus ratios. Thus, attention was shifted to the more probable stimulus and away from the less probable stimulus, which produced a shift in response bias without a change in stimulus detectability. When light was the expected stimulus, the reaction time to the visual stimulus was decreased. The ability to allocate attention between two stimuli is currently being examined following administration of selective cholinergic and noradrenergic drugs and also following frontal cortical lesions. (Supported by AFOSR-89-0481 and PHS NS08358).

46.22

VISUAL ACUITY FOLLOWING CHIASMATECTOMY IN UNILATERAL ESOTROPIC CATS. P. Bouchard* M. Ptito, F. Lepore, S. Ouessy* and J.P. Guillemot, Dép. de psychologie, Université de Montréal et Dép. de kinanthropologie, UQAM.

Unilateral esotropia surgically induced early after birth leads to amblyopia of the deviated eye (DE). This effect is usually explained in terms of binocular competition and suppression at the cortical level. In order to attenuate this competition and hence decrease the influence of the normal eye (NE) over DE, we sectioned the optic chiasma (OC) in kittens rendered strabismic soon after birth. For purposes of comparison, we also used normal cats and kittens with either unilateral esotropia or chiasmatectomy. Visual acuity (VA) was measured behaviorally using a two-choice discrimination procedure on a jumping stand. Stimuli were square-wave gratings of various spatial frequencies and thresholds were derived using two-methods: constant stimuli and staircase procedure. In normal cats, the average threshold value for monocular viewing was identical for each eye (5 cyc/deg.). In OC cats, VA was found to be lower than that of the normal cats by about a factor of 2 (2.5 cyc/deg.). In strabismic cats, VA values obtained for DE were quite low (mean=.9 cyc/deg.) whereas for NE they reached an average 3.6 cyc/deg. In strabismic cats with an OC section, VA values for DE were about .32 cyc/deg. and for NE about 2.0 cyc/deg. The results suggest that chiasmatectomy performed on otherwise normal kittens results in a lower visual acuity for each eye as previously reported by Timney and Landsdown, 1987. However, it dramatically reduces VA of both DE (which is actually almost blind) and NE (which is about two times worse than NE of ESO cats and three times worse than normal cats). the section of the optic chiasma does not seem to suppress binocular competition and has on the contrary a detrimental effect on both NE and DE.

46.23

BILATERAL ABLATION OF THE FOREBRAIN ENHANCES OPTOMOTOR RESPONSES IN GOLDFISH. M.G. Yoon. Dept. of Psychology Dalhousie University, Halifax, N. S. Canada B3H 4J1

The topographic pattern of visual projection from the eye to the brain in the goldfish can be experimentally induced to undergo two types of neural reorganizations: 1)Field Compression following a partial excision of the optic tectal lobe, and 2) Retention of the Original Topographic Polarity by tectal tissue following reimplantation after 180° rotation. We study the pattern of changes in the visuo-motor behaviors of the goldfish following the two types of microsurgical manipulations of the visual system (which result in the neural reorganizations of the afferent retinotectal pathways) by various behavioral tests. One of the innate visual behaviours we use for the tests is the optomotor response. In normal goldfish, we could hardly elicit a reliable optomotor response in tracking a single rotating bar. Two weeks after bilateral ablation of the forebrain in the goldfish, however, we find that the operated fish tend to follow the single rotating visual target with remarkably enhanced consistency. Thus, the forebrain seems to exert a distracting influence on the optomotor response generating mechanism in the normal goldfish.

SENSORY SYSTEMS—SUBCORTICAL VISUAL PATHWAYS: SUPERIOR COLLICULUS AND RELATED

47.1

COLLICULAR MAGNIFICATION IS NOT SCALED TO THE INTRARETINAL DENSITY OF AFFERENT GANGLION CELLS. D.M. Berson and J.J. Stein*. Div. Biology & Medicine, Brown Univ., Providence, RI, 02912

Magnification in central visual maps is usually thought to be proportional to the intraretinal density of afferent ganglion cells. To learn if this is true for the cat's retinocollicular projection, we made local comparisons between the densities of tectally projecting ganglion cells and areal magnification in the superior colliculus (SC) at matching retinotopic locations. Collicular magnification factors were estimated from McIlwain's map of the SC (Vision Res. 23:507, '83). Densities of afferent ganglion cells were determined from retrograde labeling patterns after deposits of fluorescent microspheres confined to the contralateral SC (n=4). For selected retinotopic sectors, we calculated the ratio of collicular areal magnification to the intraretinal density of tectopetal ganglion cells. If SC magnification were scaled to ganglion cell density, these ratios should have remained constant over the map. Instead, they varied markedly, being three to five fold higher for the area centralis than for the periphery. (Incorporating the relatively small numbers of ipsilaterally projecting retinotectal ganglion cells into our analysis would not have substantially affected this conclusion). The observed topographic variations imply that incoming retinal axons are more widely spaced in the area-centralis representation than elsewhere in the SC. This may account for anatomical and physiological evidence for topographic variations in the density of retinotectal input (see Berson et al., Exp. Brain Res. 79:459, '90). Our findings suggest that collicular magnification is specified by some mechanism other than one simply related to the density of retinal ganglion cells. The expansion of the area-centralis representation beyond what would result from peripheral scaling alone could enhance stimulus detection or localization near the center of gaze.

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47.2

ANTIBODIES TO CALBINDIN LABEL DISCRETE SUBPOPULATIONS OF INTERNEURONS IN THE CAT SUPERIOR COLLICULUS. R.R. Mize[1], C.J. Jeon[1], G.D. Butler[1], and P.C. Emson[2], [1]Dept. of Anatomy and Neurobiology, Univ. of Tennessee Health Science Center, Memphis, TN, 38163 and [2]AFRC Inst. of Animal Physiol. Cambridge, UK.

Calbindin is a calcium binding protein thought to play a role in regulating intracellular calcium. We have localized calbindin (CaBP) in the cat superior colliculus (SC) using antibody immunocytochemistry. Anti-CaBP labeled neurons were found in three distinct bands, one within the upper one-half of the superficial gray layer (SGL) , another bridging the deep optic (OL) and intermediate gray layers (IGL) and a third in the deep gray layer (DGL) where the cells sometimes formed vertically oriented patches. 48.5% of the anti-CaBP labeled cells were found in the SGL, 43.7% in OL and IGL, and 7.8% in the deepest layers of SC. Most anti-CaBP positive neurons were small (mean average diameter = 12.0 μm), but varied in morphology. Labeled neurons within the SGL had horizontal, pyriform, or stellate morphologies. Many of the labeled neurons in the OL-IGL band were stellate-like with highly varicose dendrites and broad dendritic trees. A few very large neurons within the DGL were lightly labeled. Smaller labeled neurons were also found throughout the deep layers.

Retrograde transport studies demonstrated that all but a small fraction of anti-CaBP labeled neurons were interneurons. No anti-CaBP positive neurons were retrogradely labeled after HRP injections into the predorsal bundle, dorsolateral midbrain, or lateral posterior nucleus. Only 2.4% of anti-CaBP neurons were labeled after HRP injections into the dorsal and ventral lateral geniculate nuclei. Double-labeling with calbindin and GABA antibodies showed that some but not all calbindin neurons also contained GABA. Supported by NEI Grant EY07973

47.3

ANTI-CALBINDIN LABELING IN THE LATERAL GENICULATE NUCLEUS OF THE RHESUS MONKEY AND ITS REDUCTION BY ENUCLEATION. Q. Luo[1], R.R. Mize[1], and M. Tigges[2], [1]Dept. Anatomy and Neurobiology Univ. of Tennessee Health Sci. Ctr., Memphis, TN, 38163 and [2]Yerkes Regional Primate Research Center, Emory University, Atlanta, GA 30322.

Calbindin 28Kd, a calcium binding protein, is found in selected neurons in the CNS where it is thought to regulate intracellular calcium. We have studied the distribution, density, and morphology of cells labeled by antibodies to calbindin in the lateral geniculate nucleus (LGN) of the Rhesus monkey. The effects of monocular deprivation on antibody labeling were also studied. One eye was enucleated 2 weeks prior to sacrifice in 6 monkeys, 3 of which were also monocularly occluded from birth. Another 3 animals were occluded without enucleation. In some cases, the lens of the opposite eye was removed and the aphakia corrected with a contact lens.

Anti-calbindin positive neurons were found throughout the LGN. They were densely distributed within the intralaminar zones (ILZ) and layer S and more sparsely distributed within all 6 main laminae, a pattern complementary to that of GABA-labeled neurons. Calbindin neurons had small somas and elaborate dendritic trees. Dendrites of cells in the main laminae never crossed laminar borders while those in ILZ did cross those borders.

Monocular enucleation with or without occlusion produced a significant reduction in antibody labeling in the deafferented laminae. Field measures of optical density made with an image analyzer revealed an average 12.6% reduction in optical density in each deafferented lamina compared to its adjacent layer. Calbindin neurons were still present but their labeling was sometimes reduced. Occlusion without enucleation had no effect. Thus, deafferentation but not light deprivation reduces concentrations of calbindin. Supported by NEI EY-02973, EY-05975 and EY-RR00165.

47.4

EFFERENT CELL CLUSTERS WITHIN THE CHOLINERGIC PATCHES IN THE INTERMEDIATE GRAY LAYER OF THE CAT SUPERIOR COLLICULUS. C.J. Jeon and R.R. Mize, Dept. of Anatomy and Neurobiology, Coll. of Med., Univ. of Tennessee, Memphis, The Health Sci. Ctr., Memphis, TN, 38163.

Several cortical and subcortical afferent projections to the superior colliculus (SC) have patch-like terminations in specific laminae. One of these is a cholinergic input which forms distinct patches in the dorsal intermediate gray layer (IGL) and is thought to arise from the pedunculopontine tegmental nucleus (PPTN). In this study, we have examined the relationship of these cholinergic patches to specfic efferent cell groups in the cat SC using combined retrograde transport of horseradish peroxidase (HRP) and choline acetyltransferase (ChAT) immunocytochemistry. HRP injections were made into the medial predorsal bundle (PB), the lateral tecto-pontine-bulbar pathway (TPB), and/or the PPTN in 7 adult cats. The labeled cell distributions were mapped using a computer-based microscope plotter. Distinct clusters of labeled cells were seen after two small injections which involved both the PB and TPB. Four to seven clusters were found in each section through the caudal one-half of SC. No clusters were found in more rostral sections. The cell clusters were more frequent in the medial than in the lateral portion of IGL. Most clusters consisted of 7-15 cells, all of which were small to medium size (12.2-27.1 μm in average diameter). No very large predorsal bundle cells were ever labeled within the clusters. In sections combining HRP and anti-ChAT labeling, the cell clusters were found to precisely overlap the cholinergic patches in the IGL. These cell clusters were not found after injections confined to PPTN and TPB where HRP backfilled cells were for the most part located outside of the ChAT patches. These results suggest that specific cell groups, distinguished by size and projection site, form modules that match the patch-like innervation of cholinergic afferents to SC. Supported by EY-02973.

47.5

ANTIBODIES TO CALBINDIN DELINEATE RETINAL PROJECTION ZONES WITHIN THE PRETECTUM. L. Burt Nabors and R. Ranney Mize. Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, TN 38163

The pretectum is an important visual reflex center. However, the location, boundaries, and connections of individual nuclei of the pretectum are poorly understood. Traditionally the pretectum has been divided into 5-7 component nuclei based on cell size and shape, three of which receive retinal input. This retinal input has recently been described as forming 4 projection zones running through the rostro-caudal extent of the region from medial to lateral. The relation of these zones to individual pretectal nuclei remains unclear.

We have used an antibody against the calcium binding protein, calbindin 28kd, to study the relationship between pretectal cells and the retinal projection zones in cat. Four cell clusters were labeled by calbindin within the pretectum. Reconstruction of these cell clusters also revealed zones which run the rostro-caudal length of the pretectum from medial to lateral. By combining anterograde HRP labeling of retinal terminals with calbindin immunocytochemistry, these calbindin positive cell clusters were found to overlap precisely the retinal projection zones. A percentage of these calbindin neurons were projection neurons back-filled by HRP injections into the lateral geniculate nucleus. They varied in morphology, but most were medium to large neurons. Small GABA immunoreactive cells were also found within these zones but no GABAergic cells were retrogradely labeled following LGN injections and very few colocalized calbindin.

In summary, calbindin is a precise marker of cell clusters in the pretectum which overlap the retinal projection zones. Many of these cells are large, non-GABAergic projection neurons whose target is the LGN. Whether all of the calbindin positive cells receive direct retinal input remains to be determined. Supported by NEI grant EY-02973 and an HHMI MSRF.

47.6

SENSORY-TRIGGERED OSCILLATORY ACTIVITY IN THE AVIAN TECTUM. S.Neuenschwander* and F.J.Varela
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We have recorded from tectal neurons in the optic tectum of awake pigeons whose receptive field was stimulated by a moving bar in sequences of 10 identical trials of 0.5-1.0 sec. These responses were processed to match the treatment reported for oscillatory activity in the cat's visual cortex[1,2]. Local field potential (LFP), obtained by digitally filtering the responses between 1-100 Hz, displayed oscillatory activity shortly after the stimulation began (50-100 msec). The peak frequency varied from trial to trial between 25-45 Hz (average of 30), and it was also visible in the power spectra obtained by the FFT of the LFP, as well as from their respective autocorrelograms. The average LFP preceding and following 50 msec every spike in a trial showed that spikes covary with the negative going phase of the oscillation. These oscillations were seen in both single and multiple activity and at various tectal depth. We conclude that in all these respects the tectal oscillatory activity matches remarkably well that of visual neocortex. This strengths the universality of oscillatory activity as possibly functional mechanism in the formation of neuronal assemblies, and shows that a model for it cannot rely on neocortical features such as columnarity.

[1]Gray et al. (1989) *Proc. Natl. Acad. Sci. USA* **86**:1698-1702.
[2]Eckhorn et al. (1988) *Biol. Cybern.* **60**:121-130.

47.7

HABITUATION FUNCTIONS OF MULTISENSORY NEURONS IN THE AMPHIBIAN TECTUM. M.M. Nikoletseas. Department of Anatomy, School of Medicine, University of Puerto Rico, San Juan, PR 00936

Multisensory tectal neurons integrate information from diverse sensory modalities by such processes as excitation, inhibition and habituation. In the present study quantitative analysis of the habituation of these cells was undertaken. Animals were anesthetized and paralyzed. Multimodal cells were identified and subsequently a single or a multimodal stimulus was presented repeatedly. Extracellular signals were amplified, and analyzed on-line. Spurious signals were excluded by a window discriminator and waveshape recognition filters, and histograms were constructed for immediate visualization of the habituation functions. Multisensory cells showed steeper functions when unimodal stimuli were presented. In addition a variety of firing as well as habituation functions were obtained, not allowing for easy categorization of cells. These data suggest that the known intrinsic plasticity of tectal cells is modulated not only by the various parameters of afferent stimuli but also by the presence or absence of one component of the afferent multimodal stimulus.

47.8

ALTERATION OF SENSORY RESPONSIVITY IN RAT SUPERIOR COLLICULAR NEURONS DURING STIMULATION OF THE STRIATUM OR SEPTAL NUCLEUS. J. L. Petrie* and D. A. Weldon. Dept. of Psychology, Hamilton College, Clinton, NY 13323.

Excitation of neurons in the caudate-putamen causes tectospinal/tecto-diencephalic cells to discharge vigorously. This effect has been attributed to inhibition of the GABA-ergic nigrotectal system by striato-nigral cells and suggests a possible mechanism by which orientation movements are elicited from the striatum (Chevalier et al., Brain Res. 334:215, 1985). To determine whether sensory functions of the superior colliculus (SC) are modulated by activity in the striatum, we investigated responsivity in SC neurons in urethane-anesthetized Long-Evans rats to a 300 msec flash of light or burst of white noise during electrical stimulation of the ipsilateral caudate-putamen (0.1 msec duration square wave pulses of 100μA for 100 msec at 100 Hz). We also studied the effects of stimulation of the septal nucleus on SC sensory responses. Responsivity, as measured by the number of action potentials elicited by the effective sensory stimulus, was modified in 26% of the cells by striatal stimulation and in 11% of the cells by septal stimulation. Responsivity increased in 71% and 83% of the neurons modified by striatal and septal stimulation, respectively. The results suggest a significant influence of the striatum on sensory processing in the SC.

47.9

NEURONS PROJECTING FROM THE SUPERIOR COLLICULUS TO THE PRINCIPAL TECTORECIPIENT ZONE OF THE LATERAL POSTERIOR-PULVINAR COMPLEX ARE IMMUNOREACTIVE FOR SUBSTANCE P. Jeffrey I. Hutsler and Leo M. Chalupa. Dept. of Psychology, Neurobiology and Physiology Graduate Groups, University of California, Davis, CA 95616.

Neurons within the superficial layers of the cat's superior colliculus (SC) have been shown to project to multiple visual areas of the extrageniculate thalamus (Abramson and Chalupa, 1988). We have sought to identify subpopulations of these projection neurons based upon their peptide content as demonstrated by immunocytochemical techniques. Substance P or tachykinin-related immunoreactivity has been identified in a morphologically diverse group of cells within the superficial layers, including triangular, bipolar, granular, and some pyramidal neurons. Substantial proportions of these immunoreactive cells were demonstrated to be projection neurons based upon their labeling with rhodamine latex beads following injections of this tracer into the lateral posterior-pulvinar (LP-Pul) complex. A terminal zone of substance P immunoreactivity was also observed in a discrete region in the medial portion of the LP-Pul. When adjacent thalamic sections processed for acetylthiocholinesterase staining were examined, substance P label was found to be localized within the heavily stained cholinesterase region, which has been shown to be the principal tectorecipient area of the LP-Pul complex (Graybiel and Berson, 1980). The thalamic labeling decreased markedly in animals that sustained unilateral lesions of the SC, demonstrating the collicular origin of the substance P immunoreactivity. These results suggest that substance P is involved in conveying visual information from the superficial layers of the SC to the principal tectorecipient zone of the cat's LP-Pul complex.

47.10

EFFECTS OF INACTIVATION OF THE SUPERFICIAL LAMINAE UPON THE VISUAL RESPONSIVITY OF DEEP LAYER NEURONS IN THE HAMSTER'S SUPERIOR COLLICULUS. R.D. Mooney, X. Huang, and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699.

Visual cells in the superficial superior collicular (SC) layers innervate the deep laminae. As yet, however, nothing is known about functions of this projection. We used micropressure ejection of $CoCl_2$ to reversibly block synaptic activity in portions of the superficial laminae and assessed the effects of this blockade upon the responses of deep layer cells to visual and optic chiasm (OX) stimulation. All deep layer neurons tested had receptive fields in topographic register with the part of the superficial laminae silenced by $CoCl_2$ injection. Of 28 deep layer cells tested, 21 had their visual and/or OX responses reduced by at least 50% during inactivation of the superficial laminae; the remaining 7 cells were not affected. Five cells with responses that were suppressed by $CoCl_2$ injections were filled with HRP. All had cell bodies in the stratum griseum intermediale (SGI), 3 had dendrites that ascended into the stratum griseum superficiale, one had dendrites extending into the stratum opticum (SO), and the third had dendrites that reached the SGI-SO border. We recovered one cell that was not affected by $CoCl_2$ ejection. It was a widefield vertical cell whose soma was located at the SO-SGI border. Input from the superficial laminae is thus important for the visual responsivity of many deep layer neurons in the hamster's SC. Supported by EY 08015 and EY 04170.

47.11

EFFECTS OF CORTICAL AND NIGRAL DEACTIVATION ON VISUAL NEURONS IN CAT SUPERIOR COLLICULUS.

D.D. Dunning, B.E. Stein and J.G. McHaffie. Department of Physiology, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA, 23298.

Deactivation (cooling) of posterior aspects of lateral suprasylvian cortex (PMLS, PLLS, DLS & VLS) decreased the visually-evoked responses of deep laminae superior colliculus neurons as previously reported (Ogasawara et al., J. Neurophysiol., 52:1226, 1984). The present experiments also demonstrated that some of the neurons affected by suprasylvian cortex also send descending axons into the brainstem, thereby providing the first direct evidence that cortex controls the activity of output neurons and, by inference, visual orientation behaviors mediated by superior colliculus. This is consistent with previous experiments in behaving animals (Hardy and Stein, J. Comp. Neurol., 273:527, 1988).

Deactivation (microinjected GABA) of substantia nigra pars reticulata and pars lateralis dramatically enhanced the visually-evoked activity of superior colliculus neurons but had little affect on their spontaneous activity (reflecting an unexpectedly phasic nature of nigrocollicular influence). Furthermore, despite anatomical studies indicating that the nigrocollicular projection is overwhelmingly ipsilateral, substantia nigra deactivation proved to have bilateral physiological effects. In addition, and perhaps most surprising, superficial as well as deep laminae superior colliculus neurons were modulated by the basal ganglia. Generally, the effects of deactivating cortex and substantia nigra offset one another and indicated that the balance of these inputs is critical for normal deep laminae visual activity.

Supported by NIH grant EY06562.

47.12

DISTRIBUTION OF CORTICOTECTAL CELLS IN THE SUPERIOR TEMPORAL SULCUS OF THE MACAQUE.

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The superior temporal sulcus (STS) contains a number of visual areas, including V4t, MT, MST, and STP; areas V4, TEO, and TE also extend into STS. The superior colliculus (SC) receives widespread inputs from visual cortex. However, the distribution of corticotectal cells in the different functional zones of STS is not known. We plotted the location of labeled cells in STS following injections of retrograde fluorescent tracers into SC. There were clear differences in the distribution of corticotectal cells within STS. When the injection included superficial and deep layers of SC, much of the STS contained labeled cells. Labeled cells were densely distributed through the posterior half of the lower bank of the sulcus, (areas V4, V4t, MT and MST). In the anterior half of the lower bank, the density of labeled cells decreased, so that the most anterior portions (area TE) were free of labeled cells. In the posterior half of the upper bank of STS, another area free of labeled cells extended into the densely myelinated part of MST, overlapping the region where pursuit cells have been found (Komatsu and Wurtz, 1988). Anteriorly, in area STP, labeled cells were present to the tip of STS. With an injection restricted to superficial layers, label in STS was much sparser, and confined to the lower bank. These results suggest that visual areas of the STS differ in their contributions to the corticotectal system. (Supported by EY02254, MH4213 and The Whitehall Foundation.)

47.13

SUBCORTICAL CONNECTIONS OF INFERIOR TEMPORAL CORTEX IN SQUIRREL MONKEYS.

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Patterns of cortical connections have suggested that IT cortex (area TE) of squirrel monkeys (Saimiri sciureus) contains caudal (IT_C) and rostral (IT_R) subdivisions (Weller et al., Soc. Neurosci. Abst., 15: 1108, 1989). Injections of the neuroanatomical tracers wheat germ agglutinin conjugated to horseradish peroxidase, ^{3}H-proline and leucine, Fast Blue, Fluoro-Gold, and Diamidino Yellow were made under sterile conditions in IT_C and IT_R. Both IT_C and IT_R injections labeled similar structures, including the locus coeruleus, dorsal raphe, caudate, putamen, claustrum, basal nucleus of Meynert, and lateral hypothalamus. Differences were found, however, between IT_C and IT_R in connections with the superior pulvinar and the amygdala. IT_C injections labeled the central and lateral subdivisions of the superior pulvinar, whereas IT_R injections labeled primarily the medial subdivision of the superior pulvinar. In the amygdala, IT_C injections revealed connections with the basal lateral nucleus, whereas IT_R injections labeled the lateral, basal lateral, and medial basal nuclei. These results support the hypothesis that IT_C and IT_R are separate subdivisions of cortex. Supported by EY07147 to REW.

47.14

BIMODAL VISUAL-TACTILE RESPONSES IN THE MACAQUE PUTAMEN.

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We recorded from the putamen of immobilized macaques anesthetized with N_2O. Somatotopic organization and receptive fields were essentially as described by Crutcher and DeLong for unanesthetized macaques (Exp.Brain Res., 53:233-243,1984). Cells in the face and arm area often responded to visual as well as tactile stimuli, or only to visual stimuli. The bimodal cells tended to have large, bilateral visual receptive fields, with a smaller region of best response which matched the location of the tactile receptive field. The optimal visual stimulus was usually one moving toward the monkey within 20 cm of the tactile receptive field. Five cells with tactile receptive fields on the arm only responded to visual stimuli when the arm was placed within the monkey's field of view. Cells that were exclusively visual tended to have a uniform response strength throughout their large, bilateral receptive fields.

SPINAL CORD AND BRAINSTEM

48.1

REFLEX ASYMMETRY RESULTING FROM LESIONS OF THE SPINAL CORD, AND EFFECTS THEREON OF ANESTHESIA.

J.S. Taylor, R.M. Friedman, J.X. Bao, D.P. Theele*, C.J. Vierck, and J.B. Munson. Dept. of Neuroscience, Univ. of Florida, Coll. of Med., Gainesville, FL 32610.

We are studying cellular mechanisms responsible for augmented reflexes following spinal lesions (spasticity). As a model, we are using cats with unilateral damage to the spinal cord. Reflexes are measured by recording EMG activity from both triceps surae of trained unanesthetized cats while both hind feet are flexed or extended simultaneously. Lesions of a low-thoracic dorsal quadrant (confirmed only by MRI to date) result in augmented stretch reflexes ipsilateral to the lesion. Since terminal cellular experiments will require an anesthetic procedure consistent with expression of these altered reflexes, we test these same animals similarly for the presence both of stretch reflexes and of reflex asymmetry with surgical doses of anesthetics. Brisk stretch reflexes and reflex asymmetries persist with ketamine anesthesia (30mg/kg). Supported by NS27511, NS15913, NS17474, MH15737.

48.2

QUANTITATIVE BEHAVIORAL MEASURES OF CAT TAIL REFLEXES: A MINIMALLY DISRUPTIVE MODEL TO STUDY THE CONSEQUENCES OF SPINAL CORD INJURY.

R. M. Friedman, C. J. Vierck, Jr., and L. A. Ritz. Departments of Neuroscience and Neurosurgery, Univ. of Florida, Gainesville, FL 32610.

Rhoton et al., (1988) observed qualitatively that hemisection or transection at Cal of the cat sacrocaudal spinal cord can produce a long-lasting hypertonia, cutaneous hyperreflexia and clonus of the tail. We are now developing quantitative measures of tail behaviors. As a measure of cutaneous hyperreflexia and clonus, we are characterizing the thresholds and magnitudes of responses by tethering the tip of the tail to a force transducer. For muscle tone, we determine resistance of the tail to displacement in the horizontal or vertical axis. Preliminary results indicate that spinal cord transection (n=3) or left hemisection (n=3) increase the amplitude of the cutaneous flexion reflex. In cats with transections, clonus is initiated by cutaneous stimulation. In cats with a tail that tonically deviates (n=2), there is greater resistance to movements away from the preferred position. With these sensitive assays, we hope to evaluate the behavioral consequences of spinal cord injury and repair.

Supported by NS27511 and NS07261.

48.3

SUPRASPINAL AND CERVICAL PROJECTIONS TO THE RAT SACROCAUDAL SPINAL CORD. R.L. Masson Jr., C.R. Murray* and L.A. Ritz. Depts. of Neurosurgery and Neuroscience, University of Florida, Gainesville, Fl., 32610.

The sacrocaudal spinal cord, which innervates the tail, is the subject of anatomical, physiological and behavioral investigations within our laboratory. Supraspinal and cervical neurons of the rat, projecting to the sacrocaudal spinal cord, were labeled following injection of Fluoro-Gold into the S_3-Ca_1 segments bilaterally. Supraspinal neurons were observed in the medullary and pontine raphe nuclei, the reticular nuclei, the dorsal column nuclei, the vestibular complex, noradrenergic groups, the red nuclei, periaqueductal gray, posterior hypothalamus and motor cortex. Within the cervical spinal cord, neurons were localized to laminae III-VIII and X, with the majority in the intermediate gray. Significant descending projections to the sacrocaudal spinal cord exist, with similar distributions as compared to other spinal cord levels. Functionally, important interactions involving the extremities, pelvic viscera and tail may be mediated by these connections.

This research was supported by NS27511.

48.4

DIFFERENTIAL EFFECTS OF TRAUMA ON SENSORY AND MOTOR SPINAL CORD TRACTS IN ANESTHETIZED CATS. M. Jayachandra* and V.E. Amassian, Dept. of Physiology, State University of New York, Health Science Center, Brooklyn, N.Y. 11203.

The series included 14 cats, which were anesthetized throughout with Na pentobarbital (initially 40 mg/kg I.P), supplemented by I.V. doses). Population direct (D) responses were usually recorded from the lateral corticospinal tract (CT) to contralateral motor cortical stimulation and from the posterior tibial nerve to antidromic dorsal column (DC) stimulation at C_1. A modified Allen weight drop produced the spinal cord trauma, usually between T_5-T_8. During stimulation and the weight drop, gallamine triethiodide was used as a muscle relaxant, supplemented by artificial ventilation. Responses were reduced within a second of impact; therefore, neither edema nor vascular changes accounted for the initial deficit. The deficit in the directly conducted CT usually exceeded that in the DC, although the weight fell more over the DC which also showed greater histological changes. With a submaximal drop, partial recovery could occur over a time course of, e.g., 30 min. Subsequently, a secondary depression could occur over a period of hours, suggesting more than one cause of the deficit. A DC volley transmitting through a partial block facilitated the response to local stimulation of the contused area. Facilitation was also demonstrated in CT units recorded in MI to antidromic lateral column stimulation. Summarizing, a suitable electrophysiological test system is proposed for analyzing differential functional effects of spinal trauma.

48.5

DORSAL ROOT POTENTIALS AND PRESYNAPTIC INHIBITION DURING DEVELOPMENT IN KITTENS. P. Bawa. School of Kinesiology, Simon Fraser University, Burnaby, B.C. Canada, V5A 1S6.

Experiments were carried out on kittens anaesthetised with Nembutal. Dorsal root potentials (DRPs), resulting from the stimulation of various peripheral nerves, were recorded from 1/3 distally cut L6DR. Amplitude of the DRPs was small in the first 2 weeks of age (mean approx. 50 uV) and then increased to adult values (400-500 uV) in the first 3 months of age. Duration of DRPs was longer during the first 20 days (100-400 msec) declining by 30 days of age to a range of 100-250 msec, increasing again after 3 months of age.

To examine the dependence of presynaptic inhibition on the magnitude and duration of DRPs, effect of conditioning on monosynaptic reflex (from gastroc-soleus nerve) recorded from L7VR, was evaluated. At any age, presynaptic inhibition produced by superficial peroneal nerve (a cutaneous nerve) was of much shorter duration than produced by the deep peroneal nerve (a muscle nerve) even though there was no difference in the duration of DRPs. Duration of presynaptic inhibition also depended on the receiving afferents. Supported by NSERC.

48.6

RETICULO-SPINAL INHIBITION OF RECURRENT DORSAL ROOT POTENTIALS IN THE ISOLATED NEUROAXIS OF THE FROG. H. González*, I. Jiménez and P. Rudomín. Dept. Physiol. Biophys. and Neurosci. CINVESTAV, México 07000 DF.

Most of the work concerning descending influences on segmental spinal mechanisms in the frog has been made by stimulating spinal funiculi. We now use the isolated neuroaxis to investigate the action of reticulo-spinal inputs on transmission along the recurrent pathway from motoneurons to afferent fibers (RM-AF). The reticular formation in the brain stem (RF) was stimulated with fine bipolar tungsten electrodes. Suction electrodes were employed to record DRPs and to stimulate the motor nerves from hindlimb extensors. Stimulation of the RF (3-5 pulses at 60-100 Hz) with intensities below those necessary to produce DRPs (60-200 μA), inhibited the DRPs produced by stimulation of motor nerves (VR-DRPs), to a mean value of 82 ± 6% of their control amplitude (n=5). The depression had an onset between 15-30 ms, became maximal at 40-70 ms and lasted more than 100 ms. Stronger stimulation of the RF (200-300 μA) produced DRPs lasting up to 120 ms and also depressed the VR-DRPs, but had no effect on the amplitude of the field potentials recorded in the motor pool following antidromic stimulation of motor nerves (n=8). This suggests that the reduction of the VR-DRP's produced by RF stimulation is not due to conduction block in axon collaterals of motoneurons, but rather to inhibition of impulse transmission exerted on interneurons interposed in the RM-AF. The reduction of recurrent inhibition by reticulo-spinal inputs would allow sensory input to act on motoneurons and interneurons during the execution of movements. Partly supported by grants NIH 09196, CONACyT 41739 & 891571, & UNAM fellowship (H.G.).

48.7

ACTION OF (-)-BACLOFEN ON SEGMENTAL PATHWAYS PRODUCING PAD. J. Quevedo*, J.R. Eguibar*, I. Jiménez and P. Rudomín. Dept. Physiol. Biophys and Neurosc. CINVESTAV, México, D.F. 07000 and Dept. Physiol Sci ICUAP, México.

We have shown previously (Neurosci. Abst. 15: 365. 15), that monosynaptic EPSPs produced in spinal motoneurons by activation of group Ia fibers are significantly more depressed after i.v. injection of 1-2 mg/kg (-)-baclofen than monosynaptic EPSPs produced by stimulation of the bulbar reticular formation (RF). This lead to the proposal that descending fibers also have GABAb receptors, but in lower density than Ia fibers. We have now studied in the anesthetized cat the effects of this GABAb agonist on the PAD elicited in single Ib gastrocnemius fibers. PAD was estimated by measuring the intraspinal threshold changes of the fiber. Ten minutes after 1-2 mg/kg (-)-baclofen, the PAD evoked by PBSt stimulation (3 shocks, 300 Hz, 2xT, 25 ms before the test pulse) was depressed to about 50% of control values and was completely abolished after 30-40 minutes. However, the PAD produced by RF stimulation (10 pulses, 400 Hz, 80 μA, 75 ms before the test pulse) was depressed only to about 50%. Intraspinal microstimulation may produce monosynaptic PAD by direct activation of last order interneurons synapsing onto the Ib fibers. This PAD was also depressed by (-)-baclofen but only to about 50%. Our data suggest that in addition to the effects that baclofen may have on afferent and descending fibers, the last order PAD mediating interneurons also have GABAb receptors. Those receptors located on the axon terminals of the interneurons would function as autoreceptors regulating the release of GABA. Partially supported by grants NIH 09196, CONACyT 41739 and 891571. Baclofen was a gift from Ciba-Geigy.

48.8

THE IDENTIFICATION AND CENTRAL DISTRIBUTION OF JAW-MUSCLE SPINDLE AFFERENTS IN THE RAT Dean Dessem, Division of Anatomy, Washington University Dental School, St. Louis, MO 63110

Single units were recorded from the mesencephalic trigeminal nucleus of rats deeply anesthetized with alpha-chloralose and pentobarbital. Spindle afferent types were identified by their response during ramp muscle stretches prior to and during the infusion of the depolarizing drug suxamethonium (100-200 μg/kg/min). Extracellular spike triggered averaging from clear primary and secondary afferents revealed short-latency, repeatable field potentials (3-18 μV) dorsal to and within the Nissl-stained confines of the trigeminal motor nucleus (MotV). Control averages from non-spindle cells showed no evidence of fields. Afferents which showed a small increase in dynamic index during depolarization presumably represent secondary afferents with terminations onto bag fibers. Extracellular field potentials generated by these afferents were also found dorsal to and within MotV. These results indicate that a wide diversity of jaw-muscle spindle afferent types project to MotV and to interneurons and/or distal dendrites of trigeminal motorneurons dorsal to MotV.

48.9

RESPONSE PATTERNS AND POST-SPIKE EFFECTS OF DORSAL ROOT GANGLION UNITS RECORDED DURING WRIST TORQUE TRACKING IN MONKEYS. D. Flament, P.A. Fortier & E.E. Fetz. Dept. of Physiology & Biophysics and Regnl. Primate Res. Ctr., Univ. of Washington, Seattle, WA 98195.

We documented the task-related activities and post-spike effects of peripheral afferents in dorsal root ganglia (DRG) under behavioral conditions similar to those used to study corticomotoneuronal (CM) [Fetz et al., *Prog Brain Res 80*:437, 1989] and rubromotoneuronal (RM) cells [Mewes & Cheney, *Neurosci Abst 12*:1303, 1986]. In 2 monkeys generating isometric ramp-and-hold wrist torques, we isolated 59 units in C8 and T1 DRG long enough to generate spike-triggered averages (STAs) of forearm muscle activity; 29 afferents produced post-spike facilitation (PSF) of 1 or more of the 12 EMGs. The PSFs often appeared on top of "synchrony facilitation", which began earlier than the shortest latency of electrically evoked muscle responses (3.0 ms); the PSF parameters were measured after subtracting these synchrony effects. The PSF onset latencies of DRG units averaged 4.9 ms ± 0.2 ms (S.E.) [c.f. 6.3 ms for CM cells and 5.6 ms for RM cells]. The magnitude of the PSFs, calculated as the mean percent increase of the PSF above the pre-spike baseline, was 4.6% ± 0.3 for DRG afferents [c.f. 7.0% for CM cells and 5.1% for RM cells]. DRG units facilitated 49% of the coactivated muscles [c.f. 40% for CM and 50% for RM cells]. DRG units producing PSF discharged during wrist flexion (10) or extension (13) torques or both (6). Most DRG units (52%) exhibited a tonic discharge pattern; 21% were phasic-tonic and 27% were phasic. On average, DRG unit discharge began 52 ms ± 13 prior to activation of their target muscle [c.f. -40 ms for CM and -89 ms for RM cells]. Phasic cells tended to begin earlier (-150 ms ± 59) than phasic-tonic (-72 ms ± 14) and tonic (-24 ms ± 17) cells. 7 tested DRG units responded with bursts to torque pulses which stretched their target muscles. Thus, peripheral afferents can significantly facilitate muscle activity and may be activated prior to their target muscles. **[Support: NIH grants NS12542 and RR00166 and the MRC of Canada.]**

48.10

SYNAPTIC INPUTS ONTO PUDENDAL MOTONEURONS FROM SACRAL SEGMENTAL AND BRAINSTEM PATHWAYS IN THE CAT. B. Fedirchuk, S. Hochman and S.J. Shefchyk. Depts. Med. and Physiol., Univ. of Manitoba, Winnipeg, Canada R3E 0W3.

This study examined polysynaptic input onto identified pudendal motoneurons (MNs) produced by electrical stimulation of lumbosacral segmental afferents and the pontine micturition centre (PMC). Intracellular recordings from sacral MNs antidromically activated from the external urethral sphincter (EUS) or external anal sphincter (EAS) branch of the pudendal nerve were made in chloralose anaesthetized or decerebrate male cats. Electrical stimulation at strengths 2 & 5 x threshold (T = minimum strength for activation of afferent fibres) of peripheral nerves innervating cutaneous, muscle and pelvic visceral targets was used to identify synaptic inputs to pudendal MNs. Excitatory postsynaptic potentials (EPSPs) were produced in both EUS and EAS MNs by stimulation of the ipsi- and contralateral pudendal mixed sensory nerves with no apparent difference in latency between the two sides. The latencies of EPSPs in EUS MNs (mean 3.5 ms) tended to be slightly longer than those seen in EAS MNs. Stimulation of the ipsilateral pelvic nerve (branch to the bladder) produced later excitation while stimulation of the ipsilateral superior perineal cutaneous nerve produced EPSPs at a mean latency of 2.6 ms. Mixed EPSP/IPSP were sometimes revealed using step current depolarization of the MN membrane potential. IPSPs produced by trains of stimuli to the PMC were observed in several EUS MNs but not in EAS MNs. Hyperpolarization of EUS MNs (n=2) was observed during PMC-evoked voiding while membrane depolarization or no change was observed in EAS MNs (n=2).

(Supported by the Medical Research Council of Canada and Manitoba Health Research Council.)

48.11

PERIPHERAL ACTIVATION OF THE PATTERN GENERATOR FOR HINDLIMB STEPPING IN CORDOTOMIZED, NEONATAL RATS. J.W. Commissiong. NIH, 10/5N214, Bethesda, MD 20892.

Previous results have shown that spontaneous recovery of hindlimb coordination occurs in adult rats that were cordotomized before postnatal day 14 (PN14) (Exp. Brain Res. 78: 597-603, 1989). When adult rats previously cordotomized at PN7 are placed on an inclined surface of mental bars spaced at 10 mm, the hindlimbs appear paralyzed, and dangle between the bars. Therefore, to be functional, the hindlimbs must be in contact with a surface. The present experiments were done in the decerebrate, non-anesthetized preparation to determine the role of peripheral afferents in activating the hindlinb pattern generator. The spontaneous discharge of alpha-motoneurons innervating the gastrocnemius and soleus muscles is higher in the PN7 group versus the PN14 group. The motor output, measured as an integrated electromyogram, in response to controlled stretches of varying rates and amplitudes, is greater in the PN7 versus the PN14 rats. These differences may result from augmented peripheral input (eg. Increased Gp.Ia activity) or from a greater central amplification of a normal peripheral input in the PN7 group. Future experiments will determine the contribution of these mechanisms to the recovery.

48.12

BEHAVIORAL CORRELATES OF CERVICAL SPINAL CORD CONTUSION INJURY IN THE RAT. **G.W. Schrimsher, F.J. Thompson, and P.J. Reier. Dept. of Neuroscience, U. of Florida, Col. of Med., Gainesville, FL 32610.**

The focus of this study is to establish a cervical spinal cord contusion injury model in the rat in conjunction with a battery of quantitative behavioral tests that are predictive of the extent and severity of the injury. A model of this nature will provide significant advantages as compared to midthoracic contusion models. The proximity of axonal damage relative to the cell bodies of origin of descending supraspinal systems may enhance regeneration potential. Cervical lesions will allow assessment and quantification of forelimb behavioral deficits and recovery following injury, and thus provide a new arsenal of behavioral tests that can be used to evaluate spinal cord damage. The contusion injury methodology used is an adaptation of that used by Wrathall et al. (Exp. Neuro., 88:108-122, 1985). A weight-drop contusion injury is produced by dropping a 10 gm weight from a height of 2.5 cm onto a 2.5 mm tip diameter impounder resting on the exposed dura of the C4 spinal segment. All rats that received this injury (n=10) survived and became self-sufficient within seven days of the injury. The acute (i.e. first 72 hours) recovery period is characterized by total forelimb impairment and lack of body weight support. Body weight support and locomotion usually returned within seven days (n=9). At one month, the lesion sites are characterized by a large cystic cavity involving the majority of the grey matter at C4. The cavities taper off in the dorsal columns within 3 mm of the lesion epicenter both rostrally and caudally. Rats are being examined on the following behavioral tasks: limb placement, sunflower seed shelling, horizontal reaching, force regulation, inclined plane, grid walking, and beam walking. Preliminary results (n=3) indicate at four weeks post-injury the mean crossing time increases 430% on the beam walking task and 400% on the grid walking task. Seed shelling ability was lost in 2 of 5 rats following the injury. Testing on the other tasks is in progress.

48.13

PATHWAYS CONDUCTING MOTOR EVOKED POTENTIALS IN THE SPINAL CORD OF RAT. Y.-G. PARK, J.-S. CHEON, W. H. LEE, and J. H. KIM, The Miami Project to Cure Paralysis, University of Miami School of Medicine, 1600 NW 10 Ave., R48, Miami, FL 33136.

Recently, several laboratories have used the MEP as an electrophysiological monitioring tool for predicting the degree of spinal injuries in the rat. However, the origins and the conduction pathways of these MEPs remain controversial. The goal of this study was to investigate the origins and intraspinal pathways of MEPs. Female rats (200-250 gr) were used in this study. To define the pathways conducting MEPs in the spinal cord, the following methods were utilized: (1) field mapping technique, and (2) controlled serial spinal cord lesions. The motor cortex was directly stimulated using silver ball electrodes or screws placed on the cranium. Two pairs of teflon-coated wire electrodes with an 1 mm exposed tip were implanted at T2/3 and L2/3 levels and used for recording MEPs. To construct field maps of MEPs, intracord recordings were made using glass electrodes filled with 2 M NaCl (1.5 M Ohm). Serial spinal cord lesions were made using a #11 surgical blade at T8 level. Suprathreshold stimulation produced one to four consecutive positive-negative waves which were monitored in thoracic and lumbar cord. The amplitudes and latencies of MEP components were affected very little by dorsal cord lesions including dorsal corticospinal tract and rubrospinal tract. However, lesions encompassing ventral and ventrolateral funiculi dramatically altered the amplitudes and latencies of MEP components monitored below the lesion. The results of MEP field mapping suggested that the MEPs in the rat spinal cord conducted bilaterally in the reticulospinal tracts. This study was supported by The Miami Project Research Fund.

48.14

SUPPRESSION OF SOMATOSENSORY INPUT TO THE RED NUCLEUS DURING MOVEMENT IN THE ANESTHETIZED TURTLE. *R. Sarrafizadeh, J. Keifer, and J.C. Houk.* Department of Physiology, Northwestern University Medical Center, Chicago, IL 60611-3008.

Bursts of red nucleus discharge are associated with discrete limb movements in the behaving monkey and are not modified when the movement is perturbed, suggesting that central motor programs rather than feedback from the periphery may be responsible for these signals (A.R. Gibson et al., J. Physiol. 358: 551, 1985). A recent theory of motor control suggests that sensory input may trigger the execution of preprogrammed motor commands by activating recurrent excitatory feedback in the cerebellorubral circuit (J.C. Houk *In*: Models of Brain Function, p. 309, 1989). To study sensorimotor integration in the red nucleus, we recorded extracellularly from single rubral cells in turtles *(Chrysemys picta)* anesthetized with sodium pentobarbitol (10 mg/kg).

Bursts of action potentials were obtained from the red nucleus during the execution of a scratch reflex of the contralateral hindlimb. Units increased their rate of discharge when scratching commenced, and the rate of activity returned to the spontaneous level seconds after the movement had ended. During the scratching episode, unit activity was phasically modulated with the ongoing movement as evidenced by electromyographic signals obtained from the contralateral hindlimb. These rubral units also responded to somatosensory input with excitation or inhibition which was evoked by light touch to the shell and body surface. Responses to somatosensory input typically lasted the duration of stimulation. Sensory responses, however, could not be elicited from units in the red nucleus during scratching. Rubral burst frequency and duration were indistinguishable during scratching with or without sensory perturbation. Red nucleus sensitivity to sensory input returned after the movement had ended.

This suppression of somatosensory information in the red nucleus during movement suggests that motor programs may not be instantaneously modified during the course of their execution. Sensory information may be used, however, to select appropriate motor functions prior to movement and to trigger their release.

48.15

POST-STIMULATORY INHIBITION: AN ADDITIONAL MEDULLARY INHIBITORY MECHANISM. E. Schenkel, Y.Y. Lai and J.M. Siegel. Sepulveda VAMC, UCLA Sch. Med., Sepulveda CA 91343.

Recent studies of our group have shown that medullary stimulation produces nonreciprocal inhibition of muscle tone in both the decerebrated and intact cat (Sleep Res, 18: 347, 1990). We now show that the medulla is also capable of causing inhibition by means of a poststimulatory mechanism.

Concentric bipolar electrodes were inserted in four cats, (Neurosci. Lett. 98:159-165, 1989) to targets at P 8-17, H 5-10 and L 0-2. Electrical stimulation at 26/60 stimulated sites was followed by a long latency, long lasting inhibition. For example, with stimulation in ventrocaudal medulla (200 msec trains at 200 Hz and 20 μA, n=8), the maximal inhibitory effect was observed 1-4 sec after the termination of the stimulus, with return of baseline tone 9-16 sec later. The post-stimulatory inhibition was observed at sites at which excitation appeared during the stimulus trains, as well as at sites at which inhibition appeared during trains. When the stimulation was performed in alert waking, the behavioral response consisted of a sudden turn ipsilateral to the stimulation, followed by a slow dropping of the head. Our studies suggest the existence of a long lasting inhibitory mechanism by which medullary activation may produce muscle tone suppression. (Supported by the VA and PHS grants MH43811 and HL41370.)

48.16

STARTLE RESPONSES EVOKED FROM RETICULAR FORMATION IN RATS: DI-I AND COLLISION TESTS SHOW BILATERAL CONNECTIONS. C. M. E. Hempel, Zhou Si-shun*, J. S. Yeomans. Dept. of Psychology, Univ. of Toronto, Toronto, Canada M5S 1A1.

Electrical stimulation of the nucleus reticularis pontis caudalis (RPC) with one pulse evokes startle-like responses with short latency (Davis, 1984). If a conditioning (C) pulse is delivered to one RPC site and a test (T) pulse is delivered to the contralateral RPC, collision-like effects occur at C-T intervals from 0.4 to 1.0 ms (Hempel & Yeomans, 1989). These results suggest that the neurons mediating startle have bilateral axonal connections between the two RPCs. The conduction velocities are estimated to be 7 to 40 m/s, by dividing the interelectrode distances by the difference between the refractory periods and the collision intervals. Collisions ranged from 19 to 50%.

The fluorescent tracer DiI was injected into the RPC post mortem and observed a few weeks later. DiI was taken up by myelinated fibers and transported over 10 mm into the ipsilateral longitudinal tracts of the reticular formation, the contralateral pyramidal tract and the contralateral RPC, although several weaker-labelled projections were observed. The projections to the contralateral RPC included many coarse fibers (1 to 5 um). These diameters are consistent with the behaviorally estimated conduction velocities using Hursch's (1939) ratio of 6. Therefore, post mortem DiI labels myelinated axons which may be responsible for the symmetric collision effects.

48.17

SYMMETRIC AND ASYMMETRIC COLLISION EFFECTS SUGGEST THAT MONOSYNAPTIC CONNECTIONS IN CAUDAL PONTINE RETICULAR FORMATION MEDIATE STARTLE-LIKE RESPONSES. J. S. Yeomans, C. M. E. Hempel and C. A. Chapman*, Dept. of Psychology, Univ. of Toronto, Toronto, Canada M5S 1A1.

Stimulating electrodes were placed bilaterally in the caudal pontine reticular formation (RPC) and the medulla. A conditioning (C) pulse was delivered via one electrode and a test (T) pulse was delivered via a second electrode at C-T intervals from -50 to +50 ms. A sharp decrease in the current required to evoke a startle response often occurred as C-T intervals increased from +0.4 to +1.0 ms, or decreased from -0.4 to -1.0 ms (Hempel & Yeomans, 1989). This symmetric collision suggests that the startle response is mediated by axon bundles connecting those two sites.

Between other electrode pairs, a sharp decline in required current was observed as C-T intervals rose from +0.4 to +1.0 ms, or fell from +0.2 to -0.2 ms. This asymmetric collision suggests that the axons mediating startle in these sites were interrupted by single synapses in the caudal RPC transmitting from rostral to caudal. The transmission times were estimated to be 0.3 ms, and the conduction times between RPC and medial medulla 2-3 mm caudal were near 0.1 ms. Single action potentials must be evoked with high probability across these RPC synapses. Collision effects were greater between RPC and ipsilateral, rather than contralateral, medulla. These collision tests, then, may allow the analysis of axon bundles and synapses for electrically evoked behaviors.

48.18

VERTICAL AND HORIZONTAL SACCADIC HEAD MOVEMENTS ARE ELICITED BY ELECTRICAL STIMULATION OF THE BRAINSTEM TEGMENTUM IN BARN OWLS. T. Masino and E.I. Knudsen. Dept. Neurobiology, Stanford University, Stanford, CA 94305.

Neural control of saccadic head movement in the barn owl appears to involve a transformation from a retinocentric code (retinal and tectal levels) to a Cartesian-like code in which four functionally distinct generators control orthogonal directions of saccadic movement (up, down, left, and right). As part of an effort to locate these saccade generators, we focally stimulated brainstem sites including those regions where tectal efferent fibers overlap with spinal cord afferent cell bodies. Movements were recorded using a search coil attached to the freely moving head while body movements were restricted. At each location from which movements were elicited, initial head position was varied and the dependence of movement direction on stimulus parameters (train-length, pulse-width, frequency, intensity) was determined.

Different types of head movements were evoked from different brainstem sites. However, saccadic head movements have been elicited from only two sites. From one medial region at the mesencephalic-rhombencephalic border, movements were directed vertically downward, the direction being independent of intial head position or of variations in stimulus parameters. Current thresholds were as low as 40 μA cathodal, and latencies as short as 16 msecs from stimulus onset. In contrast, stimulation applied to the other saccade generating site, in the rostral medial mesencephalon, produced horizontal movements that were directed ipsilaterally. Unlike the vertical downward movements, these horizontal movements depended on the head being initially positioned in the contralateral hemifield (relative to the body); however, large variations in starting position within that hemifield had little effect on movement direction. Current thresholds were as low as 75 μA cathodal, and latencies as short as 19 msecs from stimulus onset.

These results are consistent with the hypothesis that saccadic head movements are controlled by circuitry, residing in the brainstem tegmentum, that encodes independently horizontal and vertical components of movement. (NIH:RO1:NS27687-01)

SPINAL CORD AND BRAINSTEM: MOTONEURONS

49.1

LATENCIES OF CUTANEOUS EVOKED IPSPs AND EPSPs IN EDL MOTONEURONS OF CAT SUGGEST DISYNAPTIC CIRCUITRIES. R.G. Durkovic and J.C. Leahy. Dept. Physiology, SUNY Health Science Center at Syracuse, Syracuse, NY 13210.

Intracellular recordings were obtained from extensor digitorum longus motoneurons during electrical stimulation of cutaneous saphenous or superficial peroneal nerves in the unanesthetized, decerebrate, T-10 spinal cat. Only motoneurons with membrane potentials >55 mV and action potentials >60 mV were analyzed.

Postsynaptic potentials were complex, exhibiting either initial hyperpolarization or depolarization. Saphenous nerve stimulation most often evoked initial EPSPs. These had relatively long latencies between the cord dorsum "S" wave and EPSP onset that averaged about 4 ms. However, initial IPSPs with latencies near 1.8 ms were occasionally observed. Stimulation of cutaneous superficial peroneal nerve most often evoked IPSPs, some with latencies below 1.8 ms. Furthermore, occasional initial EPSPs of short latency (<1.8 ms) were also observed. The results suggest that the minimal circuitries for cutaneous excitatory and inhibitory inputs to certain motoneurons of the cat hindlimb involve single interneurons interposed between cutaneous afferents and these motoneurons. Supported by NSF Grant BNS 8808495.

49.2

MODULATION OF AFTERHYPERPOLARIZATION AMPLITUDE IN LUMBAR MOTONEURONS DURING FICTIVE LOCOMOTION IN THE RAT SPINAL CORD *IN VITRO*. B.J. Schmidt Dept of Medicine, Faculty of Medicine, University of Manitoba, Winnipeg Canada R3E 0W3.

Previous *in vivo* studies suggest that modulation of afterhyperpolarization (AHP) may regulate motoneuron firing during locomotion (Jordan and Shefchyk Soc Neurosci Abstr 10:184.7 1984; Brownstone et al *ibid* 12:241.2 1986; *ibid* 13:244.15 1987). In an effort to further understand the mechanisms by which cell firing is controlled during locomotion the present series employs an *in vitro* model of mammalian locomotion (Smith et al FASEB 2:2283 1988). Bilaterally intact spinal cords were transected at the C1 level and rhythmic alternating ventral root activity was induced by the application of NMDA or acetylcholine to the bath. The intracellular records of some motoneurons demonstrated spontaneous firing, predominately during the active phase of the step cycle as judged by the occurrence of the depolarizing phase of rhythmic membrane potential oscillations and ventral root discharge from the corresponding lumbar segment. Occasionally action potentials also fired during the otherwise inactive phase of the locomotor cycle. The amplitude of AHPs occurring during the peak active phase were reduced by up to 75% compared with AHPs occurring at other times in the step cycle. In other motoneurons, demonstrating rhythmic membrane potential depolarizations without spontaneous firing, action potentials were evoked by brief (<1ms) depolarizing current pulse injections delivered throughout the step cycle, followed by hyperpolarizing pulses (5ms) to test for the presence of rhythmic changes in somatic conductance. Averaged responses showed that AHPs following the evoked spikes were also reduced during the active phase of the step cycle. Motoneuron input conductance was either unchanged or increased during the inactive phase of the step cycle.

These results demonstrate that regulation of AHP during the step cycle is mediated by the spinal cord locomotor circuitry and does not depend on supraspinal neuromodulatory inputs. The data also suggest that the reduction in AHP amplitude during the active phase of the step cycle is not the result of increased somatic conductance but due to a decrease in AHP conductance. Supported by the MRC of Canada and the CLHIA

49.3

PGO CLUSTERS AND MOTONEURON MEMBRANE POTENTIAL DURING ACTIVE SLEEP. E Lopez-Rodriguez*, I. Stafford-Segert , F.R. Morales and M.H. Chase. Dept of Physiology, Dept. of Anatomy and Cell Biology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Pontogeniculooccipital (PGO) waves that occur during active sleep have been associated with the inhibition of somatic motoneurons (Morales et al., *Sleep Res.*, 18:20, 1989). This association was determined during the non-REM periods of active sleep when the predominant synaptic input to motoneurons is inhibitory rather than excitatory. However, during clusters of REMs and PGO waves, a complex pattern of motoneuron excitatory and inhibitory synaptic activity takes place (Chase and Morales, *Science*, 221:1195-1198, 1983). Consequently, the question was raised whether, within these clusters, excitatory synaptic activity is also temporally related to PGO waves. Experiments were performed in chronic cats that were prepared for intracellular recording from lumbar motoneurons during sleep and wakefulness (Morales et al., *Physiol. Behavior.*, 27: 355-362, 1981). The following relationships between PGO waves and motoneuron membrane potential were observed. (1) Clusters of PGO waves were correlated with sequences of IPSPs. (2) Excitatory potentials did not have a clear temporal relationship with the PGO clusters; they appeared even when the PGO waves were not present. These observations lead us to conclude that an active sleep-specific inhibitory system is phasically activated during clusters of PGO waves as it is during isolated PGO waves, suggesting that a single identical system is involved in motoneuron inhibition during both REM and non-REM periods. In addition, we conclude that the neuronal systems responsible for the depolarizing shifts in motoneuron membrane potential that take place during REM periods are not directly linked to those that generate PGO waves. Supported by Grants NS09999 and MH43362.

49.4

FREQUENCY POTENTIATION IN AN INHIBITORY PATHWAY TO DIGASTRIC AND MASSETER MOTONEURONS. F.R. Morales, C. Pedroarena*, P. Castillo* and M.H. Chase. Depto. de Fisiologia, Fcltd. de Med., Univ. de la Republica, Montevideo (Uruguay), Brain Res. Instit., Dept. of Physiology and Dept. of Anatomy and Cell Biology, UCLA Sch. of Med., Los Angeles, CA 90024 (USA).

A wealth of information now exists on plastic synaptic phenomena such as frequency potentiation and postetanic potentiation. Most of these studies have examined excitatory synapses. The data which are obtained are commonly extrapolated to all synapses, including inhibitory synapses, in spite of the fact that there are only a very few examples of these phenomena in inhibitory synapses, and none in vertebrates. In the present work, we examined a monosynaptic pathway from the parvocellularis reticular formation to trigeminal motoneurons to determine whether it exhibited frequency potentiation and postetanic potentiation. Experiments were performed on anesthetized cats. Intracellular recordings were obtained from antidromically identified masseter and digastric motoneurons. A region in the caudal medulla, adjacent to the hypoglossal nucleus and contralateral to the recording site, was stimulated with supramaximal current pulses (.2 ms duration, 80-120 μA). Short latency IPSPs were induced in masseter motoneurons (mean 1.16, range 1 to 1.5 ms) and in digastric motoneurons (mean 1.08, range 0.9 to 1.2 ms). These IPSPs followed frequencies between 1 to 150 cps without decrement in their amplitude. At frequencies higher than 50 cps an increase in the size of these potentials occurred. There was a sustained increase in the size of IPSPs during the application of a standardized stimulation train of 100 cps; the increase in amplitude ranged from 94 to 170%. Following the train, the IPSPs' amplitude remained augmented. In conclusion, a pathway in the brainstem was found whose inhibitory postsynaptic actions included frequency potentiation and postetanic potentiation. This research was supported by NS 09999.

49.5

ALTERATIONS IN MOTONEURON MEMBRANE CURRENTS ARE CORRELATED WITH DEVELOPMENTAL CHANGES IN THE PATTERN OF MOTOR ACTIVITY IN THE ISOLATED SPINAL CORD OF THE CHICK EMBRYO. Evelyne Sernagor and Michael O'Donovan. Laboratory of Neural Control, NINDS, NIH, Bethesda MD 20892.

Previous studies have revealed an increase in the intensity and duration of motoneuron discharge during each cycle of motor activity generated by the chick embryo spinal cord. Early in development (E7-E9) flexor motoneurons fire predominantly at the beginning of each cycle whereas at later stages (E12-E13) they fire a second burst out of phase with extensor activity. We have used the whole cell patch clamp technique to investigate developmental changes in motoneuron membrane currents which may be responsible for these changes in activity. Whole cell patch clamp recordings were obtained from antidromically identified motoneurons in the isolated spinal cord of E9 to E13 embryos.

From E9 to E13 motoneurons are depolarized throughout the cycle even when firing is inhibited. At E9 voltage clamp recordings reveal that the predominant current is a transient inward current that peaks during the flexor inhibition and reverses at -56.9±11.2 mV, leaving a small sustained inward current (mean=-2.8 pA) that is relatively insensitive to membrane potential and that we have been unable to reverse. Later in development the transient current reverses at -34.5±10.3 mV leaving a substantially larger sustained inward current (mean=-25.7 pA).

These findings suggest that the transient current is mediated by an inhibitory synaptic potential and that the sustained current is excitatory. It is not clear at present if the excitatory current is synaptic. The increase in the magnitude of the excitatory current may account for developmental changes in the firing pattern of motoneurons.

49.6

THE EFFECTS OF SELECTIVE ABLATIONS ON THE RHYTHMIC MOTOR OUTPUT OF THE ISOLATED CHICK SPINAL CORD. S.M. Ho & M.J. O'Donovan. Laboratory of Neural Control, NINDS, NIH, Bethesda, MD 20892

The isolated spinal cord of the chick embryo is capable of generating spontaneous episodes of alternating motor activity in flexor (SART) and extensor (FEM) motoneurons. Previous experiments have indicated that both classes receive a common excitatory drive and that SART motoneurons are inhibited during each cycle coincident with extensor activity. The present experiments were designed to localize the relevant interneurons by determining the effects of various ablations on the motor output of the cord. Lesions were made with sharpened tungsten needles while recording from the SART and FEM muscle nerves, or from ventral roots.

The experiments revealed a difference in the capacity of isolated rostral (T7-LS3) and caudal (LS4-LS8) portions of the cord to sustain rhythmic activity. The rostral portion generates 3-4 cycles spontaneously and up to 10 cycles in the presence of NMDA (10-25μM) whereas the caudal cord produces only 1 or 2 cycles even after NMDA application. In addition, a single segment of the rostral cord (LS1) is capable of several cycles of activity.

In the hemicord, several ablations can modify or abolish the inhibitory component of SART discharge without affecting cyclic activity. These include: isolation of LS1 and LS2 from caudal and rostral inputs, and deletion of the dorsal and medial input to T7 - LS3. However, normal alternation of flexors and extensors could be observed in some cords that had the dorsal and medial portions removed over the T7-LS3 segments *providing* that the rostral and caudal segments were left intact.

These findings suggest that the excitatory inputs to motoneurons and some of the SART inhibitory input is localized in the ventro-lateral portion of the spinal cord.

49.7

TIME COURSE OF MOTONEURONAL ADAPTATION IN RESPONSE TO EXTRACELLULAR ACTIVATION. J.M. Spielmann, Y. Laouris*, M.A. Nordstrom, R.M. Reinking* and D.G. Stuart. Dept. of Physiology, Univ. of Arizona, Tucson, AZ 85724.

We have previously reported the feasibility of studying motoneuron adaptation using sustained and repetitive extracellular activation (Spielmann et al., Soc. Neurosci. Abstr., 15:920, 1989). In the present study, we quantified several features of motoneuronal adaptation for 13 individual medial gastrocnemius motoneurons (6 FF, 1 FR, and 6 S) in response to sustained anodal extracellular stimulation (for up to 4 min). The discharge rate of the motoneurons was monitored via their EMG patterns. Motoneuronal responses usually showed an initial rapid increase in firing rate followed by a more slowly-developing adaptation. The time course of firing rate (Y) was fitted with a double exponential equation [$Y = - K_1 \cdot \exp(-t/\tau_1) + K_2 \cdot \exp(-t/\tau_2)$]: the first time constant for the initial increase was relatively short (mean ± SD; 4.1 ± 4.3 s) and may be attributable to mechanisms associated with extracellular stimulation. This initial increase is in sharp contrast to the early, rapid decrease in rate reported by Kernell and Monster (Exp Brain Res., 45:197, 1982) using intracellular current injection. The second time constant in the present work (194.1 ± 177.3 s) was comparable to the "late adaptation" described by Kernell and Monster (1982) and was correlated with the duration of discharge ($p<0.001$). Discharge duration averaged 149.5 ± 94.8 s (cf. Kernell, vide supra; 110 ± 83), with a mean total spike number of 2434 ± 1685. This study has demonstrated that sustained extracellular stimulation is a viable method for studying repetitive firing in motoneurons and may provide information that is a necessary compliment to that obtained by use of intracellular stimulation. Supported by USPHS grants NS 07309, HL 07249, NS 25077 and RR 05675. M.A.N. is a C.J. Martin Fellow of the NH & MRC of Australia.

49.8

RECURRENT INHIBITION OF MOTONEURONS IN THE RAT SPINAL CORD IN VITRO. R.E.W. Fyffe, S.P. Schneider, B.D. Gynther*, E.G. Casale. Department of Physiology, Univ. of North Carolina, Chapel Hill N.C. 27599-7545.

During studies of the mechanisms of recurrent inhibition of spinal motoneurons, we investigated morphological and functional substrates of the recurrent pathway in isolated preparations of spinal cords from rats 5 to 12 days old.

Motoneurons were identified by antidromic activation following stimulation of ventral roots. In many cells, subthreshold stimulation evoked an antidromic depolarising potential (electrotonic coupling) in addition to recurrent ipsps. The polarity of ipsps reversed near the resting membrane potential, and after intracellular injection of chloride ions. The largest ipsps were evoked by stimulating motor axons in a single ventral root; stimulation of adjacent roots produced much smaller potentials. Ipsp latency (at 27°C) was consistent with a disynaptic pathway. Ipsps were decreased by bath application of strychnine and, surprisingly, bicuculline. Motor axons had well developed recurrent collateral branches as revealed by intracellular HRP staining. Neurons located near motoneuron pools were synaptically excited by antidromic stimuli and usually responded with more than one action potential.

We conclude that the neural circuitry mediating recurrent inhibition of motoneurons is present and functional in neonatal rats and resembles that in adults of other species.

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49.9

FORCE MODULATION DUE TO FIRING RATE VARIATION IN SINGLE MOTOR UNITS DURING CENTRALLY EVOKED MUSCLE CONTRACTIONS. K.E. Tansey, A.K. Yee, and B.R. Botterman. Departments of Cell Biology & Neuroscience and Physiology, UT Southwestern Medical Center, Dallas, TX 75235.

The CNS can control muscle force by changing the number of recruited motor units and/or by altering their firing rates. Of these two mechanisms, less is known about motoneuron firing rate variation and the muscle-unit force modulation it produces. To further investigate this issue, pairs of motor axons were isolated in the cat medial gastrocnemius (MG) nerve by intracellular techniques and, following characterization of their associated muscle units' mechanical properties, their activation patterns were recorded during graded isometric contractions evoked in MG by brainstem stimulation. Subsequently, each axon was stimulated separately with its previously recorded activation pattern to approximate the unit's force output in the preceding contraction.

Once recruited, most motor units showed a steady increase in firing rate over a narrow range of increasing whole muscle force and then stabilized at a relatively high firing rate. At this point, muscle-unit force was close to maximum and was relatively insensitive to any additional fluctuations in motoneuron firing rate. Other motor units were recruited during rather steep increases in whole muscle force and fired with multiple short inter-pulse intervals before settling into a fairly constant discharge rate. This activation pattern generated a muscle-unit force profile with a fast onset that reached the unit's maximum output and then declined. Almost all of the motor units studied showed a brief decrease in firing rate and force output just before they were de-recruited. These results suggest that the firing rate modulation of a motor unit's force output contributes to changes in whole muscle force only at output levels just above that unit's recruitment threshold. Supported by NIH grant NS17863.

49.10

MOTONEURON PHYSIOLOGY AFTER H-REFLEX OPERANT CONDITIONING: INITIAL STUDIES. J.S. Carp and J.R. Wolpaw. Wadsworth Labs, NY St Dpt Health & SUNY, Albany, NY 12201.

Earlier studies showed that monkeys (Macaca nemestrina) can gradually increase or decrease the size of the triceps surae (TS) H-reflex (HR), the electrical analog of the monosynaptic spinal stretch reflex, and that such conditioning changes the spinal cord (Wolpaw & Carp, TINS 13:137-142, 1990).

In order to define these spinal cord alterations, we are recording intracellularly from motoneurons (MNs) in naive animals and in animals in which one leg's TS HR has been increased (HRup) or decreased (HRdown). Animals are deeply anesthetized during recording and sacrificed by overdose.

To date we have studied 179 MNs (130 TS MNs) in 10 naive and 5 HRup animals. TS MNs from HRup animals appear to show changes in rheobase and membrane potential that are consistent with our previous TS nerve volley studies in conditioned and naive animals (J Neurophysiol 61:563-572, 1989). Additional data are needed to confirm these preliminary findings and to assess the effects of HR conditioning on MN responses to afferent inputs.

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49.11

TRICEPS SURAE MOTONEURONS AND IA AFFERENT CONNECTIONS: FURTHER ANATOMICAL STUDIES. C.L. Lee, J.S. Carp, and J.R. Wolpaw. Wadsworth Labs, NY St Dept Health & SUNY, Albany, NY 12201.

In order to define the spinal cord alterations produced by operant conditioning of the primate (Macaca nemestrina) triceps surae (TS) H-reflex, the electrical analog of the monosynaptic stretch reflex (Wolpaw & Carp, TINS 13:137-142, 1990), we are studying TS motoneurons (MNs) and primary afferents in naive animals and in animals in which one leg's TS H-reflex has been increased or decreased.

Animals are deeply anesthetized, HRP is injected into TS MNs after assessment of their physiological properties, HRP crystals are crushed onto dorsal rootlets, and after an appropriate interval the anesthetized animals are sacrificed by overdose and perfused. Sections are processed by the conventional nickel-DAB method. MNs are reconstructed by camera lucida and afferent connections are evaluated.

In 11 TS MNs reconstructed to date from naive animals, soma diameters averaged 48 um (range 32-73) and appeared to correlate with conduction velocities. Dendritic trees had 7-13 primary dendrites and were flattened ellipsoids with longest axis usually rostral-caudal (mean 2.2 mm, range 1.7-2.5). Putative Ia connections were distributed throughout the dendritic tree and ranged from 1.0-4.9 um in diameter. Their size did not correlate with distance from the soma.

(Supported in part by NIH NS22189 & Paralyzed Veterans.)

49.12

A COMPUTER SIMULATION APPROACH TO QUANTITATE DENDRITIC MORPHOLOGY W. B. Marks and R. E. Burke. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892

At this meeting in 1989, we described the use of quantitative data from cat α–motoneuron dendrites to derive probabilities of branching and terminating per unit of branch length as exponential functions of local dendrite diameter, **d**, (**Pbr(d)** and **Ptrm(d)**, respectively). Experimentally observed distributions of individual branch lengths for any given initial diameter, $\mathbf{d_0}$, were reasonably well fit by a simulation using these empirical probabilities, plus an exponential equation relating dendritic taper to **d**. We have extended this model to build complete dendritic trees by introducing a rule to specify the diameters of daughter branches at branching points. The branch point rule takes account of the relation between parent and daughter branch diameters in motoneuron dendrites (approximating the "3/2 power rule"), as well as an observed negative dependence between daughter branch diameters. Complete trees simulated using **Pbr(d)** and **Ptrm(d)** exhibited many of the features found in real motoneuron dendrites (Cullheim et al., *J. Comp. Neurol.* 255:68, 1987) but had, on average, 3-5 fewer branch points than expected for any given stem diameter. Re-examination of the original data showed some dependance of **Pbr** and **Ptrm** on the distance, **L**, of the growth point from the branch origin. The use of probabilities, **Pbr(d,L)** and **Ptrm(d,L)**, that are strongly dependent on **d** and weakly dependent on **L**, produced better fits to the experimental data for complete motoneuron dendrites. This simulation approach is very sensitive to subtle features of dendritic architecture and may be useful for making quantitative comparisons between dendrites from different groups of neurons.

CONTROL OF POSTURE AND MOVEMENT I

50.1

SPONTANEOUS NEURONAL ACTIVITY IN THE BASAL GANGLIA-MOTOR CORTICAL LOOP IN NONHUMAN PRIMATES BEFORE AND AFTER METHYLPHENYLTETRAHYDROPYRIDINE (MPTP). A.S. Mandir, E.B. Montgomery, Jr., S.R. Buchholz, A. DeLitto, R.L. Watts, Depts. of Physiology and Neurology, Emory Univ. Sch. Med., Atlanta, GA 30322, and Depts. Neurology and Psychology, Washington Univ. Sch. Med., St. Louis, MO 63110.

The neurotoxin MPTP selectively destroys the nigrostriatal dopaminergic pathway in primates and reproduces the characteristic motor deficits of Parkinson disease. This lesion disrupts information processing in the basal ganglia-motor cortical pathway. To explore how this affects basal neuronal discharge patterns in macaques, we recorded spontaneous single cell activity in the primary motor cortex (MC;n=4 animals), supplementary motor area (SMA;n=2), putamen (PT;n=6), globus pallidus external segment (GPe;n=6) and ventrolateral thalamus pars oralis (VLo;n=2) in the normal state and weeks to months following induction of MPTP parkinsonism. No significant differences were observed in median discharge frequencies or median interspike intervals following MPTP in MC (# neurons pre-/post-MPTP, 262/228), SMA (92/21), PT (176/75), GPe (83/51), or VLo (38/16), as determined by Mann-Whitney and Kolmogorov-Smirnov statistical tests. While spontaneous single neuronal activity in the chronic MPTP parkinsonian state does not differ significantly from normal, previous work has shown that dynamic modulation of neuronal activity is disrupted.

50.2

NEURONAL, KINEMATIC AND ELECTROMYOGRAPHIC (EMG) CHARACTERIZATION OF SELF- AND STIMULUS-INITIATED MOTOR TASKS IN NORMAL AND MPTP PARKINSONIAN NON-HUMAN PRIMATES. R.L.Watts, A.S.Mandir, Depts. of Neurology and Physiology, Emory University Sch. Med., Atlanta, GA 30322 and E.B. Montgomery, Jr., Washington Univ. Sch. Med., St. Louis, MO 63110.

Two macaques were trained to perform self- and stimulus-initiated rapid wrist movements in response to visual instruction signals. Each motor task consisted of a preparation phase and an execution phase. In the stimulus-initiated task an external "GO" signal (illumination of final target light) was employed following a specific "prepare to move" signal; in the self-initiated task the animal had to generate the movement without external prompting after waiting at least 2 seconds following a different "prepare to move" visual cue. We recorded reaction time (RT), movement time (MT), maximum movement velocity (V_{max}), agonist EMG activity, and primary motor cortex (MC) movement-related neuronal activity, in the normal state and following induction of MPTP parkinsonism. In the normal state self-initiated tasks differed from stimulus-initiated tasks by exhibiting reduced maximum velocities and first agonist EMG bursts; MC activity showed a reduced rate of change and peak discharge frequency. Following MPTP, MT was prolonged, V_{max} was reduced, and EMG and MC movement-related activity were disorganized for both tasks, but to a greater extent for the self-initiated task.

50.3

RECOVERY OF LOCOMOTOR MOVEMENTS IN SPINALLY LESIONED MONKEYS. J.A. Vilensky, E. Eidelberg, A. Moore* and J.G. Walden*. Dept. of Anatomy, Ind. Univ. Sch. of Medicine and Division of Neurosurgery, Univ. of Texas Health Science Center, San Antonio, TX 78284

In a 1981 study (Brain 104:647-663) Eidelberg et al. briefly described the effects of various spinal lesions at T8 on the locomotor behavior of 10 monkeys. They concluded that sparing of pathways in the ventrolateral sector is most important for the recovery of stepping. In this report we present a detailed kinematic analysis of the recovery of 4 of these monkeys and a qualitative description of the recovery (or lack of it) for the remaining 6, as well as a reanalysis of the lesion sites. Six of the animals initially showed purely unilateral hind limb movements. Hind limb cycle durations were commonly increased during recovery whereas forelimb cycle durations were commonly reduced. Ipsilateral interlimb phase values were initially inconsistent, and even when stable values were reached they were different from preoperative values. That is, the animals adopted more of a pacing type gait. Our analysis of the lesion sites and the behavioral recovery supports the previous finding that pathways in the ventrolateral quadrant (i.e., reticulospinal and vestibulospinal) are important for locomotor recovery. However, it is noteworthy that there are cases reported in the literature of monkeys with bilateral lesions in these pathways that apparently recovered some locomotor function.

50.4

QUANTITATIVE ANALYSIS OF NECK MUSCLE ACTIVATION PATTERNS DURING CONTROLLED HEAD BEHAVIORS IN CATS. E.A. Keshner, J.F. Baker, J. Banovetz*, and B.W. Peterson. Department of Physiology, Northwestern University Medical School, Chicago, IL 60611.

When the head rotates, vestibulocollic reflexes (VCR) counteract the rotation by causing contraction of the neck muscles that pull against the imposed motion. With voluntary head rotations, these same muscles contract to assist the movement of the head. Does the CNS select a consistent and unique muscle pattern for the same head movement whether performed in a voluntary or reflex mode, or do voluntary head movements involve an infinite variety of motor patterns as is theoretically possible (Peterson et al., Amer Zoologist, 1989)? EMG activity of six neck muscles was recorded in three alert cats during sinusoidal head rotations about 23 different axes. Cats were trained to voluntarily follow a water spout with their heads. VCR responses were recorded in the same cats during rotations in an equivalent set of planes. Gain and phase of the EMG response was calculated, and analyzed to determine a vector representing the direction of rotation for which a muscle produced its greatest EMG output. The azimuth and elevation angles of these vectors were quantified and compared to see if VCR and voluntary movements involved directionally opposite (180°) maximal activation directions (MADs). MADs differed significantly ($p < 0.03$) from this hypothesis by 20° to 50°. Analysis of MAD variability within and between cats revealed small variances in the reflex task, implying that a single optimality criterion can account for VCR activation patterns. A significant feature to emerge from the tracking task was large variations in the MADs between cats, yet very small day to day variability within each cat. Consistent patterns of voluntary activation within an animal suggest that each CNS may select its own criteria to produce its unique pattern of activity. Simultaneous flouroscopic and electromyographic analyses of these movement behaviors is currently underway to determine intra-individual relations between head movements, intervertebral arrangements, and muscle activation patterns.

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50.5

INTERACTIONS BETWEEN CENTRALLY PATTERNED BEHAVIORS: RESPIRATION, MASTICATION, AND DEGLUTITION. D. McFarland, D. Veilleux*, C. Valiquette*, and J.P. Lund. Centre de Recherche en Sciences Neurologiques, Université de Montréal, Montréal, Canada, H3C 3J7.

Although many rhythmical movements occur simultaneously, little is known about the mechanisms coordinating interactions between them. We have begun to look at the interrelationship between three such behaviors: breathing, chewing, and swallowing.

Experiments were performed on awake rabbits prepared for chronic recording of the EMG activity of throat and lower jaw muscles. These signals were recorded together with movements of the rib cage and lower jaw during spontaneous breathing and the mastication of rabbit chow.

Our preliminary results can be summarized as follows. The respiratory rate increased or decreased at the start of mastication so that the masticatory and respiratory rhythms were approximately equal in frequency. However, no coupling occurred, since the phase relationship between the two rhythms was variable. Two characteristic types of swallowing patterns were observed, coincident (C) and terminal (T). C swallows occurred without a pause in the masticatory rhythm, while T swallows came at the end of a masticatory sequence. Most C swallows took place during jaw closure (97% closure, 3% opening), while the opposite was true of T swallows (18% closure, 82% opening): these proportions were significantly different (X^2=50.48, p<.0001). However, there were no significant differences in the proportion of C and T swallows coinciding with the two major phases of respiration (X^2=.1058, p=.7449), both occurred preferentially during inspiration (C: 87%, T: 85%).

Supported by the Canadian MRC.

50.6

AN ELECTROMYOGRAPHIC (EMG) ANALYSIS OF OROFACIAL MOTOR ACTIVITIES DURING TRAINED TONGUE-PROTRUSION AND BITING TASKS IN MONKEYS. E.M. Moustafa, L.-D. Lin, G.M. Murray and B.J. Sessle. Fac. Dentistry, Univ. of Toronto, Toronto, Ont., M5G 1G6.

The aim of this study was to characterize orofacial EMG activity patterns in two monkeys (M. fascicularis) trained to perform tongue-protrusion and biting tasks; EMG activities were recorded from digastric, genioglossus, masseter, platysma, zygomaticus major and orbicularis oris inferior and superior muscles. For each muscle, we assessed the mean EMG amplitude (MA) and mean area (MSA) under the integrated EMG signal during the task period (TP) and the pre-trial period (PTP), as well as the mean EMG amplitude ratio (MAR) i.e. (MA during TP)/(MA during PTP). For tongue task trials, the MA and MSA values during TP were significantly ($P<0.0001$) increased above PTP values for each of the genioglossus, orbicularis oris inferior and superior and digastric, respectively; the highest MAR occured in the former two muscles ($P<0.0001$). For biting task trials, however, a corresponding analysis showed significant increases ($P<0.0001$) for MA and MSA values during TP in the masseter, platysma, and zygomaticus; MAR was highest in the masseter ($P<0.001$). While tongue protrusion at different forces was associated with no significant change in MAR for each muscle, a significant ($P<0.0001$) change in MAR of the masseter and digastric during TP was noted at different bite forces. These EMG patterns were noted in both animals and were consistent between different recording sessions in the same animal. These data clarify orofacial muscle activity patterns during trained tongue protrusion and biting in the monkey. Supported by Canadian MRC.

50.7

NUCLEUS AMBIGUUS NEURONAL ACTIVITY DURING VOCALIZATION IN THE AWAKE MONKEY. C.R. Larson and Y. Yajima*. Comm. Sci. & Dis. and Neurobiol. & Physiol. Northwestern University, Evanston, IL 60208.

The nucleus ambiguus (NA) region contains interneurons related to vocalization, respiration and swallowing and motoneurons controlling laryngeal and pharyngeal muscles. The midbrain periaqueductal grey (PAG) also contains neurons related to vocalization, and it projects to the NA region. The present study was designed to define the unit discharge patterns of the NA as they relate to the above behaviors and to compare discharge properties of vocalization-related PAG and NA cells. Macaca nemestrina monkeys were trained to vocalize for a fruit-juice reward. EMG electrodes were surgically implanted into laryngeal muscles and a single unit recording chamber was affixed to the skull with screws and dental cement. A cuff electrode on the recurrent laryngeal nerve allowed antidromic identification of motoneurons. Some NA neurons were active only during vocalization, while others were also related to respiration or swallowing. Ensemble averages of unit activity were similar for NA and PAG neurons, but the mean firing rate was higher in the NA. Pre-vocal activity times were much shorter in NA cells than in PAG cells. Spike-triggered-averaging EMGs from NA neuron spikes revealed specific effects on laryngeal muscles with 4-5 ms latencies.

50.8

CUTANEOUS DESENSITIZATION OF THE ABDOMEN AND INNER THIGHS DISRUPTS COPULATION IN MALE RATS. C.-A. Maillard & D. A. Edwards. Dept. of Psychology, Emory University, Atlanta, GA 30322.

For a male rat, copulation involves mounting coupled with vigorous pelvic thrusting and forelimb palpation of the female's flanks. Penile insertion is almost invariably associated with a rapid, spring-like, dismount that provides an index of literal intromission, and ejaculation is easily recognized by a deep final thrust and prolonged clasp of the male to the female. Appropriately oriented mounts and pelvic thrusting provide sensory feedback undoubtedly essential for intromission and ejaculation -- the penis appears to play an important role here inasmuch as penile desensitization, produced by the topical application of a local anesthetic or transection of the sensory nerves of the penis, decreases the display of intromission and ejaculation (e.g., Adler & Bermant, J. Comp. Physiol. Psychol., 1966; Sachs et al, Neurosci. Abstracts, 1989).

We used subcutaneous injections of the local anesthetic procaine (a 2% solution in physiological saline) to study the role of non-penile somatosensory feedback in the regulation of male sexual behavior in rats. Cutaneous desensitization (produced by injections of 0.1 ml of procaine underneath the skin) of the chest or back had no effect on copulation. Desensitization of the lower abdomen and inner thighs decreased the display of intromissions and ejaculations, and dramatically increased the number of *incomplete mounts* i.e., appropriately oriented mounts without pelvic thrusting or flank palpation. These results suggests that the tactile stimulation of the abdomen and thighs associated with mounting plays an important role in the elicitation of forelimb palpation and the elicitation of the pelvic thrusting necessary for intromission and ejaculation. Supported by NSF Grant BNS-8718797.

50.9

3-D KINEMATIC ANALYSIS OF HINDLIMB WIPING MOVEMENTS IN SPINAL FROGS. L.E. Sergio, J.R. Flanagan, A.G. Feldman and D.J. Ostry. McGill University, Montreal, Canada and Institute for Information Transmission Problems, Moscow, USSR.

The 3-D kinematics of wiping movements to the back were examined in spinal frogs. The aim was to identify the elements of the frog's back wipe and their functional role. Wiping movements were elicited in spinalized adult *Rana Catesbeiana* by placing small pieces of blotting paper soaked in 5% H_2SO_4 in ten different positions covering the full area of the back. The data show that there are three essential components, or phases, of the wiping movement: a placing phase, a quick flexion of the hip and knee (absent in 20% of the wipes), and a whisk/extension phase. The first, or placing phase, appears to be the only phase dependent upon stimulus location. Furthermore, the spinal frog only takes account of stimulus location in the rostro-caudal direction (consistent with Giszter et.al., *J.Neurophys.*, 62:750, 1989). There is no hindlimb adjustment for stimulus position in the medial-lateral direction in this phase. In the second phase, previously described as the 'aiming' phase, the final endpoint position shows no dependence on stimulus location, contrary to previous findings for *Rana Temporaria* (Berkinblit et.al., *Behav.Brain Sci*, 9:585, 1986). It is proposed that the second phase serves as a preparatory movement for the extension portion of the wipe. When the wipe is partitioned into the elements described, the motion was found to be planar for the first and third phases. During the second phase there was a transition from one plane to another.

50.10

THE ORGANIZATION OF LIMB MOTOR SPACE IN THE SPINAL CORD. S. F. Giszter, F.A. Mussa-Ivaldi*, and E.Bizzi. Dept. Brain and Cognitive Sciences, M. I. T., Cambridge MA 02139.

We have examined the role of the premotoneuronal spinal areas in organizing posture and movement in spinalized frogs. We recorded the forces produced in the leg by stimulation of various spinal cord loci using a six axis force transducer attached to a spinal frog's ankle. The frog's ankle was positioned in a number of workspace locations. At each such workspace location we measured the force elicited by stimulating the same single site within the spinal cord. In this way we estimated the spatial distribution of forces and EMG activation generated by stimulation of a single spinal cord locus.

We discovered that microstimulation of the upper and middle layers of the frog spinal cord grey matter, in conjunction with positioning of the leg in different workspace locations, generated a force field with a single equilibrium point. The equilibrium point is that spatial location at which the leg would be at steady state were it free to move. In contrast, when the stimulating electrode was placed in the ventral roots or among the motoneurons, the force field found was characterized by parallel or divergent force vectors with no detectable equilibrium point. During the stimulation of the middle layers of interneurons the activation of some muscles was modulated as a function of leg location in the workspace while others were activated uniformly and were independent of the limb's location. Taken together these results indicate that activation of the spinal cord's premotoneuronal networks acts to impose a structure upon the forces generated by the limb muscles.

These results provide a neurophysiological underpinning for the equilibrium point hypothesis and its role in the control of posture and movement in the spinal cord.

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

50.11

DIRECTED MOVEMENT IN THE FROG: ELECTROPHYSIOLOGICAL FINDINGS IN THE VENTRAL MIDBRAIN. C. Smeraski and P. Grobstein. Dept. of Biology, Bryn Mawr College, Bryn Mawr, PA 19010.

Previous anatomical and lesion studies suggest that a neuronal column in the ventromedial midbrain of the frog may be an important relay for a subset of the descending tectofugal signals underlying prey orienting movements in the frog, and the origin of a signal representing stimulus location in a generalized coordinate frame. We were interested in determining whether this anatomically defined region is also physiologically identifiable and distinctive.

Vertical penetrations using tungsten microelectrodes were made through the midbrain in Rana pipiens anesthetized with MS-222. On many penetrations, there was a dorso-ventrally delimited region distinctive in having high levels of spontaneous multiunit and single unit activity. The activity could often be driven by visual and/or tactile stimuli, and occasionally by auditory stimuli. Responses, while clearly present, were labile. The dorso-ventral location of the areas of spontaneous activity was defined using electrolytic lesions visible in histological sections, and corresponded well with the previously defined ventromedial column. Medially located penetrations, which did not pass through the column, did not exhibit dorsoventrally delimited regions of spontaneous activity. The same was true of penetrations which missed the column laterally. Whether the area of spontaneous activity has sharp borders rostrally and caudally is still under investigation.

Our findings suggest that there is indeed a physiologically distinctive area which corresponds to the anatomical area previously implicated as a relay for descending tectofugal signals involved in orienting. The high levels of spontaneous activity is this area may be significant for understanding the transformation from a retinal signal to one in a generalized coordinate frame. The finding of both visual and tactile excitability in this area is consistent with prior studies indicating that visual and tactile signals converge at the level of the generalized coordinate frame circuitry.

Supported by PHS R15 NS24968, and a grant from the Whitehall Foundation.

50.12

DIRECTED MOVEMENT IN THE FROG: MOTOR EQUIVALENCE, MULTI-DIMENSIONALITY, INTERNAL FEEDBACK? P. Grobstein, J. Meyer*, and R. Egnor*. Dept. of Biology, Bryn Mawr College., Bryn Mawr, PA 19010.

Videotape study of the directed movements made by frogs in response to live mealworms has revealed two novel and significant aspects of this behavior.

The initial ballistic movement made by a given frog to a stimulus at a given 3-D location is similar on successive trials in that it aligns the longitudinal body axis with the stimulus. There are however detectable differences from trial to trial in how this similar end result is achieved. Individual movements result in both a rotation of the body axis and a 2-D body translation. Each of these components varies substantially from trial to trial; we have been as yet unable to relate the variations to any other variable, such as initial body posture. The similar end result reflects a systematic covariation of the movement components. We conclude that frogs have and use a variety of different motor patterns to orient toward a given 3-D location, and that one dimension in a Euclidean description of stimulus location corresponds to at least three dimensions in the motor output pattern. Like earlier findings, the present ones suggest that there exists in the frog nervous system a signal specifying movement objective without specific reference to motor pattern. The present findings raise new questions about how a signal related to objective is translated into a specific motor pattern, and suggest that a probabilistic element may be involved.

Following certain unilateral lesions, the initial movement of a frog in response to a lateral stimulus is forwardly rather than laterally directed, apparently because some of the information specifying objective fails to reach the stage of translation into a motor pattern. We now report that some such frogs may, by a series of movements, achieve a successful turn toward the stimulus location, even when the stimulus has been removed. The findings suggest the hypothesis that the objective signal remains present until a comparison with reafferent and/or corollary discharge signals indicates that the objective has been achieved. Such an internal feedback loop, coupled with a probabilistic element in motor pattern determination, would provide a basis for creating novel ways to achieve a particular objective.

Supported by PHS R15 NS24968, and a grant from the Whitehall Foundation.

50.13

NEURAL CONTROL OF A MUSCULAR HYDROSTAT: SIMULATION OF THE BIOMECHANICS OF A REPTILIAN TONGUE. P.E. Crago, H.J. Chiel and J.M. Mansour*, Depts. of Biomedical Engineering, Biology, and Mechanical and Aerospace Engineering, Case Western Reserve University, Cleveland, OH 44106.

We are investigating the control of a muscular hydrostat: the tongue of the lizard *Tupinambis nigropunctatus*. A functional movement, lapping, can be simulated by controlling two intrinsic muscles. A longitudinal muscle contracts to produce shortening. This muscle is wrapped by a circumferential muscle that produces lengthening by decreasing the diameter of the longitudinal muscle. The tongue is modeled as a circular cylinder of constant volume: decreasing the diameter causes an increase in length. The mathematical model is based on the mechanics of a pressure vessel. The longitudinal muscle exerts pressure on the ends of the cylinder, and this pressure is balanced by the pressure developed by the tangential forces in the cylinder walls by the circumferential muscle.

The models of the individual muscles are based on studies in cats, with parameters adjusted for the dimensions of the lizard tongue muscles. The model includes activation dynamics, the dependence of force on muscle length and stimulus period, force velocity properties, and passive stiffness.

The model tongue displays kinematics similar to published recordings of the lizard tongue, when driven by neural inputs similar to EMGs recorded in intact lizards. We are investigating the sensitivity of the motor output to the model structure and parameter values, and are focusing in particular on the role of the biomechanical nonlinearities.

Supported by NSF grant BNS-8810757

50.14

RANGE FRACTIONATION IN RESPONSES OF SPIKING INTERNEURONS THAT DETECT JOINT MOVEMENT IN LOCUSTS. S. N. Zill and S. F. Frazier. Dept. Anat., Marshall Univ. Sch. Med. Huntington, WV 25755

Two different types of reflex effects have been shown to occur when the metathoracic femoral chordotonal organ (FCO), a joint angle receptor of the locust hindleg, is mechanically stimulated. In the flexion mode, afferent firing indicating changes in joint angle in any direction excites tibial flexor motoneurons. We now show that this reflex is elicited when the FCO is stimulated in freely standing locusts and that it preferentially occurs when the leg is in positions of low mechanical advantage of tibial muscles. We postulate that this reflex serves to move the leg to a position of high stability when postural perturbations occur and have examined responses of local circuit spiking interneurons to mechanical stimulation of the FCO. These studies show that: some interneurons respond to displacements signalling changes in joint angle in any direction; these interneurons show only phasic excitatory or inhibitory responses and exhibit little hysteresis; interneurons show range fractionation and respond only in some but not all ranges of joint angle. We conclude that these interneurons preserve information provided by the FCO that could form a basis for this adaptive postural reflex. Supported by NIH NINDS grant NS22682 and the Whitehall Foundation.

50.15

HINDLIMB MOTOR ACTIVITY ELICITED BY BRAINSTEM STIMULATION IN AN ISOLATED BRAINSTEM/SPINAL CORD PREPARATION OF THE CHICK EMBRYO. G.N. Sholomenko and M.J. O'Donovan. Lab of Neural Control, NINDS, NIH, Bethesda, MD 20892.

We have developed an isolated brainstem/spinal cord (B/SC) preparation to study the development of descending pathways involved in the regulation of embryonic motor activity in the chick embryo. The cooled embryo (E12-E15) is decerebrated to the midbrain level and continuously superfused with Tyrodes solution. The ventral surface of the entire brainstem and spinal cord is exposed by laminectomy and various hindlimb muscle nerves are exposed for recording. The B/SC is then placed in a double partition bath which isolates the brainstem, cervical and lumbosacral cord into independently perfused chambers. Our results demonstrate that: 1) the isolated B/SC (E12) produces spontaneous bouts of alternating activity in flexor and extensor muscle nerves indistinguishable from the activity in the isolated spinal cord preparation 2) low threshold stimulation (0.1-1ms biphasic/25-50μA) of the brainstem consistently evoked short bouts of rhythmic neural activity closely resembling spontaneous activity. The stimulation-evoked activity could be elicited for at least 24hrs, indicating the prolonged viability of the brainstem 3) the addition of a low Ca^{++}/ high Mg^{++} to the middle bath (cervical cord) did not abolish brainstem-evoked rhythmic activity recorded from hindlimb muscle nerves. 4) NMDA (5-50μM) placed in the brainstem bath facilitated both spontaneous and brainstem-evoked lumbosacral activity.

These results suggest that descending pathways capable of initiating lumbosacral motor activity are present by stage 37 in the chick embryo.

50.16

AN EMG STUDY COMPARING FOOT SHAKING AND WALKING IN CHICKS. S. M. Woolley*, N. S. Bradley, and A. Bekoff. EPO Biology Dept., University of Colorado, Boulder, CO 80309-0334.

In chicks, foot shaking is an episodic unilateral behavior performed to remove an irritant from the foot or tarsus and is similar to the paw shake response seen in cats (Smith J. L., et al., *J. Neurophysiol.* 54:1271, 1985). In this study, we first characterized the foot shaking behavior and its underlying motor output pattern. We then compared these results to those obtained for walking.

Fine wire hook electrodes were implanted in 6 muscles of the right leg in 1- to 6-day old chicks. Foot shaking was elicited by placing a small piece of tape on the foot. Walking was elicited by enticing the chick to walk down a stationary runway. Parameters used to quantify the EMG data were: cycle period, burst durations, phase relationships and interburst intervals.

The two behaviors differ in that foot shaking involves very rapid unilateral leg movements, while walking involves alternating motions of the two legs over a wide range of cycle periods. Nevertheless, results show that foot shaking shares some common features with walking. For example, both are characterized by similar muscle synergies. Unlike walking, foot shaking exhibits similar burst durations of extensors and flexors within a cycle as well as considerable overlap of extensor and flexor activity at the onset of the flexor phase but not at the onset of the extensor phase. We suggest that the overlap of activity at the onset of the flexor phase functions to brake the rapid extensor movements of foot shaking. Supported by NIH grant NS 20310.

50.17

PROPRIOCEPTIVE MODULATION OF HEAD SCRATCHING IN CHICKS. M.B. Smith and A. Bekoff. EPO Biology Dept., University of Colorado, Boulder, CO 80309-0334.

Head scratching is one of many behaviors that are believed to be controlled by a central pattern generating circuit. This study was undertaken to determine how the influence of joint proprioceptive information modulates the motor pattern.

Six muscles of the right leg were surgically implanted with bipolar EMG electrodes in domestic chicks between the ages of 3 and 6 days. Muscles studied included the gastrocnemius lateralis (GL), tibialis anterior (TA), sartorius (SA), femorotibialis (FT), caudilioflexorius (CF), and iliofibularis (IF). EMG recordings were made 24 hours following implantation. To remove joint proprioceptive information, lidocaine hydrocholoride (2%) was injected into the right ankle joint of experimental chicks. This resulted in loss of sensation within minutes. In both normal (n=5) and experimental chicks (n=5), head scratching was elicited by placing a small piece of tape over the external ear. For each chick EMG recordings were obtained from at least three bouts of scratching.

There are no significant differences among the first four sequential cycles within a bout of scratching in either the normal or experimental condition. Although the scratch in the experimental condition appears qualitatively normal some EMG characteristics have been altered. For example, a two-way ANOVA reveals that injection of lidocaine significantly alters the duration of the cycle period (p=0.033) of each scratch. Loss of joint proprioception also significantly diminishes the number of scratches that occur within a bout of scratching. Supported by NIH grant NS 20310.

MUSCLE: MOLECULAR STUDIES

51.1

Identification and Localization of a 94 kDa Glycoprotein to the Triad Junction C.M. Knudson, K.K. Stang*, and K.P. Campbell. Howard Hughes Medical Institute and Dept. of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242.

Monoclonal antibodies have been produced against an array of rabbit skeletal muscle membrane proteins. Using subcellular fractionation of skeletal muscle membranes and immunoblot staining, two antibodies have been shown to recognize proteins (apparent MW of 90 and 94 kDa) which are greatly enriched in triads and junctional face membranes, but not detectable in T-system or surface membranes. Immunocytochemical localization of these proteins, using immunofluorescent microscopy, indicates a junctional distribution. Based upon scans of Coomasie blue stained SDS gels, the 94 kDa protein was found to be a major component (5-10%) of junctional face membrane. The 94 kDa band was identified as a glycoprotein based on Con A Peroxidase staining and treatment with Endo H. The 94 kDa band was labeled by the hydrophobic probe 3-(Trifluoromethyl)-3-(m[125I]iodophenyl)diazirine (TID), suggesting the 94 kDa protein may contain membrane spanning domain(s). An interesting but unique property of the 94 kDa complex was found when junctional face membrane was run on polyacrylamide gels under non-reducing or partially reducing conditions. Under reducing conditions with 2-mercaptoethanol, immunoblot staining with 94 kDa protein monoclonal antibodies revealed a single intense band, however, under non-reducing conditions with N-ethlymaleimide, immunoblot staining identified only multiple high molecular weight bands. No 94 kDa band was detected by the antibody or present on gels under non-reducing conditions. The localization of these proteins to the triad junction suggest that they may play a role in the egulation or modulation of sarcoplasmic reticulum Ca^{2+} release. K.P. Campbell is an Investigator of the Howard Hughes Medical Institute.

51.2

PG-1000, A LARGE CHONDROITIN SULFATE PROTEOGLYCAN IS FOUND IN THE TRANSVERSE TUBULE SYSTEM OF FISH, FROG, CHICK AND RAT SKELETAL MUSCLE. A. K. Davis* and S. S. Carlson, Department of Physiology and Biophysics, University of Washington, Seattle, WA 98195.

We have purified, biochemically characterized, and raised antibodies against a large (10^6 daltons) CS proteoglycan (PG-1000) from the extracellular matrix of the electric organ of the marine elasmobranch, *Torpedo californica* (Carlson, S. and Wight, T., 1987; Iwata, M., Davis, A. and Carlson, S., 1989). Using a monoclonal antibody that specifically recognizes PG-1000, we have detected PG-1000 immunoreactivity in skeletal muscle of a variety of organisms: electric fish, frog, chick and rat. In all four species, we observe a regular banding pattern of immunoreactivity that resembles the distribution of the transverse tubule system, the network of tubules that penetrates the muscle cell and has a lumen that is continuous with the extracellular space. PG-1000 antigenicity co-localizes with a known transverse tubule marker, the dihydropyridine receptor, confirming our hypothesis. In fish and chick, we also detect PG-1000 antigenicity in the basal lamina that surrounds muscle cells. Biochemically, the skeletal muscle antigen resembles PG-1000 isolated from electric organ. Both of the proteoglycans recognized by our antibody are similar in size to the well characterized proteoglycans isolated from cartilage. Cartilage proteoglycans occupy large hydrodynamic volumes and therefore act as aqueous space fillers. We hypothesize that PG-1000 in the lumen of the transverse tubules has an analogous structural function; we propose that it helps maintain the integrity of transverse tubules by holding them open, preventing their collapse and also providing aqueous space for diffusion.

51.3

SYSTEMATIC EXPRESSION OF ALPHA-CARDIAC MYOSIN IN MULTIPLY-INNERVATED FIBERS OF EXTRAOCULAR MUSCLE. J. Jacoby, K. Kō‡ J. Davidowitz*, C. Weiss*+ and J.I. Rushbrook*+. Dept Ophthalmol, NYU Med Ctr, NY,NY 10016 & +Dept Biochem, SUNY Downstate Med Ctr, Bklyn, NY 11203. Unlike mammalian skeletal muscle, extraocular muscles (EOMs) contain, in addition to singly-innervated fibers (SIFs), multiply-innervated fibers (MIFs) which exhibit tonic contractile properties. MIF and SIF populations, which are distributed within orbital and global layers, collectively express at least 6 different isoforms of myosin heavy chain including neonatal and EOM-specific forms. Using a type-specific McAB (RCM37) from R. Zak, we find that alpha-cardiac heavy chain, previously believed to be expressed only in heart and some muscle spindles, is present also in rabbit EOM. In EOM, RCM37 labels populations of MIFs which exhibit both acid- and alkali-stable ATPase activity, and do not co-label with a fast-specific antibody. RCM37 labels 2 populations of fibers in the global layer; one, confined to the deep global layer also co-labels with an antibody for type 1 slow myosin (S58), and the second, distributed in the intermediateglobal layer labels with RCM37 alone. In the orbital layer, RCM37 labels one population, which in the EPB labels with RCM37 exclusively, but distally co-labels with ALD180, a McAB that labels embryonic/neonatal myosin and labels the entire orbital layer. Support: NY Heart, EY 06232 and Research to Prevent Blindness.

51.4

COORDINATION OF SUCCINATE DEHYDROGENASE ACTIVITY BETWEEN MOTONEURONS AND MUSCLE FIBERS IN THE NORMAL AND FUNCTIONALLY OVERLOADED CAT PLANTARIS G. R. Chalmers, R. R. Roy, and V. R. Edgerton. Dept. of Kinesiology and Brain Research Institute. UCLA, Los Angeles, CA 90024.

Given the interdependence of motoneurons (MNs) and muscle fibers, the oxidative capacity of these two cell types might be coordinated within a neuromuscular unit. To test this hypothesis the soleus and gastrocnemius muscles in five adult cats were excised bilaterally, functionally overloading (FO) the plantaris. For 4-12 wks post-surgery the cats were exercised for 15-25 min/day. Four nonoperated cats served as controls. After treatment all cats had the right plantaris muscle injected with HRP and 96 hr later the S1-L5 spinal cord segments and left plantaris muscle were removed and frozen. Size and SDH activity of light and dark myosin ATPase (alkaline pH) muscle fibers and HRP positive MNs were measured (Chalmers and Edgerton *J. Histochem. Cytochem*, 37:1107, 1989).

There were no significant differences in MN SDH activity and soma size (mean ±SD) between the control (11.4±0.3 x10^{-3}OD/min, 3613±1154μm^2) and FO (10.5±1.1 x10^{-3}OD/min, 3910±1127μm^2) groups. Mean muscle fiber SDH activity was lower and the size larger in FO plantaris compared to control (FO:light ATPase, 6.7±2.3 x10^{-3}OD/min, 4266±1533μm^2 and dark ATPase, 4.6±1.4 x10^{-3}OD/min, 7493±3144μm^2, control:light ATPase, 14.0±1.9 x10^{-3}OD/min, 2285±421μm^2 and dark ATPase, 10.7±2.8 x10^{-3}OD/min, 3412±494μm^2). Before and after FO total SDH activity was similar in MNs (control: 39.62±9.94, FO: 38.04±9.55 OD x μm^2/min) and muscle fibers (for light and dark ATPase combined, control:32.08±1.91, FO:33.94±22.14 OD x μm^2/min).

These data indicate that although the oxidative potential is similar in MNs of a motor pool and in the muscle fibers innervated by that pool, the size and mean SDH activity of the MNs and muscle fibers can change independently. *Supported in part by NIH Grant NS16333.*

MUSCLE: GENERAL

52.1

THREE DIMENSIONAL ANALYSES AND IMAGING OF ALL FIBERS BELONGING TO A MOTOR UNIT. E.S. Hsu*, A. Garfinkel, E. Eldred, R.R. Roy, and V.R. Edgerton. Department of Kinesiology and Brain Research Institute, UCLA, Los Angeles, CA 90024-1568.

Cross-sectional area (CSA), selected enzyme activities, lengths, shapes and spatial distribution were characterized in 98 fibers belonging to a motor unit (MU) of an adult cat tibialis anterior muscle. The MU was functionally isolated, physiologically characterized and glycogen depleted as described by Bodine et al. (*J Neurophysiol* 57: 1730, 1987). The MU had a contraction time of 56 ms and a maximum tetanic tension of 3.0 g. The muscle-tendon unit was frozen *en bloc* at L_0, then cut into 1 cm blocks. The intact muscle length after freezing was 9.0 cm. Serial cross-sections were stained for glycogen by the periodic acid Schiff (PAS) method and for myosin ATPase pre-incubated under acidic and alkaline conditions. Fibers belonging to the motor unit were identified by their low level of glycogen and light myosin ATPase staining. Enzyme activity and substrate reaction products were quantified as optical density (OD) units using digital image processing (*J Histochem Cytochem* 33:1053, 1985). Fiber CSA and centroidal coordinates were obtained simultaneously at 14 different levels along the length of each fiber. In addition, serial microscope slides in 20 μm increments were carefully visualized to determine lengths and shapes at the ends of individual fibers. Motor unit fibers had consistently lower ODs in PAS staining along their lengths relative to non-MU fibers. Mean fiber length was 4.52 ± 0.4 cm. The mean of all CSA measurements of all fibers was 2308 ± 509 um^2. The MU specific tension was 1.3 kg/cm^2. Most fibers began and ended at musculotendinous junctions with modest changes in CSA along their length. Nine fibers were significantly shorter and ended mid-fascicularly, some tapering to a miniscule CSA. Spatial distribution analyses at 14 levels along the length of the fibers showed no evidence for MU fibers to cluster or be dispersed non-randomly within the unit territory. These data demonstrate the complex 3-D architecture of a MU with significant variation in total CSA along the length of the MU territory. It seems that these anatomical features could contribute significantly to the MU's mechanical output potential. ***Supported by NIH Grant NS16333.***

52.2

RELATION BETWEEN FIBER DIAMETER AND FIBER TYPE COMPOSITION IN THE RECTUS FEMORIS MUSCLE OF CATS. L.L. Glenn and P.J. Rebeta*. Department of Physiology, Ohio College of Podiatric Medicine, Cleveland, OH 44106.

The present study was conducted to determine if the difference in diameter between muscle fibers of different fiber types were related to local fiber type composition. The neuromuscular compartments of rectus femoris (RF) were identified by glycogen-depletion using PAS stain. Frozen sections (20μm thick) of RF were stained for alkaline ATPase, acid ATPase, and NADH; fiber types were determined (SO, FOG, FG) and minor diameters measured. Fibers with homogeneous diameters had higher proportions of type SO.

	RF_{pm}	RF_{pl}	RF_{dm}	RF_{dl}
SO Diameter (μm)	30.5	33.8	36.3	34.6
FG Diameter (μm)	41.4	48.5	44.2	41.4
Type SO (%)	9.4	10.6	29.4	28.3

52.3

IDENTIFICATION OF OPTIMAL INTERPULSE INTERVALS (IPIs) FOR PRODUCTION OF A RAPID RATE OF RISE OF FORCE IN RAT SOLEUS MUSCLE. S.A. Binder-Macleod. School of Life and Health Sciences, Univ. of Delaware, Newark, DE 19716.

As technology continues to improve, the increased use of electrical stimulation of skeletal muscle to aid function (FES) appears inevitable. Most functional activities (eg, walking) require muscle to generate brief submaximal levels of force with a rapid rate of rise. The combination of a rapid rate of rise and submaximal force necessitates the use of a stimulation pattern with IPIs of varying duration. This study attempts to identify the duration and number of IPIs needed at the onset of a contraction to produce a rapid rate of rise of force. Rat soleus muscle was used to define trains of pulses which most rapidly approached, but did not exceed, targeted levels of force (50-75% of maximum tetanic force). The optimal initial IPI (IPI*1) was defined as the IPI of a two pulse train which produced the greatest force and reached this peak within the shortest time. After IPI*1 was identified, it was used as the first IPI of a three pulse train while the third IPI was varied. This basic procedure was repeated until a train was developed which reached the targeted level of force. Results to date suggest that to produce targeted levels of force between 50-75% of maximum, a relatively simple pattern of stimulation is required with either 1 or 2 short IPIs (10-15 ms) needed at the onset of stimulation. The addition of one or two pulses at these short IPIs at the onset of stimulation takes advantage of the catchlike property of skeletal muscle to markedly increase the total force output of the muscle over constant frequency trains needed to produce comparable submaximum forces. Preliminary analysis has shown that the duration of these initial short IPIs must be slightly greater than the time to maximum of the first derivative of the twitch force produced by a single pulse. This work has been funded by a grant from the Foundation for Physical Therapy.

52.4

NEURAL INACTIVATION WITH TETRODOTOXIN PREVENTS RAT PLANTARIS ADAPTATIONS TO TENOTOMY OF ITS SYNERGISTS. R. N. Michel. School of Human Movement, Laurentian University, Sudbury, Ontario, Canada, P3E 2C6.

This study was constructed to examine the extent to which the morphological and functional changes that occur in the rat plantaris (PL) muscle in response to synergist tenotomy are dependent on neural activation. This was acheived by superimposing synergist tenotomy and neural inactivation. One group of Sprague-Dawley rats had the sciatic nerve of one hindlimb superfused with the sodium channel blocker tetrodotoxin (TTX) for 2 wks, and the soleus and gastrocnemius muscle tendons of both hindlimbs cut. A second group of animals that had undergone these same procedures, had the ankle of their silenced limb fixed in flexion placing the PL under passive stretch. A third group of animals served as sham-operated control. Two weeks of synergist tenotomy caused PL muscles to have greater absolute (g; 41%) and relative (mg/g body wt.; 53%) mass, and whole (18%) and individual slow-twitch (18%) muscle cross-sectional areas, compared to sham-operated controls. In these muscles, isometric contractile characteristics measured in situ in response to sciatic nerve stimulation were not improved over control. Consequently, specific twitch and tetanic tensions were 20% smaller in these muscles. Superimposition of TTX superfusion and synergist tenotomy prevented any of the morphological improvements associated with synergist tenotomy from occurring. PL in this group had reduced absolute and relative mass (58%), and whole (51%) and individual slow-twitch (60%) muscle cross-sectional areas, compared to their contralateral overloaded counterparts. Functionally, these muscles had a prolonged twitch, a decreased rate of tension development, and a reduced capacity to generate maximum forces at high, but not low, stimulation frequencies. Interestingly, fixation of the ankle had no effect on any of these parameters, and did not alter muscle rest length. The results suggest that neural activity is necessary to induce changes in the PL that are characteristic of the compensatory overload model. Clearly the mechanism of stretch-induced hypertrophy must be revisited.

52.5

NERVE-INDUCED EFFECTS ON MUSCLE PHENOTYPE IN FETAL SHEEP. J.W. Hermanson, M.J. Daood*, W.A. LaFramboise and P.W.N Nathanielsz*. Coll. of Vet. Med., Cornell Univ., Ithaca, NY 14853, Magee Womans Hospital, Pittsburgh PA. 15213 and University of Pittsburgh, Pittsburgh, PA 15217.

To study neurogenic effects on developing fetal muscle, transection of the common peroneal nerve was undertaken in early gestation age (dGA 71, N=4) and late gestational age (dGA 110:N=1; and dGA 121:N=1) fetal sheep. Animals were allowed to survive until dGA 91 and 131, respectively. Denervated and contralateral sham-operated control peroneus longus (PL) muscles were evaluated grossly, histologically, histochemically and electrophoretically. Control PL muscle samples from adults and from fetal sheep at dGA 71, 110, and 141 were similarly studied. Early-gestation age denervated muscles exhibited growth and hyperplasia relative to control dGA71 PL, but were reduced in size relative to contralateral dGA91 controls and failed to show fiber type differentiation whereas control muscle expressed type I and II fiber myosin ATPase. Denervated PL exhibited many fibers with central nuclei (approximately 15%) while contralateral control muscles contained few fibers with central nuclei (less than 4%). In two of the animals studied, denervated PL muscles contained 39,036 and 38,931 fibers, while estimated fiber numbers in contralateral control PL muscles were 45,000 and 51,340, respectively. Electrophoretic analysis of native myosin isoforms in 4% pyrophosphate gels showed a slow myosin band in late-gestation age (greater than dGA 110) muscle. Two or three fast native myosin isoforms were present in all PL muscles. Denervated extensor digitorum longus muscle (EDL) at dGA 131 were also reduced in size relative to contralateral controls and lacked the fiber type differentiation present in control EDL. Peripheral nuclear migration occurred in late-gestational age denervated as well as control EDL. These data indicate that fetal denervation reduces growth but also inhibits muscle differentiation as indicated by a failure for nuclei to migrate peripherally, and by failure to exhibit histochemical fiber type differentiation. Supported in part by NIH 21350.

52.6

Patterns of motoneurone sprouting in the rat *Tibialis Anterior* - T.Gordon, N.Tyreman*, S.Erdebil* & S.Devasahayam*, Univ.of Alberta, Edmonton,T6G 2S2

The factors that promote or limit sprouting of motoneurones following nerve injury are not completely understood. Since it is impossible to accurately measure the extent of sprouting of individual motor units -i.e., the size before and after sprouting - it has been difficult to answer these questions reliably. Using the motor unit force as a measure of the unit size, the distribution of units from partially denervated muscles can be compared with units from normal control muscles to estimate the extent of sprouting. Since, comparison of distributions is greatly improved by the number of units sampled from each muscle, we obtained a large sample of units (20-30% of the muscle) in the rat tibialis anterior by recording incremental twitch forces from bundles of axons in the spinal roots. We have determined that using the contralateral side as a control, the extent of partial denervation in experimentally denervated muscles can be reliably measured. Finally, to address the fact that motor unit force is an indirect measure of the unit size that assumes that variations in fibre size do not confound the data, we counted the number of fibres in individual units that were identified by depleting the glycogen in one unit in each muscle.

The data shows that the unit sizes change uniformly proportional to the denervation in the whole muscle therefore maintaining the size relationship after sprouting as before. The maximum sprouting capacity of the units in the rat TA seems to be a fourfold increase (75% denervation) from normal, beyond which incomplete recovery of muscle force is seen, as has also been reported for the rat soleus. The spatial distribution of the unit fibres identified by glycogen depletion shows closer clustering of sprouted unit fibres than normal, which suggests that unit territory can limit sprouting of individual units.(supported by the Rick Hansen Legacy Fund, and MRC).

52.7

PATTERNS OF MOTONEURONE SPROUTING IN THE CAT TRICEPS SURAE MUSCLES. V.F. Rafuse*, T. Gordon, R. Orozco* and M.J. Gillespie. Div. of Neuroscience, Univ. of Alberta, Edmonton, Canada T6G 2S2.

Although it is well recognized that intact motoneurones will sprout to innervate denervated fibers after partial denervation (PD), the extent and limits of sprouting of the unit population are not well understood. To determine 1) whether motoneurones increase their innervation ratio (IR) in proportion to their size as predicted by the size principle and 2) the limits to which motor unit types can sprout, we examined muscles which contain 5, 25, and 100% slow motor units, MG, LG and soleus muscles, respectively. If each motoneurone sprouts in proportion to its size, IR should increase by a constant factor y which will equal 1/N where N is the proportion (or %) of remaining units. 10-90% of the innervation was removed by unilateral section and ligation of the L7 or S1 root and the number, force, fatigability and conduction velocity of remaining motor units was determined 4- 18 months later. An average of 25% of the unit population was sampled prior to removal of the nerves and muscles for histological examination. The distribution of unit force was found to shift to progressively higher values in proportion to N for values of N>20%, if redistribution of muscle fiber size was taken into account. Since axon size did not change, the size relationship between force and axon potential amplitude was shifted by the same factor along the force axis. Thus, the data indicates that the IR of all motoneurones increases by a constant factor y, without affecting axon size. However, for N=5-20%, the smaller slower units did not increase beyond 4-5 times and the IR of remaining motor unit population showed a disproportionate increase which led to full muscle recovery in the LG and MG but not in the soleus. Thus there is a size-dependent sprouting which is more limited in the smaller than the larger motor units. (Supported by MRC and MDAC).

52.8

CYTOARCHITECTURE OF THE SOLEUS MUSCLE IN THE C57Bl/KsJ-dbm DIABETIC MOUSE David Porta and Kathleen M. Klueber, Department of Anatomical Sciences and Neurobiology, University of Louisville, School of Medicine, Louisville, KY 40292

Diabetes adversely affects both muscle and nerve. Ultrastructural evidence reveals degeneration of both the axon and myofiber thus causing denervation of type 2 myofibers (Feczko and Klueber, Am.J.Anat 182:224-240,1988). However, type 1 myofibers are thought to be more resistant to disease states. The objective of this study was to determine if type 1 myofibers of diabetic muscle exhibit similar cytoarchitectural changes observed in a type 2 muscle. Soleus muscles from 126 day old diabetic (db/db) and control (db/+) mice (n=5/group) were fixed in situ, processed using standard electron microscopic procedures and analyzed under the electron microscope. In the db/db soleus muscles, there was an increase in the lipid stores and an increase in the number of cells with displaced mitochondria. When compared to the type 2 muscle, fewer cells exhibited signs of degeneration and disruption of the mitochondria. In addition, fewer myofibers exhibited signs of denervation and reinnervation and most neuromuscular junctions appeared normal. Therefore, it appears that the soleus muscle has some ability to resist the disease state. Funded by:USHS 1R29DK41553-01

52.9

METABOLIC RESPONSE OF ADULT AND NEONATAL RAT MUSCLE TO A HIGH-FAT DIET. B.W.C. Rosser, R.M. Choksi*, B.J. Norris*, K.M. Baker* and P.M. Nemeth. Depts. of Neurology, and of Anatomy and Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Adult and neonatal rats were exposed to a high-fat low-carbohydrate diet to determine the effect of substrate availability on the metabolic phenotype of muscle. Neonatal laboratory rats normally switch from high-fat mother's milk to a high-carbohydrate lab chow diet at about 2 weeks of life and exhibit a gradual decline in lipid metabolism (Nemeth et al., J. Neurosci. 1989). By extending the exposure to high-fat, we found that β-hydroxyacyl CoA dehydrogenase (βOAC), carnitine acetyltransferase (KCAT) and thiolase activities were increased by over 50% at 1 week after weaning in soleus, EDL and diaphragm muscles. The activities continued to increase during the subsequent 5 weeks of the high-fat diet to attain levels 100% higher than controls. Kreb's cycle oxidative enzymes (MDH, SDH), glycolytic enzymes (LDH, PFK) and enzymes of high energy phosphate metabolism (AK, CK) were essentially equivalent to controls in homogenate assays.

Adult rats had a similar response with βOAC, KCAT and thiolase activities 25-100% higher than normal by 1 week after the onset of the high-fat diet. Examination of individual muscle fibers in the adult experiments revealed that increased lipid metabolism occurred in fibers of all types, with the IIb fibers having the largest increase. At 4 weeks βOAC was 45% higher than control in type I fibers, 33% higher in IIa fibers and 310% higher in IIb fibers. The IIb fibers also exhibited a 125% increase in Kreb's cycle enzymes, while these did not increase significantly in type I and IIa fibers.

The data indicate that both adult and neonatal muscles are capable adjusting their energy metabolism in response to dietary factors, and would support a dietary strategy to increase oxidative capacity. Supported by NIH grant DK38375.

52.10

VITAL MARKER FOR MUSCLE NUCLEI IN MYOBLAST TRANSFER: Q. Fang*, M. Chen*, H.J. Li*, T.G. Goodwin*, and P.K. Law. Depts. of Neurol. and Physiol./Biophys., Univ. of Tennessee, Memphis, TN 38163.

A new method is developed using Fluoro-Gold (FG) as a vital stain to label the nuclei of donor myoblasts in Myoblast Transfer studies. In culturo incubation with 0.01% FG for 16 hours resulted in 100% nuclei labelling. Intensive fluorescence persisted following nine days of subculture, when the human myoblasts were injected into the quadriceps of C57BL/6 mouse recipients immunosuppressed with cylcosporine. Injected muscles showed mosaicism of host and donor nuclei 25 days after injection, indicating (1) survival and fusion among donor myoblasts, and (2) fusion between howt and donor cells. (Supported by USPHS NS 26185, MDA, Sandoz and Walgreens).

52.11

ULTRASTRUCTURAL ANALYSIS OF CHRONICALLY ELECTRICALLY STIMULATED NERVE. B. Marts, K. Klueber, J. Brown*, and J. Hoffpauir*, Dept. Anat. Sci. & Neurobiol., Univ. of Louisville, School of Medicine, Louisville, KY 40292 and Dept. of Surgery, Indiana U. Medical School, Indianapolis, IN 46202

Are there structural changes in the nerve following chronic electrical stimulation? The objective of this study was to examine the cytoarchitecture of canine peripheral nerve after constant stimulation for 60, 150 and 365 days. The nerve to the rectus abdominis muscles of 3 female dogs per time point were stimulated electrically (2 Hz, 5.9V, 2 msec pulse duration) in order to convert Type 2 to Type 1 myofibers for use in a cardiac assist device. The implanted pacemaker did not interfere with the animals' activities. Following stimulation some myofibers exhibited signs of denervation characterized by: 1) disorganization of sarcomeres, 2) target and ring fibers, and 3) reinnervation. The stimulated nerves exhibited a decrease in the number of axons with a concomitant increase their diameter. In addition, some axons displayed signs of demyelination. A few myelin tubes appeared to contain more than 1 axon. Therefore, electrical stimulation of peripheral nerve causes axonal changes. Funded by: Surgery Dept, Indiana U. Sch. of Med. and 1R29DK41553-02.

HIPPOCAMPUS AND AMYGDALA: NEUROANATOMY

53.1

DIFFERENTIAL PROJECTIONS OF THE ANTERIOR AND POSTERIOR REGIONS OF THE MEDIAL AMYGDALOID NUCLEUS IN THE SYRIAN HAMSTER. D.M. Gomez and S.W. Newman, Department of Anatomy & Cell Biology, University of Michigan, Ann Arbor, MI 48109.

The medial amygdaloid nucleus (Me) is important for controlling mating behavior in the adult male Syrian hamster (*Mesocricetus auratus*). Chemosensory inputs from the olfactory bulbs project primarily to the anterior region of Me (MeA) whereas gonadal steroids are actively accumulated by neurons in posterior Me (MeP) and both of these signals are essential for this behavior. Not only are the sites of actions for these signals separated, but lesion studies have demonstrated functional differences between MeA and MeP. The efferent projections of MeA and MeP were investigated using an anterograde neuronal tracer, *Phaseolus vulgaris* leucoagglutinin (PHAL). Adult hamsters were iontophoretically injected with PHAL into either MeA (n=5) or MeP (n=7). After 10 days, animals were perfused with 4% paraformaldehyde. Coronal brain sections (40 um) were processed immunocytochemically for PHAL and adjacent sections were stained with cresyl-violet. Injection sites in MeP were confined; those aimed at MeA included more rostral areas but did not involve MeP. Both of these areas projected to the bed nucleus of the stria terminalis and the medial preoptic area with selective projections of MeA to the lateral, and MeP to the medial parts of these nuclei. Both areas also project to the ventromedial nucleus of the hypothalamus with MeA terminating in the core and MeP to the shell. In addition, reciprocal connections were observed between MeA and MeP. These findings suggest that both MeA and MeP have connections into distinct parts of reproductive control areas which may be important for the functional differences observed between these two regions. (Supported by NIH, NS #20629 to SWN).

53.2

CENTRAL NUCLEUS OF AMYGDALA AND BED NUCLEUS OF STRIA TERMINALIS PROJECTIONS TO SEROTONIN OR TYROSINE HYDROXYLASE IMMUNOREACTIVE CELLS IN THE DORSAL AND MEDIAN RAPHE NUCLEI IN THE RAT. D. J. Magnuson and T. S. Gray, Dept. of Cell Biology, Neurobiology and Anatomy, Loyola University Stritch Sch. of Med, Maywood, Il. 60153

The present study was designed to examine the projections of the bed nucleus of stria terminalis (BST) and the central nucleus of the amygdala to the dorsal raphe, median raphe and adjacent central gray. Injections of Phaseolus vulgaris leucoagglutinin lectin (PHA-L) were made into either the central nucleus of the amygdala or BST in anesthetized 250-350g male Long-Evans rats. Post-injection survival periods ranged from 12 to 16 d. PHA-L-immunoreactive fibers and terminals were visualized with horseradish peroxidase-brown diaminobenzidine reaction product. The tissue was then processed using standard immunocytochemistry for serotonin or tyrosine hydroxylase with a glucose oxidase-nitro-blue tetrazolium reaction product. The densest projections to the dorsal raphe and adjacent central gray were from the ventral lateral bed nucleus. A less dense projection to these regions was seen from the medial central nucleus. The median raphe nucleus was heavily innervated by the ventral lateral bed nucleus but only lightly by the medial central nucleus. Other subdivisions of the central amygdala and BST had limited or no projections to the raphe nuclei. Results demonstrate a topographically organized projection from the amygdala and bed nucleus of stria terminalis to the dorsal and median raphe nuclei. The data also indicate a quantitatively larger projection from the BST as compared to the amygdala. Supported by NS20041.

53.3

NEUROANATOMICAL MODEL OF INTRINSIC HIPPOCAMPAL CIRCUITRY IN THE RAT. A. Roskies, B. Armstrong, D.G. Amaral, and T.J. Sejnowski. Salk Institute, La Jolla, CA 92037.

Qualitative studies have revealed orderly gradients in connectivity density within and between various subfields of the rat hippocampus along the septotemporal, transverse, and radial axes (Amaral and Witter, Neuroscience 3, 1989). We have developed a method for quantifying these projections and are using these data to develop a probabilistic 3-D model of Shaffer collateral projections between CA3 and CA1. Such a model will be useful for large-scale simulations and analysis of *in vivo* and *in vitro* electrophysiological results.

We first performed densitometric analysis of projections arising from small injections in CA3 of the anterograde tracer *phaseolus vulgaris leucoagglutinin* (PHA-L). Optical density was sampled in volumes of 50μm x 50μm x 30μm. Natural coordinates of sampled areas were transformed into a coordinate system corresponding to a planar representation of an unfolded hippocampus. The modified coordinate system has major axes originating in the cell fields, corresponding to the CA2/CA1 border longitudinally, the transverse axis, and stratum oriens and stratum radiatum radially. This transformation allows mathematical analysis of connectivity in a rectangular coordinate system. A computational model based upon data from several injection sites is sufficient to predict projection probabilities from novel sites in CA3. Modeling of the connectivity density between areas CA3 and CA1 will provide the basis for predicting complex interactions between populations of cells in various portions of CA3 and their effects on cells in CA1.

53.4

ORGANIZATION OF CA1 PROJECTIONS TO THE RAT SUBICULUM: A PHA-L ANALYSIS. D. G. Amaral, P. Alvarez-Royo, C. L. Dolorfo and J. Weber. The Salk Institute and Group in Neurosciences, UCSD, La Jolla, CA 92037

We are conducting a comprehensive reinvestigation of the organization of intrinsic circuitry of the rat hippocampal formation using the anterograde tracer, *Phaseolus vulgaris leucoagglutinin* (PHA-L). In this study, iontophoretic injections have been placed at various transverse and septotemporal positions of the CA1 field and, after a survival period of 7-10 days, the brains were immunohistochemically prepared for the visualization of the lectin. The hippocampi were dissected from the brain and extended in the septotemporal axis to facilitate analysis of the longitudinal distribution of CA1 projections. Thirty-two successful experiments are currently available for analysis. CA1 projections to the subiculum terminate in the polymorphic, pyramidal and deep portion of the molecular layers. The outer portion of the molecular layer, in which the perforant path terminates, is free of CA1 input. As suggested by Tamamaki, based on his intracellular labeling studies, the CA1 projection to the subiculum terminates in a strikingly columnar fashion. CA1 cells located close to CA2 terminate in a column located distally in the subiculum, i.e. near the presubiculum. CA1 cells located close to the subiculum, in contrast, terminate in a column just across the CA1/subiculum border. CA1 cells in the mid transverse portion of the field, terminate in an intermediate position in the subiculum. While it is difficult to precisely determine the minimum transverse extent of a labeled column, in our cases it was generally about one-third of the extent of the field. The CA1 projections also appear to be fairly extensive in the septotemporal axis. In most cases, the projection extends for approximately one-third to one-half of the full septotemporal distance of the subiculum.

53.5

THE ORGANIZATION OF CORTICAL INPUTS TO THE PERIRHINAL (AREAS 35 AND 36) AND PARAHIPPOCAMPAL (AREAS TF AND TH) CORTICES IN THE MONKEY. W. A. Suzuki and D. G. Amaral. The Salk Institute and Group in Neurosciences, UCSD, La Jolla, CA 92037.

The perirhinal and parahippocampal cortices provide the major cortical projections to the entorhinal cortex of the monkey hippocampal formation. Although the perirhinal and parahippocampal cortices are reputed to be regions of polysensory convergence, a thorough examination of the cortical inputs to these regions has not yet been conducted. We have begun a series of studies investigating these inputs by placing injections of the retrograde tracers Fast Blue, Diamidino Yellow, and WGA-HRP into different levels of the perirhinal and parahippocampal cortices in the Macaca fascicularis monkey. Injections have thus far involved area 35, the temporal polar portion of area 36, and area TF. In all cases, retrogradely labeled cells were observed in both unimodal and polymodal association areas of the frontal, cingulate, temporal, insular, and parietal cortices. In our sample of injections, the visual association area TE projected more heavily to area 35 than to area TF. Conversely, the dorsolateral portion of the superior temporal gyrus (presumed auditory association cortex) and the dysgranular and granular portions of the insula (presumed somatosensory association cortices) projected more heavily to area TF. The polysensory areas labeled after injections of areas 35 or TF included the dorsal bank of the superior temporal sulcus, the cingulate cortex, the posterior parietal cortex and areas 46 and 13 of the frontal cortex. Although area 35 did not appear to receive substantial inputs from the parahippocampal cortex, there were some labeled cells in areas 35 and 36 after TF injections. In all cases, there were numerous labeled cells located in layer V of the entorhinal cortex. An understanding of the cortical inputs to the perirhinal and parahippocampal cortices is relevant to recent studies showing that these areas play a prominent role in memory function.

53.6

INTRINSIC CONNECTIONS OF THE MONKEY AMYGDALA: A PHA-L ANALYSIS OF PROJECTIONS ORIGINATING IN THE LATERAL NUCLEUS. A. Pitkänen and D.G. Amaral The Salk Institute, La Jolla, CA. 92037

We have begun a program of studies examining the intrinsic circuitry of the monkey amygdaloid complex using the lectin anterograde tracer, *Phaseolus vulgaris leucoagglutinin* (PHA-L). As a first step, PHA-L was focally iontophoresed into different portions of the lateral nucleus in different experimental animals. After a two week survival period, the brains were immunohistochemically processed to visualize the distribution of transported label. To optimize the staining in the monkey brain, several modifications were made to the procedure of Gerfen and Sawchenko. The results of our studies have confirmed many of the projections demonstrated earlier with the autoradiographic method. Projections from the lateral nucleus were observed, for example, to the accessory basal nucleus, the periamygdaloid cortex and the medial nucleus. The major new finding is that the lateral nucleus gives rise to a heavy projection to the basal nucleus of the amygdala. Fiber and terminal labeling was heaviest in the lateral part of the basal nucleus and rostral to the level of the injection site but was distributed to much of the rostrocaudal extent of the nucleus. This projection was not described in previous autoradiographic studies perhaps due to the difficulty in discriminating diffusion of isotope from labeled local projections. Ongoing studies are directed at determining the distribution and topography of intrinsic amygdaloid projections arising from different portions of the lateral nucleus. In particular, we are attempting to determine whether the two major subdivisions of the lateral nucleus (the dorsomedial and ventrolateral regions) are interconnected by intrinsic projections and whether these regions of the lateral nucleus have different projections to the other nuclei of the amygdaloid complex.

53.7

CONNECTIVITY OF THE DORSAL AND VENTRAL PRESUBICULUM OF THE RAT. I.S. Taube[1], F. Scalia[2], and D.G.Amaral[3]. [1]Department of Psychobiology, University of California, Irvine, CA 92717, [2]Department of Anatomy, SUNY Health Science Center, Brooklyn, NY 11203, and [3]The Salk Institute, La Jolla, CA 92037.

The connectivity of the dorsal and ventral portions of the presubiculum were investigated using anterograde (PHA-L) and retrograde (WGA-HRP) tracing techniques. These studies were conducted, in part, to determine whether the connectivity of the dorsal presubiculum was substantially different from that of the ventral presubiculum which would justify the use of the term "postsubiculum" for the dorsal region. Injections of PHA-L were successfully iontophoresed into various regions of the presubiculum in 25 animals and WGA-HRP was iontophoresed in an additional 20 animals.

Examination of Nissl and AChE preparations showed no clear-cut boundary between the dorsal and ventral presubiculum. PHA-L injections into all areas of presubicular cortex resulted in prominent labeling bilaterally in layer III of entorhinal cortex; additional labeling was also observed in layers I and II. All injections also led to projections to the anterior dorsal (AD), anterior ventral (AV), and lateral dorsal (LDDM) thalamic nuclei. Lighter labeling was observed in the lateral mammillary nucleus, retrosplenial and cingulate cortices, and contralateral areas of presubiculum. HRP injections into all areas of the presubiculum resulted in the labeling of cells in subicu-lum, AD, AV, and LDDM thalamic nuclei, and contralateral presubiculum. While there were minor variations in the patterns of anterograde and retrograde labeling in the various experimental cases, the connectivity of the dorsal presubiculum appeared to be very similar to that of the ventral presubiculum. These results suggest, therefore, that the dorsal presubiculum should not be considered a separate division of the subicular complex.

53.8

HETEROGENEITY OF LAYER II NEURONS IN HUMAN ENTORHINAL CORTEX. M.I. Beall, M. Akil, and D.A.Lewis. Depts. of Behav. Neurosci. and Psychiatry, Univ. of Pittsburgh, Pittsburgh, PA 15213.

Layer II neurons of entorhinal cortex (ERC), which cluster and form islands throughout most of this region, play an important role in relaying cortical information to the hippocampus. Abnormalities in these neurons have been implicated in neuropsychiatric disorders such as Alzheimer's disease and schizophrenia. However, relatively little is known about the chemical identity of the layer II neurons in normal human ERC. Left ERC was obtained at autopsy (postmortem interval 4.5-8 hrs.) from 3 individuals (ages 32-63) with no known neuropsychiatric disorders. In immunohistochemical studies, antibodies against neurofilament proteins (NF) and 2 calcium binding proteins, parvalbumin (PV) and calbindin (CB), labeled distinct subpopulations of neurons in layer II and deeper layers. In the layer II islands, a subpopulation of modified pyramidal neurons was intensely immunoreactive (IR) for NF. The number of NF-IR neurons in layer II increased in a medial to lateral gradient along the rostro-caudal axis of the ERC such that at rostral levels of ERC, NF-IR neurons were present only medially and at more caudal levels they extended farther laterally. A similar gradient was observed with smaller PV-IR neurons located in the deep half of layer II islands. A different pattern of labeling was observed with CB; layer II islands in rostral ERC contained CB-IR punctate whereas in caudal portions of ERC small CB-positive neurons were grouped between the islands. These data reveal the heterogeneity of layer II neurons in normal human ERC and may provide insight into the differential roles of these neurons in ERC function.

53.9

EFFECT OF IBOTENIC ACID LESION OF THE DORSAL HIPPOCAMPUS ON CEREBRAL DOPAMINERGIC SYSTEMS IN THE RAT. B. K. Lipska, G. E. Jaskiw, K. Farouk, J. E. Kleinman, D.R. Weinberger. CBDB NIMH Neuroscience Center at St. Elizabeths Hospital, Washington, D.C. 20032.

We have shown that medial prefrontal cortical lesions alter basal presynaptic DA metabolite indices in anteromedial caudate14 days postoperatively and permanently disrupt normal behavioral responses to mild stress. To examine whether similar effects result from dorsal hippocampal lesion, rats were lesioned bilaterally (coordinates from bregma AP -3.0 mm, ML +2.2 mm, VD -3.9 mm) with ibotenic acid (5 µg in 0.5 µl) or vehicle (buffered saline). Locomotion was assessed on the 14th and 28th days postoperatively. In ibotenic acid-lesioned rats spontaneous but not d-amphetamine-induced (1.5mg/kg) locomotion was increased on the 14th but not 28th day after lesion. The levels of DA, DOPAC, HVA assayed by GC/MS in anteromedial caudate and nucleus accumbens were not afected in lesioned in comparison to sham-operated rats. However, in rats with ibotenic acid lesions, the anxogenic ß-carboline FG 7142 and 48hr food deprivation-mild stressors - failed to attenuate locomotor exploration in comparison to sham lesioned rats. These results indicate that, unlike medial prefrontal cortical lesions, the lesions of the dorsal hippocampus do not alter basal levels of DA and metabolites, but they disturb the behavioral responses to stress in the unique way.

53.10

DISTINCT NEUROCHEMICAL FEATURES OF BRAIN METALLOTHIONEINS. M. Ebadi, and A. Earle . Depts. of Pharmacol., Neurol., and Anat., Univ. Nebr. Coll. Med., 600 S. 42nd St, Omaha, NE 68198.

Metallothioneins (MT), are low molecular weight metal binding proteins that consist of a single polypeptide chain of 61 amino acids, 25-30% of whose residues are cysteine, and that are devoid of any disulfide bonds or aromatic amino acids. We report that the brain MT possesses unique features which distinguish them from those found in the peripheral tissues. Unlike in the liver, the level of brain MT increases postnatally, and its synthesis is not induced following administration of cadmium, ethyl alcohol, or dexamethasone. On the other hand, the zinc-induced MT synthesis in the brain is associated with an accumulation of mRNA which is analogous to the zinc-induced synthesis of hepatic MT mRNA. In addition, antibodies formed against both the sheep- and rat- hepatic metallothioneins cross react completely with the hippocampal metallothioneins, suggesting that the immunologically dominant regions of the NH_2-terminal domain (residues 1-29) of neuronal metallothioneins are conserved. The regions of brains such as pineal gland, retina, and hippocampus which have high concentrations of zinc, not only synthesize MT on a continuous basis, but also have other low molecular weight zinc binding proteins of unknown nature. Since, the synthesis of brain MT is increased following icv administration of zinc and is decreased in zinc deficiency state, we postulate that zinc may regulate the synthesis of brain MT. In view of the fact that neither essential elements such as zinc and copper nor non essential elements such as cadmium and barium traverse the brain readily, it is very doubtful that the brain MTs play major roles in acute metal detoxification. On the other hand, the brain may have developed unique processes for transporting, compartmenting, releasing, and utilizing zinc, copper, calcium and other essential elements, commensurate with the diversified and vital functions endowed and invested in its various regions. (supported in part by a grant from USPHS ES-03949).

53.11

AMYGDALOID PROJECTIONS TO HIPPOCAMPAL-RECIPIENT AND -NON-RECIPIENT REGIONS OF THE ORBITOFRONTAL CORTEX IN THE MACAQUE MONKEY. C. Cavada and F. Reinoso-Suárez. Departamento de Morfología, Facultad de Medicina, Universidad Autónoma de Madrid, Spain.

In previous studies we have shown that the medial sector of the orbitofrontal cortex of macaques, including Walker's areas 14, 10, and the border region between 10 and 11, is the target of direct projections from hippocampal field CA1. This anatomical linkage suggests that the medial orbitofrontal cortex may be involved in memory function. Considering the debate about the participation of the amygdaloid complex in mnemonic processes, we have analyzed the neuronal labeling present in the amygdala in the same brains used in the hippocampal study. Multiple labeling experiments were made in both hemispheres of adult macaque monkeys by injecting retrograde tracers (HRP-WGA, FB and DY) in the various areas of the orbitofrontal cortex. Interestingly, the injections that produced the heaviest hippocampal labeling gave the weakest amygdaloid labeling, and viceversa. Moreover, we uncovered a notable topographical organization of the amygdalo-orbitofrontal projections. The hippocampal-recipient medial areas 14 and 10-11 receive sparse amygdaloid projections, that arise in the basal and accesory basal nuclei, principally in their posterior half. Area 14 is also the target of a weak projection from the posterodorsal part of the lateral nucleus. By contrast, the adjacent hippocampal-non-recipient area 13 receives an abundant projection from the lateral and accessory basal nuclei, and a less abundant one from the basal nucleus. Areas 12 and 11, that are also devoid of hippocampal input, are the targets of a heavy amygdaloid projection arising in the basal nucleus, principally in its intermediate subdivision. These findings show that the territories accessed by hippocampal and amygdaloid projections in the orbitofrontal cortex are mutually exclusive rather than convergent, arguing in favor of a functional diversity between medial and lateral orbitofrontal sectors.

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53.12

INTRAGRANULAR MOSSY FIBERS IN RATS FORM SYNAPSES WITH THE SOMATA AND PROXIMAL DENDRITES OF BASKET CELLS. C. E. Ribak and G. M. Peterson. Dept. of Anatomy and Neurobiol., Univ. of Calif., Irvine, CA 92717 and Dept. of Anatomy and Cell Biol., East Carolina Univ. Sch. of Med., Greenville, NC 27858.

Intragranular and supragranular mossy fibers arise from granule cells and occur in the dentate gyrus of hippocampi from normal and epileptic animals. Often, the intragranular fibers appear at periodic intervals perpendicular to the long axis of the granule cell layer. Rats were analyzed to determine whether such mossy fibers are associated with another cell type in the granule cell layer, the basket cell, which sends apical dendrites through this layer at periodic intervals. The brains of normal rats were prepared using the Timm's sulfide silver histochemical method that is specific for axon terminals with high levels of heavy metals and labels the mossy fibers in the dentate gyrus. The light microscopic results show that most intragranular mossy fibers are apposed to the surfaces of the somata and apical dendrites of four types of basket cell. These basket cells have different orientations of their somata and proximal dendrites but all have cell bodies in the granule cell layer. Electron microscopic results show that the labeled mossy fibers form asymmetric synapses with the somata and apical dendrites of basket cells. Previous data indicated that mossy fibers formed synapses in the hilus with only the basal dendrites of basket cells. These new data show that intragranular mossy fibers also form synapses with basket cells, a major GABAergic inhibitory cell type in the dentate gyrus. These data suggest that intragranular mossy fibers provide additional feedback inhibition of granule cells.

53.13

RECURRENT SYNAPTIC CIRCUITRY IN THE CA3 REGION OF HIPPOCAMPAL SLICES FOLLOWING FORNIX LESIONS. *P.A. Rutecki*. Section of Neurophysiology, Dept. of Neurology, Baylor College of Medicine, Houston, TX 77030.

The recurrent excitatory synaptic connections in the CA3 region of the hippocampus appear to be the substrate for the synaptic synchronization that results in epileptiform activity. To investigate these synaptic connections, Sprague Dawely rats were anesthetized and electrolytic lesions were made to deafferent the fornix of septal and commissural inputs. Animals were allowed to survive 1-10 weeks and then sacrificed for slice preparation. The lesion was demonstrated using cresyl violet staining. On average, there was a ≈70% decrease in hippocampal acetylcholinesterase activity on the side of the lesion. Following such lesions, slices from 5 of 26 animals displayed spontaneously occurring or evoked epileptiform activity in 5 mM $[K^+]_o$ saline. Using single-electrode voltage-clamp techniques in slices without epileptiform activity, stimulation of the fornix produced a robust IPSP that was primarily composed of a chloride conductance. In the presence of 10-50 μM picrotoxin, a short latency excitatory synaptic current was observed; however, in 5 of 8 neurons, the excitatory current could not be studied in isolation because of contamination by an overlapping, residual inhibitory current. In the other 3 neurons the excitatory synaptic current reversal potential was between −7 and −3 mV and was associated with a conductance of 10-20 nS.

Hippocampal slices from rats with chronic fornix deafferentation appear to be a useful preparation for studying local recurrent synaptic circuitry. Fornix stimulation evokes overlapping excitatory and inhibitory synaptic currents in CA3 pyramidal neurons. The occurrence of epileptiform discharges suggests that in some cases deafferentation results in new excitatory local circuitry. (Supported by NIH grant NS-11535 and the Klingenstein Fund.)

53.14

PROJECTIONS FROM CA_1 AND SUBICULUM TO ANTERIOR OLFACTORY NUCLEUS AND OLFACTORY BULB. Th. van Groen, C. Rodenburg*, and J.M. Wyss. Dept of Cell Biology and Anatomy, University of Alabama, Birmingham, AL 35294

Most research into the extrinsic projections of the hippocampal formation in the rat has focused on the contribution of subicular neurons and relatively little attention has been given to the contribution of CA_1 neurons. In recent studies we noted a previously undescribed projection arising from area CA_1 and subiculum to the olfactory bulb and the anterior olfactory nucleus. To further investigate this projection of the hippocampal formation, anterograde and retrograde tracing studies were conducted. For anterograde tracing experiments, small injections of PHA-L were made into area CA_1 and subiculum. Injections in the temporal third of area CA_1 labeled a dense axon and terminal plexus in the internal plexiform layer of the olfactory bulb and a less dense terminal plexus in the anterior olfactory nucleus. Following injections in the subiculum a dense plexus of axons and terminals was labeled in the anterior olfactory nucleus and a less dense terminal plexus was labeled in the olfactory bulb. Injections of the fluorescent, retrograde tracer fluorogold into the olfactory bulb labeled neurons in area CA_1 and a smaller number of neurons in the subiculum. Injections in the anterior olfactory nucleus labeled many neurons in the subiculum and only a few neurons in area CA_1. Neurons both within and superficial to the pyramidal cell layer were labeled by these injections, but all labeled neurons were confined to the temporal one-third of the hippocampal formation. These results demonstrate that the anterior olfactory nucleus and the olfactory bulb receive a projection from the temporal one-third of CA_1 and subiculum.

53.15

DYNAMIC CHANGES IN 'EFFECTIVE CONNECTIVITY' BETWEEN SIMULATED HIPPOCAMPAL NEURONS Gy. Gaal[1,2], Z. Pan[1]*, P.H. Bedenbaugh[1], G.L. Gerstein[1], [1] *Departments of Physiology and Bioengineering, University of Pennsylvania, Philadelphia,* [2] *Institute of Experimental Medicine, Hungarian Academy of Sciences, Budapest, Hungary*

Dynamic changes in the 'effective connectivity' between simultaneously observed neurons have been found by several laboratories. These can be revealed by Gerstein's gravitational clustering algorithm (Gerstein et. al., J. Neurosci. 5, 1985) and by normalization of the classical joint peristimulus time histogram (Aertsen et. al., J. Neurophys. 61, 1989). Changes of correlation have been observed in the hippocampus with a shift from the presence to the absence of theta rythm (Kuperstein et. al., Exp. Brain Res. 61, 1986), and with a shift from sleep to wakefulness (Noda et. al., Exp. Neurol., 24, 1969). However, these measurements were not corrected for rate changes.

We have constructed a model of the hippocampus using a variation of MacGregor's LRSYS32 program (Neural and Brain Modeling , Academic Press, 1987). This program simulates neurons as four state variable nonlinear systems. Hebbian changes in synaptic strength occur when pre- and postsynaptic cells are simultaneously activated. The model included a unidirectional trisynaptic excitatory pathway and feedback and feedforward inhibition within the hippocampus, reciprocal projections with the entorhinal cortex and contralateral hippocampus and a unidirectional input from the septum. The cells in CA3 were of the bursting type. The EEG was adequately simulated by defining it as the total number of neurons active at each time step. The theta rythm was simulated by periodic modulation of the septal input projection.

Changes in correlation similar to those observed experimentally have been observed in our model even when the synaptic strengths are held constant. We are investigating corrections for nonstationarity induced by the theta stimulation, and are comparing changes in effective connectivity induced by synaptic plasticity to those induced by differential activation of neural assemblies.

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53.16

IN VITRO VISUALIZATION OF THE HIPPOCAMPAL MOSSY FIBER PATHWAY WITH EXTRACELLULAR BIOCYTIN. M.M. Okazaki and J.V. Nadler. Depts. Pharmacology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

We have developed a method for visualizing dentate granule cells and their mossy fibers in the rat hippocampal slice after extracellular ionophoresis of biocytin. Horizontal 400-um thick hippocampal slices were prepared from adult male rats with a vibratome. Glass micropipettes with tip diameters of 5 um were filled with buffered 4-6% (w/v) biocytin. Biocytin was ejected into the granule cell body layer by passing positive current through the micropipette (300-600 nA for 5 min, 10 sec on/10 sec off). Uptake and transport were allowed to proceed for 3 hr, after which the slices were fixed in buffered 2% paraformaldehyde/1% glutaraldehyde at 4 °C. Slices were then cut into 50 um-thick sections with a vibratome and the biocytin was visualized with streptavidin-alkaline phosphatase (SAP) followed by the NBT/BCIP chromogen reaction (modified from King et al., 1989; Leary et al., 1983). Endogenous alkaline phosphatase activity was suppressed by preincubating the sections for 1 hr in buffered 0.5 mM EDTA. To inhibit the non-specific binding of SAP, the sections were incubated with 3% (w/v) bovine serum albumin and all glassware that contacted the SAP reagent was prerinsed in the albumin solution. This method discretely labeled a small number of dentate granule cells and all their processes with minimal background and blood vessel staining. The full extent of the mossy fiber projection, including the giant boutons, was clearly visualized. (Supported by NIH grant NS 17771.)

53.17

ORGANIZATION OF THE ENTORHINAL PROJECTIONS TO THE HIPPOCAMPAL FORMATION OF THE RAT. M.P. Witter*. Dept. of Anatomy, Vrije Universiteit, Amsterdam, The Netherlands (SPON: European Neuroscience Association).

The organization of the "perforant path" (pp) was analyzed using anterograde tracing with *Phaseolus vulgaris*-leucoagglutinin. Emphasis was on i) the terminal distribution in each of the subfields of the hippocampal formation (HF), i.e. dentate gyrus (DG), fields CA3, CA2, and CA1, and subiculum (S); ii) the putative dissociation in lateral, medial, and intermediate components of pp.

All subfields of HF are reached by fibers from the entorhinal cortex (EC). In DG and CA3/CA2, entorhinal fibers terminate along the entire transverse axis of the molecular layer, but show clearly restricted radial distributions. By contrast, in CA1 and S, fibers terminate in restricted portions along the transverse axis but fully cover of the radial width of the molecular layer. A semiquantitative analysis of the terminal patterns in the various subdivisions of HF supports the differentiation between lateral and medial pp and further indicates that artifactual labeling of an intermediate pp results from injections that involve parts of both the medial and lateral EC. The difference between DG and CA3/CA2 on the one hand and CA1 and S on the other with respect to the terminal organizations of the lateral and medial components of pp suggests a functionally different interaction between EC and the two hippocampal entities.

LIMBIC SYSTEM I

54.1

RAT BASAL NUCLEI PROJECT TO THE RETROSPLENIAL CORTEX VIA THE FORNIX. S.L.Gage,S.R.Keim, and W.C.Low. Prog. in Med. Neurobiol. and Dept. of Physiol. and Biophys., Indiana Univ. Sch. of Med., Indpls., IN 46202.

The cholinergic input to the retrosplenial cortex (RSC) has been defined by previous investigators as a single pathway extending primarily from the medial septal nucleus (MSN) and the diagonal band of Broca (DBB). This pathway has been shown to extend from these nuclei anteriorly and superiorly around the genu of the corpus callosum, and then posteriorly towards the RSC in a bundle positioned dorsal and medial to the cingulum. Our preliminary studies to confirm the nature of the cholinergic input to the RSC support the presence of this pathway, but also suggest the existance of an additional cholinergic projection. The retrograde tracer, Fluoro-Gold was injected into the right RSC of 12 male rats (-5.8 mm AP; 0.7 mm ML; -2.2 to - 1.0 mm DV). Prior to injection, 4 rats received bilateral aspiration lesions of the cortex and corpus callosum immediately posterior to Bregma, including all medial grey and white matter laterally through the cingulum. Four additional rats received lesions of the cortex as described, as well as lesions of the underlying fornix. Survival time was two weeks. Following fixation, sections were vibratomed at 50 um. Sections were either mounted on slides and dried for fluoroscopic (UV) inspection, or incubated with acetylthiocholine for 18 hr, reacted with sodium sulfide, mounted on slides and covered slipped for inspection of acetylcholinesterase (AChE) staining pattern by light microscopy. Non-lesioned animals displayed the typical laminar pattern of AChE staining in the RSC and numerous intensely fluorescent cells bilateraly in the MSN and DBB. The number of fluorescent cells was much greater in the basal nuclei ipsilateral (I) rather then contralateral (C) to the injection site. Animals with the cortex plus fornix lesion consistantly showed a complete loss of AChE staining bilateraly in the RSC, as well as a complete bilateral lack of fluorescent cells in the MSN and the DBB. Animals with the cortical lesion alone displayed a partial loss of AChE staining. This decrease from the normal AChE staining pattern was more notable in the anterior RSC and became less significant in the posterior RSC. In addition, each cortically lesioned animal displayed numerous fluorescent cells bilateraly in the MSN and the DBB (I>C). No fluorescent cells were noted in the substantia innominata or the nucleus basalis of any animal denoting the localization of the injections to medial cortex. This evidence indicates that there exists a pathway from the MSN and DBB that is not mediated by the previously documented cortical route and is most probably mediated by the fornix. While the present study does not prove the nature of the transmitter involved in this pathway, the combination of the AChE results and the location of the cells of origin imply that this pathway is at least partially cholinergic.

54.2

DIFFERENCES IN CONNECTIVITY WITHIN THE CINGULATE CORTEX IN RAT. D. Zeng and S.L. Stuesse, Dept. of Neurobiology, N.E. Ohio Univ. College of Medicine, Rootstown, OH 44272.

Rat cingulate cortex occupies a large portion of anterior-medial cortex and has been subdivided into three areas based on cytoarchitectonics (Zilles, 1985). We compared afferent and efferent connections of two of these areas, Cg1/Cg2 versus Cg3, by iontophoresing small quantities of wheat germ agglutinated horseradish peroxidase into either of these two divisions and identifying efferent connections and the cell groups which projected to these areas. 50 μm serial sections were processed using Mesulam's tetramethylbenzidene method. Within the thalamus, areas with Cg3 connections tended to be medial to those of Cg1/Cg2. Cg3 received projections from the mediodorsal, ventral reuniens, paraventricular, and paratenial nuclei while Cg1/Cg2 was connected to the mediodorsal, paratenial, portions of the ventrobasilar complex, and many of the posterior and pretectal nuclei. Cortical projections were more widespread to Cg3 than to Cg1/Cg2 and included the infralimbic, perirhinal, agranular insular, dorsoendopiriform, and other parts of "limbic" cortex. Cg1/Cg2 was interconnected primarily with retrosplenial cortex. Cg1/Cg2 was connected with the claustrum while Cg3 was not. Cg3 received input from the CA1 layer of the hippocampus, the lateral hypothalamus, amygdala, globus pallidus, and portions of the ventral striatum. In the brainstem, both received input from Barrington's nucleus, dorsal tegmental nuclei, and raphe dorsalis. Cg3 received input from raphe linearis while Cg1/Cg2 received it from the rostral nucleus of the medial longitudinal fasciculus. Projections to the brainstem from Cg1/Cg2 targeted deep layers of the superior colliculus, reticularis gigantocellularis, nucleus cuneiformis, the paramedian pontine reticular formation, and the A5 region. Thus Cg3 should be considered a "limbic" area while Cg1/Cg2 has more "motor" connections and may contain a portion of the frontal eye fields. Supported by NIH MH43363.

54.3

ANTERIOR CINGULATE CORTEX PROJECTIONS TO RETROSPLENIAL AND POSTSUBICULAR CORTICES. G. Fisk, Th. van Groen and J.M. Wyss. Dept. of Cell Biology and Anatomy, University of Alabama at Birmingham, Birmingham, AL 35294

Small iontophoretic injections of the anterograde tracer PHA-L were made into the anterior cingulate cortex (infraradiata cortex; IRα, anterior; IRβ, posterior) to characterize the efferent projections of these areas. IRβ injections labeled dense terminal plexes in the retrosplenial and postsubicular cortices. Following dorsal IRβ (IRβc) injections the densest labeling was consistently in the postsubicular and retrosplenial agranular (Rag) cortices, with much less dense labeling in rostral retrosplenial granular a cortex (Rga) and in the ventral half of the retrosplenial granular b cortex (Rgb). The projection to Rag terminated in the dorsolateral one-third of this cortex and extended into the adjacent part of area 18. The results suggest that the anterior to posterior position of IRβc neurons correlates with the anterior to posterior position of the terminal plexes in the postsubiculum. Following ventral IRβ (IRβa and IRβb) injections, the densest labeling was consistently in Rgb and Rga cortices, with less dense labeling in Rag and postsubicular cortices. Compared to IRβ injections, injections into IRα cortex labeled fewer axons and terminals in retrosplenial and postsubicular cortices. These projections primarily originated in the caudal part of IRαc and terminated in the rostralmost postsubiculum. Injections made into rostral IRαc labeled dense terminal plexes in the contralateral, homotopic cortex, but did not label terminals in postsubicular or retrosplenial cortices.

54.4

ANALYSIS OF PIRIFORM CORTEX NEURONS IN THE GUINEA PIG WITH INTRACELLULAR STAINING TECHNIQUE. K. Kishi, and K. Murakami*. Dept. Anat. Toho Univ. Schl. of Med. Tokyo, Japan.

This study is aimed at quantatative analysis on the morphology of piriform cortex neurons in the adult guinea pig anesthetized with pentobarbital. Biocytin (Sigma, 4-6% solution) was intracellularly injected into piriform cortex neurons at the level of anterior commissure. Survival time ranged from 4-24 hr. Eighty micron thick frozen sections were stained with ABC reagent. Three cell types were morphologically identified: pyramidal neuron in layer II, pyramidal neuron in layer III, and multipolar neuron in layer III and endopiriform nucleus. Pyramidal neurons in layer II exhibited a single apical dendrite and one to three basal dendrites. Apical dendrite branched into a large number of secondary dendrites in the layer II, and the terminal branches reached to the layer I a. The basal dendrites distributed in layers III and II. The axon arose from the deep side of the cell body or from the stem dendrite, and run caudally in the depth of layer III or to the endopiriform nucleus. Axon collaterals were emitted at first in the layer III of the vicinity of cell body, and then at relatively regular intervals (around 500 μm intervals) through the course of main axon. These preliminary results suggest that majority of pyramidal neurons of layer II at the level of anterior commissure project their axons to the caudal layer III.

54.5

90^0 PHASE LAG IN OUTPUTS OF EXCITATORY AND INHIBITORY NEURONS CHARACTERIZES CORTICAL NEGATIVE FEEDBACK LOOPS
W. J. Freeman and F. H. Eeckman, Department of Molecular and Cell Biology, University of California at Berkeley CA 94720

Oscillations about "40 Hz" (the gamma range of 20 to 90 Hz) in "spontaneous" and evoked activities of single neurons in the visual, auditory, somesthetic and olfactory cortices raise the question: Are they the property of individual neurons that become entrained, or the property of populations of excitatory and inhibitory neurons, that are coupled by synapses?

Analysis of pulse trains of cortical neurons yields unequivocal answers. On the one hand, the mean firing rates of most cortical neurons are far less than the gamma range, and the pulse intervals are not periodic. Hence they are not individual oscillators and cannot entrain each other. On the other hand, interneuronal feedback loops require that two classes of neurons exist in each area of cortex, that show oscillation at the same frequency, but with a quarter cycle of phase lag by the activity of the inhibitory neurons behind the excitatory neurons. These two classes have been seen in every area of cortex where they have been sought, including the "spontaneous" EEG and averaged evoked activity of the olfactory bulb and the pyriform cortex (Freeman,1975), the anterior nucleus and entorhinal area (Eeckman & Freeman,1990), the hippocampus (Horowitz, et al.,1968), and the visual cortex (Gray, 1989). Where ever the inhibitory neurons are identified, their output lags. These findings are necessary and sufficient experimental proof of interneuronal negative feedback and indicate that it is widespread in the cerebral cortex. NIMH06686.

54.6

KINETICS OF RO 5-4864-INDUCED CHANGES IN LIMBIC EVOKED POTENTIALS IN THE FREELY BEHAVING RAT.
D. E. Woolley, K.-S. DAI* and H.L. Drummer. Dept. of Animal Physiol., Univ. of Calif., Davis, CA 95616.

To learn more about the mechanisms underlying the convulsive effects of the atypical benzodiazepine Ro 5-4864, a dose (15 mg/kg i.p. in DMSO) which produced only minimal seizure activity, e.g., myoclonus, 7-30 min after administration was studied in 17 rats. Amplitude of the population slow wave (SW) evoked in the dentate gyrus (DG) by stimulation of the prepyriform cortex (PPC) was already increased at 20 min (the first recording), with a maximum increase (80%) at the lowest intensity 2 hr after administration. With increasing stimulus intensity the percent increase was not as great (50-70%), but the increase lasted longer, e.g., through 24 hr instead of 6 hr. Thus, the maximal increase in amplitude of the PPC-evoked DG response occurred one or more hr after seizure activity. Amplitude of the population spike produced in the DG by stimulation of the dorsal perforant path was increased several-fold by 20 min or even earlier, but was back to control levels by 6 hr, and so more closely paralleled seizure activity. Because the SW did not change, Ro 5-4864 appears to have enhanced synaptic efficacy.

54.7

MAPPING SEPTAL (S) EFFECTS ON HIPPOCAMPAL (H) POPULATION SPIKES IN CA1 OF RAT.
P.G. Newlon, and S.K. Gudeman*, Dept. of Neurosurgery, Eastern Virginia Med. Sch., Norfolk, Va. 23507-1912.

Septal facilitation of intrinsic hippocampal population responses has been reported both in the presence and absence of S-induced field potentials in the hippocampus. We examined S-H interactions when S stimulation was aimed at 2 sites within the S in each of 20 rats: One in which S stimulation produced a field potential in CA1, and a deeper site in which no field was evoked in CA1. Paired S and H (CA3) pulses were presented over interstimulus intervals (ISI) from 0-1.5 sec, and changes in the CA1 population spike amplitude were examined. When a population response was present in CA1 with S stimulation, facilitation was obtained at ISI's /= 100 msec, and depression at shorter ISI's. However, at S sites that produced no CA1 field potential, depression was common. It seems probable that septohippocampal facilitation in the presence of a field response may represent activation of CA1 via S-H input to CA3 rather than by monosynaptic S-H input to CA1. The source of the depressant effect is unknown, and deserves exploration. This work was funded in part by an R. Clifton Brooks, Jr., Fellowship and the Jeffress Trust.

54.8

PHARMACOLOGICAL MECHANISMS UNDERLYING HIPPOCAMPAL RSA.
D.K. Bilkey and A.J. Heynen. Dept. of Psychology, University of Otago, Dunedin, New Zealand.

Using the in vitro model of hippocampal rhythmical slow activity (RSA) developed by Bland and coworkers (1986), we have confirmed the report of McVicar and Tse (1989) that carbachol-induced RSA is rarely observed in slices from adult rats. When the GABA antagonist picrotoxin (PTX) is introduced into the bathing medium, however, carbachol (CARB) reliably produces RSA in adult slices. Furthermore, RSA could also be induced by coinfusion of glutamate (GLU) and PTX, while neither of these substances alone produced rhythmicity. In vitro RSA, therefore, requires both the excitation produced by CARB or GLU, and coincident disinhibition produced by PTX. Similar mechanisms may operate in vivo. In a test of this hypothesis we found that microinfusion of CARB into the rat dentate gyrus produces RSA. This can be blocked by inactivating the medial septum with a procaine microinfusion. The subsequent cannulation of PTX into the dentate produces a recovery of rhythmicity. Similarly, RSA can also be induced in vivo by concurrent microinfusion of GLU and PTX into a rat with a procained septum. The disinhibitory effects produced by PTX may be provided in situ by the septo-hippocampal circuit described by Bilkey and Goddard (1985). The excitation produced by GLU and CARB may model pharmacological events occurring during type 1 and type 2 RSA, respectively.

54.9

A RE-EVALUATION OF NEURONAL CONFIGURATIONS IN THE RAT BED NUCLEUS OF THE STRIA TERMINALIS (BNST).
C-J. Shi, L. Roberts and M.D. Cassell. Dept. Anat., Univ. of Iowa, Iowa City, IA 52242.

Recent studies (Ju and Swanson, 1988) indicate that the rat BNST consists of at least 20 cyto-architectonically distinct subdivisions. In view of the limited amount of information available on neuron morphology in this area, we have evaluated this proposed cytoarchitectonic division of the BNST using the section-Golgi method. The anterodorsal BNST contains mainly small, round moderately spiny neurons with those in its central core resembling the larger, multipolar type characteristic of the medial central amygdaloid nucleus. The rostral part of the anterolateral BNST (oval nucleus) contains medium-sized spiny neurons resembling those of the caudate-putamen. The caudal part of this subdivision contains larger, but more moderately spiny cells. The anteroventral BNST contains mostly medium-sized, fusiform cells, with larger, sparsely spinous cells resembling preoptic neurons, in the magnocellular division. In the principle nucleus, small neurons with densely spinous secondary dendrites predominate. The interfascicular and transverse nuclei contain medium to large-sized cells with moderately spiny dendrites. Overall, the data indicate that each subdivision of the BNST contains a characteristic, if heterogeneous, population of neurons. The presence of different types of cells within specific regions of cytoarchitectonic divisions suggests that further fractionation of the BNST may be warranted. Supported by NIH Grant NS25139.

54.10

DISTRIBUTION PATTERNS OF RAT CENTRAL AMYGDALOID (Ce) EFFERENT NEURONS.
M.D. Cassell, N. Sun and L. Roberts*. Dept. Anat. Univ. of Iowa, Iowa City, IA 52242.

Previous studies (Veening et al., 1984; Cassell et al., 1986) have reported that Ce neurons projecting to the dorsal vagal complex (DVC), parabrachial complex (PB) and bed nucleus of the stria terminalis (BNST) distribute in overlapping medial/lateral trends that do not correspond to the major cytoarchitectonic divisions of the Ce. In contrast, many Ce afferents (e.g., from the insular cortex), neuropeptides and histochemical markers (e.g. Timm stain) show close correspondence with these divisions. We have therefore re-evaluated the distribution of Ce efferent neurons using double injections of bisbenzimide, Fast Blue and Fluorogold into various combinations of sites in the BNST, PB and DVC. Overall the data indicate that rather than following general medial/lateral trends, the distribution patterns of Ce efferent neurons show a strong correlation with the underlying cytoarchitecture. Ce neurons projecting to BNST distribute over the lateral, lateral capsular, ventral and intermediate subdivisions with numbers of cells located in a distinct caudal part of the medial subdivision. However, cells in individual Ce subdivisions project to distinct regions of the BNST. Ce neurons projecting to the DVC present an almost exact complementary distribution in the rostral and middle parts of the medial subdivision. Ce neurons projecting to the PB are almost exclusively confined to the lateral subdivision where they are intermingled with cells projecting to the BNST. The complementary patterns of BNST and DVC neurons correspond precisely with the differential distribution patterns of insular cortical afferents, dark/light Timm's staining and enkephalin/pro-opiomelanocortin immunostaining. Supported by NIH Grant NS25139.

54.11

Input-Output Connectivity of the Sublenticular Continuum of the Basal Forebrain: Introducing the Localization of Fluorescent Axonal Tracers at both the Light and Electron Microscopic Level. L. Schmued and L. Heimer, Otolaryngology Dept., University of Virginia Health Science Center, Charlottesville, VA 22908.

Extending horizontally beneath the lentiform nucleus lies a continuum which shares many common features including extensive internuclear connections, extensive reciprocal connections with brainstem autonomic nuclei, major inputs from ventral temporal cortical areas, and immunoreactivity to αangiotensin II. This sublenticular continuum (SLC) includes the amygdala central nucleus, sublenticular substantia innominata, and lateral bed nucleus of the stria terminalis. The purpose of this study is to examine potential monosynaptic connections of the SLC. To do this, the recently introduced anterograde fluorescent tracer, Fluoro-Ruby, was injected into either allocortical sites, lateral amygdala nuclei, or the parabrachial nuclei. The retrogradely transported fluorescent tracer, Fluoro-Gold, was injected into either the nucleus of the solitary tract or parabrachial nuclei. Examination of the SLC with the fluorescent microscope reveals retrogradely labeled cells following Fluoro-Gold injection into the nucleus of the solitary tract which overlap labeled terminals resulting from Fluoro-Ruby injection into either the parabrachial nucleus or the basolateral amygdala. Only a few fibers could be found in the SLC following Fluoro-Ruby injection into allocortical regions, in contrast to the basolateral amygdala which contained dense terminal plexi. We are presently examining the first two cases at the electron microscopic level to look for monosynaptic connections. We have been able to visualize the fluorescent axonal tracers electron microscopically by combining an antibody to Fluoro-Gold (H. Chang) with a photo conversion procedure we have recently developed for detection of Fluoro-Ruby. (Supported in part by NIH grants NS-17743 and HD07323.)

54.12

GABA TERMINALS FORM SYMMETRIC SYNAPSES PRIMARILY WITH DOPAMINERGIC NEURONS IN THE RAT VENTRAL TEGMENTAL AREA: GABA-INPUT INVERSELY PROPORTIONAL TO THE LEVEL OF TYROSINE HYDROXYLASE IMMUNOREACTIVITY. V. E. Bayer and V. M. Pickel. Div. of Neurobiology, Dept. of Neurology & Neurosciences, Cornell Univ. Med. College, NY, NY 10021.

We examined the ultrastructural basis for GABAergic modulation of dopaminergic (DA) neurons in the rat ventral tegmental area (VTA). Single sections were labeled with rat GABA-antiserum and a rabbit antiserum against the catecholamine-synthesizing enzyme tyrosine hydroxylase (TH) using peroxidase-antiperoxidase and immunoautoradiographic markers. Of 96 GABA terminals, 66% were in contact with TH-containing cell bodies or dendrites. All junctions with recognized membrane specializations were of the symmetric type, indicative of inhibition. Some GABA-terminals provided dual inputs onto two unlabeled, two TH-labeled, or labeled and unlabeled soma and/or dendrites. Analysis of TH-labeled soma and dendrites confirmed our recent observation (Bayer and Pickel, J. Neurosci., in press) that neurons exhibit differences in levels of detectable TH immunoreactivity and that the more intensely labeled neurons receive more numerous contacts from unlabeled terminals. However, the number of contacts by GABA-labeled terminals was proportionally greater on the dendrites that contained lower levles of TH-immunoreactivity. These results suggest that in the rat VTA, synaptic input from GABAergic terminals may not only inhibit DA neurons, but may influence the level of TH needed for dopamine synthesis. (Supported by grants NIMH MH00078 and MH40342 and NIDA DA04600 to V. M. P.)

54.13

The Oncogene *c-fos* As An Index Of Mesostriatal And Mesocortical Dopamine System Activity R.P. Dilts, T.E. Helton and J.F. McGinty Dept. of Anatomy, ECU School of Medicine, Greenville, NC 27858

The detection of the oncogene *c-fos* or its protein product, Fos, has been proposed as an index of metabolic changes produced by cellular activity. Unlike previous techniques, the detection of Fos, or Fos related antigens (FRAS), occurs within the nucleus, allowing for the histochemical characterization of the identified neuron. The distribution of FRAS within the rat has been determined by immunocytochemical techniques two hours after the subcutaneous administration of apomorphine (5 mg/kg) or haloperidol (2 mg/kg). When compared to vehicle treated or niave animals apomorphine produces a patch distribution of Fras immunoreactivity (IR) within the striatum. Haloperidol treated animals display a more uniform distribution of FRAS-IR within the striatum which is accompanied by increases in FRAS-IR within the nucleus accumbens, olfactory tubercle and frontal cortex. Additionally, unilateral lesions of the A10-A8 dopamine neurons were performed using 6-hydroxydopamine (6 ug/1.5 ul) administered in the rostral A10 region. Apomorphine (5 mg/kg, s.c.) produces a patch distribution on the contralateral side to the lesion and a robust increase in FRAS-IR on the ipsilateral side. Attempts are currently being made to semi-quantitate these results and to histochemically identify the neurons expressing FRAS-IR within the striatum and other terminal regions of the mesolimbic dopamine system following pharmacological manipulation. Supported by DA03982.

54.14

AN ULTRASTRUCTURAL STUDY OF MET-ENKEPHALIN AND BETA-ENDORPHIN IN THE RAT PARAVENTRICULAR THALAMIC NUCLEUS. L.J. Freedman[1] and M.D. Cassell[2]. [1]Neuroscience Program and [2]Department of Anatomy, University of Iowa, Iowa City, IA 52242.

Immunocytochemical studies reveal that the paraventricular thalamic nucleus (PVT) contains a wide variety of peptidergic fibers. However, it is not clear whether these fibers synapse in the PVT or if they pass through, merely connecting other areas rich in peptides. To address this question, we conducted an immunocytochemical study of the rat paraventricular thalamic nucleus at the electron microscope level using antibodies to met-enkephalin and beta-endorphin.

Both peptides were located primarily in the lateral part of the nucleus and were found in unmyelinated axons and terminals making synaptic contacts. Enkephalin synapses were usually symmetric, with electron dense regions on both the pre- and post-synaptic membranes, and tended to be on dendritic spines. Endorphin terminals also made symmetric contacts, but these usually did not have synaptic densities on either side, and were more frequently on dendritic shafts. The largest endorphin terminals were about twice the size of the largest enkephalin terminals.

These results indicate that beta-endorphin and met-enkephalin are likely to play a role in the function of the PVT. For example, they may be involved in the stimulation-produced analgesia some investigators have described there (Kupers et al., 1988).

COMPARATIVE NEUROANATOMY: FISH AND AMPHIBIA

55.1

THE PRESUMPTIVE NUCLEUS PEDUNCULOPONTINUS OF THE ATLANTIC STINGRAY IS NOT PART OF THE MESENCEPHALIC LOCOMOTOR REGION. R.L. Puzdrowski and R.B. Leonard, Marine Biomedical Institute, University of Texas Medical Branch, Galveston, TX 77550.

The functionally defined midbrain locomotor region (MLR) in the Atlantic stingray, Dasyatis sabina, shares many topological characteristics with the functionally defined MLR in mammals. Nuclei in the region of the mammalian MLR include cuneiformis and pedunculopontinus (PPN). There is debate as to which of these cell groups constitute the anatomical substrate for the functionally defined MLR. We have identified a nucleus in the rostral rhombencephalon, in close association with the cerebellar peduncle, that shares histochemical markers with the mammalian PPN. This nucleus is positive for acetylcholine esterase and more importantly NADPH-diaphorase activity (a characteristic unique to the PPN in this region of the mammalian brain). The presumptive PPN in stingrays lies outside the region identified as the MLR. If additional experiments support the identification of this nucleus as the homolog of the mammalian PPN, they will also support the assertion that the PPN is not a part of the vertebrate MLR. Supported by NS07185 and NS11255.

55.2

GNRH IMMUNOREACTIVITY IN THE BRAIN OF THE ROUND STINGRAY, UROLOPHUS HALLERI. L.S Demski and D.E. Wright, University of Kentucky, Lexington, KY, 40506

GnRH-immunoreactivity (ir) was studied using antisera to salmon GnRH. Beaded GnRH-ir fibers were located in the terminal nerve (TN), dorsal and midventral portions of the telencephalic hemispheres, and the septopreoptic area; more diffuse fibers were found in the optic tectum, tegmentum, and spinal cord. Many ir-cells were located in the TN ganglia. In some cases, dense-cored vesicles in the cells and their processes were labeled using EM-immunogold techniques. Few perikarya were seen in the septopreoptic area, although scattered GnRH-ir fibers were present in the median eminence and hypothalamus. Over 800 GnRH-ir cells (10-20μM) were found in the midbrain extending rostrocaudally (1.5mm) along the midline near the oculomotor nerve; the nucleus diverges ventrolaterally at its most rostral level. The presence of well-developed midbrain and TN GnRH systems coupled with a seeming reduction of septopreoptic GnRH cells may be a unique feature of elasmobranchs related to the physical separation of the hypothalamus and the gonadotropin-rich ventral lobe of the pituitary.

55.3

SEROTONERGIC, ENKEPHALINERGIC, AND CATECHOLAMINERGIC CELLS IN THE BRAIN OF A SHARK, HETERODONTUS FRANCISCI. S.L. Stuesse, and W.L.R. Cruce. Dept. of Neurobiology, N.E. Ohio Univ. College of Medicine, Rootstown, OH 44272.

The location of immunopositive cells within the brainstem of elasmobranchs may give us clues to phylogeny. For example, nucleus raphe dorsalis (RaD), the largest serotonergic cell group in mammals, is not present in rays, skates, or guitarfish. We chose heterodontid sharks, a sister group to these batoids, for an out-group comparison of this and other characters. We identified cells in the brainstem of *Heterodontus francisci* which were immunopositive for tyrosine hydroxylase, serotonin, or leu-enkephalin and compared the distribution of these antibodies to descriptions in mammals and other elasmobranchs. The majority of tyrosine hydroxylase-positive cells were found in the midbrain tegmentum (A9, A10) and the hypothalamus, and putative A1, A2, A5, A7 cell groups were found in the metencephalon and myelencephalon. Serotonin-positive cells were found both in midline raphe nuclei and scattered lateral to these nuclei. We identified probable homologs to raphe pallidus, obscurius, magnus, and centralis superior (B8). A small cluster of cells dorsomedial to the medial longitudinal fasciculus was a probable homolog of rat RaD. Brainstem nuclei which were positive for leu-enkephalin usually contained serotonin-like immunoreactivity, and the distribution for these two substances was similar. Besides the raphe nuclei, reticular groups which contained both 5-HT+ and LENK+ cells included reticularis (r.) ventralis, r. magnocellularis, r. paragigantocellularis lateralis, r. pontis caudalis, and r. pontis oralis medialis and lateralis. Thus, this shark contains many of the major brain stem raphe and catecholaminergic cell groups described for rats, but the relative distribution of the immunopositive cell groups is different. Supported by NS25895 and OBOR Research Challenge Funds.

55.4

GONADOTROPIN HORMONE-RELEASING HORMONE (GnRH)-IMMUNOREACTIVITY IN THE BRAINS OF PRIMITIVE BONY FISHES. D.E. Wright and L.S. Demski. School of Biological Sciences, University of Kentucky, Lexington, KY 40506.

GnRH immunoreactivity (ir) was determined in the brains of two species of brachiopterygian fishes (Polypterus sp. and Calamoichthys calabricus) using antisera to salmon and mammalian GnRH. Small ir-perikarya (<10μm) with processes extending rostrocaudally were dispersed along the ventral surface of the caudal olfactory bulb and rostral telencephalon. Larger, unipolar ir-cells (15-20μm) were located in the rostral midbrain immediately ventral to the ventricle in the region of the posterior tubercle. Beaded ir-fibers were most abundant in the olfactory bulbs, several areas of the ventral telencephalon, dorsolateral surface of the pallium, diencephalon, habenula, optic tectum, and tegmentum. Fewer ir-fibers were present in the medulla and spinal cord. Ir-fibers could be followed along the ventral surface of the telencephalon and hypothalamus into the pituitary. The largest fascicles of ir-fibers were present in the rostral telencephalon. They probably correspond to projections of the terminal nerve.

55.5

ORGANIZATION OF THE FOREBRAIN IN *EPTATRETUS STOUTI* (MYXINOIDEA). H. Wicht and R.G. Northcutt, Neurobiology Unit, SIO & Dept. Neurosciences, A-001, UCSD, La Jolla, CA 92093

Cytoarchitectonic analysis of the forebrain of the Pacific hagfish revealed eight major regions: olfactory bulb, pallium, preoptic area, central prosencephalic nucleus, epithalamus, dorsal thalamus, ventral thalamus, and hypothalamus, with approximately 40 divisions and subdivisions. The pallium has a division comprising five laminae, further subdivided into seven areas, and a nonlaminated division subdivided into three areas. Histochemical analysis (AchE, 5HT, SP, Met-ENK, Leu-ENK, ∝ -MSH, FMRF, and LHRH) confirmed the major regions as well as many of their divisions and subdivisions. Topography and secondary olfactory input indicate that the bulk of the laminated pallium is the homologue of the lateral pallium of other craniates. The nonlaminated pallium may be the homologue of the dorsal and/or medial pallium. The central prosencephalic nucleus is the most enigmatic cell group in the myxinoid brain. This conspicuous cytoarchitectonic formation has previously been regarded as the homologue of the medial pallium or the eminentia thalami, but its histochemical composition (high content of AchE, Leu-ENK, Met-ENK, and SP) and topography (continuity with the ventral thalamus) make this unlikely. The present data suggest that it may be homologous to the striatum or to a rostral diencephalic cell group of other craniates. Alternatively, it may be an autapomorphy of hagfishes.
Supported by the DFG (Wi909/1-2) and the NIH (NS24869).

55.6

RETINOPETAL AND RETINOFUGAL PROJECTIONS IN LARVAL AND ADULT LAMPREYS: AN IN VITRO STUDY WITH HRP AND FLUORESCENT DEXTRAN-AMINES. B. Fritzsch and R. G. Northcutt. Neurobiol. Unit, SIO, and Dept. of Neurosciences, A-001, UCSD, La Jolla, CA 92093.

The retina of adult lampreys projects bilaterally to the tectum and receives a bilateral retinopetal input from cells of the tegmental midbrain. In birds, which have a comparably large efferent contingent (chickens, 0.5%; lampreys, 2%), the size of the ipsilateral efferent projection reportedly depends on the size of the ipsilateral retinofugal projection (D. D. M. O'Leary and W. M. Cowan, Dev. Brain Res., 12: 293, 1984). We studied the afferent and efferent projections of the retina in larval and postmetamorphic lampreys to obtain insights into developmental patterns leading to the adult organization. HRP or fluorescent dextran-amines were applied to the cut optic nerve or needle injected in the midbrain. In vitro brains and eyes were kept up to three days in a fish filled with cold Ringer's solution before they were fixed and processed. Application of tracers to the optic nerve in larvae revealed bilateral efferents in the midbrain but a completely crossed retinofugal projection. After metamorphosis, injections into the midbrain revealed ipsilateral ganglion cells concentrated at the nasal and temporal retinal margin; this suggests that they were formed late in development. In contrast to birds, lampreys develop a bilateral retinopetal projection before a bilateral retinofugal projection. Moreover, retinopetal and retinofugal fibers do not interact in the chiasm. Supported by the DFG (Fr572) and the NIH (NS24869).

55.7

TOPOGRAPHIC ANALYSIS OF SUBPOPULATIONS OF RETINAL GANGLION CELLS IN THE FLORIDA GARFISH, *LEPISOSTEUS PLATYRHINCUS*. S. P. Collin and R. G. Northcutt. Dept. of Neurosciences A-001 University of California San Diego, La Jolla CA 92093 USA

The topography of the retinal ganglion cell layer of the florida garfish, *Lepisosteus platyrhincus* was investigated using retrograde labelling with horseradish peroxidase (HRP) and cobaltous-lysine from the optic nerve. After precipitation and intensification, three subpopulations of ganglion cells were characterised on the criteria of soma size and position, dendritic stratification and primary branching pattern. The majority of ganglion cells (95%) lie in a single sublamina and range in size between 5 and 14 μm (soma diameter). At least three further subclasses of cells could be differentiated within this group but, counted collectively in wholemount, their density changes substantially throughout the retina. This was reflected by a ventral horizontal streak (9400 cells per sq mm) and a temporal area centralis (5900 cells per sq mm) with density gradients of 6:1 and 4:1, respectively. In contrast, a population of giant ganglion cells (with a soma diameter of 18-20 μm and a dendritic field diameter of up to 800μm) that lies within a closely apposed sublamina, is relatively evenly distributed (32 cells per sq mm). The garfish also possesses a distinct population of displaced ganglion cells lying at the border of the inner plexiform and inner nuclear layers ranging in soma diameter from 5 to 9 μm. The topography of these displaced cells (3.75% of the total population) reflects that of the major population although cell densities in both the ventral horizontal streak and the temporal area centralis peak at 625 cells per sq mm and possess a centro-peripheral gradient of 25:1.

This data provides the basis for continuing studies on the central projections of subpopulations of ganglion cells with special emphasis on ipsilateral input to the optic tectum.

Supported by the AAEF and NH&MRC (SPC) and NIH (NS24869).

55.8

AN IMMUNOCYTOCHEMICAL COMPARISON OF PURKINJE CELLS AND ACOUSTICOLATERAL PYRAMIDAL (CREST) CELLS IN WEAKLY ELECTRIC GYMNOTIFORM TELEOSTS. M. J. Lannoo, L. Maler, and R. Hawkes. Prog. Neurosci., Ohio Univ. Athens, OH 45701; Dept. Anatomy, Ottawa Univ., Ottawa, Ont. K1H 8M5; Dept. Anatomy and Neuroscience Research Group., Univ. Calgary, Calgary, Alberta.

Morphological and functional similarities have suggested a relationship between cerebellar Purkinje cells and acousticolateral pyramidal (crest) cells. We extend this comparison by using two monoclonal antibodies generated against the hindbrain of the weakly electric gymnotiform fish *Apteronotus leptorhynchus*.:
i) *anti-zebrin II* recognizes a 36 kD protein present in Purkinje cell subsets over a range of species including birds and mammals (Brochu et al. '90 *JCN* 291: 538-552). We now report that *zebrin II* in gymnotiforms is also expressed *transiently* during development by ampullary organ-receptive, but not tuberous organ-receptive pyramidal cells. Thus, *anti-zebrin II* can be used to distinguish between two types of electroreceptor inputs at the molecular level. This is the first time *zebrin II* has been seen in a non-Purkinje cell.
ii) in contrast, *anti-type I* recognizes a 47 kD protein present in the adult in all Purkinje cells and all pyramidal cells (as well as in Mauthner and electric organ pacemaker neurons). This is true only in the genus *Apteronotus*, surprisingly *Eigenmannia* spp. and *Sternopygus* spp. are unreactive. That *anti-zebrin II* and *anti-type I* label both Purkinje and pyramidal cells supports the idea that these cells are related either through homology or common function.

55.9

NEWBORN CELLS IN THE BRAIN OF ADULT WEAKLY ELECTRIC KNIFE-FISH. G.K.H. Zupanc. Department of Neurosciences, University of California at San Diego, La Jolla, CA 92093.

In the central nervous system of warm-blooded vertebrates, neurogenesis is thought to take place predominantly in prenatal or early postnatal development. In the brain of weakly electric knifefish *(Eigenmannia sp.)*, however, new cells continue to be generated extensively also during adulthood. Fish were injected with 5-bromo-2'-deoxyuridine (BrdU) which is incorporated into replicating DNA. After a survival time of 12 hrs, mitotic S-phase cells were localized by a specific monoclonal anti-BrdU antibody (Amersham).

BrdU-labelled cells were found in the periventricular zones of the following brain regions (for nomenclature, see Maler et al., *J. Chem. Neuroanat.*, in press): Subdivision 1 and 2 of the dorsomedial part of the telencephalon; ventral and dorsal subdivisions of the ventral area of the telencephalon; anterior subdivision of the nucleus preopticus periventricularis; eminentia thalami; medial subdivision of caudal dorsal posterior area of the telencephalon; central-posterior nucleus; hypothalamus ventralis; medial subdivision of the nucleus recessus lateralis; hypothalamus dorsalis; nucleus posterioris periventricularis; periventricular nucleus of posterior tuberculum; nucleus tuberis lateralis pars anterior; nucleus tuberis lateralis; nucleus of the posterior recess. Labelling with ^{3}H-thymidine and survival periods of 2 weeks indicate that part of these newborn cells migrate away from their sites of origin to other areas in the brain.

55.10

ONTOGENY OF SEXUAL POLYMORPHISMS IN MONOAMINERGIC INPUTS TO THE SOUND-GENERATING MOTOR SYSTEM OF A VOCALIZING FISH. M. Marchaterre, H. Baker, and A. Bass. Section of Neurobiol. & Behavior, Cornell Univ., Ithaca, N.Y. 14853, Lab. Molecular Neurobiology, Cornell Univ. Medical College, White Plains, N.Y. 10605.

In the plainfin midshipman (*Porichthys notatus*), large, nest-guarding males ("Type I") generate long duration (up to 1 hr) acoustic communication signals called "hums" during the breeding season. Sonic activity has not been observed in females or a second group of smaller reproductively active males ("Type II"). The sound's fundamental frequency is determined by the synchronous firing of sonic motoneurons whose rhythmic, oscillatory-like discharge is established by nearby pacemaker neurons. Electron microscopic studies indicated the presence of dense core vesicles in synaptic terminals within the sonic motor nucleus (SMN) and thus suggested the presence of monoaminergic inputs. Neurochemical studies were thus initiated to evaluate the ontogeny of possible sexual dimorphisms in the position and extent of serotonin (5HT)- and tyrosine hydroxylase (TH; indicative of catecholamines)-like immunoreactivity (IR) in and around the SMN. Only the adult, vocalizing Type I males were consistent in having a high density of 5HT- and TH-IR fibers within the SMN. The onset of high levels of 5HT- and TH-IR was correlated with gonadal hypertrophy in males as revealed in studies of non-reproductive juveniles. 5HT-and TH- IR cells were outside the SMN and did not correspond to pacemaker neuron position. Thus, it is unlikely that monoamines are excitatory transmitters of the pacemaker system. However, given the documented effects of monoamines on other motor systems, including their oscillatory properties, we propose that monoaminergic inputs modulate and/or sustain levels of sonic motoneuron activity in vocalizing Type I males and thus contribute to their ability to generate hums. Supported by NSF and NIH.

55.11

FREEZE-FRACTURE STUDY OF THE TELEOST MENINGES. H.J. Carruncho & R. Anadón. (SPON: European Neuroscience Association). Dept. Fundamental Biology; 15706- Santiago de Compostela (Spain).

The structure of the meninges of lower vertebrates differs considerably from that of mammals. Because there are only a few works about the fish meninges, we have studied the ultrastructure of the meninges of the rainbow trout hypothalamus using the freeze-fracture technique.

Four layers can be differentiated in the meninges at this level. Blood vessels are found only in the outermost and the innermost layers. Vessels are non-fenestrated and characteristically have abundant caveolae (20-40 nm in size) in the endothelial cell surface.

The meningeal cells of the outermost layer (layer 1) do not present intercellular junctions between their processes. In layer 2, the outermost cells are flattened and have intermediate to strong tight junctions (according to the classification of Claude, P. and Goodenough, D.A., J. Cell Biol., 58:390, 1973) between their lateral processes. The inner cells of this layer and the cells of the layer 4 have weakly tight junctions (1-3 opened particle strands) that are generally associated with small gap junctions. The layer 3 has five type of cells, none of which have intercellular junctions.

In conclusion, the teleost meninges are well developed and differentiated into four layers; one of them (layer 2) resembles the barrier layer of mammals, although in fish the subarachnoid space is absent.

55.12

WHY ARE NEUROFILAMENTS PRESENT IN FROG OLFACTORY AXONS BUT NOT IN THOSE OF THE TIGER SALAMANDER? M.A. Wentz* and P.R. Burton. Dept. of Physiology and Cell Biology, University of Kansas, Lawrence, KS 66045.

EM studies show that olfactory axons of the tiger salamander, Ambystoma tigrinum, rarely contain neurofilaments (NFs), whereas those of the bullfrog, Rana catesbeiana, contain many NFs. Other axons of the salamander nervous system show typical NFs. Using monoclonal antibodies, comparative immunoblot studies of NF proteins were carried out in SDS/PAGE preparations of olfactory nerve and spinal cord extracts of the two species. The studies indicate that the nominal Mr of the presumed high (H), medium (M), and low (L) NF proteins from Ambystoma spinal cord are 230, 160, and 77 kD, respectively, and the equivalent proteins from Rana are 207, 160, and 110 kD. Salamander olfactory axons, which lack NFs, primarily contain the M subunit protein with only trace amounts of the H and L subunits. However, olfactory axons of the frog contain all three subunit proteins. Diameter measurements of salamander and frog olfactory axons show that the former are, on average, larger than those of the frog by about 30%. These studies indicate: 1) epitopic heterogeneity among NF proteins in the amphibian nervous system, 2) that the absence of NFs in Ambystoma olfactory axons may be due to the relative lack of two of the NF subunit proteins, and 3) that the structural and dimensional integrity of these axons are not dependent on assembled NFs. Supported by NIH grant NS25518 and a grant from the K.U. General Research Fund.

55.13

DISTRIBUTION OF TYROSINE HYDROXYLASE IMMUNOREACTIVITY IN THE SALAMANDER OLFACTORY BULB. K.A. Hamilton. Cellular Biology and Anatomy, LSU Medical Center, Shreveport, LA 71130.

In the salamander olfactory bulb, cells in several synaptic laminae appear to contain GABA and/or dopamine and may inhibit mitral/tufted output cells in response to odor stimulation (see K.A. Hamilton and J.S. Kauer, J. Neurophysiol., 62: 609, 1989). In the present study, the distribution of catecholaminergic cells was further investigated using immunocytochemical methods.

Tyrosine hydroxylase immunoreactivity was observed in a population of granule-layer cells. Smooth dendrites extended from the cell bodies and gave rise to numerous spiny branches in the external plexiform layer and periglomerular region. As in fish, but not mammals, periglomerular cell bodies were unstained (see J.R. Alonso et al., Brain Behav. Evol., 34: 318, 1989). Additional antisera are presently being used to determine which TH-immunoreactive cells in the salamander bulb might contain dopamine.

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55.14

VOMERONASAL SYSTEMS DURING AQUATIC PHASES IN SALAMANDERS WITH DIFFERING LIFE-HISTORIES. H.L. Eisthen, D.R. Sengelaub, and J.R. Alberts*. Program in Neural Science, Indiana University, Bloomington, IN 47405.

The axolotl (Ambystoma mexicanum) is a neotenic salamander that remains aquatic throughout its life. The axolotl's nasal cavity contains separate populations of sensory receptors along the medial wall and in a lateral diverticulum, corresponding to the loci of the olfactory and vomeronasal receptors in terrestrial salamanders. The medially-located receptors project to the main olfactory bulb, and the those in the lateral diverticulum project to an accessory olfactory bulb (AOB), indicating that axolotls possess both an olfactory and a vomeronasal system (VNS). These data contradict Bertmar's (1981) hypothesis that the VNS evolved as an adaptation to terrestrial life.

We have examined the nasal cavities of both adult and larval tiger salamanders (A. tigrinum), a transforming species from which axolotls are thought to have evolved. The nasal cavities of both larvae and adults possess a lateral diverticulum containing sensory receptors, and the forebrains appear to contain AOBs. Similarly, we have found a receptor-lined lateral diverticulum and AOB in the larva of another ambystomid, the Jefferson's salamander (A. jeffersonianum). These findings suggest that the VNS of the adult axolotl is a characteristic of larval ambystomids. We have also examined adults of a secondarily-aquatic species, the Eastern red-spotted newt (Notophthalmus viridescens), and have found similar evidence of a VNS. Our work in progress with the neotenic, aquatic mudpuppy (Necturus maculosis) indicates that this species possesses a lateral diverticulum but not an identifiable AOB.

Because the VNS is present during the aquatic phases of the life cycle in salamanders with diverse life histories, our data suggest that the VNS did not evolve as a terrestrial adaptation.

55.15

THE *OLFACTORETINALIS* SYSTEM DOES NOT CORRESPOND TO THE "TERMINAL NERVE" AS ASCRIBED TO F.PINKUS. T.Szabo, J.-P. Denizot*, S.Bläser*, M.Véron-Ravaille*, D.Rouilly*, Dept. Neurophysiol. Sens., Lab. Physiol. Nerveuse C.N.R.S., F-Gif/Yvette Cedex.-Anatomie und Zytobiologie d. Universität, 6300 Giessen GFR.

The *olfactoretinalis* system (ORS) is widely considered to be the terminal nerve. However, the characteristics of the "terminal nerve" as described by F.Pinkus (1894), are quite different from those of the ORS. "Rising as a fine unmyelinated nerve from the diencephalic brain level, the 'terminal' nerve courses cranialwards on the ventral brain surface with the olfactory nerve. Beyond the olfactory epithelium, it disappears in a cell group on the dorsal wall of the anterior nares".

We could demonstrate immunohistochemically a similar structure in several species of gymnotiform fish (Szabo et al., 1989). In *Eigenmannia* a substance P containing fiber bundle accompanies the olfactory nerve. It originates in a group of ganglion cells located between the olfactory epithelium and the nares. Centrally it penetrates into the olfactory bulb (OB) and forms a glomerular terminal area at the mediocaudal pole of the OB. Peripheral, the ganglion cells seem to project to a particular structure in the epithelial wall of the nares.

Since in the same fish a distinct ORS could be demonstrated at the OB border with anti FMRF-amide antiserum, the described structure should be considered as the terminal nerve.

55.16

SEPTAL AREA CONNECTIONS IN RANID FROGS. T.J. Neary and R.G. Northcutt. Anatomy Division, Creighton Univ., Omaha, NE 68178 and SIO Neurobiology Unit/UCSD Department of Neurosciences, La Jolla, CA 92093.

The connections of the septal area were studied in bullfrogs, R. catesbeiana, following applications of either HRP or WGA-HRP. Most structures connecting with the septal area were labelled bilaterally, but a few, indicated (I), were consistently labelled only ipsilaterally. Labelled cells were found in the main olfactory bulb (MOB)(I), the lateral (I), dorsal (I), and medial pallial fields, the olfactory tubercle/diagonal band nucleus, and the medial and lateral amygdalar nuclei; anterior (ATh) and central (CTh)(I) thalamic nuclei, ventromedial thalamic nucleus (VM), anterior preoptic area (POa), magnocellular preoptic nucleus (Mg), suprachiasmatic nucleus (SCh), ventral hypothalamic nucleus (VH), and posterior tuberculum (TP); nucleus of the MLF (NMLF), and principal toral nucleus (I); isthmal raphe, lateral isthmal reticular formation, and secondary isthmal nucleus. Labelled fibers/terminals were found in the MOB and accessory olfactory bulb (I), the medial, dorsal and lateral pallia (all I), and contralateral septal area; ventral habenula (I), ATh, CTh, VM, POa, Mg, SCh, VH, TP, and NMLF; the pretectal and pretoral grey, deep tectal layers, toral laminar nucleus, and periventricular tegmentum.

Supported by a BRSG to Creighton University (TJN) and NS 24869 (RGN).

55.17

CONNECTIONS BETWEEN LIMBIC AND NEUROENDOCRINE NUCLEI IN THE FOREBRAIN OF HYLA CINEREA. J. D. Allison, W. Wilczynski and J. A. Morgan. Dept. of Psychology, University of Texas, Austin, TX, 78712.

The preoptic area and ventral hypothalamus act as a bipartite control system for pituitary and gonadal hormone secretion. In addition, these areas are important in the control of reproductive behavior. We previously examined auditory inputs to each of these areas (Allison and Wilczynski, Soc. Neurosci. Abst. 13:869, 1987; 15:374, 1989). In this study we examined connections between telencephalic limbic areas and these neuroendocrine control centers. HRP was iontophoretically injected into either the medial pallium (MP), medial and lateral septal nuclei (MS, LS), preoptic area (POA), or ventral hypothalamus (VH) of adult green treefrogs. After a 7-day survival time, the animals were sacrificed under anesthesia and the brains extracted, fixed, cut at 40u, mounted, and reacted with standard techniques using TMB as the chromagen. The heaviest connection noted was a bilateral, reciprocal connection between the POA and the MS and LS. The POA is also reciprocally connected to the MP and VH. In contrast, the VH receives a light projection from the dorsal MP and the ventral portion of the LS. Each of the projections to the VH were minor in comparison to the projections to the POA. Finally, a projection from the ventromedial portion of the MP to the MS was noted. (Supported by NIMH grant RO1 MH45350)

55.18

EXPERIMENTAL EVIDENCE THAT AMPULLARY ORGANS OF SALAMANDERS DERIVE FROM PLACODAL MATERIAL. R.G.Northcutt, B.Fritzsch, and K.Brändle*. Neurobiol. Unit, SIO, and Dept. of Neurosciences, A-001, UCSD, La Jolla, CA 92093 and J.W.Goethe-Univ., Frankfurt, FRG.

To test the hypothesis that neuromasts and ampullary organs develop from the same placode (Northcutt, Soc.Neurosci.Abstr., 12:103,'86), we extirpated or transplanted supraorbital placodes in wild or albino axolotl embryos between stages 26 and 35. Orthotopic or ectopic (i.e. belly) transplants from wild (pigmented) embryos onto albino embryos allowed us to follow development by using a natural marker. After appropriate survival times, the animals were fixed, and the skin was removed, flat mounted on slides and counterstained. Extirpation of the supraorbital placode resulted in marked reduction or loss of both ampullary and neuromast organs. Both ectopic and orthotopic transplanted pigmented placodes differentiate into ampullary and neuromast organs in the unpigmented albino hosts. Our data suggest early commitment (at least 10 stages before ampullary organs can be recognized morphologically) and autonomous differentiation of ampullary progenitors. They also suggest that the progenitors of ampullary and neuromasts organs are interspersed within a placode. In axolotls there is no evidence that ampullary organs arise from embryonic sources other than dorsolateral placodes. (Supported by grants from the NIH and DFG.)

55.19

ORGANIZATION OF BRAIN STEM RETICULAR NUCLEI IN AN AMPHIBIAN, THE NORTHERN LEOPARD FROG, RANA PIPIENS. W.L.R. Cruce and D.S.H. Adli. Neurobiology Department, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272 and Zoology Department, University of Malaya, 59100 Kuala Lumpur, MALAYSIA.

Based on classical neuroanatomical methods only a small number of distinct subdivisions have been recognized in the reticular formation of frogs. Some of these subdivisions (Potter, J.Neurophys., 1965) have been useful for neurophysiological studies yet the subdivisions have no obvious homologies in other vertebrate brains. We used immunohistochemistry and tract-tracing techniques in combination with cyto- and dendro- architectonics to study reticular nuclei in Rana pipiens. We subdivided the reticular formation based on descriptions of mammalian reticular nuclei (Newman's nomenclature for rat, J.Hirnforschung, 1985). The features used for characterization were neurochemical localization (enkephalin, substance P, somatostatin and serotonin), cell morphology (dendritic arborization, soma shape, soma size, and soma orientation), and descending connections to the spinal cord. In the rhombencephalon we found reticularis (r.) dorsalis, r. ventralis pars alpha and pars beta, r. magnocellularis, r. parvocellularis, r. gigantocellularis, r. paragigantocellularis lateralis, r. paragigantocellularis dorsalis, r. pontis caudalis pars alpha and pars beta, nucleus visceralis secundarius/Kolliker-Fuse complex, raphe obscurus, raphe pallidus, raphe magnus and raphe pontis. In the mesencephalon we found locus coeruleus-subcoeruleus complex, r. pontis oralis pars medialis and pars lateralis, r. pedunculopontinus, r. cuneiformis, r. subcuneiformis, raphe dorsalis-raphe centralis superior, and raphe linearis. This study supports the hypothesis that the reticular formation is a phylogenetically old and conservative part of the brain. Supported in part by N.I.H. grant NS25895 and Ohio Board of Regents Research Challenge funds.

56.1

REPEATED INTRACEREBRAL MICRODIALYSIS FOR THE MEASUREMENT OF STIMULATED DOPAMINE RELEASE. Dianne M. Camp and Terry E. Robinson. Department of Psychology and Neuroscience Program, The University of Michigan, Ann Arbor, MI 48109

We previously reported that with repeated intracerebral microdialysis the basal extracellular (EC) concentration of dopamine (DA) remains stable over two probe insertions separated by two weeks (Camp et al. *Soc. Neurosci. Abst. 15*, 1989, 559). This suggests that the basal EC concentration of DA is not sensitive to the tissue damage produced by multiple probe insertions. Whether or not the increase in EC DA produced by *d*-amphetamine (AMPH) is similarly unaffected by tissue damage produced by repeated probe insertions was addressed in this experiment.

Microdialysis in freely moving male rats was performed twice on the same animals, separated by one week. The effect of AMPH (2.0 mg/kg) on EC DA was greatly attenuated following the second probe insertion, regardless of whether animals received AMPH or saline during the first probe insertion. As reported previously, the basal EC concentration of DA did not differ between the two insertions.

It is suggested that it may not be feasible to use a within-subjects design using multiple probe insertions to characterize the dopaminergic response to repeated AMPH treatment. However, repeated microdialysis has been used to study the effects of repeated cocaine administration on EC DA, and therefore, this may not be the case for other drugs. Experiments are currently in progress to address whether the effects observed are unique to AMPH.

56.2

CIRCADIAN CHANGES IN BEHAVIOR AND EXTRACELLULAR DOPAMINE IN THE NUCLEUS ACCUMBENS DURING AMPHETAMINE WITHDRAWAL. Pamela E. Paulson and Terry E. Robinson. Department of Psychology and Neuroscience Program, The University of Michigan, 1103 East. Huron Street, Ann Arbor, MI 48109.

Rats show a significant decrease in nocturnal locomotor activity during withdrawal from amphetamine (AMPH). This nocturnal hypoactivity (behavioral depression) persists for 1-2 weeks following the discontinuation of pretreatment with escalating, but non-neurotoxic, doses of AMPH, and is accompanied by changes in the postmortem tissue concentrations of dopamine (DA) and norepinephrine. It has been suggested that post-AMPH withdrawal behavioral depression may be related to the "distress syndrome" seen in humans during AMPH withdrawal, which is associated with symptoms of depression, dysphoria and changes in catecholamine function. The present experiment was designed to further characterize the behavioral and neurochemical consequences of AMPH withdrawal in rats. To do this, the extracellular concentrations of DA and its metabolites in the nucleus accumbens will be continuously monitored over 20 minute intervals across the day-night cycle in AMPH-pretreated and control rats at different points in time following the discontinuation of AMPH pretreatment, using a fully automated microdialysis system. At the same time, locomotor activity, feeding patterns and body temperature rhythms will be quantified. This experiment is in progress and the results will be presented at the meeting.

56.3

PERSISTENT EFFECTS OF NEUROTOXIC DOSES OF METHAMPHETAMINE ON THE EXTRACELLULAR CONCENTRATION OF DOPAMINE IN THE NUCLEUS ACCUMBENS AND NEOSTRIATUM. S.T. Ferrell, D.M. Camp, and T.E. Robinson. Dept. of Psychology and Neuroscience Program, University of Michigan, Ann Arbor, MI 48109.

Repeated exposure to high doses of methamphetamine (MA) is toxic to dopamine (DA) neurons, producing a large, long-lasting depletion of striatal DA in postmortem tissue due to the degeneration of DA terminals. In contrast, MA does not significantly reduce the resting extracellular concentration of DA in the striatum, and MA-pretreated rats are able to greatly increase the extracellular concentration of DA in response to a d-amphetamine (AMPH) challenge [Robinson et al. *Neurosci. Lett.*, 1990: *110*, 193]. This normalization of extracellular DA may be responsible for the absence of pronounced behavioral deficits in animals depleted of DA by MA. However, behavioral abnormalities were observed in MA treated animals in response to an AMPH challenge. Relative to controls, MA treated rats were hypersensitive to the locomotor-activating effects of an amphetamine challenge. We hypothesized that this accentuated behavioral response may be due to sensitized DA release in the nucleus accumbens, which is less depleted of DA by neurotoxic doses of MA. To test this hypothesis, *in vivo* microdialysis will be used to measure the extracellular concentration of DA simultaneously in the nucleus accumbens and the neostriatum of freely moving rats pretreated with either saline or neurotoxic doses of MA, before and after an amphetamine challenge. The experiment is in progress and the results will be presented at the meeting.

56.4

ESTROGEN AND STRIATAL DOPAMINE RELEASE: A MICRODIALYSIS STUDY. S. A. Castner and J. B. Becker. Department of Psychology, Neuroscience Laboratory Building, The University of Michigan, Ann Arbor, Michigan 48104-1687.

There are sex-related and estrous cycle-dependent differences in amphetamine (AMPH)-stimulated behaviors and striatal dopamine (DA) release. Intact female rats exhibit a greater behavioral response to AMPH than do males. In addition, following ovariectomy (OVX) AMPH-induced behavior is attenuated, as is the striatal DA response to AMPH *in vitro* . Estrogen treatment reinstates both responses to that of intact females when given to OVX female rats (**Behav. Brain Res.** 19:27, 1986). The present study was designed to determine if there are sex differences in the effect of estrogen on the striatal DA response to AMPH in adult rats as assessed using microdialysis in freely moving rats.

Male and female rats were castrated (CAST) or OVX for at least 1 month prior to the experiment. Dialysis probes were inserted through chronic guide cannulae aimed at the striatum. On the day of dialysis,12 to 18 hours after dialysis probe insertion, striatal dialysate samples were collected at 15 min. intervals. After baseline DA release was determined, animals received either estradiol benzoate (5 µg) or oil; 30 min later they received AMPH. DA concentrations were assayed by high performance liquid chromatography with electrochemical detection. Following AMPH administration, behavior was videotaped, and stereotypy ratings were scored from the tapes (stereotyped head/forelimb and sniffing were each rated from 0-4 at 5 min intervals). Preliminary results suggest that estrogen treatment potentiates striatal DA release. Additional results will be presented at the meeting.

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56.5

THE STIMULUS PROPERTIES OF AMPHETAMINE GENERALIZE TO INJECTIONS OF MORPHINE INTO THE VENTRAL TEGMENTAL AREA BUT NOT THE NUCLEUS ACCUMBENS J.P. Pruhan and J. Stewart. Center for Studies in Behavioral Neurobiology, Concordia University, Montreal, Quebec, Canada, H3G 1M8.

The present study investigated whether injections of morphine into either the ventral tegmental area (VTA) or the nucleus accumbens (NAS) could produce discriminative stimuli similar to those of the psychomotor stimulant, amphetamine (AMPH). Rats were trained to discriminate 1.0 mg/kg AMPH from saline using a two-lever successive discrimination procedure with food pellets available on a VI-30 sec schedule. Generalization tests then were conducted during 5 min extinction sessions. When tested with a range of AMPH doses (saline, 0.25, 0.5 and 1.0 mg/kg, ip) the rats showed graded increases in drug-lever responding (20 to 80%) with increases in the AMPH dose. Increases in AMPH-lever responding also were observed following subcutaneous injections of the dopamine receptor agonist apomorphine (0.10, 0.15, 0.20, 0.25 mg/kg), although the response levels reached a maximum of only 57% responses on the AMPH-lever. Bilateral injections of morphine sulphate into the VTA (2.5, 5.0 and 10.0 ug/0.5 ul/side) produced increases in AMPH lever responding to the same extent as apomorphine (39 - 54%). In contrast, these doses of morphine had no effects on AMPH-lever responding when they were injected into the NAS, although bilateral injections of amphetamine sulphate into the NAS (2.5, 5.0 and 10.0 ug/0.5 ul/side) did increase responding on the AMPH-lever (42 - 56%). These results suggest that activation of opiate receptors within the VTA can produce subjective effects similar to those produced by systemic injections of AMPH. Given the apparent role of meso-accumbens dopamine neurons in mediating the stimulus properties of AMPH, it is likely that these neurons also mediate the AMPH-like stimuli produced by intra-VTA morphine.

56.6

DYSKINETIC EFFECTS OF HALOPERIDOL ON AGED AND 6-HDA LESIONED RATS. S.K. Johnson, J.Lee*, H. Fisher* and G.C. Wagner. Depts. of Psychology and Nutritional Sciences, Rutgers University, New Brunswick, N.J. 08903

Neurochemical and behavioral effects following a high dose (25 mg/kg) haloperidol treatment were compared in young and aged male rats as well as in rats previously treated with a 6-HDA lesion of the striatum. Rats were administered 2 injections of haloperidol 3 weeks apart and observed for 14 weeks. Aged rats as well as the 6-HDA-treated rats exhibited 10-40% depletions of striatal dopamine relative to controls. In addition, both aged and 6-HDA lesioned rats showed increases in chewing behavior as early as 3 days after the first injection. Drug challenge revealed that aged and 6-HDA lesioned rats showed an increased sensitivity to apomorphine-induced chewing. Finally, both aged and 6-HDA lesioned rats showed a long-lasting haloperidol-induced increase in dopamine. Increasing age is the most powerful predictor of tardive dyskinesia. The present studies suggest that both a dopamine receptor proliferation and a haloperidol-induced elevation in central dopamine may be acting to increase dyskinetic behaviors.

56.7

EFFECTS OF METHAMPHETAMINE-INDUCED DOPAMINE LESIONS ON RAT FORCE LEVER PERFORMANCE. G.C. Wagner, G. Woertwein*, and M. Forman*. Psychology Dept. Rutgers Univ. New Brunswick, NJ 08903

Two groups of water deprived, adult, male Long-Evans rats were trained to press a recessed lever with between 10 and 15 g of force for a duration of 1.0 sec in order to procure a water reinforcer. Responses of less than 10 or greater than 15 g of force reset the duration timer. One group of rats (n = 6) had previously been treated with 12.5 mg/kg of methamphetamine administered SC once every 2 h for four injections while the second group (n = 5) received comparable injections of saline.

It was observed that the methamphetamine caused a 34% depletion of striatal dopamine lasting one year. In addition, both groups acquired the force lever response at approximately the same rate. Under baseline conditions, an average of 10.6 or 4.0 force band entrances per reinforcer were made by lesioned or control rats, respectively. Furthermore, lesioned rats were significantly more sensitive to challenge with IP oxotremorine (0.1, 0.2, 0.4 mg/kg, 20 min presession) than controls. These observations are discussed in reference to parkinsonian tremor.

56.8

THE EFFECTS OF METHAMPHETAMINE-INDUCED NEUROTOXICITY ON MOTOR PERFORMANCE IN THE RAT. Walsh S.L. and Wagner G.C. Psychology Dept., Rutgers University, New Brunswick, NJ 08903.

Methamphetamine (MA) is a potent neurotoxin which produces degeneration of both dopaminergic and serotonergic fibers in non-human primates and rodents. The following studies were designed to evaluate the effect of high dose administration of MA on motor performance in the rat.

Long-Evans male rats were trained on either a one-way active avoidance task, the rotorod task or to traverse the balance beam. When the subjects had reached a criterion, they were treated subcutaneously with either saline or MA at 12.5 mg/kg (4 injections with 2 hours between each). Following one week of recovery, the subjects were returned to the task. In the active avoidance task, the MA-treated subjects took significantly longer to respond to the conditioned stimulus in comparison to the controls. This deficit was observed for up to 8 weeks following recovery. No impairments were observed on the rotorod task. However, the MA-treated subjects were significantly impaired on the balance beam for up to 5 weeks post-recovery. The MA-treated subjects made over twice as many footslips on the beam as their control counterparts and this effect was reversible through the administration of l-dopa (100 mg/kg) 30 minutes prior to the test session. Additionally, the MA-treated subjects were significantly less sensitive to the effects of fenfluramine on active avoidance performance and rotorod performance than the controls. The MA treatment produced a significant decrease in the striatal content of both dopamine (47%) and serotonin (39%) in comparison to control subjects.

These results indicate that methamphetamine administration in high doses produces long-lasting impairments in motor performance in the rat including impairments in response time and balance. These deficits may be, in part, attributable to the reduction of dopamine levels resulting from this treatment.

56.9

A68930 AND L-DOPA RAPIDLY DIMINISH THE ROTATION RESPONSE TO D1 AGONISTS IN 6-OHDA LESIONED RATS. P. Curzon, J.W. Kebabian and D.R. Britton. Neuroscience Res., 47U, Pharmaceutical Discovery, Abbott Laboratories, Abbott Park, IL 60064.

Degeneration of dopamine neurons results in the development of supersensitivity of dopamine receptors in both Parkinson's disease and in the animal model using unilateral 6-OHDA lesions of the nigro-striatal pathway (Ungerstedt, 1971). We have investigated the effects of sub-chronic treatment of unilaterally lesioned rats with either l-dopa (+ carbidopa) or with the selective D1 antagonist, A68930. L-dopa (509 umol/Kg, po) produced continued rotation over 4 consecutive days of treatment. Rats treated with l-dopa for 3 days followed by either SKF-38393 or A68930 on day 4 showed a decrease in responsiveness to the D1 agonist. When animals were treated with A68930 (1.0 umol/Kg, sc) on day 1 there was a robust rotation response which was virtually eliminated on the 3rd day of such treatment. Dose response challenges on day 4 following 3 days of treatment with 1.0 umol/Kg substantiated the conclusion of a rightward shift in responsiveness to D1 agonists. Locomotor responsiveness of intact animals to similar doses of A68930 showed no change over 5 days of treatment. These data suggest that both l-dopa and A68930 reduce the supersensitivity of the denervated D1 receptor mediating rotation.

56.10

D1 RECEPTORS IN THE MPOA FACILITATE COPULATION AND EX COPULA ERECTIONS. E. M. Hull, R. C. Eaton, V. P. Markowski, L. A. Lumley, J. T. Thompson, and J. Moses. Department of Psychology, SUNY at Buffalo, Amherst, NY 14260.

We have reported that stimulation of D1 and D2 receptors with apomorphine in the medial preoptic area (MPOA) of male rats enhanced copulation and genital reflexes. Blocking D1 and D2 receptors with cis-flupenthixol impaired copulation and reflexes. Recently we reported that the D2 agonist quinelorane (LY-163502) in the MPOA delayed the onset of copulation, but then facilitated ejaculation. Furthermore, D2 stimulation decreased ex copula erections and increased seminal emissions. We suggested that D2 receptors in the MPOA shift the balance of autonomic influence to favor sympathetically mediated seminal emission at the expense of parasympathetically (and somatically) mediated erection.

We now report that the thienopyridine D1 agonist 4-(3',4'-dihydroxyphenyl)-1,2,3,4-tetrahydrothieno[2,3-c]-pyridine (THP), in the MPOA, increased ejaculations in copula and increased erections in restrained supine male rats. Finally, the D1 antagonist SCH-23390 decreased erections ex copula and increased seminal emissions.

These data are consistent with our hypothesis (Hull et al., JPET, 1989) that the ratio of D1 to D2 stimulation in the MPOA regulates the balance of autonomic influence on genital reflexes. Specifically, D1 receptors appear to enhance erectile mechanisms and inhibit seminal emission, whereas D2 receptors enhance seminal emission and inhibit erection.

Supported by NIMH grant MH-40826 to EMH. The D1 agonist was kindly donated by Dr. David E. Nichols, Purdue Univ.

56.11

IN VIVO VOLTAMMETRY IN FREELY MOVING RATS: EFFECTS OF DOPAMINE AGONISTS AND NEUROLEPTICS ON NEOSTRIATAL ASCORBATE AND BEHAVIOR. R.C. Pierce, S. E. Yount, D.B. Reising*, D.W. Miller*, S.L. Hammes*, S. Davis*, and G.V. Rebec. Prog. Neural Sci., Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Electrochemically modified carbon-fiber electrodes were used to assess the role of dopamine (DA) agonists on ascorbate (AA) release in the neostriatum of awake, behaving rats. Relative to controls, indirect agonists (2.5 mg/kg d-amphetamine or 20.0 mg/kg GBR-12909) produced marked behavioral activation concomitant with a significant increase in AA. Comparable effects were observed following the combined administration of selective D1 (10.0 mg/kg SKF-38393) and D2 (2.0 mg/kg quinpirole) agonists, but not after either of these drugs alone. Thus, behavioral activation and AA release were closely related to stimulation of both D1 and D2 DA receptors. The amphetamine-induced rise in AA was studied further in separate animals treated with various known or putative neuroleptics, including 1.0 mg/kg haloperidol (D2>D1 antagonist and sigma antagonist), 2.0 mg/kg SCH-23390 (D1 antagonist), 30.0 mg/kg (±)sulpiride (D2 antagonist), 20.0 mg/kg clozapine (atypical neuroleptic), 10.0 mg/kg BMY-14802 (sigma antagonist), and 4.0 mg/kg MK-801 (NMDA antagonist). All antagonist treatments attenuated various components of the amphetamine behavioral response and rapidly lowered neostriatal AA to baseline levels, suggesting that neostriatal AA release is linked to behavioral activation.

Supported by NSF BNS 87-11240.

56.12

ACUTE AND LONG-TERM AMPHETAMINE TREATMENTS ALTER EXTRACELLULAR ASCORBATE IN NEOSTRIATUM BUT NOT NUCLEUS ACCUMBENS OF FREELY MOVING RATS. G.V. Rebec, S.E. Yount*, and P.E. Langley*. Prog. Neural Science, Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Amphetamine elicits a characteristic series of behaviors, including locomotion and, with increasing dose, a period of focused stereotypy. Although dopaminergic input to both neostriatum and nucleus accumbens has been implicated in these behavioral changes, ample evidence suggests that ascorbate (AA), which is found in high concentrations in both regions, may play a modulatory role (White et al., Psychopharmacology, 1988, 94:284). Preliminary data from the neostriatum of freely moving rats indicate that amphetamine produces a dose-dependent elevation in AA that increases in animals treated for seven consecutive days, paralleling a progressive augmentation of behavior (Rebec et al., Soc. Neurosci. Abstr., 1989, 15:558). The present study extended this research to the nucleus accumbens. Electrochemically modified carbon-fiber electrodes were positioned in this site, and the voltammetric wave for AA was monitored following acute or long-term amphetamine treatment. Neither 1.0 nor 5.0 mg/kg d-amphetamine altered AA release in nucleus accumbens even when the treatment was continued for up to two weeks. These results suggest that amphetamine-induced AA release may be site-specific and that extracellular AA in neostriatum and nucleus accumbens is regulated by different mechanisms.

Supported by NSF BNS 87-11240.

56.13

DOPAMINE- AND SIGMA-RECEPTOR ANTAGONISTS IMPAIR REACTION-TIME CONDITIONED AVOIDANCE RESPONDING IN RATS. I.M. White, J.L. Haracz, P.E. Langley*, M.T. Ciancone*, and G.V. Rebec. Prog. Neural Science, Dept. Psychol., Indiana Univ., Bloomington, IN 47405.

Haloperidol and other neuroleptics impair performance on conditioned-avoidance-response (CAR) tasks in rats at doses that parallel their clinical potency. In the present study, we used a version of this task--the rapid, conditioned-forepaw-withdrawal response--to compare haloperidol, a D2>D1 dopamine antagonist and sigma antagonist, with clozapine, an atypical neuroleptic, and with BMY-14802, a selective sigma antagonist. Some animals also were prepared for recording single-unit activity during the CAR task. Haloperidol (0.01-0.25 mg/kg), clozapine (0.5-5.0 mg/kg), and BMY-14802 (1.0-10.0 mg/kg) produced comparable decreases in the number of successful avoidances and also increased response latency in a dose-dependent manner. These results suggest that sigma-receptor blockade mimics the effects of some currently used neuroleptics on CAR performance, lending support to the notion that BMY-14802 may exert antipsychotic effects in humans (Taylor et al., In: Schizophrenia, Tamminga and Schulz, eds, 1990). Preliminary single-unit data from the neostriatum, which contains a high density of both dopamine and sigma receptors, indicates the presence of neurons that change activity in response to various components of the CAR task. Further analysis of these cells in response to dopamine- or sigma-receptor antagonists promises to shed light on the neuronal mechanisms of action of these drugs.

Supported by NIDA (DA02451) and Scottish Rite Foundation.

56.14

TREMULOUS CHEWING MOVEMENTS IN RATS INDUCED BY HALOPERIDOL, PILOCARPINE AND STRIATAL DOPAMINE DEPLETION. J.D. Salamone, R.E. Steinpreis, M.E. Taylor and G. Jicha. Department of Psychology, University of Connecticut, Storrs, CT, 06269-1020.

Tremulous chewing movements in rats have been reported to occur in various drug treatments, but it is not clear how these movements are related to human motor syndromes. Acute administration of 0.4 or 0.8 mg/kg haloperidol (HP) produced only slight, nonsignificant increases in chewing. However, repeated injection of 0.4 mg/kg HP over 10 days significantly increased chewing responses. Acute administration of pilocarpine increased chewing, and combination of 0.4 mg/kg HP with 0.5 mg/kg pilocarpine produced significantly more chewing than either drug alone. Depletion of striatal dopamine with bilateral injection of 6-hydroxydopamine into the ventrolateral striatum also increased chewing resonses. These data indicate that tremulous chewing movements in rats share some characteristics with human Parkinsonian symptoms.

56.15

INDIVIDUAL VARIABILITY IN EXPLORATORY BEHAVIOR CORRELATES WITH DOPAMINE RELEASE BY AMPHETAMINE IN NUCLEUS ACCUMBENS. C.W. Bradberry, R. Gruen, and R.H. Roth. Dept. of Psychiatry, Yale Univ.Sch. Med., New Haven, CT 06510.

Dopamine (DA) function in the nucleus accumbens has been linked to exploratory behavior (EB). We wished to determine whether individual variation in nucleus accumbens DA function correlates with individual variations in EB. Rats were placed in a novel environment and EB was rated over a 10 min session. Within one week, animals were anesthetized and implanted with microdialysis probes into the nucleus accumbens. Following attainment of stable basal DA levels, animals were administered 3 mg/kg d-amphetamine sulfate i.p. The increase in DA caused by the amphetamine was compared with the previous behavioral scores for the same animal. A significant correlation was found to exist ($R=0.43$; $P < 0.05$) between the maximal increase in DA caused by amphetamine and the duration of EB seen earlier. Basal DA levels did not significantly correlate with EB. These results suggest that presynaptic variability in the nucleus accumbens DA innervation may underlie variations in EB. Possible DA neuronal characteristics responsible include altered distribution of DA between intraneuronal pools, altered regulation of release and/or synthesis, or differences in density of DA innervation. Supported by MH-14092 and DA-05119.

56.16

LOCOMOTOR EFFECTS OF CHRONICALLY CO-ADMINISTERED HALOPERIDOL AND NICOTINE IN RATS. L.L. Wing, R. Li*, R.J. Wyatt and D.G. Kirch. Neuropsychiatry Branch, NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032.

In a previous study, we found that in the rat striatum and hippocampus chronic co-administration of nicotine and haloperidol significantly decreased DA and 5-HT turnover compared to saline-treated animals. In the present study, behavioral associations of these biochemical effects were observed during 6 wks of treatment. Fifty-two male Sprague-Dawley rats (375g +15g) were implanted with Alzet 2ML4 osmotic pumps. Half the animals received pumps filled with isotonic saline, and half with pumps containing nicotine (free-base, 12 mg/kg). Thirteen of the saline controls and thirteen of the nicotine treated animals were given 21 mg/kg, IM haloperidol decanoate at 2 wk intervals, and the rest saline. Animals were tested for 1 hr in Omnitech activity monitors at 1, 3 and 6 weeks after implantation.

Haloperidol decreased locomotor activity, an effect which was potentiated by nicotine during the first 30 min of the first week. This potentiation had disappeared by week 6. These data have clinical implications given the widespread concurrent use of these drugs by schizophrenic patients, especially those having dyskinesias.

56.17

THE BEHAVIORAL EFFECTS OF DIHYDREXIDINE, A HIGH POTENCY, FULL EFFICACY D_1 DOPAMINE RECEPTOR AGONIST. **K.J. Darney, Jr.[1], M.H. Lewis[1,2], W.K. Brewster[4], D.E. Nichols[4], and R.B. Mailman[1,2,3]. Brain and Dev. Res. Ctr.[1], Depts. of Psychiat.[2] and Pharmacol.[3], Univ. of North Carolina, Chapel Hill, NC, 27599 and School of Pharmacy[4], Purdue Univ., West Lafayette, IN, 47907.**

An understanding of the role of D_1 dopamine receptors and D_1/D_2 receptor interactions in mediating behavior has been hampered by the lack of a potent, full efficacy D_1 dopamine agonist. We assessed the behavioral effects of dihydrexidine, a novel D_1 receptor agonist (trans-10,11-dihydroxy-5,6,6a,7,8,12b-hexahydrobenzo-[a]phenanthridine) which has a ten-fold higher affinity for the D_1 vs. D_2 receptor, and which is as efficacious as dopamine at stimulating D_1 mediated cAMP synthesis in striatal membranes. In the first study, the dose-response relationship of dihydrexidine was examined using male Sprague-Dawley rats (n=48), each administered a subcutaneous injection of 0.0, 0.3, 1, 3, 10 or 30 mg/kg. The behavioral effects of dihydrexidine were assessed using an observational method that quantified the modified frequency of multiple behavioral topographies. The frequency of three behaviors (grooming, sniffing, and locomotion) was altered significantly by dihydrexidine. Grooming was increased at 1.0 and 3.0 mg/kg, but returned to control levels at higher doses. The failure of dihihydrexidine to stimulate grooming at the two higher doses may well be due to activation of D_2 dopamine receptors. Dihydrexidine increased locomotion and sniffing in a dose dependent fashion over the whole dose range tested. Other behavioral topographies such as licking, gnawing and rearing were not systematically affected by drug administration. In a second study, rats (n=80) were pretreated with either a selective D_1 antagonist (0.1 mg/kg SCH23390), a selective D_2 antagonist (1 mg/kg remoxapride), or vehicle. Thirty min later rats were administered either dihydrexidine (3 mg/kg) or vehicle and their behavior coded by direct observation as above. SCH23390 antagonized the effects of dihydrexidine on grooming, locomotion, and sniffing. Conversely, remoxapride blocked dihydrexidine-induced locomotion, but had no effect on dihydrexidine-induced grooming or sniffing. Numerous behaviors are believed to be mediated by the interaction of D_1 and D_2 receptors. These data indicate that dihydrexidine, as a full efficacy D_1 agonist with significant D_2 properties, will be a powerful tool to characterize the nature of these interactions in the mammalian brain. **(Supported by HD38970, MH40537, and MH42705).**

56.18

EFFECT OF COCAINE AND STRESS CROSS-SENSITIZATION ON EXTRACELLULAR DOPAMINE RELEASE IN RAT NUCLEUS ACCUMBENS. B.A. Sorg and P.W. Kalivas†, Department of Psychology and Department of VCAPP†, Washington State University, Pullman, WA 99164-4820

The *in-vivo* dialysis technique was used to evaluate the effects of daily cocaine treatment (15 mg/kg) on the ability of acute footshock (0.45 mA/200m sec/sec for 20 min) to alter extracellular levels of dopamine and its metabolites, DOPAC, and HVA. The reciprocal experiment, in which the ability of daily shock to alter dopamine release upon acute cocaine treatment, was also performed. The levels of dopamine, DOPAC and HVA in the rostral nucleus accumbens were measured by HPLC-EC. Dopamine and DOPAC levels were only slightly increased in cocaine- vs. saline-pretreated animals upon acute footshock. In contrast, in the reciprocal experiment, dopamine release by cocaine was increased in shock vs. sham-shock pretreated rats (maximum levels were 125% and 69% above baseline levels, respectively). In addition, the maximum level of dopamine release occurred earlier in shock-sensitized animals. Concurrent measures of locomotor activity showed a similar increase in motor behavior in shock vs. sham shock animals. Both shock and sham shock groups showed a slight decrease in DOPAC and HVA levels upon acute cocaine injection.

56.19

MICRODIALYSIS AND TISSUE LEVELS OF BASAL DOPAMINE ARE UNCHANGED AFTER CORTICAL LESIONS, BUT ACUTE AMPHETAMINE-EVOKED EFFLUX IS ELEVATED AND DISSOCIATED FROM ROTATIONAL BEHAVIOR. E. Castañeda, I. Q. Whishaw, and S. D. Oddie. Department of Psychology, University of Lethbridge, Lethbridge, Alberta T1K 3M4.

It has been proposed that neocortical damage leads to acute and chronic changes in rotational behavior and subcortical dopamine (DA) activity. We gave rats hemidecortications and evaluated: (1) rotational behavior, (2) extracellular dopamine levels (using dialysis) during spontaneous and amphetamine-induced activity, and (3) postmortem tissue DA concentrations. Extracellular and tissue DA levels were unchanged acutely and chronically. After amphetamine administration (1.5 mg/kg, s.c.) acute hemidecortication produced ipsiversive circling and extracellular DA was elevated bilaterally. Chronically, hemidecorticate rats were not different from control rats. The results suggest that: (1) cortical damage does not produce asymmetrical changes in DA activity and (2) injury-induced rotation is unrelated to DA activity. The unexpected finding of acutely elevated amphetamine-stimulated release suggests that cortical injury produces a nonspecific acute suppression of DA turnover.

56.20

INTRACRANIAL SELF-STIMULATION ON FR15 ENHANCES THE BEHAVIORAL RESPONSE TO LOW DOSES OF AMPHETAMINE SULFATE IN THE RAT. P. R. Hartley and D. B. Neill. Dept. of Psychology, Emory University, Atlanta, GA 30322.

Systemic administration of amphetamine is well known to induce increased responding for intracranial self-stimulation (ICSS). We observed that rats performing lever-press ICSS at lateral hypothalamic electrodes on a fixed ratio 15 (FR15) schedule of reinforcement completely ceased bar-pressing at 2 mg/kg *d*-amphetamine sulfate (AMPH). Dose-response analysis revealed a shift left for FR15 rats compared to FR1 rats. Rats on the FR1 schedule showed either an increase or decrease in responding at 2 mg/kg, dependent on nondrug response rate; however, unlike the FR15 rats, all continued lever-pressing.

Observation of the FR15 rats at 2 mg/kg showed them to be in a form of stereotypy that consisted of sniffing, rearing, and head-bobbing. Preliminary investigation of ambulatory behavior obtained immediately following the ICSS session revealed that the FR15 rats showed less of a response than the FR1 rats; this is consistent with the enhanced stereotypy in the FR15 rats. In addition, post-session baseline ambulatory activity was higher in the FR15 rats. We propose that the FR15 schedule induced an elevated dopamine release; the addition of AMPH to this elevated baseline resulted in stereotypy and cessation of ICSS.

DRUGS OF ABUSE: ALCOHOL I

57.1

EFFECTS OF CHRONIC ETHANOL ADMINISTRATION AND WITHDRAWAL ON NUMBERS OF VASOPRESSIN-CONTAINING NEURONS. GP Kozlowski and JH deSchweinitz*. Dept. of Physiology, University of Texas Southwestern Medical Center, Dallas TX, 75235-9040.

Vasopressin (VP) enhances learning and memory which are reduced in alcoholics. Previous studies using the PAP technique (Neurosci Abstr 15,416) showed that ETOH given 30 days, reduced the numbers of VP neurons by 62% in comparison to control (CTRL) rats. Here, we compare numbers of VP-containing neurons in rats on either CTRL (n=5) or ETOH (n=5) liquid diets using a simultaneous pair-feeding method for 30 days followed by 10 days Lab Chow and water *ad lib*. The more sensitive ABC Elite kit (Vector Labs) was used for immunocytochemistry of serial-sectioned rat brains. Means of cell numbers of CTRL vs. ETOH were: ant hypothal area-767±74 vs. 750±83; ant commissural nuc- 52±21 vs. 63±27; supraoptic nuc- 2064±192 vs. 1835±185; suprachiasmatic nuc-1169±189 vs. 811±140; paraventricular nuc- 1046±70 vs. 1060±105; and retrochiasmatic supraoptic nuc-385±90 vs. 345±111. Means of total numbers of neurons/brain were 5482±458 CTRL vs. 4865±425 ETOH. There was no statistical difference between the two treatment groups indicating that VP synthesis resumes within 10 days after ETOH treatment. Supported by NIAAA AA-06014.

57.2

EFFECT OF ETHANOL ON NEURONS IN FRONTAL AND PREFRONTAL CORTEX AND MEDIAL DORSAL (MD) THALAMUS IN AWAKE, BEHAVING RATS. Kosobud, A.E., and Chapin, J.K. Department of Physiology and Biophysics, Hahnemann University, Philadelphia, PA 19102.

Although ethanol (EtOH) has profound depressant actions, it has been shown to be a stimulant at low doses, and in initial stages of intoxication. It has been proposed that multisynaptic processess are particularly vulnerable to EtOH, and that the loss of descending inhibition from higher order cortical association areas may be responsible for some EtOH-induced stimulation. Prefrontal and frontal cortex play a role in sensorimotor integration, in planning and selection of appropriate behavior, and in short-term task-related memory. In the present experiments, we examined the effects of ethanol on single unit activity in MD thalamus and various sites in medial frontal and prefrontal cortex. Multiple bundles containing 4-6 microwire electrodes were chronically implanted in female rats, 250-300 g. In general, all implants were in one hemisphere, but in some cases, symmetrical bilateral implants were made. Continuous recordings were obtained from cells in rats locomoting on a timed treadmill before and after EtOH administration (1 g/kg i.p.). A videocamera was used to record behavior throughout the experimental session. In general, cells in the frontal and prefrontal cortex showed higher rates of firing either during locomotion (treadmill on) or rest (treadmill off), but in addition, displayed some more specific responses. For instance, one cell showed increased activity when the animal was at rest, but right hindlimb movement-related firing during locomotion. Most thalamic cells showed higher activity rates during movement. EtOH tended to inhibit the firing rate of cells in both cortex and thalamus. Furthermore, the difference in firing rate between locomotion and rest was reduced or eliminated, especially in the first 10 minutes of intoxication. Thus, it is possible that the disruption in higher cortical function induced by low doses of EtOH reflects a loss of selective activity, rather than a pure inhibition. Supported by grants NS26722, AA06965, K02-AA00089 and AFOSR 90-0266

57.3

EFFECTS OF ACUTE ETHANOL TREATMENT ON THE FIRING THRESHOLD OF HIPPOCAMPAL GRANULE NEURONS. G.L.F.Yuen, M.Patil and D.Durand. Applied Neural Control Lab., Dept. of Biomedical Engineering, Case Western Reserve University, Cleveland, Oh 44106.

Although low dose, acute exposure of ethanol has been reported to affect the unit activities and field potentials of hippocampal neurons (Folger and Klemm 1978; Wiesner, Henriksen and Bloom 1987), the cellular basis for these changes are unclear. As a step towards clarifying the underlying mechanism(s), detailed intracellular recordings were performed in hippocampal granule neurons with control and ethanol (50mM) media using the *in vitro* slice preparation. Recordings obtained from 12 cells showed that 91% of the cells displayed specific changes in the firing properties of the soma and/or dendrites after an exposure time of 15 minutes or more. 4 out of 6 neurons (67%) showed an *increase in the orthodromic firing threshold* measured at various pulse-widths of excitation. This effect was reversible in 2 out of 3 cells (67%). Such an increase in the dendritic firing threshold was observed simultaneously (3 out of 4 cells) with a decrease in the amplitude of the EPSPs (53-71%) evoked by identical stimuli after ethanol treatment. 7 out of 9 cells (77%) showed *increased somatic excitability* as measured by the strength-duration relationship and repetitive firing responses. These effects were reversible in 2 out of 3 cells. Although more recovery data are needed, these somewhat opposite effects of ethanol on the soma and dendrites of these neurons may help to account for some of the inconsistent results from previous single cell studies. An increase in the dendritic firing threshold is also consistent with recent voltage-clamp studies showing a reversible depression of the NMDA-induced current by ethanol in dissociated hippocampal neurons (Lovinger, White and Weight 1989) and the demonstration of a role of the NMDA receptor in the EPSPs of normal hippocampal granule neurons (Lambert & Jones 1989). Supported by NIAAA grant# 5R1AA06773-05.

57.4

DISSIMILAR EFFECTS OF ETHANOL AND CHLORDIAZEPOXIDE ON INHIBITION IN THE FASCIA DENTATA AND HIPPOCAMPUS REGIO SUPERIOR Steffensen, S.C. and Henriksen, S.J., Research Institute of Scripps Clinic, La Jolla, CA 92037.

Acute intoxicating levels of ethanol increase paired-pulse (PP) inhibition in the dentate while having no effect on population spike (PS) amplitudes evoked by perforant path stimulation suggesting that ethanol specifically targets recurrent mechanisms in the dentate (Wiesner and Henriksen, *Neurosci. Letts.* 79:169, 1987). In the present study, we describe and compare the effects of ethanol and the benzodiazepine, chlordiazepoxide, on evoked PSs, PP responses, spontaneous activity and interneuronal single-unit excitability in the dentate gyrus and CA1. In the dentate, chlordiazepoxide had no effect on PS amplitudes, decreased dentate granule cell (DGC) spontaneous firing rate (65%) and increased PP inhibition, effects similar to those of ethanol in the previous study. In CA1, ethanol decreased PS amplitudes (31%) evoked by Schaffer collateral stimulation, decreased pyramidal cell (PC) spontaneous firing rate (42%) and had no effect on PP responses. Chlordiazepoxide produced similar effects on these measures. Since GABA interneurons are believed to be activated by recurrent collaterals from DGCs and PCs, these results suggest that ethanol, like the benzodiazepines, potentiates GABA-mediated inhibition in the dentate and CA1. However, ethanol and chlordiazepoxide appear to have dissimilar effects on the excitability of putative interneurons recorded in these regions. Interneurons were categorized by conventional criteria; however, the most stringent criterion was that these cells showed non-decremental impulse discharges following stimulation of their respective afferents. Impulse discharges were evident in CA1 after single shocks whereas, in the dentate, impulse discharges were evident only after paired-shocks. In all cells studied, ethanol markedly increased (200-800%) and chlordiazepoxide abolished the number of interneuronal discharges in both the dentate (N=18) and CA1 (N=4). These results suggest that the inhibitory effects of ethanol are produced by increases in interneuronal discharge and that benzodiazepines inhibit by a facilitatory action at the GABA receptor/chloride ionophore complex.

57.5

ETHANOL AND MK-801 DIFFER ON BEHAVIORAL AND ELECTROPHYSIOLOGICAL MEASURES. P.E. Simson, K.B. Johnson, H.E. Criswell, and G.R. Breese. Brain and Development Research Cntr., Univ. of North Carolina, Chapel Hill, NC, 27599.

A number of studies have suggested that ethanol (ETOH) blocks N-methyl-D-aspartate (NMDA) receptors. In this study, we compared the effects of ETOH and the NMDA antagonist MK-801 on behavioral and electrophysiological measures. In a behavioral test measuring spontaneous activity, ETOH, in doses up to 1.5 g/kg, i.p, decreased spontaneous activity while MK-801, in doses up to 0.3 mg/kg, i.p., increased spontaneous activity. In an electrophysiological test, single unit recordings of medial septal neurons were obtained from urethane-anesthetized rats, and the effects of ETOH and MK-801 on the excitation of these neurons by iontophoretically-applied NMDA were observed. At the highest dose tested, ETOH (2.5 g/kg, i.p.) blocked NMDA-evoked activity in only 56% of neurons tested; lower doses of ETOH blocked correspondingly smaller proportions of neurons. In contrast, MK-801 (0.6 mg/kg, i.p.) blocked NMDA-evoked activity in 100% of neurons tested. While these data indicate that some of the effects of ETOH may occur through blockade of NMDA receptors, this action may not explain all of the effects of ETOH. Supported by AA-08024, MH-33127, NS-26595 and HD-03110.

57.6

ETHANOLAMINE CONTROL OF LIPID PEROXIDATION CAUSED BY ETHANOL. F. Poldrugo, A. Fiore*, G.Z. Abakumov*, I.L. Bykov*, Yu.A. Tarasov*. Alcohol Research Group, Univ. of Trieste, 34100 Italy; Inst. of Biochemistry, BSSR Acad. Sci., Grodno 230009 USSR

The activation of lipid peroxidation (LPO) during ethanol (ETOH) intoxication is well documented. In this research the effect of a natural bioantioxidant: ethanolamine (EA) on the acute and withdrawal effect of ETOH has been investigated.

In the acute studies, male albino rats were injected i.g. with a 25% ETOH solution at doses of 1g/kg b.w. The alcohol withdrawal syndrome (AWS) was obtained after discontinuation of exposure to ETOH vapors (30mg/l) for a 14 day period. EA (100 mg/kg b.w.) was injected i.p. one hour before ETOH injection and before withdrawal evaluation (10 hours after ETOH withdrawal).

Hydroxyperoxide concentrations and the antioxidative activity (AOA) of EA (measured through chemiluminescence parameters) were detected in plasma. Plasma hydroxyperoxide concentrations were increased by 207% vs control rats after acute ETOH injection. EA administration counterbalanced this effect. LPO was not increased during AWS. However, plasma AOA was decreased both during acute ETOH intoxication and AWS. EA increased AOA in both conditions.

Thus, the antioxidative property of EA has been proven to counteract ETOH oxidative effects.

57.7

INTERACTION OF ETHANOL AND L-GLUTAMATE IN THE GUINEA PIG ILEUM G.D. Frye Medical Pharmacology, Texas A&M Col. of Med., College Station, TX 77843

CNS depressant actions of ethanol (ET) appear to involve inhibition of excitatory amino acid receptors as shown by blockade of N-methyl-D-aspartate (NMDA) activated currents in hippocampal neurons by ET (Lovinger et al., Science 243: 1721-1724, 1989). Whether this interaction occurs in the peripheral nervous system was tested in the longitudinal muscle-myenteric plexus preparation (LMMP). Both ET (Frye et al., J. Pharmacol. Exp. Ther. 252:474-481, 1990) and L-glutamate (GLU; Moroni et al. Neurosci. Lett. 68:57-62, 1986) induce brief atropine-labile contractions in LMMP. Confirming previous reports, GLU responses were found to be 2-amino-5-phosphonovalerate (APV; 100uM) and Mg^{2+} sensitive. Preincubation with ET (30-100mM) for 2 min inhibited contractions to GLU (60uM) and caused additive inhibition with 100uM Mg^{2+}. ET (65mM) did not inhibit contractions to acetylcholine (0.1uM), serotonin (0.1uM) or histamine (0.3uM). On the other hand ET contractions were not blocked by either APV or Mg^{2+}. These results suggest that ET may selectively inhibit peripheral NMDA receptors as well as those in the CNS. Supported by PHS grants AA00101, AA06322.

57.8

CHRONIC ETHANOL TREATMENT AND HIPPOCAMPAL CELL RESPONSES TO CARBACHOL. L. Taylor, G.D. Frye, W.H. Griffith. Dept. of Med. Pharmacol., Texas A&M Col. of Medicine, College Station, TX 77843

Chronic ethanol (ET) irreversibly damages forebrain cholinergic neurons (Arendt et al., Neurosci. 33:435-462, 1989) and may lead to persistent increases in muscarinic receptors in the hippocampus (HIP). To test this, rats were fed a nutritionally complete ET liquid diet for 3 months, were withdrawn from ET for 4 months and then pre- and postsynaptic sensitivity of CA1 pyramidal cells to carbachol (CCh) was examined. In both ET treated or control HIP slices, bath applied CCh (1-10uM) similarly reduced dendritic field EPSPs following stimulation of the Schaffer collateral. CCh (0.02-2 uM) also inhibited slow afterhyperpolarizations (AHPs) in CA1 cells following a train of action potentials. Based on the number of cells where AHPs were inhibited by 0.02-0.2uM CCh, ET treatment appeared to increase sensitivity to CCh compared to pairfed controls or young (2 mo old) diet naive animals. Although preliminary, these results suggest that longterm ET treatment may cause persistent increases in postsynaptic sensitivity to muscarinic agonists in CA1 neurons. Supported by PHS grants AA00101, AA06322, AG07805, NS22456.

57.9

INFLUENCE OF CHRONIC ETHANOL TREATMENT ON SLEEP TIME AND HIPPOCAMPAL CALCIUM CURRENTS OF LONG- AND SHORT-SLEEP MICE. S.P. Aiken, G.-J. Huang, and J.J. McArdle New Jersey Medical School (UMDNJ), Newark, NJ 07103.

Since tolerance to ethanol is associated with an increase in the number of dihydropyridine binding sites in rat cortex (Ann. N.Y. Acad.Sci. 560:465), we measured hippocampal Ca^{2+} currents (I_{Ca}) in Short(SS)- and Long-sleep (LS) mice which differ in their sensitivity to ethanol. Specifically, SS mice lost their righting reflex for 78 min after an i.p. injection of 5g/kg of ethanol. Sleep time for LS mice was 131 min in response to only 4 g/kg. Whole cell recording of I_{Ca} from hippocampal cells, enzymatically isolated from normal adult SS and LS mice, revealed equivalent ($p>0.01$) maximal values of 463 and 527 pAmps, respectively. In order to evaluate the effect of chronic ethanol treatment on I_{Ca}, we placed SS and LS mice on a calorically controlled liquid diet containing 3.8% ethanol. Subsequent measurement of sleep time or I_{Ca} was made 24 hrs after temporarily placing the mouse on a normal liquid diet. Within 7 days of beginning this treatment, sleep time had declined to a plateau of 52% and 50% of control for LS and SS mice, respectively. Since I_{Ca} of the tolerant LS mouse (600 pAmp) was significantly ($p<0.01$) greater than I_{Ca} of the SS mouse (400 pAmp), we suggest that exposure to chronic ethanol is associated with an increase in the number of calcium channels in LS but not SS mice. (supported by grant R01 AA08025-01 from the NIAAA).

57.10

EFFECTS OF ETHANOL ON THE HINDBRAIN OF DEVELOPING AXOLOTLS. M. W. Egar, V. J. McAdoo, and D. M. Schroeder. Anatomy Dept., Indiana University School of Medicine, Indianapolis, IN 46202 and Medical Sciences Program, Indiana University, School of Medicine, Bloomington, IN 47405

Behavioral effects of the recessive lethal gene (matilda) indicated possible cerebellar or hindbrain dysfunction. These behavorial effects are only expressed after the larvae have reached approximately 12 weeks of age. This coincides with the maturation of the hindbrain and cryptic metamorphosis. In an attempt to produce phenocopies, larval axolotls (Ambystoma mexicanum) 4-10 weeks after spawning, were grown for 4 weeks in 1% ethanol. Control and experimental groups of 20 animals each were selected from non-matilda spawnings. All animals were fed brine shrimp following the daily change of their solution. For the experimental groups the alcohol was added to the 1:4 concentration of Holtfreter's solution. Observations throughout the experimental period indicated an abnormal gill posture similar to that seen in homozygous matilda larvae. The ethanol treated larvae also showed an erratic swimming pattern. These animals were smaller than the controls at the end of the 4 weeks when all animals were sacrificed. The hindbrains were fixed and processed for light and electron microscopy. An apparent increase in the amount of lipids in the ependymal layer of the treated hindbrains may be due to the delayed development caused by the ethanol. The matilda hindbrains showed an even more pronounced accumulation of lipids.

57.11

THE EFFECT OF TREATMENT PARADIGM ON THE CONTRIBUTION OF LEARNING TO ETHANOL TOLERANCE. J.L. Weiner* and J.M. Khanna. Dept. of Pharmacology., Univ. of Toronto, & Addiction Research Foundation., Ontario, Canada.

We demonstrated previously that cycloheximide, an amnestic drug, could block the development of chronic tolerance to ethanol when measured on the moving belt test but had no effect on tolerance assessed by the tilt-plane and hypothermia tests using a gavage treatment paradigm.

A second study was done to characterize the effect of treatment paradigm on the contribution of learning to the development of ethanol tolerance using the hypothermia test. For this purpose, a conditioning treatment protocol was used. Four groups of male, Sprague-Dawley rats were treated daily with saline or ethanol (2.0 g/kg) preceeded by saline or cycloheximide (0.3 mg/kg) in an environment made distinct from the colony room by the addition of white noise and dim lighting. The group receiving saline/ethanol displayed significant tolerance when tested in the distinct environment, a conditioned hyperthermic response when given a placebo injection in that same room but no tolerance when tested in the colony room. In contrast, the cycloheximide/ethanol group showed no tolerance in either test room and failed to exhibit the conditioned compensatory response.

These studies demonstrate that the treatment paradigm used in the induction of tolerance can significantly affect the contribution of learning to tolerance development.

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57.12

TIME COURSE AND PATTERN OF ASTROCYTE HYPERTROPHY IN THIAMINE DEPLETED RATS. P. Nellis*, M. Khurgel, N.W. Milgram and G.O. Ivy. Div. Life Sci., Univ. of Toronto at Scarborough, Ont. M1C 1A4 Canada

We have previously shown that chronic thiamine depletion leads to abnormal neuronal responsiveness within 5 days and to motor seizures by 12 days. We have also shown that heightened neuronal activity causes astrocyte hypertrophy (AH). We thus decided to examine AH and other anatomical changes in thiamine depleted rats. Rats were maintained on a thiamine depleted diet supplemented with pyrithiamine for 5, 8 or 12 days, followed by normal diet for 1d, 1 wk or 1 mo prior to sacrifice. Astrocyte hypertrophy (AH) was visualized using immunoreactivity for anti-glial fibrillary acidic protein; nissl stains were also used. AH was present in hippocampus, pyriform cortex and amygdaloid nuclei (with the exception of the posterior basolateral nucleus) in all rats with 1d recovery from thiamine depletion. AH appeared to be increased in these areas in all rats with 1wk survival times and persisted for at least one month. In addition, all 12d treated rats which underwent motor seizures incurred extensive neuronal death in thalamus. It remains to be determined if AH precedes the thalamic necrosis and why the first brain regions to undergo AH are not the regions which experience neuronal death. Similar morphological changes may occur in humans with severe malnutrition or following alcohol withdrawal seizures.

57.13

GABA MEDIATION OF ETHANOL'S ANTI-CONFLICT EFFECT

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Previous results have shown that the anti-conflict effect of ethanol (EtOH) was antagonized by isopropylbicyclophosphate, a ligand that binds at or near the chloride ionophore at the picrotoxinin site of the GABA/benzodiazepine ionophore complex (*Alcohol* 5 p 437 1989). The purpose of this study was to further evaluate the reactivity of this receptor complex to GABA-selective neuropharmacolgical agents during acute EtOH treatment in male Wistar rats. A modified Geller-Seifter conflict test measured food reinforcement paired with incremental shock and unpunished responding (random interval-30 sec schedule). In separate experiments we tested: (1) the non-competitive GABA antagonist picrotoxin (0, 0.25, 0.50, 1.0 mg/kg ip 20 min pretreatment; (2) the quarternary competitive antagonist bicuculline methiodide (0, 50, 100, 200 ng icv 20 min pretreatment); and (3) the GABA transaminase inhibitor amino-oxyacetic acid (AOAA 0, 7.5, 15 , 30 mg/kg ip 90 min pretreatment) for their ability to mediate the anti-conflict effect of EtOH (0.75 g/kg 15 min pretreatment ip). As shown previously, EtOH increased punished responding, and here this effect was antagonized by picrotoxin (1.0 mg/kg) and bicuculline methiodide (200 ng). In contrast, the depression of unpunished responding produced by EtOH was not reversed by either treatement. AOAA (30 mg/kg) enhanced EtOH's effects on conflict responding. When subjects were tested without EtOH treatment, neither GABA antagonist affected responding, and AOAA (30mg/kg) attenuated unpunished responding. These results sugest that GABA may interact with the "anxiolytic" effects of EtOH, but at the doses tested, antagonism of GABA receptor activity did not antagonize EtOH's sedative properties (Supported in part by NIAAA 05297 and NIAAA 06420).

57.14

ALCOHOL AFFECTS FEEDFORWARD BUT NOT FEEDBACK INHIBITION OF HIPPOCAMPAL CA1 NEURONS IN VIVO.

J.R. Criado and R. Thies. Depts. of Psych. and Behav. Sci. and of Physiol. and Biophysics, Univ. of Okla. Health Sci. Ctr., Oklahoma City, OK 73190.

We studied the effects of acute alcohol (1.5 g/kg, i.p.) on field potentials evoked by orthodromic and antidromic stimulation of CA1 neurons. Metal electrodes recorded from the hippocampal CA1 field in male rats anesthetized with 1.0% halothane anesthesia. Bipolar concentric stimulating electrodes produced synaptic excitation and feedforward inhibition from the Schaffer collaterals. Electrodes in the alvear region produced antidromic excitation and feedback inhibition. Alcohol increased the amplitude of field potentials elicited by synaptic excitation, but this was not sensitive to dose. The response to a second stimulus given 40 msec after the first is decreased. This was further decreased in the presence of alcohol, suggesting enhanced inhibition. Alcohol did not affect the amplitude of antidromic field potentials, or of responses to second stimuli given antidromically. These findings suggest that inhibitory interneurons in the Schaffer collateral pathway are more sensitive to the effects of alcohol than interneurons located in the recurrent pathway.

57.15

ETHANOL LEVELS IN PREGNANT MICE AND FETUSES. L.D. Middaugh and W.O. Boggan. Dept. Psychiatry, Med. Univ. S.C., Charleston, SC 29425

Offspring of pregnant C57BL/6 mice fed liquid diets containing 25% ethanol (E) derived calories have increased neonatal mortality, growth deficits extending into adulthood, and behavioral deficiencies as adults. Control procedures established that the observed effects are not due to nutritional deficiency; however, ethanol concentrations attained in the dam ingesting the diet and in her fetuses are not published. The present study characterized the drinking pattern and ethanol exposure in pregnant mice and fetuses maintained on the E-diet. In Experiment 1, pregnant mice increased diet ingestion across pregnancy to maintain a rather constant dosage (23.6 to 22.1 g/Kg/Day on Gestation 5-7 vs 15-17). Ethanol and pair-fed sucrose controls ingested equivalent volumes of diet over a 24-hr period; however, controls consumed 90% of their diet within 14 hr of the diet change at 0800 hr compared to 50% for mice fed the E-diet. In Experiment 2, ethanol concentrations in samples taken at intervals after the diet was changed indicated no detectable ethanol during the day time (0800, 1200, 1600 hr). At night (2000, 2200, 2400, & 0200 hr), maximum ethanol levels were 130 mg% for maternal plasma, 1243 ug/g for maternal brain, and 1121 ug/g for fetal brain. The highest individual sample values were at the 2200 hr sampling; however, the ethanol levels were least variable and the group average was highest at 2000 hr (1 hr after lights out). Maternal plasma values correlated well with maternal and fetal brain values (r^2=0.99). (Grant #AA06611).

57.16

BEHAVIORALLY-RELEVANT DOSE OF ETHANOL STIMULATES VASOPRESSIN RELEASE FROM MOUSE NEUROINTERMEDIATE LOBE, IN VITRO. T. van Teunenbroek* and D. L. Colbern. Department of Physiology and Biophysics, University of Illinois at Chicago, IL 60680.

We have reported that post-training administration of moderately high doses of ethanol (2-3 g/kg) improves passive avoidance conditioning in mice (Colbern, *et al.*, 1980, 1986). Retrograde facilitation of memory by ethanol has also been observed in humans for both visual (Parker, *et al.*, 1980, 1981) and verbal information (Mueller, *et al.*, 1983). Ethanol affects many neurotransmitter and endocrine systems implicated in memory processes. We are investigating whether the release of vasopressin (VP) is involved in the facilitation of memory by ethanol.

In the present study, the neurointermediate lobe of the pituitary (NIL) was rapidly dissected from 16 male Swiss-Webster mice killed by decapitation. The NILs from two mice were placed in each superfusion chamber (200µl) and perifused (200µl/min) for two hours with oxygenated Krebs Ringer Bicarbonate Buffer (KRB, 37°C) to achieve a stable baseline of VP release. For the next 24 min, the NILs were perifused with either 50 mM ethanol in KRB or KRB alone. Superfusate was collected every 6 min and VP was measured by radioimmunoassay. Superfusion with ethanol resulted in a dramatic increase in VP release (mean ± SD, 11.83 ± 1.01 pg/50µl) compared to basal release (1.35 ± .48 pg/50µl).

These data are consistent with our earlier studies in which ethanol produced marked VP release from rat neurointermediate lobe. Further *in vitro* and *in vivo* investigation will focus on factors mediating VP release by ethanol and the possible role of this neuropeptide in the effects of ethanol on memory. *Supported by PHS Grant AA06747 to DLC.*

57.17

ETHANOL INHIBITS CALMODULIN-DEPENDENT ACTIVATION OF DIHYDROPYRIDINE-SENSITIVE CALCIUM CHANNEL IN NEURONAL MEMBRANE. Paul Y.Sze, Alex Canda* and Byung H. Yu*. Dept. of Pharm. & Mol. Biol., The Chicago Medical School, North Chicago, IL 60064.

Specific binding of [^{3}H] PN-200-110 to particulate preparations from rat brain was increased by 3-fold after incubation of the membrane with 50 uM ATP, 5 mM Mg, 1 mM Ca and 1 uM calmodulin. The presence of all four components was necessary for the activation; addition of 1 mM EGTA or 0.2 mM trifluoperazine prevented the activation. The radioligand binding was also increased by incubation of the membrane with MgATP and the catalytic subunit of bovine heart cAMP-stimulated protein kinase (50 units/ml). MgATP together with 1 mM cAMP was ineffective, indicating that addition of an exogenous cAMP-stimulated protein kinase was required in the activation. These results are consistent with the interpretation that the L-subtype calcium channel in brain could be activated by both a calmodulin-dependent and a cAMP-dependent mechanism.

Ethanol at 25-200 mM had no effect on the basal level of [^{3}H] PN-200-110 binding to the membrane (i.e., without the activating system). However, the presence of ethanol markedly inhibited the calmodulin-dependent activation of the radioligand binding, whereas the cAMP-dependent activation was not affected. At 150 mM, ethanol inhibited more than 50% of the activation. Thus, from the in vitro characterization, it appears that ethanol does not act directly on the calcium channel; instead, its action is on calmodulin-dependent mechanisms regulating the channel activity. These data are in support of our hypothesis that calmodulin plays a pivotal role in mediating ethanol actions on membrane function in brain.

(Supported by AA07230)

LEARNING AND MEMORY—PHARMACOLOGY: ACETYLCHOLINE I

58.1

INTRASEPTAL INFUSION OF THE GABA-A AGONIST MUSCIMOL IMPAIRS THE ACQUISITION AND RETRIEVAL OF MEMORY. A.H. Nagahara and J.L. McGaugh. Department of Psychobiology & Center for the Neurobiology of Learning and Memory, Univ. of California, Irvine, CA 92715.

We previously reported that intraseptal infusion of muscimol (1, 5 nMol) decreases high-affinity choline uptake in the hippocampus and impairs acquisition in a water-maze place learning task (PLT). The present experiments investigated the effects of intraseptal injections of muscimol on the learning and retention of an inhibitory avoidance task (IAT) and a PLT.

Sprague-Dawley rats with implanted intraseptal cannula received buffer or muscimol (1 or 5 nMol) either 5 min prior or immediately after training. The first set of experiment examined the effects of pretraining and posttraining injection of muscimol on the IAT and PLT. In both tasks, pretraining, but not posttraining, injections of muscimol impaired memory retention.

The second set of experiments examined the effects of pretraining intraseptal muscimol on memory over various retention delays on the IAT (15 s, 15 min, 48 hr-retest) and on a single-trial PLT (15 s, 5 min). On the IAT, the 5 nMol muscimol group showed normal retention at 15 s, but was impaired on the 15-min test and 48-hr retest. On the single-trial PLT, the 1 nMol muscimol group showed normal memory on the 15-s retention test, but was impaired on the 5-min retention test. These findings indicate that intraseptal muscimol impairs long-term memory, but not short-term memory performance.

The third set of experiments examined the effects of pretest (5 min prior) intraseptal muscimol on retention performance on an IA task and on a free swim probe after acquisition of a PLT. Intraseptal muscimol impaired retention performance in the IA task (5 nMol) and on the PLT (1, 5 nMol). These sets of experiments suggest that the GABAergic system in the MSA and its regulation of the septohippocampal cholinergic system play a role in the acquisition and retrieval of long-term memory.

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58.2

INTRASEPTAL ADMINISTRATION OF BICUCULLINE PRODUCES MEMORY DEFICITS IN THE RAT. J.J.Chrobak & T.C.Napier, Dept. of Pharmacology, Loyola Univ. Chicago Sch. Med., Maywood, IL 60153.

Septohippocampal neurons are critical to neurophysiological processes within the hippocampus and thus support the cognitive processes subserved by activity within that structure. Acute pharmacological manipulations within the septum can alter performance in working memory tasks. Infusion of the GABAergic agonist muscimol produces deficits in the performance of a delayed-non-match-to-sample (DNMTS) radial arm maze (RAM) task. The present report indicates that acute septal infusion of the GABAergic antagonist bicuculine (BIC) can also produce a deficit in the performance of this task.

Male Sprague-Dawley rats, trained to perform a DNMTS-RAM, were implanted with a single cannula aimed at the medial septal nucleus. A within-subjects design was utilized to examine the effects of BIC (0.5ug) on performance of this task with one and four hr retention intervals. Administration of BIC immediately following the first four choices produced an impairment in maze performance at both retention interval. This treatment also produced an increase in latency per choice. BIC-induced impairments were not observed when administered two hours following the predelay session. These data support previous observations that pharmacological manipulation of GABAergic activity within the septum modifies working memory processes. (This work was supported by a Illinois Department of Public Health grant to JJC, and a BRSG grant to TCN).

58.3

CHLORDIAZEPOXIDE-INDUCED WORKING MEMORY IMPAIRMENT: SITE SPECIFICITY AND ANTAGONISM WITH RO15,1788. R.W. Stackman and T.J. Walsh. Rutgers University, Deptartment of Psychology, New Brunswick, NJ 08903.

The following experiments examined (1) the site-specificity of CDP induced memory impairments and (2) whether the BDZ antagonist RO15,1788 would attenuate these impairments.

Adult Sprague-Dawley rats were trained to perform a working memory version of the eight arm radial maze (RAM) task, in which a one hr delay was imposed between the fourth and fifth arm choices. Following acquisition of this task, animals were implanted with either a single guide cannulae in the medial septum (MS) or bilateral cannulae in the amygdala. Following surgery, rats were retrained on the task and injected immediately following the pre-delay session with CDP or vehicle. Intraseptal injection of 30, but not 15, nmoles of CDP impaired working memory performance. No memory impairments were observed when CDP was injected into the MS 15 mins following the pre-delay session or when CDP (10, 20, 30 nmoles) was bilaterally injected into the amygdala.

In the final experiment, rats were injected intraperitoneally with either an amnestic dose of CDP (5 mg/kg) or saline immediately following the pre-delay session. They were then infused, into the MS, with either 10 nmoles of RO15,1788 or the 5% polyethylene glycol vehicle. Systemic injection of CDP produced a working memory impairment that was blocked by administration of the BDZ antagonist into the MS. These data suggest that CDP-induced amnesia is mediated by BDZ receptors in the MS. Supported by BRSG grant 07058 to TJW.

58.4

GABAergic CONTROL OF BASAL FOREBRAIN CHOLINERGIC NEURONS AND MEMORY. M. Sarter and P. Dudchenko*. Dept. of Psychology, The Ohio State University, Columbus, OH 43210.

The involvement of the GABAergic innervation of basal forebrain cholinergic neurons in the rats' conditional visual discrimination performance was examined. Performance in such a task is based on the subject's ability to retrieve information about response rules. Following the acquisition of the task, guide cannula were implanted bilaterally into the substantia innominata (SI) of both hemispheres. Administration of the $GABA_A$-agonist muscimol into the SI (0, 25, 50 ng/0.5 µl/ hemisphere) dose-dependently decreased the number of correct responses, increased the number of errors of omission, and increased response latency. Systemic co-administration of the cholinesterase inhibitor physostigmine (0, 0.1, 0.2 mg/kg; i.p.) exclusively interacted with the effects of muscimol on correct responding, producing both increasing and attenuating effects on the impairment induced by muscimol. These results support the hypotheses that the activity of basal forebrain cholinergic neurons is under inhibitory control by a GABAergic input, and that this neuronal link is involved in mnemonic processing.

58.5

EFFECTS OF DM-9384, A NEW COGNITION-ENHANCING AGENT, ON CHOLINERGIC SYSTEM IN RAT CORTEX.
S. Watabe, H. Yamaguchi* and S. Ashida*. Research Institute, Daiichi Pharmaceutical Co. Ltd., Tokyo 134, Japan.

We have reported that DM-9384(N-(2,6-dimethylphenyl)-2-(2-oxo-1-pyrrolidinyl)acetamide) accelerates both the metabolic turnover of GABA and ACh uptake in rat cortex(Soc. Neurosci. abstr.,1989). DM-9384 also activated choline acetyltransferase(ChAT) activity in rat cortex. These results indicate the possibility that DM-9384 acts on GABAergic and cholinergic systems. In the present study, we have examined the effects of DM-9384 and its congeners (aniracetam and oxiracetam) on both [^{3}H]-ACh release(in virto and ex vivo) and GABA-induced [^{3}H]-ACh release from rat cortical slices. The amount of released [^{3}H]-ACh from slices was significantly increased (65%) by oral adminstration of DM-9384 (3mg/kg), although DM-9384(10^{-7}M) had a little effect in vitro. GABA showed a dose-dependent increase in ACh release. The GABA-induced ACh release was enhanced by DM-9384 (10^{-7}M). Aniracetam and oxiracetam had a similar but slight effect on the ACh release. These results suggest that DM-9384 stimulates ACh release through acting on GABAergic system in rat cortex.

58.6

NIMODIPINE ENHANCES LEARNING OF THE 8-ARM RADIAL MAZE IN YOUNG RATS.
A. Levy, R. M. Kong*, H. R. Lieberman and T. M. Rauch*. U. S. Army Research Institute of Environmental Medicine, Natick, MA 01760.

Several investigators have reported that the calcium channel blocker nimodipine improves cognitive performance in aged and brain-damaged animals. In the present study, 20 mg sustained-release (3 weeks) nimodipine pellets were implanted subcutaneously into young Fischer-344 rats (5 month old; 290 g). Training in the 8-arm radial maze began two days later. Nimodipine significantly improved learning of the maze as compared to placebo (n=12; P<.001 for main drug effect on correct choices out of the first 8). Nimodipine treated rats also made twice as many choices per unit time during the first week of training (P=.005). Their average serum nimodipine concentration on day 17 was 7 ng/ml. Hippocampal microdialysis, using a 3 mm probe placed at the CA1 and DG regions (AP=-3.8; L=1.6; V=1.5-4.5 with respect to Bregma), was performed on days 18-20 of the treatment. Thirty ul dialysate samples were collected at 2 ul/min and analyzed for acetylcholine and choline content on an HPLC-EC, using the BAS assay kit. Significantly higher extracellular ACh levels were found in nimodipine treated rats (71.4±3.6 nM; n=4) as compared to controls (52.5±2.5 nM; n=5) (P=.003).

58.7

EARLY ONSET OF IMPAIRED LEARNING AND MEMORY IN SENESCENCE ACCELERATED MICE (SAM).
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The SAM P/8JF mouse strain has a 40% shorter life span than other mice such as the C57BL/6J strain. When SAM mice were trained on left-right T-maze footshock avoidance task, they showed an age-dependent decrement of learning and memory. Whereas C57BL mice have been reported to show impaired learning and memory starting at 24 months of age, the SAM mice showed such decrements beginning at 10 months of age for learning and at 8 months of age for retention. Normal retention for simple passive avoidance conditioning and an analysis of footshock escape latencies suggests that age-related difference in footshock sensitivity did not account for impaired retention in T-maze conditioning. Similar age-related impairments were found in learning to avoid shock in a shuttlebox and in learning to press a lever for milk reinforcement. A battery of drugs was tested to determine if abnormal functioning of any specific neurotransmitter system might account for the impaired learning and memory. SAM mice showed improved retention for drugs effecting dopamine, norepinephrine, GABA and opioids but compounds that effected serotonin or acetylcholine failed to improve retention. Present data indicates that higher doses of arecoline, an ACh agonist, were needed to improved retention in SAM mice. The memory impairment maybe related to alterations in the cholinergic and serotonergic systems. SAM P/8JF mice show an early onset of impaired learning and memory in spite of good general health.

58.8

CHOLINERGIC MODULATION OF MEMORY AND PERFORMANCE OF LONG-EVANS RATS IN THE T-MAZE.
J.M. Ordy, T.M. Wengenack, G.J. Thomas, A.S. Howell*, and W.P. Dunlap*. Fisons Pharmaceuticals, Univ. of Rochester, Rochester, NY 14623, and Tulane Univ., New Orleans, LA 70118.

Studies with Alzheimer's (AD) patients, normal humans, and animal models have shown enhancement of memory by physostigmine (PH) and arecoline (AR), and memory impairment by scopolamine (SC). Reversal of SC memory impairment by indirect or direct agonists has been used in animal models of drug development for memory impairment in AD. Conflicting findings exist concerning: 1) dissociation of drug effects on memory from effects on performance, and 2) efficacy and selectivity of cholinergic agonists used for reversal of SC memory impairment. In dose-response and time-course studies, the aims were to compare the effects of SC, methyl scopolamine (MESC), PH, and AR on spatial-working memory and on performance of adult Long-Evans rats in the T-maze. In "double-dissociation" comparisons, SC impaired memory independent of effects on performance, MESC impaired performance independent of effects on memory. Both PH and AR reversed SC memory impairment independent of effects on performance. The cholinergic dose-response and time-course findings indicated a potential utility for the use of animal models for studying drug modification of memory impairment in AD.

58.9

INTENSITY-DEPENDENT EFFECTS OF SCOPOLAMINE IN PASSIVE AVOIDANCE.
S. E. Cruz-Morales, M. Durán-Arévalo and R. A. Prado-Alcalá. Behav. Pharmacol. Prog., ENEP-Iztacala, Anahuac Univ., and Physiol. Dept., Med. Sch., National Univ. of México.

Anticholinergic-induced amnesia, can be prevented by increasing the magnitude of the reinforcers during learning. It is not known wheter this is a gradual or an "all-or-none" effect. To test these possibilities, rats were trained in a one-trial passive avoidance task; retention was tested 24 hr later. During training different footshock intensities were used: 2.5, 2.6, 2.7, 2.8, 2.9, and 3.0 mA; half the groups were injected with 8 mg/kg of scopolamine (SCOP) and the other half was not Streated. Isotonic saline or methylscopolamine (8 mg/kg) were injected to two additional groups, trained with 2.5 mA. Injections were given 5 min posttraining (i.p.). SCOP produced amnesia in the 2.5, 2.6 and 2.7 mA groups; all other groups showed near-perfect performance. These results suggest that once a certain level of training-related stimulation is reached, the cholinergic system is switched-off, and other neurochemical systems mediate the consolidation process. Supported by CONACYT (P228CCOX891608)

58.10

NEW METHODS TO DETERMINE IN VIVO ACTION OF REVERSIBLE ACETYLCHOLINESTERASE INHIBITORS: TACRINE AND E2020.
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The reported efficacy of tetrahydroaminoacridine (THA) in ameliorating Alzheimer dementia focussed attention on a distinct class of acetylcholinesterase (AChE) inhibitors--centrally active drugs which inhibit the enzyme allosterically by reversible binding. Inhibition of AChE by these drugs is more potent at lower ACh concentrations; therefore, they may be better able to selectively reverse age- and Alzheimer-related hypofunction without producing cholinergic hyperactivity in unaffected brain areas. However, reversible inhibitors such as THA (in contrast to carbamates or organophosphates) readily dissociate from AChE upon dilution for brain homogenization and assay, making it difficult to quantify their action in vivo. Using minimal tissue dilution, we compared the inhibition of AChE measured radioenzymatically in discrete rat brain regions and blood after systemic THA. These results and direct fluorometric HPLC assay of THA indicate that this drug selectively accumulates in certain brain regions, and reaches concentrations (6.5 μM peak) which inhibit AChE over 90% in rat cortex after 5 mg/kg s.c., a dose without marked locomotor inhibitory action. AChE inhibition in cortical homogenates diluted 8-fold at assay is only 50-60%, but declines more slowly than THA level. A novel, reversible and specific AChE inhibitor, E2020 (Yamanishi et al, Soc.Neurosci.Abst. 14:59, 1988), is also characterized; and a new approach is presented for quantifying the in vivo effect of reversible AChE inhibitors in brain--protection against the irreversible AChE inhibitor diisopropylfluorophosphate (DFP). After 2 mg/kg s.c. E2020, maximum inhibition of AChE measured ex vivo in 5-fold diluted cortex is 70% and AChE inhibition declined steadily from 1 hr to 5 hr after drug. However, this dose of E2020 results in over 90% protection of AChE from irreversible inhibition by DFP for up to 3 hr in cortex homogenized directly in buffer with DFP (1 μM final), and 60% protection from DFP remains at 5 hr after E2020, a time by which AChE inhibition is barely detectable in the ex vivo assay. Thus, DFP antagonism provides a sensitive measure of the in vivo interaction of E2020 with AChE involving less reversal by dilution (only 2-fold final) than ex vivo AChE assay (5-fold minimum dilution for brain). Correction of ex vivo AChE inhibition for dilution, brain THA assays and measurement of DFP antagonism provides concordant evidence that the reversible, allosteric inhibitors result in near maximal inhibition of brain AChE at doses without adverse cholinergic side-effect.

58.11

STAUROSPORINE FACILITATES RECOVERY FROM THE BASAL FOREBRAIN-LESION-INDUCED AMNESIA AND DEFICIT OF CHOLINERGIC NEURON IN RATS. T.Nabeshima[1,2], S.Ogawa[1*], H.Nishimura[1*], K.Fuji[1*], T.Kameyama[1*] and Y.Sasaki[3*]. [1]Dept. of Chem. Pharmacol., Fac. Pharmac. Sci., Meijo Univ., Nagoya 468, [2]Dept. Hospital Pharmacy, Nagoya Univ. Sch. of Med., Nagoya 466, and [3]Biochem. Res. Lab., Bio-Sci. Inst. Life Sci. Center, Asahi Chem. Industry Co., LTD., Nobeoka 882, Japan.

Alzheimer's disease is characterized by the loss of cholinergic neurons in the nucleus basalis of Meynert and by a primary loss of memory function. Since staurosporine has been reported to induce differentiation in the human neuroblastoma cell in vitro, we have investigated whether an administration of staurosporine in vivo attenuates the amnesia induced by basal forebrain-lesion in rats. The multiple dosage of staurosporine at the doses of 0.05 and 0.1 mg/kg (i.p.) attenuated the impaired performance of water maze and passive avoidance tasks, although the drug-administration was started 2 weeks after basal forebrain-lesion. Moreover, staurosporine (0.1 mg/kg) partially reversed the decrease of choline acetyltransferase activity in the cortex induced by basal forebrain-lesion. These results suggest that the administration of staurosporine attenuates amnesia through reversal of deficits in cholinergic neurons induced by basal forebrain-lesion. This evidence indicated that neurotrophic factor-like substances may open the way for novel therapeutic approaches to Alzheimer's disease.

58.12

QUISQUALIC ACID LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS (NBM) DIFFERENTIALLY AFFECT WORKING AND REFERENCE MEMORY IN RATS. S.L. Biggan*, R.J. Beninger, J. Cockhill, K. Jhamandas & R.J Boegman. Dept. of Psychol. and Dept. of Pharmacol. & Toxicol., Queen's University, Kingston, K7L 3N6, Canada.

Excitotoxic lesions of cholinergic cell bodies of the nbm have been shown to produce memory loss like that seen in both normal and pathological aging. The purpose of the present study was to investigate the effects of quisqualic acid lesions of the nbm on working and reference memory in a double-Y maze. Rats were trained to an 88% correct criterion, and were then given either bilateral quisqualic acid lesions (60 nM, 0.5 ul) or sham lesions (0.9% saline, 0.5 ul) of the nbm. One week post-surgery, rats were tested on the double-Y maze task with delays of 0, 5, or 30 seconds being introduced prior to both the working and reference memory choice. Nbm lesions produced a 63.2 ± 6.2% decrease of cortical choline acetyltransferase (ChAT) compared to shams. Delays affected only the working memory of the sham group. Rats with lesions showed a significant impairment of working memory at all delays, but no change in reference memory. Results indicate that quisqualic acid lesions of the nbm that produce significant reductions in cortical ChAT selectively impair working memory. (funded by NSERC)

58.13

CHOLINERGIC MODULATION OF INTRINSIC FIBER SYNAPSES MAY INCREASE AUTO-ASSOCIATION MEMORY CAPACITY OF RAT PIRIFORM (OLFACTORY) CORTEX.

M.E. Hasselmo and J.M. Bower, Div. Biology 216-76, Caltech, Pasadena, CA, 91125.

Computational modeling in our laboratory suggests that piriform cortex may function as an auto-association memory (Wilson and Bower, 1988, *Neural Information Processing Systems*. D. Anderson ed. AIP Press, N.Y., pp. 114-126). In simulations, the efficiency of such a memory is enhanced when there are differences in the physiology and neurochemical modulation of afferent and intrinsic fiber synapses. Using *in vitro* slice preparations, we have found physiological differences between these two populations of synapses (Hasselmo and Bower, J. Neurophysiology, in press). To study possible differences in their neurochemical modulation, we tested the effect of the acetylcholine analog carbachol on afferent and intrinsic fiber synaptic potentials.

Carbachol selectively suppressed synaptic potentials elicited by stimulation of intrinsic fibers in layer 1b (average 82.8% suppression at 100μM, I.C. 50 near 5μM) while having little effect on potentials evoked by stimulation of afferent fibers in layer 1a (average 10.5% suppression at 100μM). Carbachol did not change the time course of intracellularly recorded potentials, but increased intrinsic fiber paired-pulse facilitation measured extracellularly (average 44.4% increase at 100μM), suggesting a presynaptic mechanism of suppression. Cells also showed an increase in excitability, which may compensate for reduced synaptic input.

Recent modeling shows that selective cholinergic modulation of intrinsic connections may increase the sparseness of pyramidal cell activity by reducing the spread of excitation, thus allowing a stronger effect of lateral inhibition. This greater sparseness of activity would increase the capacity and efficiency of auto-association storage. This model might provide a theoretical framework for the memory deficits associated with the loss of cortical cholinergic innervation in Alzheimer's disease.

(Supported by ONR grant N00014-88-K-0513 and NIH training grant NS07251.)

58.14

INVOLVEMENT OF NICOTINIC CHOLINERGIC SYSTEMS IN SHUTTLE-ESCAPE DEFICITS PRODUCED BY INESCAPABLE SHOCK IN RATS. S.R. McGurk, T.R. Minor, and L.L. Butcher, Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563.

Exposure to inescapable tail-shock produces subsequent deficits in shuttle-escape responding in rats. Other investigatiors have shown that central muscarinic cholinergic systems are involved in this response. We now report that nicotinic cholinergic systems are involved as well.

Male rats were exposed to inescapable tail-shock and on the next day tested for shuttle-escape performance. Before escape testing, rats were given either scopolamine (0.1. or 1.0 mg./kg); mecamylamine (5, 10, 20 mg/kg); hexamethonium (10 mg/kg); or saline. Shock-induced deficits in escape performance were significantly attenuated by scopolamine (1.0 mg/kg) and mecamylamine (10 and 20 mg/kg). Hexamethonium was ineffective, indicating the importance of central nicotinic systems in this response.

Finally, a group was given a combination of subthreshold doses of scopolamine + mecamylamine (0.1 and 5 mg/kg, respectively). The combination treatment attenuated shuttle escape deficits. That subthreshold doses of nicotinic and muscarinic antagonists summate suggests both are acting on the same or interconnected neural circuits underlying escape responding. (Supported by grant NS10928 to L.L.B.)

58.15

RADIO-FREQUENCY LESIONS OF THE NUCLEUS BASALIS MAGNOCELLULARIS AND CHOLINERGIC DRUGS IMPAIR DIFFERENTIAL CONDITIONING IN RATS. J. E. Dencoff, A. E. Butt, B. G. Cooper*, K. Nopp-Dvorak*, G. K. Hodge. Department of Psychology, University of New Mexico, Albuquerque, NM 87131.

Deficits in differential conditioning performance resulted from bilateral radio-frequency lesions of the nucleus basalis magnocellularis that were exacerbated by scopolamine but were not attenuated by physostigmine. Twenty-nine male white rats were trained on a differential conditioning bar-press task and tested for performance following injections of the cholinergic drugs scopolamine (0.125 mg/kg) and physostigmine (0.03 mg/kg), alone or in combination. Rats were then given bilateral lesions of the nucleus basalis, allowed a short period of recovery, and again tested for performance under scopolamine or physostigmine. Lesions of the nucleus basalis resulted in significant task impairments, including reduced success rates and prolonged task completion times. Performance deficits were exaggerated by administration of scopolamine, but were not alleviated by physostigmine. These results may be explained by factors such as lesion specificity, drug dosage, task complexity, drug supersensitivity, and animal age. Results suggest that although lesions of the nucleus basalis interfere with cholinergic systems, which may be involved in the processes of learning and memory, cholinergic replacement treatment alone may be insufficient to remedy neuronal loss in the nucleus basalis.

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58.16

LEARNING DEFICITS IN RATS (NBM LESION) ARE ATTENUATED BY A NOVEL ACETYLCHOLINESTERASE INHIBITOR 3-PYRIDINOL DIMETHYLCARBAMATE. M.E.Bach*, M.Sano*, L.J.Cote*, S.Ginsburg and R.Mayeux. Dept.of Neurology, College of P&S, Columbia University, New York, NY 10032.

Lesions of the nucleus basalis magnocellularis (nbm) result in decreased cholinergic activity, and learning and memory deficits in humans and animals. The performance of lesioned rats on a new learning task, the repeated acquisition paradigm (Peele & Baron, JEAB.,49:275-290, 1988) employing an 8 arm rotary maze, was compared to sham and control groups. Quinolinic acid (120 nmol in 1ul) or saline (1ul) was delivered unilaterally at the following coordinates (Paxinos and Watson): AP 7.5mm, LAT+/-2.4mm and DV 2.9mm to lesioned and sham groups respectively. The nbm lesions were confirmed via acetylcholinesterase staining. Each session of the repeated acquisition task, a new set of 4 arms were baited with food reinforcements and the rats were required to learn, across 10 trials, which arms were baited. The lesioned rats required significantly more sessions to acquire the task when compared to sham and control rats. Physostigmine (.09 and .12mg/kg) and 3-pyridinol dimethylcarbamate, "Norpyridostigmine" (10mg/kg) both diminished the acquisition deficit in the lesioned rats when administered ip 15 min prior to testing. Norpyridostigmine was as effective as physostigmine in attenuating the learning deficit associated with nbm lesions. The repeated acquisition task is sensitive to cholinergic manipulations and therefore a useful model for evaluating potential treatments for memory deficits of dementia.

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59.1

NUMBER OF REM PERIODS AND WEIGHT CHANGE IN MAJOR DEPRESSION. T. Hsu, M.D.*, J. E. Shipley, M.A, M.D., Al. S. Eiser, Ph.D., R. F. Haskett, M.D., L. J. Grunhaus, M.D., A. C. Pande, M.D.
Department of Psychiatry, Univ. of Michigan Sch. of Med., Ann Arbor, MI 48109.

In a previous study, we reported that depressed patients with comparable depressive severity differed with respect to a number of measures of EEG sleep, depending on whether they reported loss or gain of weight during their depressive episode. Because of recent reports linking REM sleep with metabolic function, we examined the association between self-reported weight change and the number of REM periods per night in a sample of patients with MDD.

All 107 patients in the study had MDD by RDC. Subjects were divided into three groups according to 1) whether they had 1-2, 3-4, or 5-6 mean REM periods per night, and 2) whether they reported a loss or gain of weight during their current depressive episode. These groupings were similar with respect to age, sex, in- vs. out-patient status, HDRS, and MDD subtype. All recordings of EEG sleep were made after two weeks drug-free, and the data from two consecutive nights were averaged.

The three REM-period groups differed significantly with respect to their distribution within the three weight-change groups ($p<.002$ by Chi-square); weight-losers exhibited significant variation while weight-maintainers and weight-gainers had very little variation. Comparison of the REM-period groups with respect to REM latency and other selected measures of EEG sleep by multiple ANOVAs did not reveal any significant differences. These results suggest that further metabolic characterization of depressive patients may be of value in defining depressive subtypes.

59.2

DOSAGE FOR THE SALIVARY DST IN DEPRESSED PREPUBERTAL CHILDREN. I.L. Extein and D.R. Porter*.
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The recognition of major depression in children and the utility of antidepressant medications underline the importance of accurate diagnosis. Nonsuppression of serum cortisol on the dexamethasone suppression test (DST) has been suggested as a marker for major depression in children as in adults, but the multiple blood samples required has limited the application of the DST in children. Since serum and salivary cortisol levels are highly correlated in depressed adults and children, a salivary DST has been developed. We studied two dosages of dexamethasone to determine optimal parameters for the salivary DST in children - the 1.0 mg standard for adults, and a reduced 0.5 mg dosage. Dexamethasone was administered at 11 PM to hospitalized children. Saliva was obtained the next day at 8 AM, 12 N, 4 PM, and 11 PM for assay of cortisol by radioimmunoassay (RIA). A cortisol >90 ng/dl at any time point defined non-suppression. The first 13 patients (10 major and 3 bipolar depression by DSM III-R; ages 8-13, mean age 10) received 1.0 mg. One was a nonsuppressor. The next 14 patients (12 major and 3 bipolar depression; ages 6-13, mean age 10) received 0.5 mg. Six were nonsuppressors. The sensitivity of the 0.5 mg salivary DST for depressive disorders of 43% was significantly higher than that of 8% for the 1.0 mg test by Chi-Square, and similar to the reported sensitivity of the DST in adults. Specificity needs to be studied.

59.3

NEUROPHYSIN RESPONSE TO ELECTROCONVULSIVE THERAPY IN DEPRESSED PATIENTS.
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Our previous studies have shown a strong association between the neurophysin response to ECT and outcome in depression. Serum neurophysins were measured at the first and last treatments in a course of ECT monitored by EEG given to 17 depressed patients. The findings confirm that the release of oxytocin-associated neurophysin (hNpII) after the first ECT correlated with the extent of eventual improvement but this was not a useful predictor of recovery. hNpII release did not change between first and last treatments and hNpII release was not related to EEG measures of seizure activity or improvement in depression. The hypothesis is advanced that hNpII release is a measure of electrical stimulation of mid brain. (Scott et al, Lancet 1986 i 1411: Whalley et al, Psychol.Med. 17: 312, 1987).

59.4

PITUITARY AND ADRENAL ALTERATIONS IN MAJOR DEPRESSION: MRI AND CT STUDIES. C.B. Nemeroff, N.R. Dunnick*, O. Boyko*, G. Figiel*, D. Reed, P.M. Doraiswamy and K.R.R. Krishnan.
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It is now well established that a large percentage of patients that fulfill criteria for major depression exhibit hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis. We present here the results of two studies: (1) evaluation of the size of the adrenal gland in 37 depressed patients and 12 normal controls by computed tomography (CT). We hypothesized that depressed patients would exhibit adrenal enlargement secondary to hypersecretion of ACTH, and perhaps other POMC products,(2) evaluation of the size of the pituitary gland by magnetic resonance imaging (MRI) in 19 depressed patients and 19 normal controls. There is considerable evidence from preclinical studies that hypothalamic hypophysiotropic hormones such as CRF are trophic to anterior pituitary cells. We hypothesized that if CRF and TRH are hypersecreted in major depression, depressed patients would exhibit pituitary enlargement. The results obtained supported both hypotheses: depressed patients exhibited adrenal enlargement (35% of depressed patients exhibited adrenal hypertrophy) and pituitary enlargement (depressed patients exhibited increased pituitary volume). Studies in progress will determine whether these changes are reversible, or related to clinical or neuroendocrine features of depression. (Supported by NIMH MH-42088, MH-40189 and MH-44716).

59.5

CEREBRAL GLUCOSE UTILIZATION IN HUNTINGTON'S DISEASE: INFERIOR FRONTAL HYPOMETABOLISM IDENTIFIES PATIENTS WITH MAJOR DEPRESSION. HS Mayberg, SE Starkstein, CE Peyser*, SE Folstein*, RF Dannals*, MF Folstein*, HN Wagner, Jr.* Johns Hopkins Medical Institutions, Baltimore, MD 21205.

Positron Emission Tomography (PET) studies in patients with Huntington's Disease (HD) have identified decreases in glucose metabolism (CMRGlc) in the striatum, cingulate and frontal cortices. While depression is present in more than 40% of HD patients, the relationship between the mood disorder and regional CMRGlc abnormalities have not been directly assessed. CMRGlc was measured using ^{18}FDG and PET in 10 HD patients (4 depressed, 6 non-depressed) and 7 controls. All patients (mean age 41$\pm$13 years) had < 7 years duration of chorea and comparable scores on measures of apathy, irritability, cognitive function, neurological impairment, overall disability, and cortical and subcortical atrophy. Significant decreases in striatal and cingulate CMRGlc were seen in both HD groups compared to the normal subjects. Normalized CMRGlc (region/whole brain) was reduced in orbital and inferior prefrontal cortex (Fr) in the depressed patients (Fr=0.87$\pm$.12) compared to both the non-depressed patients (1.06$\pm$.13) and the controls (1.09$\pm$.05) (group x region $F(2,9)=7.63$, $p=.0001$). After adjustment for age, there was a significant correlation between depression severity (PSE score) and Fr CMRGlc ($r^2=0.72$, $p< .02$). These selective metabolic changes suggest that dysfunction of specific cortico-striatal pathways may underlie the affective disorder common in HD.

59.6

CEREBRAL HEMISPHERE ASYMMETRIES IN DEPRESSION AFTER STROKE R.G.Robinson, S.E.Starkstein, J.B.Bryer*, M.L.Berthier*, B.Cohen*, T.R.Price. Dept. Psychiatry, Johns Hopkins Univ. Sch. Med., Balto., MD 21205

We examined the association between post-stroke depression and the pattern of brain asymmetries visualized on computerized tomography (CT) scan, in patients with a single acute cerebrovascular lesion. Based on CT scan measurements, patients were divided into those with typical (N=39) or reversed (N=17) frontal and/or occipital asymmetries. Among patients with a typical occipital asymmetry, those with left frontal or left basal ganglia lesions showed a significantly higher frequency of major depression (i.e., 7 of 8, 88%) and significantly higher depression scores than patients with similar lesion location but reversed occipital asymmetry (i.e., 0 of 9) or those with a typical asymmetry and lesions in other (left or right) brain areas (i.e., 5 of 31, 16%) (Fisher Test $p<.005$). Among patients with a reversed occipital asymmetry, 3 of 8 (38%) with right hemisphere lesions but 0 of 9 with left hemisphere lesions had major depression ($p<.1$).Similar findings were observed when patients were divided into those with typical or reversed frontal plus occipital asymmetries. This study demonstrates that the previously reported significant association between post-stroke major depression and lesion location is restricted to patients with a typical occipital asymmetry, and is not present in patients with a reversed occipital asymmetry.

59.7

NEUROPEPTIDE Y CONCENTRATIONS IN POST-MORTEM BRAIN FROM VICTIMS OF SUICIDE AND CONTROLS P.S. Widdowson*, G.A. Ordway and A.E. Halaris. Depts. Psychiatry, Neuroscience, Pharmacology, Case Western Reserve Univ. and MetroHealth M.C., Cleveland, OH 44109.

Neuropeptide Y (NPY) is found in large concentrations in human brain. The presence of specific binding sites for NPY in human brain suggests that NPY may play an important role in neurotransmission. In brain, NPY coexists with norepinephrine, serotonin and GABA, transmitters which have been implicated in suicide. We measured NPY in four regions of brains from victims of suicide (S) (mean age 46.3 ± 3.5; n= 14) and sudden death controls (C) (mean age 40.6 ± 4.3; n= 19). NPY was extracted from tissue by boiling in acetic acid, sonication and centrifugation. Lyophilized supernatants were stored at -80 °C and NPY measured by RIA and expressed as pmol/g tissue. Means and S.E.M. were calculated and data analyzed by two-tailed Student's t-test. NPY was reduced significantly in frontal cortex by 23% (C, 86±4; S, 66±3; $p<0.005$), in temporal cortex by 25% (C, 76±4; S, 57±1; $p<0.05$), in caudate by 47% (C, 168±16; S, 98±19; $p<0.05$), but not in cerebellum (C, 2.6±0.6; S, 3.1±0.7). NPY concentrations did not correlate with either subject age or post-mortem interval suggesting that these factors did not contribute to the NPY reduction. This data provides evidence for a role of NPY in suicide.

59.8

NEUROCHEMISTRY OF THE HUMAN LOCUS COERULEUS IN SUICIDE AND DEPRESSION, G.A. Ordway, P.S. Widdowson*, K. Streator-Smith* and A.E. Halaris. Depts. Psychiatry, Pharmacology and Neuroscience, Case Western Reserve Univ. and MetroHealth Medical Center, Cleveland, OH 44109.

A role of norepinephrine (NE) in depression has been debated for many years. The principal source of NE in brain is the locus coeruleus (LC), which projects to many brain regions including the limbic system. Activation of alpha-2 adrenoceptors (AARs) located in the LC modulates release of NE and activity of LC cells. We obtained post-mortem brain tissue from victims of suicide (n=13) and age-matched sudden death controls (n=13) to study AARs and NE in the LC. AARs were quantitated by autoradiography of binding of [^{125}I]iodoclonidine (ICLO) to sections through the rostral LC. NE was measured by HPLC-EC in adjacent sections. No significant differences in ICLO binding (mean±S.E.M.) between control (29±4 fmol/mg protein) and suicide tissue (29±2 fmol/mg protein), or in NE concentration in LC between control (6.1±0.8 ng/mg protein) and suicide tissue (5.0±0.4 ng/mg protein) were observed. Comparisons of ICLO binding and NE in LC from those suicides with a history of depression (n=7) or violent suicides (n=7) to their respective age-matched controls revealed no differences. These data suggest that if the central NE system is perturbed in depression or suicide, then alterations in AARs or NE may be detected in terminal fields, but not in the LC.

59.9

ALPHA$_2$-ADRENERGIC BINDING IS NOT ALTERED IN SUICIDE. *V. Arango, P. Ernsberger, M.D. Underwood and J.J. Mann.* Labs. of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA 15213.

We have previously shown that there is an increase in 5-HT$_2$ receptor binding in the prefrontal (PFC), but not in the temporal cortex of suicide victims compared to controls (*Arch Gen Psychiatry*, 43:954-9, 1986; *Arch Gen Psychiatry*, in press). In addition to the well-documented changes in a variety of indices of the serotonergic system, other neurotransmitter systems seem to be involved in suicidal behavior. In the noradrenergic system we found that β-adrenergic receptor binding is increased in PFC of suicide victims compared to controls, and that α_1-adrenergic binding is increased in layers IV-V of PFC in the suicide group. Using quantitative autoradiography we now sought to study α_2-adrenergic binding in PFC of controls dying acutely from causes other than suicide and compare it to suicide victims. Both groups were paired for assay by matching for postmortem delay, age and gender (N=12 pairs). Toxicology was negative for centrally active drugs. Sections (15 µm) were pre-incubated, pre-washed and incubated in 4nM ^{3}H-*p*-amino clonidine (^{3}H-PAC) to label α_2-receptors. Non-specific binding was defined by parallel incubation with 100 µM epinephrine. Tissue sections and ^{3}H-standards were exposed to Hyperfilm for 16-18 weeks, developed in GBX and quantified using a PC-based image analysis system (Imaging Inc.). The distribution of α_2-adrenergic receptors was the same in both groups and formed 5 isodensity bands corresponding to layer I, layer II, upper layer III, layers III-IV and layers V-VI. Binding was densest in layer I and upper layer III and lowest in layers V-VI. The suicide group did not differ from the control group in the level of α_2-adrenergic binding in any of the cortical layers. The binding did not correlate with age (39 ± 6 y) or postmortem delay (15 ± 2 h). We conclude that, despite binding alterations in other adrenergic receptor subtypes, α_2-adrenergic binding is not altered in PFC of suicide victims. This work was supported in part by PHS grant MH40210.

59.10

BETA-ADRENERGIC BINDING IN SUICIDE VICTIMS. K.Y. Little, G.E. Duncan and G.R. Breese. University of North Carolina, Chapel Hill, NC 27599.

The catecholamine theory of Schildkraut and Prange postulates that changes in noradrenergic functioning lead to mood disorders. Although only 50% of suicide victims suffer mood disorders, studies of beta-adrenergic receptors in the brains of uncategorized suicides have provided mixed evidence generally suggesting increased numbers. In this study, we explored beta receptor binding in Broadmann's area 10 of 15 suicides versus matched controls, using homogenated membranes and I^{125} pindolol, a specific beta receptor ligand. Autoradiography of hypothalamic beta binding was also done in 5 pairs.

Brain tissue was obtained at autopsy as authorized by the Chief Medical Examiner and quick frozen with dry ice. Control subjects were chosen to match suicides by age, sex, race, post mortem interval, and suddenness of death. About 20mg of tissue from the middle frontal gyrus at the medial border of the cerebral convexity was examined in each subject. The assay conditions were: tissue concentration 5mg/ml, total tube volume .2ml, 24°C for 30 minutes, dilution with cold buffer and rapid filtration. 150 pM of I^{125} pindolol and 100 mM of isoproterenol were used. Specific binding averaged 70%. Autoradiography of hypothalamic sections was done with the same ligand and competition concentrations, and quantified using optical densitometry.

Our results in suicides demonstrate a significant decrease in cortical beta-receptors compared to controls ($p = .01$), an increase in hypothalamic beta receptors, and an inverse correlation between this binding within individuals. There appeared to be racial differences in the regulation of beta receptors.

59.11

SPONTANEOUS ALTERNATION: AN ANIMAL MODEL FOR OBSESSIVE-COMPULSIVE DISORDER? E. Yadin, D. Pilchak,* E. Friedman and W.H. Bridger. Dept. of Psychiatry, The Medical College of Pennsylvania, Philadelphia, PA 19129.

This study entailed the adoption of a well-known behavioral paradigm, spontaneous alternation, as a possible animal model for some of the symptoms observed in obsessive-compulsive disorder (OCD) in humans. Food-deprived rats were run in a T-maze in which both a black and a white goal box were equally baited with a small chocolate chip. Each rat was given 7 trials every other day during which it was placed in the start box and allowed to make a choice. The mean number of choices till an alternation occurred was recorded. After a stable baseline of spontaneous alternation was achieved the effects of manipulating the serotonergic system were tested.

Both the nonselective 5-HT agonist 5-MeODMT (1 mg/kg) and the more selective 5-HT$_{1A}$ agonist 8-OH-DPAT (3 mg/kg) disrupted spontaneous alternation, a change that was reversed by the nonselective 5-HT antagonist methiothepin (1 mg/kg). A course of chronic treatment (2x5 mg/kg for 21 days) with the selective 5-HT reuptake blocking agent fluoxetine had a protective effect on the 5-MeODMT-induced disruption of spontaneous alternation behavior.

Serotonergic manipulations of spontaneous alternation may be a simple animal model for the perseverative symptoms (or indecisiveness) seen in people diagnosed with OCD.

59.12

ORBITAL GYRUS METABOLISM CORRELATES WITH EXPRESSED DISTRESS/ANXIETY AND TREATMENT RESPONSE IN OCD PATIENTS. JM Schwartz, LR Baxter*, ME Phelps, BH Guze*, JC Mazziotta, MP Szuba*, J Barrio*. UCLA School of Medicine, Los Angeles, CA 90024

Work by several groups using PET demonstrates increased metabolic rates (MR) in the orbital gyrus (OG) of patients with obsessive-compulsive disorder (OCD). We are studying a series of patients with OCD, attempting to demonstrate changes in regional MR prior and subsequent to treatment with either pharmacologic or cognitive biobehavioral therapy. Commensurate clinical responses were seen for both of these treatments.

Preliminary data analysis (N=14) shows significant correlations ($p \leq .02$) between right (rt) OG MR with respect to ipsilateral hemisphere MR (Hem) (OG/Hem) and Profile of Mood States (POMS) Anger/Hostility, Tension/Anxiety, and Depression/Dejection scales. Further, 10 subjects studied prior and subsequent to treatment (5 medication, 5 behavioral) showed a significant correlation between % change on Yale-Brown Obsessive Compulsive Scale scores and change in rt OG/Hem ratio ($r_s = .57$, $p=.045$; $tau=.41$, $p=.05$).

These data indicate that orbital gyrus may be involved in the mediation of sensations relevant to symptom expression in OCD.

59.13

ELEVATION IN PAIN THRESHOLDS IN BULIMIA NERVOSA. P. L. Faris, N. C. Raymond*, L. A. Howard*, R. McCollister*, J. E. Mitchell* and E. D. Eckert*. Department of Psychiatry, University of Minnesota, Minneapolis MN 55455.

Converging lines of evidence suggest that some components of nociception and food intake may be subserved by common neurochemical mechanisms. In particular, short-term satiety is known to involve peripheral responses to the ingestion of a meal which are mediated in part by afferent vagal fibers. The vagus nerve has also been implicated in mediating a portion of the analgesic response to peripherally administered opioids. Other neurotransmitters, e.g. CCK, are thought to function in both short-term satiety and in nociception. Thus, we hypothesize that eating disorders involving disruption of short-term satiety mechanisms may also be accompanied by alterations in nociceptive processing.

Bulimia nervosa is characterized by prolonged feeding bouts, without a feeling of satiety; in other words, an abnormality in short-term satiety mechanisms. We have investigated nociceptive and tactile responsivity in 28 subjects fulfilling DSM-IIIR criteria for bulimia nervosa and 31 normal volunteer. Tactile thresholds were determined with Von Frey fibers using an ascending/descending method of limits. Tactile thresholds were not found to differ between the two groups (t=.237). Nociceptive thresholds were determined using a Ugo Basile analgesia meter, which steadily increases the force placed on the dorsal surface of a finger. Both pain detection (DT) and pain tolerance (TT) thresholds were determined for each finger. Bulimic subjects were found to have a consistent elevation in both DT and TT compared to normal controls ($p < .004$, DT; $p < .006$, TT). [Supported by MH 43077, MH43296 (JEM), & RSDA MH00595(PLF)]

59.14

PHENYTOIN IMPROVES BRAIN FUNCTIONS AMONG IMPULSIVE AGGRESSIVE PRISONERS. E. Barratt, T. Kent*, S. Bryant, A. Felthous, M. Stanford. Dept. of Psychiatry and Behavioral Sciences, Univ. of Texas Medical Branch, Galveston, TX 77550.

Phenytoin has been shown to reduce impulsive aggressive behaviors (Barratt, et al., J. Clin. Pharm., 29:851, 1989). In the current extension of this research, phenytoin significantly changed cortical event related potential topomaps (ERPT) toward a more normal profile. Prisoners (N=20) with a history of aggressive behaviors were administered phenytoin (300mg/day) or a placebo (6 weeks each) in a double-blind cross over study. ERP's were recorded during several tasks including an "off-target" task designed to study impulsiveness. Data were analyzed using topographical and related statistical analyses. In contrast to medical students (N=20) and adult controls (N=20), the prisoners had well defined frontal lobe and diffuse right hemisphere positivity during the window for the late positive component (LPC)(250-600ms) in their baseline recordings. Phenytoin significantly reduced the frontal lobe activity and diffuse right hemisphere activity with a better focused posterior LPC. The placebo did not. These data are consistent with the hypothesis that impulsiveness is a frontal lobe function and suggests, further, a right hemisphere involvement.

NEUROMUSCULAR DISEASES

60.1

SKELETAL MUSCLE ULTRASTRUCTURAL PATHOLOGY IN ACUTE POLYRADICULONEUROPATHY. A. Márquez*, H.J. Finol, B. Muller* and I. Montes de Oca*. Medicine, Sciences and Dentistry Faculties. Universidad Central de Venezuela. Apartado 50587. Caracas 1050. VENEZUELA.

Although motor disturbances are the main features in acute polyradiculoneuropathy (Guillain-Barré syndrome)and muscular atrophy and pain can be present in it, the skeletal muscle pathology of this syndrome has been poorly studied. In this work we report the ultrastructural alterations observed in a muscle biopsy made for diagnostic purposes in a patient with an acute polyradiculoneuropathy, a 15 years old girl who developed an ascending, bilateral muscle weaknees which produced a nearly complete flaccid paralysis from the neck down. The patient needed respiratory assistance. Lumbar puncture showed an increase in protein content and no pleocytosis. The study exhibited the typical abnormalities of neurogenic atrophy (loss of myofibrils, alterations of sarcotubular system and folding of muscle basement membrane) and several findings not usually observed in denervated muscle as fiber necrosis, capillary alterations and mononuclear cell infiltration. The histopathological picture we observed is similar to that in the muscle compromise of autoimmune diseases. This work suggests the possibility for a common autoimmune mechanism in the muscular and nervous lesion in this disease. Supported by grants from the CDCH of UCV, the British Council Venezuela and Fundación Polar.

60.2

EXPERIMENTAL DIABETIC NEUROPATHY: EARLY FEATURES AND EFFECTS OF MICROVESSEL MANIPULATION D.W. Zochodne, L.T.Ho*. Department of Medicine, Queen's University, Kingston, Ontario K7L 3N6.

Studies of streptozotocin-induced diabetic neuropathy (ESDN) at 4 months have identified endoneurial oligemia and hypoxia - features which might respond to microvessel manipulation. In normal nerve, guanethidine adrenergic sympathectomy enhances perfusion. We studied 6 week ESDN in Sprague-Dawley rats: controls, diabetics and diabetics with concurrent guanethidine sympathectomy. In diabetic animals there was slowed mixed caudal conduction and resistance to ischemic conduction failure (RICF). Oligemia was not present, but oxygen tensions were shifted in the hypoxic direction. In the sympathectomized animals, microvascular resistance was not lowered, but there was less shift of the oxygen tension histogram. Mixed caudal conduction velocity and RICF values were similar to non-sympathectomized diabetics, but motor amplitudes recruited from caudal fibers were higher. In summary, our studies suggest that hypoxia may be more prominent than oligemia in early ESDN and sympathectomy may improve the oxygen profile. (Supported by CDA, MDA and MRC).

60.3

MUSCLE PROTEIN SYNTHESIS IN CHRONIC ETHANOL-FED ADULT RATS. I. Held, H. Yeoh* and K. Price*. Neuroscience Research, VA Hospital, Hines, IL 60141 and Dept. Biochem. Loyola Univ. Med Sch., Maywood, IL 60135.

Alcoholic myopathy frequently occurs during chronic alcoholism, but the biochemical alterations that lead to the loss of muscle proteins are not clear. The objective of this preliminary study was to determine the effect of long-term ethanol ingestion on protein synthesis in different types of skeletal muscle of adult rats. The ribosomal activity and protein content were determined in the soleus and extensor digitorum longus (EDL) from 16-month-old Fisher 344 rats fed a nutritionally complete liquid diet containing 6.7% ethanol for 2, 4 and 6 months, and from weight-matched control rats pair-fed an isocaloric liquid diet. Evaluation of muscle ribosomal activity was based on the *in vivo* incorporation of 3H-puromycin during elongation of peptide chains on the ribosome. The level of 3H-label (DPM) incorporated into trichloroacetic acid-precipitable material extracted from the whole muscle was measured at 30 min after tail-vein injections of 3H-puromycin (2.5 μCi/μmole; 3 μmoles/100 gm rat), and the specific radioactivity was expressed as dpm/mg muscle protein. Altogether, the results suggest that the ethanol-induced loss of muscle protein observed at 6 months may be due to earlier alterations in protein synthesis at the ribosomal level.

60.4

NEUROFILAMENT PHOSPHORYLATION AND UBIQUITINATION IN FROG MOTONEURONS AFTER VENTRAL ROOT AXOTOMY S. Murayama, D. L. McIlwain & K. Suzuki. Depts of Pathology and Physiology, Univ. Of North Carolina, Chapel Hill, NC, 27599-7525

Phosphorylation of neurofilament and ubiquitination were studied immunocytochemically in frog motoneurons after axotomy. The ninth and tenth ventral roots of grass frogs (Rana pipiens) were transected unilaterally on the left side and sacrificed at 10, 20 or 40 days after operation. The corresponding segments of the spinal cord were fixed in 4% paraformaldehyde for two hours and frozen sections were immunostained with avidin biotin complex method by monocloncal anti-neurofilament (SMI31 and 06-17, Sternberger) or anti-ubiquitin antibody (DF2, Mori et al 1987). Both SMI31 and 06-17 recognize phosphorylated epitopes of the middle molecular weight subunit of frog neurofilament on immunoblots. Perikaryal staining of motoneurons with SMI31 or 06-17 was observed on the operated, but not the control side, and reached a peak by 20 days post-axotomy. Perikaryal staining of injured motoneurons with DF2 also reached a peak by 20 days, while it remained weak on the control side. Our study demonstrates axotomy-induced phosphorylation of neurofilament and ubiquitination in perikaryal cytoplasm of motoneurons. This system may be a good model for the study of human motor neuron disease, where lower motoneurons contain epitopes for phosphorylated neurofilament and ubiquitin.

60.5

REGULATION OF EXCESSIVE INTRACELLULAR CALCIUM ACCUMULATION (EICA) AND MUSCLE DEGENERATION BY DILTIAZEM (DTZM) IN DYSTROPHIC HAMSTERS (DH). S.K. Bhattacharya, P.L. Johnson, T.A. Adamec*, and D.R. Shanklin*. Departments of Surgery, Neurobiology & Pathology, U. of Tenn., Memphis, TN 38163.

Membrane-mediated EICA plays a fundamental pathogenetic role in muscular dystrophy. Oral DTZM (25 mg/kg BW/day) reduced EICA, plasma CK, and histopathology in heart (HT) and rectus femoris (RF) of DH (Muscle & Nerve, 5:73, 1982). DTZM was reported safer and more effective than Nifedipine or Verapamil. Since $t_{1/2}$ of DTZM in circulation is short, we studied i.p. DTZM (80 mg/kg BW/day) in CHF-146 strain DH and CHF-148 normal hamsters (NH), for 7 months starting at age 37 days; untreated hamsters served as controls. HT and RF were biopsied for Ca and Mg quantitation, histology and EM. Relative cardiac enlargement (RCE) was measured in mg HT/100 gm BW. EICA in HT and RF, plasma CK, RCE, and mortality of DTZM treated DH were reduced ($p<0.001$). Muscle degeneration, Ca deposition, fatty infiltration, and fibrosis were also reduced. EM of dystrophic myocardium revealed calcified mitochondria and destruction of normal striations, whereas treated HT had no calcification. The larger dose of i.p. DTZM, compared to oral, produced much superior and lasting effects in DH. Higher dose and improved delivery may enhance therapeutic prognosis in DMD boys treated with 8 mg of DTZM/kg BW/day (Neurology, 38:609, 1988). These data reconfirm that DTZM has profound cardioprotective and overall salutary effects in DMD. NIH Grant #AR-38540.

60.6

IMMORTALIZATION AND DIFFERENTIATION OF MDX MOUSE MYOBLASTS

DE Bredesen, MD Seelig*, J Yang*, and N Kedersha*. UCLA Neuropsychiatric Institute, Los Angeles, CA 90024-1769.

Therapy for Duchenne's muscular dystrophy (DMD) may now be feasible because of (1) identification of the dystrophin gene (Koenig et al., Cell 1987;50:509) and (2) conversion of muscle fibers of mdx mice from dystrophin-negative to dystrophin-positive by injection and fusion of normal myoblasts (Partridge et al., Nature 1989;337:176). One of the remaining problems is the production of large numbers ($>10^8$) of autologous dystrophin-positive myoblasts, since Duchenne's myoblasts obtained at biopsy have a reduced mitotic capacity (Blau et al., PNAS 1983;80:4856).

We and others have recently found that temperature-sensitive (ts) immortalized neural cells may be utilized to produce genetically engineered neural transplants (Whittemore et al., Neurosci Abs 1988:233.1; Bredesen et al., Neurol 1989;39:124; Bredesen et al., Ann Neurol 1990;27:205). We have used a similar approach with mdx myoblasts. Control C57Bl/6 and mdx myoblasts were cultured at postnatal day 1, and infected with a recombinant retrovirus constructed by Frederiksen et al. (Neuron 1988;1:439) that effects expression of a ts large T antigen of SV40 (tsA58). Cells from both C57Bl/6 and mdx selected in G418 proliferated at 34°; at 38°, the cells fused to form myotubes, then exhibited frequent contractions. After 7 days at 38°, striations began to appear. Immunocytochemical staining for spectrin, for a cytoskeletal marker, and for vaults (a ribonucleoprotein structure) was indistinguishable from control primary cultures. PCR of total RNA was performed using primers flanking the mdx point mutation. Dystrophin sequences were detected in both groups at 34°, increasing in both cases with fusion at 38°.

We hope that a similar approach may be useful for the expansion, modification, and reimplantation of autologous cells from patients with degenerative disorders such as DMD.

60.7

A NOVEL MECHANOTRANSDUCING CHANNEL IN SKELETAL MUSCLE FROM THE *mdx* MOUSE. A. Franco[1] and J.B. Lansman[2]. [1]Graduate Program in the Neurosciences and [2] Department of Pharmacology, UCSF, San Francisco, CA. 94143.

An alteration of myoplasmic calcium homeostasis involving elevated intracellular Ca is characteristic of Duchenne's muscular dystrophy. The mechanism by which calcium accumulates is not known. We recently described the presence of a novel mechanotransducing channel in myotubes from the *mdx* mouse, an animal model for human Duchenne's muscular dystrophy (Nature 344: 670-673, 1990). Unlike previously described mechanotransducing channels in normal muscle which open in response to stretching the membrane, a subset of channels in *mdx* myotubes are open continuously at rest and become silent when the membrane is stretched. Both the stretch-activated and stretch-inactivated channels observed in *mdx* myotubes have identical single-channel conductances and selectivities for divalent and monovalent cations, but are ~5 times more permeable to Ca^{2+} than to monovalent cations. Furthermore, both channel types are equally sensitive to stretch regardless of whether it opens or closes the channel and both types of channel open in response to membrane depolarization. In some instances, conversion of stretch-activated to stretch-inactivated gating can be observed while recording from a single patch. We propose that stretch-inactivated channels appear in *mdx* myotubes through the modification of existing stretch-activated channels and that these channels may provide a pathway for calcium leakage into dystrophic muscle.

60.8

A Sarcolemmal Glycoprotein Linked to Dystrophin is Deficient in Dystrophic Muscle. J.M. Ervasti, K. Ohlendieck, S.D. Kahl* and K.P. Campbell. Howard Hughes Medical Institute and Dept. of Physiology and Biophysics, University of Iowa College of Medicine, Iowa City, IA 52242.

We have previously shown that dystrophin can be purified from detergent solubilized rabbit skeletal muscle membranes using WGA Sepharose because of its tight association with a sarcolemmal glycoprotein (Campbell, K.P. and Kahl, S.D. Nature, 338:259-262, 1989). Here, we report that dystrophin from rabbit skeletal muscle is part of a large (~18S) oligomeric complex as determined by sucrose gradient centrifugation. In addition to dystrophin, the complex contains a 59 kDa protein triplet and four glycoproteins of 156 kDa, 50 kDa, 43 kDa and 35 kDa, all in stoichiometric concentrations relative to dystrophin. Monoclonal antibodies specific for the 156 kDa or the 50 kDa glycoprotein showed immunofluorescence staining only on the cell periphery of cryostat sections which indicates a restricted localization of the complex to the sarcolemma of skeletal muscle. Immunoaffinity beads specific for dystrophin or the 50 kDa glycoprotein selectively adsorb the dystrophin-glycoprotein complex indicating that the complex is tightly associated. Of particular interest was the marked reduction of the 156 kDa glycoprotein in muscle from mdx mice and patients afflicted with Duchenne muscular dystrophy. In dystrophic muscle, thus, the absence of dystrophin may lead to the loss of a dystrophin-associated glycoprotein(s) which may be the first step involved in the molecular pathogenesis of muscular dystrophy. The elucidation of the function of these proteins should help to define the function of dystrophin and explain how its absence results in Duchenne muscular dystrophy. K.P. Campbell is an Investigator of the Howard Hughes Medical Institute. J.M. Ervasti is an NINDS post-doctoral fellow (NS07247-04). This work was also supported by MDA.

60.9

Characterization of the Dystrophin Glycoprotein Complex in Isolated Skeletal Muscle Sarcolemma. K. Ohlendieck, J. M. Ervasti and K. P. Campbell, Howard Hughes Med. Inst., Dept. of Physiology & Biophysics, Univ. of Iowa Col. of Med., Iowa City, IA 52242.

A library of monoclonal antibodies that are specific for the sarcolemma, the sarcolemma and transverse tubular system, or the transverse tubular system of rabbit skeletal muscle have been prepared and used in the development of a isolation procedure for skeletal muscle sarcolemma. Briefly, light muscle membranes were treated with 0.6M KCl and a crude surface preparation was isolated by sucrose density gradient centrifugation. A highly purified preparation of sarcolemma was isolated from the crude preparation by wheat germ agglutination. The purified sarcolemma was enriched in the sarcolemma marker proteins and including the components of the dystrophin glycoprotein complex. The dihydropyridine receptor, a t-tubule marker, and the Ca^{2+}-ATPase of sarcoplasmic reticulum were greatly diminished in the sarcolemma preparation. SDS-PAGE and densitometric scanning indicated that dystrophin comprised one percent of the total protein in the sarcolemma preparation. Furthermore, immunofluorescence labeling localized components of the dystrophin glycoprotein complex to the cell periphery of skeletal muscle cells. Fast and slow muscle fibres exhibited the same intensity of immunofluorescence staining in the sarcolemma. Our results demonstrate that the dystrophin glycoprotein complex is exclusively localized in the sarcolemma and that dystrophin is the major component of the cytoskeletal network underlying the sarcolemma of the skeletal muscle cell. KPC is an Investigator of the Howard Hughes Medical Institute. JME is an NINDS postdoctoral fellow.

60.10

DOES DYSTROPHIN ACCUMULATES PRE OR POSTSYNAPTICALLY AT THE NEUROMUSCULAR JUNCTION? J.P. Tremblay, L.P. Fortier, J. Huard, C. Labrecque, G. Dansereau. Lab. of Neurobiology, Hôp. Enfant-Jésus and Laval Univ., Québec, P.Q., Canada, G1J 1Z4.

Muscle cross sections of female mouse heterozygote for the mdx (dystrophic) gene were examined for the colocalization of dystrophin an acetylcholine receptors. The site of neuromuscular junctions (NMJs) were localized by revealing the acetylcholine receptors with α-bungarotoxin coupled to cascade blue. The presence of dystrophin on the same cross sections was revealed by an immunoperoxidase method. Four different observations were made in these heterozygote animals for the coexistence of AChR and dystrophin. First, α-bungarotoxin sites (i.e. NMJs) were observed on dystrophin positive muscle fiber cross sections with an accumulation of dystrophin at these sites. Secondly, α-bungarotoxin sites were observed on dystrophin positive fibers without a dystrophin accumulation at NMJs. In a third type of observations, there was a coexistence of α-bungarotoxin and dystrophin labelling at NMJs on muscle fibers which remaining perimeter was negative for dystrophin. The fourth observation was the presence of NMJs, identified by α-bungarotoxin, on muscle fibers completely negative for dystrophin even at the NMJ. These observations suggest that either dystrophin is initially expressed by the muscle fiber near the NMJ possibly due to a trophic effect of the nerve terminal or that dystrophin is present not only in the muscle membrane but also in the presynaptic nerve terminals.

60.11

EFFECTS OF BETA-ADRENERGIC AGONISTS IN MUSCULAR DYSTROPHY OF THE CHICKEN. R.K. Entrikin, R.T. Abresch* and D.B. Larson*. Univ. of California, Davis, CA 95616.

Of over 1,000 compounds evaluated previously for beneficial effects on hereditary avian muscular dystrophy, glucocorticoids were the most effective. Unfortunately, they improved muscle function only at doses that reduced growth rate. Isoproterenol, however, increased muscle function at doses that had little effect on growth rate. In the present study two specific beta-2 agonists, salbutamol and metaproterenol (Sigma, St. Louis), were administered i.p., once-daily to dystrophic Line 413 chicks, beginning on day two ex ovo, according to an approved animal use protocol. Effects were assessed by exhaustion scores (ES, the consecutive number of times a chick could rise from the supine in immediate succession during a single trial) and plasma creatine kinase (CK) activity. In brief, beta-2 agonists were equally effective to isoproterenol. ESs of dystrophic birds were increased 15-fold (to 75% of normal Line 412 control levels), and plasma CK activity was reduced by up to 90%. Compounds with beta-1 effects have not been evaluated in human Duchenne muscular dystrophy (DMD), mainly due to concerns over the known cardiac abnormalities in DMD patients. Since beta-2 agonists have fewer adverse cardiac effects, they seem more promising as candidates for human trials. (Supported by NIDRR Grant #H133B80016 and the Muscular Dystrophy Association.)

60.12

DYSTROPHIN EXPRESSION IN THE BRAIN. F.M. Boyce[1], M.P. Rosenberg[2], A.H. Beggs[1] and L.M. Kunkel[1]. [1]Children's Hospital and Howard Hughes Medical Institute, Harvard University, Boston, MA 02115, and [2]Dept. of Molecular Biology, Squibb Institute for Medical Research, Princeton, NJ 08543.

Dystrophin is a large (427 kD) protein with homology to the spectrin/ -actinin family of cytoskeletal elements. Dystrophin is expressed in all types of muscle tissue, and absence of dystrophin in muscle results in Duchenne muscular dystrophy, a relatively common and lethal inherited disease of young males. The only other tissue that contains significant levels of dystrophin is the brain. The function of dystrophin in the brain has not been established, but about 30% of Duchenne patients exhibit some degree of mental retardation. This raises the possibility that dystrophin may play a role in brain independent of its function in muscle.

We have isolated the genomic region which encodes an alternative promoter for dystrophin in the brain. This region is located over 90 kb 5' to the promoter used in muscle. Cellular transfection experiments using established cell lines have suggested that the brain promoter is much weaker than the muscle promoter in each cell line tested. To more accurately assess the transcriptional activity of each promoter, we have constructed several lines of transgenic mice containing either the brain or muscle promoter linked to a luciferase reporter gene. The existence of a separate transcriptional apparatus for dystrophin in the brain is further evidence of a functional role of dystrophin in the brain.

60.13

MONOCLONAL ANTI-TITIN IgG AUTOANTIBODIES PRODUCED BY MYASTHENIC THYMIC B CELLS. C. L. Williams, J. E. Hay*, T. W. Huiatt*, V. A. Lennon. Mayo Clinic, Rochester, MN 55905 and Iowa State University, Ames IA 50011.

Striational autoantibodies (StrAb) binding to contractile elements of muscle occur in patients with myasthenia gravis (MG) or thymoma. We reported previously that monoclonal IgM StrAb from MG patients bound to actin, α-actinin and myosin (J Exp Med 164: 1043, 1986). We now have isolated myasthenic thymic B cell clones secreting IgG StrAb that bind to titin, a major myofibrillar protein unique to striated muscle. Titin specificity was established by 1) immunoblot reactivity with bovine skeletal muscle titin and 2) immunofluorescent staining of myofibrils and cultured muscle cells in a pattern identical to that produced by polyclonal rabbit anti-titin IgG. IgG anti-titin antibodies were similarly identified in serum from four patients with MG and thymoma (including the donor of the B cell clones), but not in serum from four patients without thymoma. Our data implicate titin as a major specificity of StrAb associated with thymoma. **Supported by NIH grant NS 23537 (VAL) and an MDA grant (TWH).**

60.14

INHIBITION OF NEUROMUSCULAR TRANSMISSION IN THE LIZARD BY SERUM FROM PATIENTS WITH LAMBERT-EATON MYASTHENIC SYNDROME (LEMS). C.A. Lindgren and D.R. Mellon*. Dept. of Biology, Allegheny College, Meadville, PA 16335.

Lambert-Eaton Myasthenic syndrome (LEMS), a human autoimmune disorder characterized by a decrease in acetylcholine release at the neuromuscular junction, is thought to be caused by the production of antibodies which react with calcium channels in the motor nerve terminals. Previous studies have demonstrated that blood serum from affected humans can passively transfer the symptoms of LEMS to mouse and rat. The present study shows that LEMS effects can be induced at the neuromuscular junction of the lizard (Anolis carolinensis) by applying serum from patients with LEMS in vitro. Although sera from humans without demonstrated LEMS symptoms had no effect, sera from certain patients diagnosed with LEMS significantly impaired neuromuscular transmission. The exact time course of the effect was variable; however, impaired transmission typically appeared within 4-6 hours of the application of serum.

62

SYMPOSIUM. HAIR CELLS OF THE INNER EAR: STRUCTURE, TRANSDUCTION, AND ACTIVE MOTION. D.P. Corey, Mass. Gen. Hosp. (Chairman); L. Tilney*, Univ. Penn.; A.J. Hudspeth, Southwestern Med. Ctr.; J.F. Ashmore, Univ. Bristol.

Two recent advances in sensory neuroscience have been a greater understanding of the active biomechanics of the auditory system, and a growing appreciation of mechanically activated ion channels as a third class of channel, distinct from voltage- and ligand-gated channels. This symposium will pull together these areas by discussing sensory transduction and force generation by hair cells, the receptor cells of the auditory and vestibular systems.

Recent work has given us a good model for transduction by hair cells: The mechanically sensitive organelles of the cell are the stereocilia, each one a membrane-bounded, ordered array of actin filaments cross-linked by fimbrin. A remarkable developmental sequence produces the variable lengths and arrangement of stereocilia within the bundle. A fine filament extends from the tip of each stereocilium to the side of the tallest adjacent stereocilium; these "tip links" are thought to be directly attached to ion channels near the tips of the stereocilia. The geometry of the bundle is such that displacement of the bundle in the excitatory direction stretches each tip link, increasing tension on the channels and thereby increasing their probability of opening. The movement associated with the conformational change of opening has been measured as about 4 nanometers. The increased tension initiates an adaptation process that restores the channel's open probability to its resting level; this seems to involve a movement of each tip-link attachment point along the side of each stereocilium by an active cytoplasmic motor, which has some similarities to myosin. Two observed mechanical correlates of the adjustment are a change in bundle stiffness with the same timecourse as adaptation and an active movement of an unrestrained bundle with manipulations that affect the adaptation process. Outer hair cells of mammalian cochlea have an additional active movement that is a rapid, longitudinal contraction with depolarization; this may mediate mechanical amplification of vibration by the basilar membrane.

65.1

TETANIC STIMULATION CAUSES AN APV-SENSITIVE INCREASE IN cAMP LEVELS IN RAT HIPPOCAMPAL CA1 REGION. D.M. Chetkovich and J.D. Sweatt. Div. of Neuroscience, Baylor College of Medicine, Houston, Texas, 77030.

Cyclic AMP has been implicated as being important in learning and memory in *Drosophila* and *Aplysia*, as well as in playing a role in the induction of long-term potentiation (LTP) in the dentate gyrus and CA3 region of the hippocampus. The aim of the present study was to identify changes in cAMP in rat hippocampal CA1 region after tetanic stimulation and to investigate possible mechanisms for these changes. High frequency stimulation (100 Hz for 1 sec, 3x at .2 Hz, sufficient to induce LTP in >95% of slices) of the Schaeffer collaterals caused a 46±9% (n=16) increase in cAMP levels in the CA1 region. This increase was blocked by bath application of 50 μM D,L-2-amino-5-phosphonovaleric acid (APV) (6±8% increase in cAMP, n=5), suggesting that the NMDA subtype of excitatory amino acid (EAA) receptor mediates the cAMP increase. Furthermore, a 10 minute bath application of 5 mM glutamate caused a 121±9% increase in cAMP (n=3), and this response was attenuated (10±5% increase above control, n=4) by bath application of 50 μM APV. This finding further substantiates a role for NMDA receptors in controlling cAMP levels. To determine the mechanism by which EAA receptors might elicit an increase in cAMP levels, adenylyl cyclase (AC) activity was assayed in membranes prepared from rat CA1 region. 10 μM GTP and 10 μM forskolin were found to elevate AC activity 45±5% and 1300±320% (n=4), respectively, while 5 mM glutamate did not affect AC activity when applied alone or with 10 μM GTP. However, in the presence of 400 nM calmodulin, Ca^{++} affected AC activity in a biphasic manner. AC was inhibited when free Ca^{++} was above 100 μM but stimulated to 600% above control when free Ca^{++} was in the low micromolar range. These data suggest that NMDA receptor activation causes an increase in cAMP secondary to increases in intracellular Ca^{++}. Thus, tetanic stimulation and the resultant NMDA receptor activation may cause an elevation in cAMP levels in hippocampal CA1 region via this mechanism. Overall, these data suggest the possibility that cAMP is involved in the induction of LTP in the CA1 region of the hippocampus.

65.2

PERSISTENT ALTERATION OF PROTEIN KINASE ACTIVITY DURING THE MAINTENANCE PHASE OF LONG-TERM POTENTIATION. E. Klann and J.D. Sweatt. Div. of Neuroscience, Baylor College of Medicine, Houston, Texas, 77030.

Various reports have suggested a role for persistent kinase activation as a mechanism for the maintenance of long-term potentiation (LTP) in the hippocampus. To test this hypothesis, we measured protein kinase activity in homogenates of the CA1 region of rat hippocampal slices 45-60 minutes after the induction of LTP. LTP was induced by tetanic stimulation of the Schaffer collateral pathway (100 Hz for 1 sec., 3 trains delivered 5 min. apart, 181±15% of control EPSP slope, n=16). Slices were frozen, dissected, and homogenized in buffer containing 50 mM HEPES, pH 7.4, 10 mM $MgCl_2$, 1 mM EGTA and 1 mM EDTA. Homogenate was added to reaction mixtures containing 100 μM ^{32}P-ATP and 2 μg of the exogenous kinase substrate myosin light chain (MLC). After SDS-PAGE, incorporation of ^{32}P into MLC was quantitated using autoradiography and densitometric scanning. We have detected an LTP-induced increase in phosphorylation of both MLC (215±29% of control, n=9) and endogenous substrates using this assay. As the assay mixture contained EGTA, the elevated phosphorylation is independent of Ca^{2+}. The increase in Ca^{2+}-independent substrate phosphorylation is due to increased kinase activity rather than decreased phosphatase activity as sodium pyrophosphate (1-2 mM) did not alter this increase (326±64% of control MLC phosphorylation, n=7). Treatment of the slices with 50 μM D,L 2 amino 5-phosphonovaleric acid (APV) blocked both the induction of LTP (111±6% of control EPSP slope, n=3) and the induction of the alteration in protein kinase activity (90±24% of control MLC phosphorylation, n=3), indicating NMDA receptor activation is necessary for the establishment of the long-term change in protein kinase activity. We also have found that the increased kinase activity in homogenates is blocked by the addition of PKC (19-36), a selective peptide inhibitor of protein kinase C (126±29% of control MLC phosphorylation, n=5). Thus, we conclude that the maintenance phase of LTP is associated with NMDA receptor-mediated production of a Ca^{2+}-independent form of protein kinase C.

65.3

THE ROLE OF THE CALPAINS IN LONG-TERM POTENTIATION. J.B. Denny*, J. Polan-Curtain*, A. Ghuman*, M.J. Wayner and D.L. Armstrong. Division of Life Sciences, The University of Texas at San Antonio, San Antonio, TX 78285

While the calpains have been proposed to play a role in memory, their action in the development of long-term potentiation (LTP) has heretofore not been defined. We show that LTP is blocked by the extracellular application of two newly-developed, highly potent calpain inhibitors. Incubation of hippocampal slices with 6 micromolar N-acetyl-leu-leu-norleucinal or N-acetyl-leu-leu-methioninal for 1 hr blocked the induction of LTP in the Schaffer collateral-CA1 synaptic zone but had no effect if applied 1 hr after tetanization. Injection of the above peptides or leupeptin into the intact rat brain blocked the induction of LTP in the perforant path-granule cell synaptic zone. Baseline synaptic transmission in all cases was unaffected by the presence of the inhibitors. Although the calpains have been shown to convert purified protein kinase C to protein kinase M, we find that this conversion is not required for LTP maintenance since application of the membrane-permeable PKM inhibitor staurosporine has no effect on established LTP. The action of the calpains on other substrates is required, however, and these substrates may be cytoskeletal proteins and possibly other kinases. Supported by NIH grants GM07717-10 and RR08194.

65.4

MODULATION OF AMPA/QUISQUALATE (A/Q) RECEPTORS BY PHOSPHOLIPASE A_2 (PLA_2): A NECESSARY STEP IN LTP? G. Massicotte, P. Vanderklish*, G. Lynch and M. Baudry. NIBS Program, University of Southern California, Los Angeles, CA 90089-2520.

The expression of LTP in area CA1 of hippocampus has been proposed to result from an increased responsiveness of the AMPA/quisqualate receptors, while the induction of LTP is triggered by the activation of Ca-dependent processes including possibly calpain and PLA_2. Systemic administration of kainic acid (KA) produces a rapid activation of calpain in the hippocampus and we used this procedure to explore the relationships between calpain, PLA_2 and LTP. We determined both the degree of LTP in hippocampal slices and the effect of PLA_2 on the binding properties of the A/Q receptors in brain membranes from control and KA-injected rats. In both hippocampal and cerebellar membranes from control rats PLA_2 treatment produced a significant increase (40%) in the binding of ^{3}H-AMPA to the quisqualate receptors. The effect of PLA_2 on ^{3}H-AMPA binding to hippocampal membranes was markedly reduced in KA-treated rats, while the effect was not significantly modified in the cerebellum. A large reduction in the magnitude of LTP induced by a theta burst stimulation paradigm was observed in area CA1 of hippocampal slices prepared from KA-treated rats. Injection of KA was accompanied by an important breakdown of spectrin, a substrate for calpain, in the hippocampus but not in the cerebellum. Treatment of telencephalic membranes with calpain also blocked the PLA_2 effect on the ^{3}H-AMPA binding. These findings suggest that the PLA_2-induced modification of A/Q receptors is a necessary step in the development of LTP.

65.5

KINETICS OF PKC ACTIVATION CONSTRAIN THE TEMPORAL SPECIFICITY OF PKC-BASED NEURONAL PLASTICITY: A QUANTITATIVE MODEL. C. Chen. Computation & Neural Systems Program, 216-76 Caltech, CA 91125.

Protein kinase C (PKC), activated by phospholipid, calcium and diacylglycerol (DG), underlies various neuronal plasticity mechanisms. However, the kinetic interaction among these activators remains unclear. Based on various experimental data, a quantitative two-step model is proposed for PKC activation kinetics. PKC activation consists of: (1) a calcium- and phospholipid-dependent translocation from the cytosol to the membrane, and (2) a DG-dependent activation within the membrane. Phospholipid is rich near the membrane, and therefore is not a limiting factor. Three calcium kinetics (slow, intermediate and fast), representing a wide range of calcium dynamics in living cells, are used to test the model along with the DG kinetics empirically derived from experiments in human platelets (Takai, Y. et al., Adv. in Cyclic Nucleotide Res., 14:310, 1981). The model predicts that the degree of PKC activation depends on the timing between DG and calcium. Maximal PKC activation occurs when the peak of calcium lags DG for 5-10 sec. Consequently, the model indicates that the cooperative nature of PKC activation constrains the temporal window in which two intracellular messengers can induce PKC-based neuronal plasticity, such as associative LTP in hippocampal neurons.

65.6

EVIDENCE FOR A PRESYNAPTIC ROLE OF PROTEIN KINASE C IN HIPPOCAMPAL MOSSY FIBER SYNAPTIC TRANSMISSION. **D.M. Terrian, D.K. Ways* and R.L. Gannon. Depts. of Anatomy and Medicine, East Carolina University School of Medicine, Greenville, NC 27858.**

It has been suggested that long-term potentiation in the hippocampal mossy fiber (MF) synapse involves fundamentally different presynaptic mechanisms from those employed by other hippocampal synapses, since both protein kinase C (PKC) and phosphoprotein F1 appear to be absent in MF terminals. In the present study we evaluated this proposal by directly comparing the metabolic properties of hippocampal MF synaptosomes and a conventional P_2 synaptosomal fraction prepared from the same hippocampal tissue. PKC-dependent histone phosphotransferase activity was found to be comparable in MF and P_2 synaptosomes. Western blot analyses were performed to confirm this unexpected finding and the results demonstrate that the alpha, beta and gamma subspecies of PKC are all present in relatively equivalent amounts in these two different subcellular fractions. However, an SDS-PAGE analysis of the endogenous substrates phosphorylated by PKC indicated that protein F1 is not present in MF synaptosomes. A functional role for PKC in the hippocampal MF nerve endings seems to be indicated by the finding that phorbol-12,13-dibutyrate (PDB) and phorbol-12,13-diacetate produce a dose-dependent potentiation of the K^+-evoked increase in the availability of cytosolic free calcium and the concomitant release of endogenous glutamate and dynorphin B. The biologically inactive 4-α-phorbol was without affect on any of these parameters and the PDB enhancement of Ca^{2+}-dependent release was blocked by the PKC antagonist staurosporine. It is concluded that hippocampal MF nerve endings possess a variety of PKC isoforms and that their activation is sufficient to have an important influence on MF synaptic transmission and plasticity. **Supported by AFOSR 89-0531.**

65.7

TWO FORMS OF LTP IN HIPPOCAMPAL CA3 PYRAMIDAL CELLS
R.A.Zalutsky and R.A. Nicoll, Dept. of Pharmacology, U. of California, San Francisco, CA 94143

We have investigated the mechanisms of LTP at the associational-commissural (a/c) and the mossy fiber (mf) synapses onto the same CA3 pyramidal cells using intracellular, whole cell, and field potential recordings in guinea pig hippocampal slices.

Intracellular recordings confirmed the observation on extracellular fields (Harris & Cotman,Neurosci. Letts. 70:132-137,1986) that a/c but not mf LTP is blocked by the NMDA antagonist APV. Further intracellular experiments injecting current or calcium chelators into cells demonstrated that the induction of a/c LTP depends on post-synaptic calcium concentration and membrane potential , as does NMDA dependent LTP in CA1 pyramidal cells. However, mf LTP in the same cells was not affected by these manipulations nor by more drastic alterations of the cells' interior using whole cell recording and voltage clamp with high concentrations of BAPTA and fluoride in the recording pipettes.

As has been previously shown for NMDA dependent LTP (McNaughton, J.Physiol. 324:249-262,1982) paired pulse facilitation (PPF), a presynaptic phenomenon, is not altered during a/c LTP. On the other hand PPF is clearly reduced in mf LTP (measured under voltage clamp with whole cell recording).

Our results indicate that CA3 pyramidal cells express two fundamentally different forms of LTP. The results suggest that in a/c LTP induction is postsynaptic and the consequent increase in synaptic strength is postsynaptic (or perhaps presynaptic but independent of PPF), and in mf LTP both induction and the increased synaptic strenth are presynaptic.

65.8

AN APV INSENSITIVE COMPONENT OF LTP IN AREA CA1 OF RAT HIPPOCAMPUS.
L.M. Grover & T.J. Teyler, Neurobiology Department, N.E. Ohio Univ. College of Medicine, Rootstown, OH 44272.

Long-term potentiation (LTP) of Schaffer collateral/commissural synapses in area CA1 of hippocampus is sensitive to NMDA receptor antagonists. We have observed a component of LTP at these synapses that is selectively induced by high frequency (200Hz) tetanic stimulation, and that is not prevented by the competitive NMDA receptor antagonist APV (50-200μM). We studied this APV insensitive component of LTP using intra- and extracellular recordings. This component of LTP is induced under conditions where NMDA receptor mediated synaptic responses are demonstrably blocked, has a delayed onset beginning 2-5 min post-tetanus, and develops gradually over 15-20 min. Intracellular injection of the calcium chelator BAPTA prevents both the APV sensitive and insensitive components of LTP, suggesting that an increase in $[Ca^{2+}]_i$ is essential for induction of the APV insensitive component, as has been previously shown for that APV sensitive component. Bath application of the dihydropyridine Ca^{2+} channel antagonist nifedipine (10μM) reduces the APV insensitive component of LTP, suggesting that certain patterns of tetanic stimulation may be capable of promoting sufficient Ca^{2+} influx through voltage dependent Ca^{2+} channels to induce LTP. Supported by the ONR.

65.9

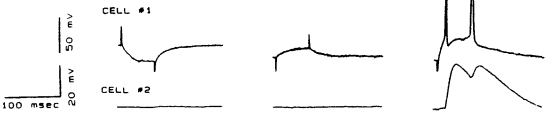
ARACHIDONIC ACID-INDUCED, ACTIVITY-DEPENDENT POTENTIATION OF SYNAPTIC TRANSMISSION IN THE HIPPOCAMPUS.
T.V.P. Bliss* and J.H. Williams* (SPON: ENA). National Institute for Medical Research, Mill Hill, London NW7 1AA, U.K.

Arachidonic acid (AA) when transiently perfused into the dentate gyrus *in vivo* or *in vitro* produces a persistent potentiation of the population EPSP if the frequency of synaptic activation is increased during the period of exposure to exogenous AA (Williams et al., Nature, 341, 739, 1989). A similar effect is seen in area CA1 *in vitro*. Population EPSPs were simultaneously recorded from str. radiatum in CA1 and from the molecular layer of the dentate gyrus. Perfusion for 30 min with medium containing AA (50μM), together with an increase in the rate of stimulation from 1/30 Hz to 1/4 Hz was followed by a slow potentiation of the EPSP in both pathways, which reached a plateau in 30-90 min and which showed no decline in the next 1-3 h. In neither area was induction of the effect blocked by D(-)APV (50μM) or NDGA (100μM). Occlusion experiments suggest a shared mechanism for the expression of AA-induced plasticity and LTP. Similar activity-dependent potentiation was not produced by perfusion with other fatty acids (linolenic, linoleic, oleic, palmitic or docosahexaenoic acids; 50μM in each case).

65.10

Intracellular recordings from pyramidal cell pairs in cultured rat hippocampal slices J.A. Kauer and R.W. Tsien, Dept. of Molecular and Cellular Physiology, Stanford University Medical Center, Stanford, CA 94305.

Hippocampal slices were cultured for 1-4 weeks (B.H. Gahwiler, *J. Neurosci. Meth.* 4: 329-342, 1981). PSP responses from neurons were recorded in response to stratum radiatum (s.r.) stimulation. Many similarities to acutely prepared slices were observed: 1) HRP staining showed normal cell morphology; 2) 10 μM CNQX abolished EPSPs; 3) EPSP duration increased with depolarization and reversed near 0 mV; 4) epileptiform burst firing was sometimes observed; 5) pairing depolarization to 0 mV with stimuli to s.r. (which triggers LTP in acute slices) produced EPSP potentiation lasting over 35 minutes in about 40% of neurons. Finding this potentiation suggests that all factors and transmitters required for LTP in acute slices are intrinsic to the hippocampus. Some differences from acute slices were also noted. Spontaneous PSPs were prominent and paired pulse facilitation was often not observed. Tetanic stimulation did not cause potentiation. Constant stimulation to s.r. elicited a wide range of EPSP sizes, from failures to PSPs of 10 mV or more. The EPSPs recorded simultaneously from two unconnected CA1 or CA3 cells in response to s.r. stimulation often covaried throughout an experiment.



Pairs of CA1 or CA3 neurons were examined for synaptic interactions. There was no electrotonic coupling between pairs, and the EPSPs reversed near 0 mV. Whereas in acute slices, the chance of synaptic connectivity within CA1 or CA3 is less than one in 100, it was roughly one in four in our cultured slices. The EPSP size averaged over 10 mV, ten times that reported in acute slices. The relatively frequent and strong synaptic connectivity in this system may be favorable for explorations of presynaptic mechanisms of synaptic plasticity and neuronal function.

65.11

PRESYNAPTIC ENHANCEMENT DURING LONG-TERM POTENTIATION (LTP) REVEALED BY WHOLE-CELL RECORDINGS.
R.W. Tsien & R. Malinow, *Depts. of Molecular & Cellular Physiology, Stanford University and Physiology & Biophysics, University of Iowa.*

LTP is triggered postsynaptically in the CA1 region of the hippocampus, but the locus of persistent modification of synaptic transmission remains controversial. To test for possible changes in presynaptic function, we have analyzed the variability in synaptic currents elicited by minimal stimulation (0.25 Hz) before and during LTP. Whole cell recordings were obtained from CA1 neurons 2-3 cell diameters below the surface in rat hippocampal slices, cut 400-500 μm thick. This method allows recordings from cells with largely intact dendritic structures, while also providing much better signal resolution and biochemical access than conventional microelectrode recordings. We found that robust LTP could be evoked by pairing a steady postsynaptic depolarization to ~0 mV with continued minimal activation of the test pathway (40 stimuli at 2 Hz). Typically, mean synaptic current (M) was increased 3-fold after pairing. Prolonged dialysis of the postsynaptic cell blocked the triggering of LTP, with no effect on expression of LTP. Synaptic currents displayed a large trial-to-trial variability, reflecting the probabilistic nature of transmitter release. Block of postsynaptic receptors with CNQX attenuated the responses but left their relative variability unchanged. In contrast, maneuvers that alter presynaptic release (elevated $[Ca^{2+}]_o$, lowered $[Mg^{2+}]_o$, added 4-AP or increased stimulus strength to recruit more fibers) produced expected alterations in synaptic variability: M^2/variance increased, and the histogram of synaptic current amplitudes shifted from a distribution skewed to small currents, toward a symmetrical bell-shape. We found consistent and qualitatively similar changes during LTP (14 experiments). In addition, the proportion of synaptic failures sharply decreased with LTP when failures could be clearly resolved (5/5 recordings). While not excluding the possibility of some change in postsynaptic responsivity, increases in M^2/variance and decreases in failures both support an enhanced likelihood of transmitter release during LTP.

65.12

LONG-TERM POTENTIATION OF SYNAPTIC TRANSMISSION BETWEEN INDIVIDUAL CA3 AND CA1 NEURONS IN RAT HIPPOCAMPAL SLICES.
R. Malinow & R.W. Tsien, *Depts. of Physiology & Biophysics, University of Iowa and Molecular & Cellular Physiology, Stanford University.*

LTP has been studied extensively between populations of neurons. A general but unproven assumption has been that enhanced transmission is a property expressed by single synapses, rather than an emergent property of many synapses. However, this assumption has been questioned by the most complete study to date (Friedlander et al., J. Neurosci., 1990). Apart from its significance to theories of learning, finding LTP at one-to-one connections between presynaptic and postsynaptic neurons would facilitate analysis of statistical properties of transmission and the cellular mechanisms that underly LTP.

We have found robust LTP of transmission between pairs of individual CA3 and CA1 neurons. The CA3 neuron was impaled with an intracellular electrode, and the postsynaptic CA1 neuron was accessed with a whole-cell pipette. Potentiation was induced by pairing postsynaptic depolarization with presynaptic activity. Enhancement was observed in 3/4 pairs with detectable synaptic connections, and in 1 of 5 pairs with no apparent connection prior to pairing. The increase in mean synaptic current was as large as 10-fold and lasted for the duration of the recording (up to 1.5 hr). Transmission in a control (non-paired) pathway did not change, suggesting that the enhancement was synapse-specific. After pairing, we noted a marked decrease in the proportion of synaptic failures, and the amplitude distribution, which was initially skewed toward small currents, changed toward a more symmetrical shape with LTP. Thus, we conclude that (1) LTP can be expressed at connections between individual neurons (although without anatomical analysis we cannot say whether the connection occurs at a single synapse), and (2) expression of LTP in one-to-one connections, as with minimal stimulation, involves a presynaptic modification that increases the likelihood of transmitter release.

65.13

HOMO- AND HETERO-SYNAPTIC LONG-TERM POTENTIATION IN THE OLFACTORY-HIPPOCAMPAL CIRCUIT IN THE ADULT GUINEA PIG ISOLATED BRAIN MAINTAINED IN VITRO. M. de Curtis, A. Alonso[1], and R. Llinás. Dept. of Physiolgoy & Biophysics, N.Y.U. Medical Center, 550 First Ave, N.Y., N.Y., 10016 and Dept. of Neurology & Neurosurgery, Montreal Neurological Inst., McGill Univer., Montreal, Quebec, Canada, H3A 2B4[1]

Long-term potentiation (LTP) was investigated along the olfactory system from the lateral olfactory tract (LOT) to the piriform cortex, entorhinal cortex, and hippocampus. Experiments were performed in the arterially perfused adult guinea pig isolated brain maintained in vitro (Llinás, et al., *J. Physiol.* 414:16P, 1989; de Curtis and Llinas *Soc. Neurosci. Abstr.* 15, 660, 1989). Trains of electrical stimuli (4 msec, 100 Hz bursts) were delived to the LOT at 5-8 Hz for 1-3 seconds. These produced a clear potentiation of the monosynaptic and associative evoked potentials in the piriform and anteromedial entorhinal cortices. As opposed to most research on LTP, this potentiation did not required the use of picrotoxin for its generation. Direct stimulation of the periamygdaloid cortex (PAC) induced potentiation of the mono- and disynaptic EPSPs elicited in the entorhinal cortex, while basolateral amygdala (BLA) stimulation induced LTP mainly in the lateral entorhinal cortex. Following addition of 2-APV (100 µm) to the perfusate, long-lasting potentiation of the mono- and disynaptic associative EPSPs could not be evoked. 2-APV also blocked the LTP induced in the entorhinal cortex by PAC stimulation, indicating that NMDA-mediated mechanisms are responsible for both the induction and the expression of LTP in the entorhinal cortex (Llinas and Alonso,Soc Neurosci. Abstr.16, 1990). Prolonged potentiation of evoked potentials in both the entorhinal cortex and dentate gyrus were obtained after application of a tetanizing train to the PAC. Once potentiated, the amplitude of the entorhinal cortex and dentate gyrus responses evoked by BLA stimulation were also increased, indicating that hetero-synaptic long-term potentiation is present in the entorhinal cortex-dentate gyrus circuit in the in vitro isolated brain preparation. Supported by grant 13742 from NINDS and by Italian CNR grant 2042856.

65.14

IN VITRO HEBBIAN AND NON-HEBBIAN LTP IN ENTORHINAL CORTEX LAYER II STELLATE CELLS. R. Llinás and A. Alonso[1], Dept. of Physiology & Biophysics, N.Y.U. Med. Ctr, 550 First Ave., N.Y., N.Y. 10016 and Dept. of Neurology & Neurosurgery, McGill University, Montreal, Canada, H3A 2B4[1]

Long term potentiation (LTP) of synaptic transmission following single stimulation of the white matter (WM) and following direct, rhythmic subthreshold membrane potential change was studied intracellularly in the entorhinal cortex (EC) in rat brain slices in the presence of picrotoxin (50-100µM). EPSPs evoked by WM stimulation comprised an early and late component. APV (50 µM) reduced the amplitude of the early component and abolished the late component (which also demonstrated "anomalous" voltage-dependent properties). These results indicate that part of the fast component and all of the slow component of the EPSP are mediated through activation of MNDA receptors. Tetanic or rhythmic single-shock WM stimulation (5-10 Hz for 10-20 sec) induced LTP in 19 of 27 cells tested. Similar results were found in field potential and intracellular recordings in the isolated brain preparation where LTP may be evoked in the EC in the absence of picrotoxin (deCurtis et al.,*Soc,.Neurosci. Abst.*, 16, 1990). Also, in 15 of 23 cells tested in slices the EPSP was enhanced for as long as 3 hrs by intracellular stimulation for 20 sec with a train of subthreshold, 10-12 mV, 100 ms current pulses delivered at 5 Hz (without pairing with white matter stimulation). While present in the early component, the enhancement was most prominent in the late component. LTP was prevented by APV superfusion. More significantly, LTP, induced by white matter or single cell direct stimulation, was blocked by APV. However, the block of acquired LTP was reversed by washing indicating that this drug did not interfere with the maintenance of LTP, but only with its expression. These results indicate the presence of Hebbian and non-Hebbian NMDA-induced and expressed LTP in the entorhinal cortex. Supported 13742 from NINDS and Fogarty Foundation.

STRESS, HORMONES AND THE AUTONOMIC NERVOUS SYSTEM I

66.1

EFFECTS OF STRESSORS ON CARDIOVASCULAR SYSTEM, PLASMA AND TISSUE CATECHOLAMINE LEVELS IN RATS. H. M. Rhee. Dept. of ORU Med. Sch., Tulsa, OK 74137.

Various types of stress cause an increase in systemic blood pressure, which eventually produce cardiac, renal, and other end organ damage. The elevation in blood pressure is usually caused by release of adrenocorticotropin from the anterior pituitary gland, of norepinephrine from sympathetic nerve terminals and of epinephrine from the adrenal medulla. Studies show that stress alters adrenal catecholamine synthesis which is controlled by primarily neuronal and humoral factors. Adrenal catecholamines are co-released stoichiometrically with enkephalins upon stress, which suggests an involvement of opioid receptors in stress physiology. Young male Sprague-Dawley rats were anethetized for a chronic cannulation of the carotid artery and jugular vein. After 3 days recovery from the surgery the rats were subjected to either immobilization, heat stress or treadmill exercise for various durations. Heat stress under a red light for 5 min, three times with 5 min interval, increased blood pressure and heart rate. Immobilization and forced running also increased the parameters and plasma catecholamines. Although a pretreatment of the animals with naloxone or beta-funaltrexamine did not produce always uniformed effects against the stressors, the data suggest that opioid receptors in the central as well as in the periphery modulate adrenergic and cardiovascular activities under stressful conditions.

66.2

PLASMA DOPA AND TISSUE TYROSINE HYDROXYLASE DURING ACUTE AND REPEATED IMMOBILIZATION STRESS R. Kvetnansky, D.S. Goldstein*, V.K. Weise*, G. Baggy*, K. Szemeredi*, C. Holmes* and I.J. Kopin. Clinical Neuroscience Branch, NINDS and Hypertension-Endocrine Branch, NHLBI, NIH, Bethesda, MD 20892.

Recent studies suggest that plasma dopa levels reflect in vivo catecholamine synthesis, but the relationship between tyrosine hydroxylase (TH) and dopa levels has not been assessed directly. In this study, plasma concentrations of catechols were measured by a liquid chromatographic-electrochemical technique and adrenal and splenic TH by a CO_2-trapping technique in conscious intact or adrenal-demedullated (DEMED) rats during acute and after repeated 2 hr intervals of immobilization stress (IMO). Dopa levels increased beginning within 1' of IMO and plateaued after 5', but adrenal and splenic TH was unchanged. Even 1' of gentle handling increased plasma dopa. After IMO or handling, dopa levels decreased to baseline within 20'. Increases in dopa were similar in intact and DEMED rats. Catecholamine levels increased more rapidly than dopa levels. After 7 daily 2 hr intervals of IMO, dopa levels were the same as before the first interval of IMO, whereas TH was increased significantly. The results indicate that plasma dopa levels increase rapidly during IMO. The increases are independent of adrenomedullary activation and are not simultaneous with increases in catecholamine levels. Since dopa levels were increased when TH was not, and since TH was increased when dopa levels were not, increases in plasma dopa levels during IMO stress in rats do not directly reflect alterations in tissue levels of tyrosine hydroxylase.

66.3

EFFECTS OF BENZODIAZEPINES ON METABOLIC STRESS IN HUMANS. A. Breier, O. Davis*, R. Jones*, B. Kirkpatrick, R. Buchanan*. Outpatient Program, Maryland Psychiatric Research Center, Baltimore, MD 21228

Several preclinical studies have demonstrated that benzodiazepines affect a number of systems involved in the stress response such as HPA axis activation and increases in catecholamine turnover. There exists comparatively little information about the effects of benzodiazepines on the stress response in humans. In the present study, we examine the effects of the benzodiazepine, alprazolam (1.5mg), on the neuroendocrine, neurochemical and physiologic effects of metabolic stress in healthy volunteers (N=11). Metabolic stress was induced by double-blind, placebo controlled IV administration of 2-deoxyglucose (2DG) (50mg/kg) which competitively inhibits glucose metabolism resulting in intracellular glucoprivation. 2DG alone produced significant increases (mean±sem; pre-to 2hr post-2DG infusion) in plasma cortisol (13.5±2 to 26.6±2 mg/dl) and glucose (65±3 to 137±13 mg/dl). Alprazolam pretreatment attenuated 2DG-related increases in plasma cortisol and glucose by 25% and 20%, respectively. These data suggest that the GABA/BZ receptor may play a role in modulating glucoprivic stress in humans. The effects of alprazolam on 2DG-induced changes in plasma catecholamines, physiologic function (HR, BP, temperature) and behavioral responses will be presented.

66.4

TOLERANCE TO NICOTINE MAY BE MODULATED BY CORTICOSTERONE. A. C. Collins, E. U. Grun and J. R. Pauly. Institute for Behavioral Genetics, Univ. of Colorado, Boulder, CO 80309.

Chronic treatment with nicotine often results in tolerance to the behavioral and physiological effects elicited by this drug. Several studies have demonstrated that chronic nicotine treatment also results in increases in the number of brain receptors that bind nicotine but other studies have failed to detect alterations in receptor numbers in animals that showed tolerance. This finding suggests that factors other than changes in receptor numbers may regulate tolerance. In the present study, the effects of chronic saline or nicotine injection (three times daily for 12 days, 2 mg/kg) on nicotine sensitivity were determined in DBA/2 and C57BL/6 mice. Chronic nicotine injected mice showed reduced sensitivity to nicotine but brain nicotinic receptors were not changed. This tolerance persisted at least 4 weeks after the last chronic nicotine injection. Plasma corticosterone (CCS) levels were also measured. Chronic nicotine treatment resulted in tolerance to nicotine-induced CCS release, but pre-injection levels of CCS rose as injection continued. Because previous studies from our laboratory have demonstrated that elevated CCS levels results in reduced sensitivity to nicotine, we suggest that the tolerance to nicotine that occurs following chronic injections is due, in large part, to the chronically elevated CCS levels.
Supported by DA-05131, DA-00116 and an award from the R. J. Reynolds Tobacco Company.

66.5

ADRENAL MEDULLECTOMY EFFECTS ON CHRONIC RENAL HYPERTENSION. T.C. Herzig and J.R. Haywood. Department of Pharmacology, University of Texas Health Science Center at San Antonio, San Antonio, Texas 78284-7764.

The goal of this study was to determine the effects of adrenal medullectomy on chronic renal hypertension. Rats were made hypertensive with a one-kidney, figure-8 renal wrap; sham operation consisted of unilateral nephrectomy. Four weeks later, catheters were inserted into the femoral artery and vein for blood pressure (BP) and heart rate (HR) measurements, and administration of drugs. After 24 hours, BP and HR were recorded for a control period; then, rats underwent adrenal medullectomy (MX) or sham operation (MS), producing four groups: wrap-MX, sham-MX, wrap-MS, sham-MS. BP and HR were recorded daily for 7 days. The contribution of the sympathetic nervous system to the maintenance of BP was assessed on the seventh day pharmacologically. Demedullation produced a transient fall in BP to normotensive levels in the wrapped animals (155±7 mmHg to 127±6 mmHg); by day 3, BP rose to hypertensive levels. BP in the sham-MX group was unaffected by this manipulation. MS had no affect on BP in either the wrap-MS or sham-MS groups. MX decreased plasma epinephrine while plasma norepinephrine tended to rise on day 2 post-MX in wrap and sham animals. Ganglionic blockade was greater in the wrapped animals compared to the sham animals in both the MX (-46±3 mmHg vs -22±2 mmHg) and MS (-45±3 mmHg vs -30±2 mmHg) groups. These observations indicate that the adrenal medulla contributes significantly to the maintenance of renal wrap hypertension. (Supported by HL36080.)

66.6

BIOCHEMICAL ISOLATION, CHARACTERIZATION AND PARTIAL PURIFICATION OF CORTICOTROPIN-RELEASING FACTOR (CRF) RECEPTORS FROM RAT BRAIN. D.E. Grigoriadis, J.A. Heroux, D.M. Pearsall*, and E.B. De Souza. NIDA / ARC, Baltimore, MD 21224

The primary role of CRF in regulating pituitary-adrenocortical secretion is well established. Recent studies in the laboratory have focused on elucidating the biochemical characteristics of CRF receptors in brain, endocrine and immune tissues and on purification of CRF receptors in brain. Central and peripheral CRF receptors differ in their molecular size (58,000 Da and 75,000 Da, respectively) as determined by SDS-PAGE. These differences in molecular size do not appear to be due to structural differences in the proteins themselves but rather due to alterations in the post-translational modification of the glycosylation profiles. In order to purify CRF receptors we used ^{125}I-oCRF affinity cross-linked rat brain membranes, solubilized in 1% digitonin. Cross-linked solubilized CRF receptors (2 g protein) were applied to 40 ml wheat-germ agglutinin, washed and eluted with 250 mM β-N-acetylglucosamine. Eluted receptors (40 ml) were concentrated to 10 ml and applied to a DEAE ion-exchange column. The matrix was washed and eluted with a gradient of 0 - 500 mM NaCl. Samples of the eluates of both columns were electrophoresed on SDS-PAGE confirming that both the WGA-purified and DEAE-purified receptor had a molecular weight of 58,000 Da. In parallel, eluted samples were processed for 2-dimensional electrophoresis. Radiolabeled proteins (400 µg) were electrophoresed on a pH gradient (3 - 10) in 7.5 % polyacrylamide tube gels before being electrophoresed in the second dimension, dried and exposed to X-ray film for autoradiography. The use of the affinity cross-linked CRF receptors as a tracer will be a valuable tool in the purification and identification of the native receptor protein.

66.7

OVARIAN STEROID MODULATION OF CRH STIMULATION OF CORTISOL IN RHESUS MONKEYS. L. E. Heisler, R. L. Reid and D. A. Van Vugt. Departments of Physiology and Obstetrics and Gynecology, Queen's University, Kingston, Ontario, Canada K7L 3N6

Ovarian steroids appear to modulate cortisol secretion in a positive manner in rhesus monkeys since reduced cortisol concentrations in ovariectomized monkeys can be reversed with ovarian steroid replacement. To examine the site of action of ovarian steroids in this regard we compared the cortisol response to administration of 100 ug CRF in ovariectomized and intact female rhesus monkeys. In addition, we determined cortisol concentrations in both groups while restrained in primate chairs. Cortisol is elevated during this condition presumably due to increased CRH secretion. CRH administration caused an elevation of cortisol in intact monkeys from 14.7 ± 1.46 ug/dl to 33.2 ± 3.292 ug/dl 75 minutes post-injection. In ovariectomized monkeys cortisol rose from 11.9 ± 1.18 ug/dl to 32.5 ± 2.77 dl. The increase in cortisol concentration was not significantly different between intact and ovariectomized monkeys. Similarily, the rise in cortisol levels in response to chair restraint was not significantly different between these two groups. Cortisol levels rose to 33.5 ± 3.78 ug/dl from the normal midday level of 18.2 ± 2.51 ug/dl in intact monkeys while in ovariectomized monkeys the rise was from 12.6 ± 0.56 ug/dl to 33.6 ± 3.781 ug/dl. These data indicate that ovarian steroids do not influence pituitary-adrenal responsiveness to CRH and suggest that during basal states, ovarian steroids modulate circulating cortisol levels by an action on hypothalamic CRH neurons. Since chair restraint stress caused similar cortisol elevations in intact and ovariectomized animals, we conclude that this effect of ovarian steroids on CRH release can be negated by this form of acute stress.

66.8

ACUTE HYPOGLYCEMIC STRESS INFLUENCES THE ACTIVITY OF NEUROPEPTIDE Y AND GALANIN NEURONS IN THE RAT HYPOTHALAMUS. J.I. Koenig, R.M. Strauss* and L.M. Kaplan, Physiology Dept., Georgetown Univ. Sch. of Med., Washington, DC and Gastrointestinal Unit, Massachusetts General Hosp., Boston, MA.

The induction of hypothalamic-pituitary-adrenal axis activity by hypoglycemic stress is well-characterized. We have recently observed that galanin (Gal) inhibits, and NPY stimulates, pituitary ACTH secretion. Because these effects appear to be mediated by peptide actions within the hypothalamus (HT), we sought to determine the response of HT Gal and NPY neurons to hypoglycemic stress. Fasted, male Sprague-Dawley rats were sacrificed at various times after the injection of insulin (5 IU/kg, ip). Plasma ACTH levels increased 15-fold within 30 min after insulin injection. Gal and NPY concentrations in HT fragments were determined by RIA and mRNA levels by blot hybridization analysis. HT Gal concentrations were unchanged at 30 min. However, 90 min after insulin treatment, the concentration of Gal in the median eminence (ME) increased 55%. Concurrently, Gal concentrations decreased 33% in the HT fragment (POA) containing the paraventricular (PVN) and supraoptic nuclei, and Gal mRNA increased 2-fold exclusively in the basal HT fragment (MBH). Insulin treatment increased ME NPY concentrations 2-fold over 90 min, but consistently reduced POA NPY levels by 55-60%. In contrast, MBH NPY levels were reduced 45% at 30 min but tripled to 145% of control by 90 min. These observations suggest NPY and Gal neurons are differentially affected by hypoglycemic stress. NPY projections from the MBH to the PVN and ME appear to be activated early and may be involved in stimulating ACTH secretion, whereas Gal neurons in the MBH are activated later and likely attenuate the early increase in ACTH secretion.

66.9

NERVE GROWTH FACTOR DOWN-REGULATES ANGIOTENSIN II RECEPTORS IN PC12 CELLS. R.C. Speth and K.H. Kim, Dept. of VCAPP and Program in Biochem. Biophys. Genetics and Cell Biol., Washington State Univ., Pullman, WA 99164-6520.

Adrenal chromaffin cells, which are derived from the neuroectoderm, do not display a neuronal phenotype. They contain angiotensin II (AII) receptors that mediate catecholamine release. The PC12 cell line derived from a rat pheochromocytoma offers a tool to study the adrenal chromaffin cell under varying influences. PC12 cells (gift of Dr. Robert Perlman) contain ^{125}I-sarcosine^{1}, isoleucine^{8} AII (^{125}I-SI AII) binding sites in a high concentration 804 ± 127 fm/mg protein. In the presence of mercaptoethanol, binding affinity for ^{125}I-SI AII is enhanced, 152 ± 25 pM, vs. 402 ± 96 pM in the absence of this sulfhydryl reducing agent. Competition assays indicate the following order of potency for ^{125}I-SI AII binding: AIII > SI AII > AII > p-aminophenylalanine^{6} AII = AI > 3-8 hexapeptide AII. This suggests that PC12 cells contain the AII_{β} receptor subtype (see Grove et al., this volume). When the cells are grown in the presence of retinoic acid (RA, 10^{-6} M) they retain an endocrine cell phenotype, while addition of nerve growth factor (NGF, 5 ng/ml) causes them to develop a neuronal phenotype. RA did not significantly alter the density or affinity of ^{125}I-SI AII binding in the PC12 cells, but NGF decreased the density of ^{125}I-SI AII binding sites by 70% without significantly altering the binding affinity. Thus, development of a neuronal phenotype is associated with a reduction in AII receptor density. Supported by grants from NIH (NS 21305) and American Cancer Society (IN-119K).

66.10

A ω-CONOTOXIN-SENSITIVE Ca^{2+} INFLUX PATHWAY IS SELECTIVELY ACTIVATED BY ANGIOTENSIN II (AII) IN BOVINE ADRENAL MEDULLARY (BAM) CELLS. M. K. McMillian, R. K. Tuominen, P. M. Hudson, H. H. Suh and J. S. Hong. LMIN, NIEHS/NIH, Research Triangle Park, NC 27709.

ω-Conotoxin blocks Ca^{2+} influx and neurotransmitter release in response to depolarization in sympathetic neurons, but has little effect on Ca^{2+} and exocytotic responses to KCl or nicotine in cultured BAM cells. AII stimulates catecholamine secretion from both sympathetic nerve endings and BAM cells. We find that conotoxin blocks AII-stimulated, but not nicotine- nor bradykinin-stimulated [^{3}H]NE and [^{32}P]ATP release from cultured BAM cells. AII-stimulated $^{45}Ca^{2+}$ uptake is also inhibited, but Ca_i responses (Fura 2) to AII are not blocked, unless intracellular Ca^{2+} pools are depleted with ionomycin or cells are pretreated with phorbol myristate acetate. [^{3}H]Inositol phosphate accumulation in response to AII is unaffected by conotoxin. Since bradykinin stimulates [^{3}H]inositol phosphate accumulation more effectively than AII, yet none of the bradykinin responses are blocked by conotoxin, it seems likely that the conotoxin-sensitive Ca^{2+} influx in response to AII is independent of phospholipase C activation.

66.11

C-FOS IMMUNOREACTIVITY IN HYPOTHALAMIC NUCLEI AFTER DEHYDRATION STRESS. J. M. Ding, P. J. DeCoursey*, and J. Buggy. Depts. of Physiology and Biology, Univ. of South Carolina, Columbia, SC 29208

The proto-oncogene c-*fos* codes for a nuclear phosphoprotein (Fos) that binds to DNA and appears to act as a transcription regulator coupling physiologically relevant stimuli to long-term responses by altering gene expression. Since water deprivation has been reported to increase Fos immunoreactivity (Fos-IR) in nuclei of neurons of the paraventricular (PVN) and supraoptic (SON) nuclei, this study in rats examined other dehydration stimuli. In both Sprague-Dawley and Brattleboro rats, acute intracellular dehydration (hypertonic NaCl, sc) induced Fos-IR in several hypothalamic nuclei associated with body fluid regulation including the OVLT and median preoptic nucleus, subfornical organ, and nucleus circularis as well as SON and PVN. Intracerebroventricular injection (ICVT) of carbachol (which produces responses similar to intracellular dehydration) also induced Fos-IR in SON and PVN. In contrast, hypovolemia or ICVT angiotensin did not potently induce Fos-IR in the above brain areas. These results suggest that osmotic but not volemic hydrational stimuli activate Fos in several hypothalamic nuclei associated with regulation of body fluids.

ALZHEIMER'S DISEASE: COGNITIVE AND CLINICAL STUDIES

67.1

NEUROPSYCHOLOGICAL TEST PERFORMANCE OF ALZHEIMER'S DISEASE PATIENTS WITH AND WITHOUT EXTRAPYRAMIDAL SIGNS. K. Edwards*, D. Salmon, D. Galasko, N. Butters and L. Thal. UCSD School of Med., San Diego, CA 92093.

A variant of Alzheimer's disease (AD) has been described in which the neuropathological changes typical of AD occurred concomitantly with diffusely distributed neocortical and brain stem Lewy bodies. Patients with the Lewy body variant (LBV) evidenced mild extrapyramidal signs (EPS) on neurologic examination and were more impaired than patients with "pure" AD on neuropsychological measures. This study suggested that clinically identified AD patients with EPS differ neuropsychologically from AD patients without such signs. To address this issue, the neuropsychological test performance of 10 clinically diagnosed AD patients with EPS (AD-EPS) (i.e., rigidity, bradykinesia, and masked facies) was compared to that of 10 clinically diagnosed AD patients without EPS. Although the two patient groups were matched for age, education and overall level of dementia, the AD-EPS patients were significantly more impaired than the AD group on a test of verbal fluency and a visuospatial/constructional task but performed similarly on tests of language. The results suggest that AD patients with EPS have a pattern of neuropsychological deficits distinguishable from typical AD patients, and that the deficit profile is similar to that reported for patients with neuropathologically verified LBV.

67.2

NEUROPSYCHOLOGICAL-NEUROPATHOLOGICAL CORRELATES IN ALZHEIMER'S DISEASE. D. Salmon, E. Masliah, R. DeTeresa*, L. Hansen*, N. Butters, R. Katzman and R. Terry. UCSD Sch. of Medicine, San Diego, CA 92103.

Quantification of synapses by microdensitometry of tissue sections reacted with anti-synaptophysin was performed on the midfrontal (MF), inferior parietal (IP) and superior temporal (ST) brain regions of 13 patients with Alzheimer's disease (AD). Plaques, neurofibrillary tangles (NFT), large neurons and ChAT levels were also quantified. Prior to death these patients had been assessed neuropsychologically with Blessed's Information-Memory-Concentration (IMC) test, the Mini-Mental State (MMS) exam, and the Dementia Rating Scale (DRS). Significant correlations were obtained between density of MF synapses and IMC (r=-.72), MMS (r=.67) and DRS (r=.58) scores. MF tangles (r=.65) and large neurons (r=-.58) correlated significantly with only IMC scores. In the IP region, synapse density correlated significantly only with the MMS (r=.60). However, IP NFT correlated significantly with the IMC (r=.79), MMS (r=-.66) and DRS (r=-.63), and IP large neurons with the IMC (r=-.57) and DRS (r=.64). MF and IP ChAT levels correlated significantly with all three neuropsychological tests. These results suggest that the loss of neurons, synapses and acetylcholine play as important a role as plaques and NFT in the development of the dementia associated with AD, and may be the primary determinant of cognitive loss.

67.3

JUDGEMENT OF RECENCY IN ALZHEIMER'S DISEASE. D.S. Knopman and Bret Haake*. Dept. of Neurology University of Minnesota Hospital, Minneapolis, MN 55455.

To quantitate working memory processes in Alzheimer's disease (AD), a judgement of recency test (JOR) was given to 17 AD patients (pts) and 21 normal elderly (NE) subjects. In the JOR test, drawings of common objects were repeated after 0-9 intervening items. Subjects estimated the lag (number of intervening items plus current one) each time a drawing was reshown. Both groups overestimated true lag for items of< lag 4 for AD pts and< lag 6 for NE. Above CL, both groups underestimated true lag, with estimated lags all of approximately (CL + 1).Thus, AD pts judged recency qualitatively similar to NE, but the CL below which the AD pts differentiated one lag from others was much lower than for the NE. Beyond CL, AD pts did not perform randomly: their responses suggested that they based their estimates on weak but still extant memory traces, the weakness of which they interpreted as being approximately one item beyond CL. Alternatively, the defect in AD could represent a contraction of working memory space for intervening items.

67.4

IMPLICIT AND EXPLICIT MEMORY FUNCTIONS IN ALZHEIMER'S DISEASE AND HUNTINGTON'S DISEASE. C. Randolph* and C. Hagger. Clinical Brain Disorders Branch, NIMH Neuroscience Center, Wash. DC 20032

Implicit and explicit memory functions were studied in normal controls, patients with Alzheimer's (AD) and Huntington's disease (HD) using a priming paradigm involving a word-stem completion task. This paradigm has been previously used to demonstrate "preserved" implicit memory function in amnestics. It has also been reported that AD patients do not exhibit this preserved susceptibility to priming. A measure of explicit memory was obtained in the present study, under the same conditions that the implicit measure was made. Explicit and implicit recall were positively correlated in all groups. After controlling for explicit recall ability through ANCOVA, AD patients were found to be normally susceptible to the effects of priming on implicit recall. HD patients, however, demonstrated increased susceptibility to priming. The results suggest that implicit memory performance is dependent in part on explicit memory ability, and that cognitively impaired patients may adopt response strategies on indirect measures of memory that differ from those of normal controls.

67.5

INITIAL SYMPTOMS AND LATER NEUROPSYCHOLOGICAL PERFORMANCE IN ALZHEIMER'S DISEASE. D. Freed, C. Hoss*, A. Piccinin* and V. Henderson. Department of Psychology and Alzheimer's Disease Research Center, University of Southern California, Los Angeles, CA 90089.

This study examined the possible relationship between initial symptoms and later neuropsychological performance in a group (N=23) of patients with probable Alzheimer's disease (AD). Two independent raters classified (when unambiguous) caregiver-reported initial symptoms as primarily involving either language or visuospatial function. Inter-rater reliability approached r=.80. For the 13 patients classified with early language (n=4) or visuospatial (n=9) impairment, initial symptoms predicted relative performance on two analogous neuropsychological tests (WMS-R subtests Digit Span and Visual Memory Span) administered an average of 4.8 years after caregiver-reported onset of symptoms. A chi-square analysis indicated a significant association ($p < .05$) between initial symptoms and later neuropsychological performance. These results suggest that initial symptoms may predict cognitive impairment observed years later in AD patients.

67.6

ACTIVATION OF REGIONAL CEREBRAL BLOOD FLOW (rCBF) IN EXTRASTRIATE CORTEX DURING A FACE MATCHING TASK IN PATIENTS WITH DEMENTIA OF THE ALZHEIMER TYPE (DAT). C. Grady, J. Haxby*, B. Horwitz, M. Schapiro*, R. Carson*, P. Herscovitch, S. Rapoport. Lab. of Neurosciences, Nat. Inst. on Aging, Bethesda, MD 20892.

We showed in young and old healthy subjects that rCBF, as measured by [15O]-water and positron emission tomography, is activated bilaterally in occipitotemporal extrastriate cortex during a visual face matching task (Grady et al., J Cereb Blood Flow Metab, 1989, 9:S574). In this study we measured rCBF in 4 patients with mild-moderate DAT (mean age 65±5 yrs) and in 10 healthy older controls (66±5 yrs) during face matching and a sensorimotor control task. All patients performed well above chance on the tasks. The DAT patients did not have significantly lower whole brain CBF in the control task (32.9±3.4 ml/100g/min) compared to controls (36.7±4.5), but did show regions of low flow in temporal cortex. Pixel-by-pixel difference images were computed by normalizing flow values to whole brain CBF and subtracting the control task images from the face matching images. The areas of activation in the difference images from all subjects were then mapped to a standard brain. Patients and controls showed activated rCBF in the same regions of occipitotemporal extrastriate cortex during face matching. In addition, the mean activation in the patients was not significantly different from controls (22±10% vs. 13±7% in controls). These results show that the appropriate areas of extrastriate cortex are activated during object vision in DAT patients even though these regions have low flow, suggesting that the mechanism recruiting these regions for cognitive processing is functionally intact.

67.7

VISUAL EVOKED POTENTIAL COMPONENTS IN DEMENTIA: SELECTIVE DELAY OF FLASH P2 IN PROBABLE ALZHEIMER'S DISEASE. K.L. Coburn and J.W. Ashford. Brain Mapping Lab, Southern Illinois Univ. Medical School, Springfield, IL 62702.

Earlier studies have shown that the P2 component of the flash visual evoked potential (VEP) is delayed, while earlier flash and pattern reversal VEP components are not, in etiologically mixed groups of demented patients compared to nondemented patients or normal controls. This study tested the hypothesis that the flash P2 delay is characteristic of Alzheimer's disease (AD) rather than a general feature of dementia by comparing probable AD (n=23) and unlikely AD (n=8) demented patients to elderly normal controls (n=12). The flash P2 was found to be the only delayed VEP component and the delay was found only in the probable AD group. These results imply that earlier findings were due to inclusion of AD patients in the etiologically mixed demented patient groups, and that VEP testing may be of value in the differential diagnosis of AD within demented populations.

67.8

DISCORDANCE AND CONCORDANCE OF ALZHEIMER'S DISEASE (AD) IN MONOZYGOTIC TWINS INDICATE TWO MODES OF TRANSMISSION. **S.I. Rapoport and M.B. Schapiro. Lab. of Neurosciences, National Institute on Aging, NIH, Bethesda, MD 20892**

Studies indicate that genetics play an important role in the etiology of AD, but the extent of this role is controversial. Two forms of AD have been suggested, a familial hereditary form (with estimated frequencies of 50 to 80% or more); and a sporadic nonheritable form. To test the hypothesis that both forms exist, we determined the incidence of affected first degree relatives of pairs of monozygotic twins concordant or discordant for AD. A total of 29 pairs of twins (4 from our laboratory, 25 from the literature) were evaluated, where disease, monozygosity and family history were documented. Of 14 concordant twin pairs, 11 had affected first degree relatives, whereas 3 did not. Of 15 discordant pairs, 3 had affected first degree relatives, whereas 12 did not. Fisher's exact test indicated that the concordant twins had a significantly more frequent family history of AD than did the discordant twins, ($p < 0.003$). The results suggest that concordant but not discordant twins are more likely to have a heritable form of AD. AD in discordant twins may result from environmental influences or a post-zygotic somatic chromosomal change.

67.9

LINKAGE OF LATE-ONSET FAMILIAL ALZHEIMER'S DISEASE ON CHROMOSOME 19. A.D. Roses, J. Bebout*, L. Yamaoka*, P.C. Gaskell*, W-Y. Hung*, A.P. Walker*, M.J. Alberts*, C. Clark*, K. Welch*, N. Earl*, A. Heyman* and M.A. Pericak-Vance*. Dept. of Medicine and Dept. of Neurobiology, Div. of Neurology, Duke Univ. Med. Ctr., Box 2900 Durham, NC 27710.

We have studied 28 families with late-onset Alzheimer's Disease (LOAD; mean=69.8 yrs). The linkage analysis included 258 family members (129 affected). Twenty-five families had 3 or more affecteds and 15 were autopsy confirmed. Due to the late-onset, the mode of inheritance (i.e., recessive vs. dominant AD) could not be clearly determined so the data were initially examined with the Affected Pedigree Member Method of Weeks and Lange (Am. J. of Hum. Genet., 42:315-326, 1988) which detects linkage and/or association but does not depend on the mode of inheritance and uses data on affecteds only. This analysis produced significant test results for two markers on chromosome 19: BCL3 and D19S13. These markers were then tested using standard likelihood methods assuming autosomal dominant inheritance. When the analysis was limited to the affecteds only, making no assumptions concerning the disease status of other family members: BCL3, z=2.5 at $\hat{\theta}$=0.00; for D19S13, z=1.84 at $\hat{\theta}$=0.05. Another polymorphic probe, ATP1A3 was then selected for testing because of its location between the other two probes on chromosome 19. ATP1A3 analysis gave z=1.73 at $\hat{\theta}$=0.05. Multipoint analysis (MA) which combines data from several loci (BCL3-ATP1A3-D19S13): gave z=4.6 for affecteds only analysis, inclusion of at-risk individuals in the analysis after the adjustment for age of onset gave z=2.48 Six additional probes from the proximal long arm of chromosome 19 are under investigation. D19S19, D19S29, Cyp2a/2b, D9S9, ApoC2, CKMM. To date (4/90) each probe has demonstrated positive lod scores. Updated lod scores including MA analysis will be presented.

67.10

NICOTINE AND HUMAN COGNITION

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The reduction in nicotine receptors in the neocortex and hippocampus of patients dying with dementia of the Alzheimer type (DAT) suggests that stimulating the remaining receptors with nicotine might enable an alternative approach to cholinergic treatment of patients with mild/moderate DAT. A preliminary study of subcutaneous nicotine in groups of young normals and patients with DAT (all n=7) is described. Tests of rapid visual information processing (RIP)(non-spatial working memory), short term spatial working memory and cortical arousal (critical flicker fusion test (CFF)) were modified to allow the baseline performance of the DAT patients to approximate that of normal subjects. Nicotine (0.4, 0.6, 0.8 mg s.c.) produced dose-dependent improvements in the DAT patients in the accuracy and speed of responding in the RIP task, reduced the threshold for CFF, but had no beneficial effect on the test of spatial short term memory. These preliminary observations are extended in two ways: by studying the effects of acute nicotine on larger populations of subjects, including equivalent numbers of smokers and non-smokers; and by investigating the effects of chronic nicotine, administered in the form of chewing gum, on similar measures, but also including an evaluation of effects on indices of everyday living.

67.11

DRINKING HABITS OF EARLY AND LATE ONSET ALZHEIMER'S PATIENTS. M.Bidaut-Russell and G.T.Grossberg*. Dept.of Psychiatry and Human Behavior, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Alcohol as a causative or contributing risk factor for Alzheimer's disease (AD) has been previously evaluated (Amaducci, L.A. et al.,Neurology., 36:922-931, 1986; Heyman, A. et al., Ann Neurol., 15:335-341, 1984). We reviewed 52 deceased patients (35 women and 17 men) with an autopsy-confirmed clinical diagnosis of AD to see whether certain drinking patterns could affect the time of onset of AD in male and female patients. Information about drinking habits was obtained through a questionnaire answered by a patient's next of kin. Ten patients (4 men and 6 women) had early onset AD (EOAD) (first symptoms appearing before age 65). Forty two patients (13 men and 29 women) had late onset AD (LOAD)(first symptoms after age 65). Patients were divided into non drinking, occasional, light, moderate and heavy drinking categories. Four men (23%) and 11 women (31%) did not drink. Forty six percent of women were considered occasional drinkers, 20% light drinkers and 3% moderate drinkers. Twelve percent of men were described as light drinkers, 47% as moderate drinkers and 18% as heavy drinkers. LOAD/EOAD ratio were calculated for male non drinkers (3) and drinkers (3.33) and for female non drinkers (4.5) and drinkers (5). In the female group, occasional drinkers with LOAD outnumbered those with EOAD by a 15 to 1 ratio while light drinkers with LOAD outnumbered those with EOAD by a 2.5 to 1 ratio. Only 1 woman belonged to the moderate drinker category and she had EOAD. All male light drinkers were LOAD patients. Male moderate and heavy drinkers with LOAD outnumbered those with EOAD by a 3 to 1 and 2 to 1 ratio respectively.

These results suggest that, although male patients were more likely to develop early onset AD than female patients, different drinking patterns had little influence on the time of onset of AD within the male and female patients groups.

67.12

ANXIETY AND LOCUS CERULEUS CELL COUNT IN ALZHEIMER DISEASE P.D.Fleming, J. Shaw*, K. Burbank*, P. Engel*, R.Carraway* U.Mass.Med.Sch.,Worc.,MA 01655,U.Conn.,N.Britain Gen.Hosp., N.Brit.CT, Harvard Med. Sch.,McLean Hosp.,Belmont MA 02178

The noradrenergic system may mediate anxiety in part but the nature and extent of this mediation remains unknown. Alzheimer disease (AD) creates a partial lesion of locus ceruleus (LC) in many cases and provides an opportunity to investigate the consequence of noradrenergic cell loss. We studied the LC in 25 AD and 5 control cases.After formalin fixation, the pons was sectioned at ~2 mm intervals, and 3 serial sections were macroscopically selected at sites of greatest LC density, embedded in paraffin, cut at 8 μ sections,stained with hematoxylin and eosin. In each of the 3 sections all LC cells and all pigmented cells were counted bilaterally by two investigators. Hospital records of each case were reviewed and rated for presence of anxiety and agitation each on a 0-5 scale for 3 time periods: before onset of dementia, early in dementia, and middle to late in the dementia. Ratings were also performed on three subtests of Hamilton Depression Scale for agitation, psychic anxiety and somatic anxiety. 15% AD cases showed evidence of anxiety and 10% agitation before the onset of dementia, and 50% anxiety and 75% agitation after the onset for an increase of 35% for anxiety and 65% for agitation. LC cell counts range from 11-109/unilateral nucleus/section for AD group,(mean = 58.3±25.7 SD),and 73-112 (88.4±13.7) for the control group. Initial statistical analysis by correlation suggests the following possible trends:a possible positive correlation between pigmented LC cell count and anxiety before onset of illness, and possible negative correlation between LC cell count and agitation before onset and early in the course of the illness.

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS I

68.1

THE ROLE OF THE BASAL LAMINA MATRIX ON AXONAL GROWTH AND GUIDANCE IN THE RETINA. W. Halfter. Dept. of Neurobiology, Anatomy, and Cell Science, Univ. of Pittsburgh, Sch. of Med., Pittsburgh, PA 15261.

During early development of the retina optic axons navigate with high precision toward the optic disc. The localization of the neurites close to the vitreal surface suggests that axonal guiding molecules are found at this site. To approach this question, the vitreal surface of the retina (inner basal lamina, covered by a monolayer of ventricular endfeet) was isolated. Other basal laminae were obtained from the pigment epithelium and skin. The vetricular endfeet and all basal laminae proved to be excellent substrata for neurite outgrowth, however, guidance cues were not found in all of these preparations. Antisera raised against the various basal laminae inhibited neurite extension not only on the basal laminae but also on the ventricular endfeet. Moreover, the antisera disturbed the morphology and the fasciculation of the neurites. The results show that 1. neurite extension and axonal navigation are independently regulated, 2. basal laminae and ventricular endfeet contain similar or even identical neurite promoting molecules, and 3. matrix components also have a function on the morphology and fasciculation of the neurites.

68.2

NEURITE-INHIBITING PROTEOGLYCANS MAY HELP DETERMINE UNIDIRECTIONALITY OF NEURITE GROWTH IN THE DEVELOPING OR REGENERATING CNS. Diane M. Snow and Jerry Silver. Department of Neurosciences, CWRU, Cleveland, Ohio 44106.

In many regions of the nervous system, axons elongate unidirectionally. However, the mechanisms that control this stereotyped axon guidance are unknown. In the developing eye, the retinal ganglion cells (RGC), positioned at the center of the retina, generate an axon preferentially toward the optic fissure. Similarly, in the dorsal root entry zone (DREZ) of the spinal cord during regeneration of transected/reimplanted roots, axons can exit the CNS into the root, but cannot travel from the PNS into the CNS along the same pathway. What is the reason for these unidirectional growth patterns?

Previous work in our laboratory has shown that a chondroitin-sulfate-containing proteoglycan (KS/CS-PG) is present in functional barriers *in vivo* (e.g. the roof plate) and is inhibitory to DRG neurite outgrowth in a concentration-dependent manner *in vitro*. In the present study, we examined whether this molecule may be involved in the patterning of unidirectional outgrowth in the retina and spinal cord.

Using immunocytochemical techniques, we found that C-6-S is expressed in the central retina of E13 rats and gradually moves peripheral to the optic fissure as the ganglion cells differentiate. By E17, the level of C-6-S is significantly reduced. At the DREZ, C-6-S is expressed on astrocytes in amounts far exceeding controls following root injury. In both examples, axons grow away from or avoid regions which express this molecule.

To test the effect of a CS-PG on directional behavior of neurites in culture, we grew E9 chick dorsal root ganglia on dishes coated with various concentrations of KS/CS-PG mixed with laminin (LN) on one half and only LN on the other half, since LN is expressed both in retina and in the DREZ. The growth pattern in both paradigms was analyzed. DRG neurites which begin on LN stop abruptly at the border of LN and PG/LN. However, the subpopulation of DRG neurites which initiate on the mixture of PG/LN grow unidirectionally toward the LN side of the dish. This same paradigm is currently being used to test direction of growth of RGC neurites.

These *in vitro* and *in vivo* data suggest that C-6-S may play a role in the choice of direction of neuronal elongation. These data also provide evidence that the response of a neurite to CS or other such molecules may depend upon whether the neurite was initiated on the PG or approached the PG after first elongating on a growth-promoting substrate.

68.3

ANTI-ASTROTACTIN BLOCKS NEURONAL MIGRATION *IN VITRO*. G. Fishell and M.E. Hatten. Dept. of Pathology, College of Physicians and Surgeons of Columbia University, NY, N.Y. 10032

The interplay of neurons with glia is critical in the morphogenic placement of neurons during development. Previous work suggests that astrotactin plays a central role in mediating developmental neuron/glia interactions. astrotactin expression is developmentally regulated on *in vivo* and *in vitro* migrating neurons. Furthermore, a series of *in vitro* assays has demonstrated that antisera against astrotactin can prevent the formation of neuron/glia contacts (Edmondson *et al*, J. Cell Biol. 106:505, 1988), and significantly reduce neuron/glia adhesiveness (Stitt and Hatten, Soc. Neurosci Abtr. 15:568 1989). To study neuronal migration *in vitro*, we have developed an assay which allows us to simultaneously track hundreds of migrating neurons in the presence or absence of a variety of immune activities. Culture conditions optimized for neuronal migration were generated by taking granule cell enriched cerebellar preparations and plating them at low density on matrigel. Using this assay we have demonstrated that anti-astrotactin is able to reduce neuronal migration to less than 10% of control levels. In contrast, Fab fragments against L1, NCAM and TAG, antisera against the β_1 integrin chain or combinations of these Fabs and antisera have no effect on the rate of neuronal migration. To investigate the mechanism by which anti-astrotactin blocks neuronal migration, we have recorded actively migrating neurons at high power using DIC optics in a Berg chamber and subsequently micro-perfused anti-astrotactin into the chamber. Within 1hr of the addition of antibodies migration was completely arrested and the cytological features of a migrating neuron (ie the presence of a leading process, a caudally positioned nucleus and cytoplasmic streaming into the leading process) disappeared. This suggests that astrotactin may not only help mediate neuron/glial adhesion but may also orchestrate the events involved in neuronal migration.

68.4

ENTACTIN IS EXPRESSED IN THE EARLY EMBRYONIC AVIAN BRAIN. V. L. Miller and S. A. Moody. Department of Neuroscience, University of Virginia, Charlottesville, VA 22908.

Entactin is an RGD bearing, sulfated glycoprotein that forms stable, non-covalent complexes with laminin in basement membranes. Laminin also occurs along forming axon tracts and may play a role in axon guidance. Therefore, we examined the developmental course of the expression of entactin in order to determine whether it might play a similar role. Tissue sections were labeled with polyclonal antibodies made against recombinant entactin (gift of Albert Chung, Univ. Pittsburg). The entire st 12-13 brain was immunopositive, with outlines of virtually all of the neuroepithelial cells visible in a radial array. The st 15-16 brain continued to be brightly labeled, however staining was most intense in the ventricular layer where cells were still proliferating. Staining was reduced in the intermediate and marginal zones of the hindbrain, which correspond to areas of cell migration and axon tract formation, respectively. By st 17 there was a rostral to caudal gradient in entactin expression; labeling in rostral regions was similar to earlier stages, while that in the hindbrain was markedly reduced. By st 18/19 cellular elements in the brain were less discernable and the entactin immunofluorescence was homogenous in appearance. At st 20 the brain was stained less intensely than the surrounding mesenchyme. At st 24 staining in the brain was not above control levels. We have demonstrated that entactin is expressed early in the developing avian brain. However, its expression is not coordinated with that of laminin: entactin expression is ubiquitous and is not restricted to forming axon tracts, regions of cell migration or the basement membrane. Highest levels of entactin expression are associated with those time periods and locations in which the neuroepithelium is a cohesive germinal zone. Therefore, entactin may play a role in cell-cell attachment.

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68.5

BIOCHEMICAL CHARACTERIZATION OF THE ROCA1 ANTIGEN: A MOLECULE EXPRESSED IN ROSTRO-CAUDAL GRADIENTS IN THE MAMMALIAN NERVOUS SYSTEM.

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Spinal cord axons display a rostrocaudal, positional bias in their innervation of sympathetic ganglia and intercostal muscles (Wigston, D.J. and Sanes, J.R., J. Neurosci., 5:1208-1221, 1985). To examine the molecular basis of this positional specificity, mAbs were produced using the immunosuppression technique which bind preferentially to rostral sympathetic ganglia. We previously reported that the staining of one of these mAbs, ROCA1 (ROstroCAudal), is highest in rostral sympathetic ganglia and intercostal nerves and declines in a graded manner in the caudal segments. ROCA1 stains satellite cells in the ganglia and Schwann cell sheaths in the nerves, suggesting that molecular rostrocaudal differences exist on glial surfaces. We have used an immunoblot analysis to identify the ROCA1 antigen as a 60 kD protein present in membrane fractions of newborn and adult rat sympathetic ganglia and intercostal nerves. Quantification of the rostrocaudal distribution of the antigen in intercostal nerves demonstrates a gradient like that seen with the immunohistochemistry, with about four-fold more protein present in rostral than in caudal nerves. Deglycosylating membrane preparations with ENDO-H or ENDO-F does not eliminate the binding of ROCA1 to the 60 kD protein, and fails to alter the mobility of the 60 kD protein on SDS-PAGE.

68.6

CLONING OF THE NEURON-GLIA LIGAND ASTROTACTIN USING cDNA LIBRARIES FROM EARLY POSTNATAL GRANULE NEURONS

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In the developing cerebellum, we have identified astrotactin and shown that it is the major ligand forming contacts between young granule neurons and radial processes of Bergmann glia, implementing glial guided migration into the internal granule layer (Edmondson et. al., J. Cell Bio 106:505, '88; Stitt et. al., submitted). It is first expressed in mouse just before birth, is active during the first postnatal week and is absent in the adult. We sought to clone astrotactin for structural and functional analyses by first producing mouse cDNA libraries, directionally cloned into a modified λgt11 vector, starting from from $2x10^8$ granule cells taken at postnatal day 3-5 and purified on Percoll gradients In the primary library, approximately 10^7 recombinants, of average size 2 kb and with defined orientation, were obtained. After amplification, 10^6 recombinant phage were screened with astrotactin antibody which, for the tertiary screen, had been purified by absorption with PC12 cells and adult cerebella. Of 3 clones selected, AN 1 contains a 500 bp insert which has a DNA sequence not found in our searches of GenBank. Moreover, antibody eluted from plaque lifts of this phage produces a 100 kD band on Western blots, corresponding to the molecular weight of authentic astrotactin. We conclude that AN 1 is a likely candidate cDNA coding for astrotactin and are now generating the full length species.

68.7

DEFASCICULATION OF NEURITES INVOLVES THE MANNOSE-CONTAINING EPITOPE OF THEIR SPECIFIC 130 KD SURFACE GLYCOPROTEIN.

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In leech neuronal development, bundles of sensory afferents enter the CNS and defasciculate, dispersing to their connections. This defasciculation is inhibited by three independent perturbation experiments that target the interaction between the sensory afferents' unique 130 kD glycoprotein and a carbohydrate-binding protein within the neurites' environment. The experiments are performed in the germinal plate of 10-11 day embryos. The macromolecular permeability of the germinal plate allows us to experimentally manipulate neuronal growth in a virtually intact nervous system. This is important since cell-type specific surface glycoproteins can be differently regulated *in vivo* and *in vitro* (Barakat et al. Biology of the Cell, 66, 1989). The binding Lan3-2 Fab fragments (6-12 nM) to the mannose-containing epitopes of the 130 kD glycoprotein forces the neurites to remain associated in a tightly fasciculated axon tract. A similar result is obtained by deglycosylating the 130 kD glycoprotein with N-Glycanase (4 units/300 ul). Addition of mannosylated BSA (1 uM), which competes for the mannose-binding protein, also inhibits the defasciculation of sensory afferents. These results suggest that carbohydrate recognition, which is well documented outside the nervous system, plays a major role in regulating neuronal architecture in the CNS.

68.8

CHEMOTROPIC INDUCTION OF AXON COLLATERAL BRANCH FORMATION BY TARGET TISSUE IN VITRO

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The corticopontine projection, which arises from layer 5 of neocortex, develops by delayed interstitial budding of collaterals from corticospinal axons, rather than by a direct ingrowth of primary axons or by a bifurcation of the growth cone; branches form in the axon tract overlying the basilar pons (O'Leary & Terashima 1988 *Neuron* 1:901). When co-cultured with explants of cortex in 3-dimensional collagen gel matrices, basilar pons elicits the directional growth of cortical axons and collaterals (Heffner, Lumsden & O'Leary 1990 *Science* 247:217). Here we present quantitative evidence that basilar pons induces interstitial branching of layer 5 cortical axons in the cortical explant and in the collagen gel. Full thickness pieces of P0-P1 rat motor cortex were explanted into collagen gels. 24 hrs later, P0 "target" explants of basilar pons or a control tissue (olfactory bulb) were placed in the gel lateral to axons already extending from the ventricular surface of the cortical explant. After 48 total hrs, the cultures were aldehyde fixed and DiI was injected into the "target" explant, or directly into the collagen in the path of the outgrowing axons in cases in which no "target" explant was added, to retrogradely fill cortical axons, branches and their cells of origin. In cultures of cortex alone or with control tissue later added, branches are not seen in the collagen gel but occasional branches form randomly to right or left in the cortical explant; labeled cell bodies are distributed through all cortical layers with 29% in layer 5. In cultures with basilar pons added, cortical axons branch in the collagen gel with all branches directed toward the pons, and many branches are seen in the cortical explants with 93% directed toward the pons; 86% of labeled cortical neurons are in layer 5. We conclude that basilar pons releases a diffusible tropic signal that acts preferentially on layer 5 neurons to direct axon growth, and also induces the budding of axon collateral branches, within both a neural and collagenous substrate. Support: NEI grant EY07025.

68.9

ANTIBODIES TO 9-O-ACETYLATED GANGLIOSIDES CAUSE RETRACTION OF NEUROBLASTOMA AND DRG GLIAL PROCESSES.

I.E. Friedman, S.M. Fortune* and M. Constantine-Paton. Dept. of Biology, Yale Univ., New Haven, CT

The JONES monoclonal antibody (MAb) recognizes a family of gangliosides identified as 9-O-Acetylated-GD3 and -GQ1c (*Lipids* 24:680,1989). These antigens are expressed along both spatial and temporal gradients in the developing rat embryo nervous system (*Nature* 324:459,1986), suggesting a role in neuronal guidance. We have asked whether the application of JONES interferes with process extension. B65 neuroblastoma cells which express both A2B5 and JONES antigens were selected by "panning". Cells were plated at a very low density (1-10,000 cells/ml) on collagen or poly-L-lysine (PLL) coated coverslips. After several hours, when cells had sprouted processes, we recorded using video-enhanced microscopy. Approximately 50% of the cells are quiescent, 35% are extending processes and 15% are oscillating - oscillating cells grow for 15-20 minutes and then retract at about the same rate. Continuously growing cells do so for >3 hrs. We taped single cells for 20-30 minutes to determine if they were growing, non-oscillating cells. The medium was replaced with one containing JONES (0.5 mg/ml). Within 5-10 minutes a rapid retraction of growing processes could be observed. Usually within 40 min. the cells rounded up but continued to extend and retract filopodia. This rapid retraction was not observed on PLL coated coverslips or with similar concentrations of MAb A2B5, which recognizes several gangliosides including GD3 and GQ1c. In primary DRG explants grown on fibronectin, addition of the JONES MAb caused elongated GFAP positive cells to round up. Neurites were not affected directly by JONES. However, glial rounding was associated with collapse of the neurite web. Addition of the anti-integrin β1-subunit antibody induced retraction of neurites only and A2B5 had no effect. We are continuing our investigations with both the neuroblastoma and DRG explants using different substrates. We propose that the gangliosides 9-O-acetylated-GD3 and -GQ1c can modulate adhesion and thus, by virtue of their topological and temporal distribution, influence the circuitry of the developing nervous system.

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68.10

OLIGODENDROCYTES CULTURED FROM ADULT RAT ARE PERMISSIVE FOR RETINAL NEURITE OUTGROWTH.

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To test the hypothesis that oligodendrocytes inhibit neurite outgrowth (and therefore axonal regeneration in CNS), explants of embryonic day 15 rat retina have been co-cultured with oligodendrocytes from adult rat spinal cord. Dissociated spinal cord cultures were treated with fluorodeoxyuridine for 2 wks then replated onto polylysine-coated coverslips. Cells were labeled with antibodies to galactocerebroside (>90 % of cells positive) and glial fibrillary acidic protein (<5 % positive), showing the cultures to be mostly oligodendrocytes with few astrocytes present. Retinal explants placed on these cell layers all extended neurites within 5 days (n=44). Explants placed directly on polylysine did not extend neurites (n=23). Neurites, identified by antibodies to neurofilaments or by phase contrast, grew both on the comparatively rare astrocyte surfaces and on the galactocerebroside-positive processes of oligodendrocytes, which spread to cover most of the area of the cultures. Neurites neither followed oligodendrocyte processes as if guided by them nor avoided them. It is concluded that oligodendrocytes in some circumstances can provide a permissive substratum for neurite outgrowth. Supported by NIH BRSG 2 S07 RR05386.

68.11

IN VITRO INTERACTIONS BETWEEN MERKEL CELLS AND APPROPRIATE (SENSORY) AND INAPPROPRIATE (SYMPATHETIC) NEURONS. Randall N. Pittman, Weslia Patterson*, Steve Pakola*, and Peter Vos. Dept. of Pharmacology, Univ. of PA School of Medicine, Philadelphia, PA 19104.

Cultured Merkel cells contain NGF and provide trophic support for NGF-dependent sympathetic and sensory neurons. Sensory neurons extend neurites onto Merkel cells in vitro and branch extensively following contact. Neurites of sympathetic neurons routinely do not grow onto Merkel cells, and growth cones often collapse and retract following contact with Merkel cells. Fixation of Merkel cells with paraformaldehyde does not alter the number of contacting sensory or sympathetic neurons; however, the extensive branching of sensory neurons is decreased to control levels. Studies are underway to investigate the effects of altering levels of NGF produced by Merkel cells on the extent of neurite arborization on Merkel cells, and to determine the molecular mechanisms responsible for the selective contact of Merkel cells by sensory neurons and the avoidance of Merkel cells by sympathetic neurons. Supported by the American Paralysis Association and McKnight Foundation.

68.12

PROMOTION OF PROCESS OUTGROWTH FROM ADULT FROG MOTONEURONS IN CULTURE BY A MUSCLE ECM EXTRACT Damien P. Kuffler and Theres Luethi*. Institute of Neurobiology, Univ. of Puerto Rico, Old San Juan, Puerto Rico 00901; *Dept. of Pharmacology, Biocenter, Basel, Switzerland.

Motoneurons isolated from adult frogs, Rana pipiens, survive and sprout for four weeks in culture in a defined medium (Erulkar et al., '90, J. Physiol., in press). Macrophages cultured along with the motoneurons interact with the motoneuron processes to bring about their elongation. Contact by a migrating macrophage with the tip of a motoneuron process, but not along its length, causes the process to elongate in association with the continued migration of the macrophage.

In a further series of experiments the influence of various substrates on process outgrowth from adult motoneurons have been tested. Substrates tested include polylysine, Con A, mouse EHS laminin and a 10 mM EDTA extract of innervated skeletal muscle extracellular matrix (ECM). Of these, muscle ECM promotes the best process outgrowth. Motoneurons attach to this substrate and extend processes after 1.5 days and the processes reach an average length of 150 μm by 14 days. We have now partially purified an ECM fraction that is far more effective in promoting attachment of motoneurons and process outgrowth. On this extract process outgrowth starts within 12 hours and the processes achieve a ten fold greater length, 1.5 mm by 14 days. When run on a SDS gel bands are seen to co-migrate with mouse EHS laminin under reduced and non-reduced conditions. These results suggest that the ECM fraction might contain a laminin-like molecule. We are presently attempting to characterize this ECM factor.

68.13

TEMPORAL AND SPATIAL REGULATION OF SIALATED GANGLIOSIDE (GQ)-LIKE IMMUNOREACTIVITY ON A STEREOTYPED GROWTH CONE ARRAY. J. Jellies. Neurobiology Research Center & Dept. of Physiology & Biophysics, Univ. of Alabama, Birmingham, AL, 35294.

Each oblique muscle organizing cell (Comb- or C-cells) in the embryonic medicinal leech (*Hirudo medicinalis*) projects a parallel array of about 70 growth cones that navigate through the germinal plate in a stereotyped fashion. These growth cones exhibit two distinct phases of motility; an early, slowly advancing phase as they align parallel and second, a rapidly advancing phase where all growth cones extend toward the edges of the germinal plate. One might predict that molecules favoring the different motility phases would be differentially expressed. The IgM mAb A2B5 recognizes a sialated ganglioside -GQ- (Eisenbarth, et al., PNAS, 76:4913, 1979), has been used extensively as a cell-specific marker of vertebrate neurons and type 2 astrocytes (Raff, Science, 243:1450, 1989) and recognizes most, if not all embryonic leech axons as well as the C-cells in acid/alcohol-fixed whole-mounts. Embryos from each of three groups of siblings were dissected and fixed at 24 hour intervals from embryonic day 9 to 14 then at 48 hour intervals to day 18. The GQ-Like Immunoreactivity (GQLI) is temporally regulated - C-cells exhibit no GQLI during the slowly advancing phase but do label intensely just after the onset of, and during the rapidly advancing phase. Furthermore, GQLI on C-cells is largely restricted to the growth cones, with little staining of processes or somata. In negative controls, sibling embryos processed without primary mAb, or with a different IgM show no specific staining. Although the function of GQ is not understood, and the antigen recognized by mAb A2B5 in leech has not yet been identified, carbohydrate moieties have been implicated in neuronal pathfinding and recognition. The spatial and temporal distribution of GQLI on C-cell growth cones is that predicted for a molecule associated with the ability of growth cones to advance through an otherwise highly adhesive environment rather than one involved in the early interactions orienting growth cones. (Supported by NIH NS28603-01 to JJ; mAb's A2B5 and HNK-1 were the kind gifts of E. Geisert).

CONTROL OF POSTURE AND MOVEMENT II

69.1

REGIONAL CEREBRAL BLOOD FLOW CHANGES IN IPSILATERAL AND CONTRALATERAL MOTOR AREAS DURING UNILATERAL MOVEMENTS. T. Zeffiro and M. Hallett. Human Motor Control Section, NINDS, National Institutes of Health, Bethesda, MD 20892.

In order to determine the relative involvement of cortical and subcortical motor areas ipsilateral and contralateral to limb movement, we have examined the changes in regional cerebral blood flow (rCBF) in subjects performing unilateral flexion and extension movements of the wrist. We studied 12 subjects with positron emission tomography utilizing 30 mCi injections of H_2O^{15}. Emission scanning continued for 1 minute following injection of the tracer. Each subject underwent 6 scans: 2 at rest, 2 during alternating wrist movements and 2 during passive vibration of the palm. All movements were self-paced, with an average rate of 1 cycle per second. For analysis, each scan was scaled to a mean of 100 and then active and rest scans were subtracted to obtain a difference image representing the net change in rCBF. Circular regions of interest (8mm diameter) were examined in both subcortical and cortical motor areas.

The largest increases were observed in cortical areas, including: the ipsilateral and contralateral sensorimotor cortex, premotor cortex, posterior parietal cortex and the supplementary motor area. There was a strong relationship between the contralateral and ipsilateral increase in sensorimotor cortex in each subject, such that the ipsilateral increase was 30% to 40% of the corresponding contralateral change. Increases in premotor and supplementary motor areas were less lateralized.

These results demonstrate a previously unreported activation of ipsilateral motor cortex during the performance of a relatively simple, unilateral motor task. The involvement of ipsilateral cortical motor areas in the planning or execution of simple, alternating limb movements may be more extensive than previously thought.

69.2

"VIRTUAL TRAJECTORIES" OF SINGLE-JOINT MOVEMENTS SHOW TWO BASIC STRATEGIES. M.L.Latash and G.L.Gottlieb. Rush-Presbyterian-St. Luke's Medical Center, Chicago, IL 60612

We have recently described a method for reconstructing shifting joint compliant characteristics (JCCs) during slow movements. Further processing of the data leads to reconstructing "virtual trajectories" (VTs) of the movements. Calculating total torque as a sum of inertial and external components allowed applying this method to movements of higher speeds. In our experiments, the subjects learned a simple elbow flexion movement against a torque bias and were asked to reproduce the learned command irrespective of possible external torque changes. In different series, the amplitudes were about 18°, 36°, and 54°, and movement times were about 250 ms, 500 ms, and 800 ms. Slow torque changes (loadings or unloadings over 300, 500, or 800 ms) took place in half of the trials starting 100 ms after the tone signal to initiate the movement. The individual trials were later aligned according to the moment of visible deflection of the acceleration trace corresponding to the beginning of the voluntary movement (t_0). Acceleration, angle and torque values were measured at different times t_i after t_0. Angle and total torque for each t_i were plotted in the torque-angle plane and linear regression equations were computed (coefficients of linear correlation over 0.8 for more than 50% of the sets). The regression equations let one assess changes in the JCC threshold and slope during a movement and calculate a VT. For slow movements, the VT was monotonic and ahead of the averaged "actual trajectory". VTs of fast movements were N-shaped irrespective of movement amplitude. There was a considerable increase in the JCC stiffness in the middle of the fast movements. Fast movements over different amplitudes (Speed-Insensitive Strategy) had coinciding VTs during the initial phase. Both ascending arms of the N-shape were directly scaled with the amplitude. VTs for movements with different speeds (Speed-Sensitive Strategy) diverged at the very beginning. The method allows reconstruction of VTs in a wide range of movement speeds.

The study was supported by NIH grants AR 33189 and NS 15630 and American Paralysis Association grant LA1-8901-1.

69.3

GUIDANCE OF VOLUNTARY MOVEMENT. P.R. Burgess and T.A. Cooper*. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108.

Two strategies might be used to move an object from one place to another. Model A: The voluntary motor system uses preselected torque-angle relationships to guide a load to a particular final position. Any unanticipated difference in the size of the load will produce a position error whose magnitude is determined by the steepness of the torque-angle profiles and the size of the difference, assuming that sensory feedback is not used to make a voluntary shift to a more appropriate torque-angle relationship. Thus, the greatest accuracy is achieved with the steepest profiles (the stiffest joint). Model B: If involuntary torque-angle relationships are not steep but relatively flat, even a small unanticipated difference in lifted weight, if uncorrected by an appropriate change in effort, would result either in little or no movement or an overshoot error that could easily be many degrees in amplitude. As a result, Model B is highly dependent on feedback to regulate voluntary commands. When subjects are asked not to intervene voluntarily during an abrupt change in load, Model A is supported. When they are asked to make the same effort in isometric tests at different joint angles, Model B is supported. What are the implications of these observations for the role of high level sensory feedback in the guidance of voluntary movement?

69.4

KINESTHETIC TRIGGERING MECHANISMS IN MOVEMENT SEQUENCES. P. J. Cordo, R. S. Dow Neurological Sciences Institute, Portland, OR 97209

This experiment examined how the nervous system processes kinesthetic input from 1 joint to trigger movement at another joint in movement sequences. Human subjects opened the right hand without visual feedback as the right elbow was passively extended through a target angle 25 deg from the starting angle. Seven constant velocities (18-85 deg/s) were presented in random order. Two variations of this experiment included rotations which either stopped or changed velocity before reaching the target.

The results indicate that:
1] Subjects can use kinesthetic input from the passively rotating elbow to open the hand with great accuracy. Depending on the velocity, the average error ranged from 0.5-3 deg.
2] The latest time at which kinesthetic input can influence the timing of the hand movement is 100 ms before the hand begins to open.
3] During the elbow rotation, the nervous system continuously calculates the angle in front of the target at which the motor command must be triggered so the hand opens at the target angle.
4] The calculation of this "triggering angle" is based on kinesthetic input related to the elbow velocity. Presumably, this triggering angle is compared to elbow angle during the rotation.

69.5

PREMOVEMENT MODULATION OF THE SOLEUS H REFLEX IS NOT ALTERED BETWEEN NON-TRACKING, TRACKING, AND PERTURBED TRACKING TASKS. **D.F.Collins, J.D.Brooke, W.E.McIlroy.** Neurophysiology Lab., School of Human Biology, University of Guelph, Guelph, Ontario, Canada, N1G 2W1.

Plasticity of gating of the homonymous Ia afferent pathway to Soleus (Sol) motoneurons just prior to bilateral ankle movement was studied in six subjects. The research investigated if the characteristic agonist and antagonist premovement modulation could be altered when the "need" for that afferent input was changed. Non-tracking, tracking, and perturbed tracking tasks were used as low, medium and high "need" situations respectively. Each subject took part in two sessions; in one, the movement was plantarflexion (Sol agonist), and the other, dorsiflexion (Sol antagonist). The perturbations rotated the ankle in the direction of movement, requiring correction by the antagonist, and were delivered after the response signal and before movement onset. They were introduced before approximately 20% of the trials within a block, in a random order. Sol H reflexes were evoked between the response signal and movement onset in all trials. Only H reflexes from the non-perturbed trials were collected for analysis. They were separated into 20 ms bins, relative to contralateral EMG onset, and averaged (95% CI=±15%). Control reflexes were collected during each experimental session, prior to the delivery of the warning signal. Stimulation intensity was maintained at the lowest level at which the H reflex could be evoked concommitantly with a small but stable M wave. Results indicate that this task "need" does not alter the pre-movement modulation. Also, comparison of the control reflexes indicates no change in the ongoing tonic control exerted on this pathway between these tasks. The lack of plasticity in the afferent gating shows that adaptations for such task changes are achieved through other inputs to the motoneurons.
Supported by NSERC Canada Grant #A0025.

69.6

REFLEX REVERSAL DURING HUMAN RUNNING: DIFFERENTIAL CONTROL OF BICEPS FEMORIS AND TIBIALIS ANTERIOR. J.Duysens, A.A.M.Tax, M.Trippel and V.Dietz. Dept. of Med. Physics & Biophysics, K.U.Nijmegen, 6500 HB Nijmegen, The Netherlands and Dept. of Clin. Neurology & Neurophysiology, Univ. of Freiburg, D-7800 Freiburg i. Br., F.R.G.

By electrically stimulating the sural nerve at the ankle one obtains ipsilateral reflex responses with a latency between 57 to 85 ms in biceps femoris and tibialis anterior in humans at rest. This coactivation suggests that both muscles participate in a common flexor reflex. During running the two muscles differ in their spontaneous activity pattern and the question arises whether reflex coactivation would persist under these conditions. Therefore the modulation of these reflex responses to sural nerve stimulation during the step cycle was studied in human volunteers running on a treadmill.

Biceps femoris showed a small amount of spontaneous activity during early and middle stance, while a large burst occurred in the second half of the swing phase. In contrast, the reflex responses in this muscle reached quite a large amplitude at the beginning and near the middle of the stance phase while being small during swing. Tibialis anterior was active at the start and the end of the swing phase and this roughly corresponded to the period of maximum reflex activations. Hence the ipsilateral biceps femoris had responses which were out of phase both with its spontaneous activation during running and with the responses in the tibialis anterior. This reversal indicates that reflex pathways to these two muscles are controlled indepently during running. Equivalent results were obtained in the leg contralateral to the stimulation: crossed biceps femoris responses with a latency of about 80 ms occurred predominantly during the contralateral stance phase.

69.7

FUNCTIONAL CHARACTERISTICS OF REFLEX COACTIVATION IN MAN. F. Lacquaniti, N.A. Borghese* and M. Carrozzo*. I.F.C.N., C.N.R., 20131 Milano, Italy.

The behavior of stretch reflexes in man has most often been investigated during tasks involving position control. Only recently has it begun to investigate reflex behavior during mechanical interaction of the limb with an object. Thus, it was found that during a catching task the reflex responses evoked by ball impact on the hand violate the law of reciprocal innervation, since antagonist muscles are coactivated. To discriminate between central preset and peripheral effects (e.g. skin stimuli) on the reflex, we studied here the behavior of stretch reflex responses evoked by torque motor perturbations applied before and during catching. Under basal conditions, such responses do conform to the law of reciprocal innervation. Thus, we considered that if reflex coctivation was contigent on the peripheral stimulus of ball impact, no changes in the stretch reflex evoked by torque pulses would occur prior to impact, while if reflex coactivation can be centrally preset, a reversal of the stretch reflex would occur prior to impact. The latter case was experimentally found. By computing the temporal changes of the mechanical impedance of the hand during catching, we found that this parameter is effectively maximized around impact time.

69.8

KINEMATIC AND KINETIC CHANGES DURING THE LEARNING OF A MULTI-ARTICULAR KICKING MOVEMENT. R.P. Young and R. Marteniuk. Dept. of Kinesiology, University of Waterloo, Waterloo, Ont., Canada, N2L 3G1.

The present study examined the changes in kinematics and kinetics that accompany the learning of a kicking task. Subjects performed 15 blocks of 16 right-legged kicking movements with a 1.67 kg weight attached to the sole of their foot. Subjects attempted to perform all movements as close as possible to a goal MT of 400 ms. Changes in temporal accuracy indicated that subjects learned the movement. Movement kinematics, as measured by end-effector path and hip-, knee-, and ankle-angle trajectories, changed minimally as the movement was acquired. In contrast, net muscle moments, as calculated using inverse dynamics, became stereotypic as the movement was learned, even after temporal accuracy had stabilized. Also, net-muscle-moment trajectories for adjacent joints became more coordinated as the movement was learned. Therefore, subjects learned to perform the movement by producing a preferred set of motor commands, in the form of coordinated net-muscle-moment trajectories.

69.9

BIOMECHANICS OF HUMAN HEAD MOVEMENT CONTROL IN THE SAGITTAL PLANE. W. Graf, D.H. Wang, C. de Waele*, P.P. Vidal and B. Jaenisch*. The Rockefeller University, New York, NY 10021; Lab. Physiologie Neurosensorielle, CNRS, 75270 Paris, France; Schildautal-Klinik, 3370 Seesen/Harz, FRG.

The range of motion of the head-neck joints was measured form X-ray photographs taken at rest, in extreme flexion and extension. Movement strategies of flexion/extension movements were obtained via fluoroscopy imaging. Most of the data could be retrieved from the published literature.

As in other vertebrates, including monkeys, there is only a limited range of motion between most cervical vertebrae with the largest excursions possible in the atlanto-occipital articulation and at the cervico-thoracic junction. As in monkeys, the range of motion of the atlanto-occipital articulation is very limited (8-13 deg). However, unlike in other vertebrates, all cervical vertebrae in human are much more brought into play during the observed flexion/extension movements. In particular, significant translational excursions are obvious. Nevertheless, flexion/extension movements are largely constrained to occur at the atlanto-occipital articulation and at the cervico-thoracic junction. In particular, the latter is used for most flexion/extension movements. Similar to the monkey, this may be a consequence of the limited range of motion of the atlanto-occipital articulation. Thus, large flexion/extension movements of the head-neck ensemble are only possible by moving the entire cervical column in the sagittal plane. - Supported by USPHS grant EY-04613.

69.10

QUANTIFICATION OF ELECTROMYOGRAPHIC ACTIVITIES USING CIRCULAR STATISTICS. D. Bourbonnais, M. Goyette*, J. Filiatrault*, J. Gauthier*, A.B. Arsenault, and D. Gravel*, Montreal Rehabilitation Institute and School of rehabilitation, University of Montreal, Montreal, Quebec, Canada, H3C 3J7.

Circular statistics were used to characterize electromyographic activity (EMG) recorded in the lower limb of normal subjects. Subjects (n=12) were required to maintain a static torque (approximately 30% Max) at the hip in abduction(0°), adduction(180°), flexion(90°), extension(270°) and in intermediate directions (45°, 135°, 225°, 315°). EMG activity of each muscle was represented by a vector. The vector's magnitude was determined by the average value of the rectified EMG and its angle was determined by the direction of the torque. The EMG vectors, in all of the eight directions, were summed for each subject. The resultant vector represented the mean angle and mean EMG magnitude. Among subjects, the mean EMG activity of the gluteus medius, rectus femoris, gracilis, biceps femoris and gluteus maximus was observed at 28°, 70°, 150°, 274° and 325° respectively. The Hotelling two-sample test revealed that activity of these muscles was significantly different amongst themselves ($p<.05$). These results indicate that circular statistics are a potential tool to characterize direction and magnitude of muscle activities.

This project is funded by NHRDP, MRC and FRSQ.

69.11

RED NUCLEUS DISCHARGE DURING REACHING IN CATS. K.M. Horn, P.L.E. van Kan and A.R. Gibson. Barrow Neurological Institute, Phoenix, AZ, 85013.

Magnocellular red nucleus (RNm) contains discrete populations of neurons that discharge during movements of the forelimb or hindlimb. This study was undertaken to determine if a coordinated whole limb movement recruits activity in a high proportion of RNm neurons. To date, we have recorded from 17 forelimb RNm neurons in a cat trained to reach out and retrieve a lever. The lever was mounted on a torque motor so that loads and perturbations could be introduced during movement.

All units have shown large modulations in discharge during the task. Mean firing rate increased from 25 ± 18 imp/sec at rest to 161 ± 88 imp/sec during movement. While the responses of RNm neurons were consistent across repeated reaches, individual neurons preferentially responded to different aspects of the task. Surprisingly, no unit has shown a response to perturbations introduced during movement, and only one unit has shown a load-related response (a firing decrease with increased load). The reaching paradigm promises to be a valuable motor task for analyzing the contribution RNm makes in the control of forelimb movements.

69.12

NOCICEPTIVE INFLUENCES UPON RUBROSPINAL CELLS IN THE CAT. H. Steffens* and Y. Padel. Lab. Neurosciences Fonctionnelles, Equipe "Mécanismes Sensori-Moteurs", CNRS, 13009 Marseille, France.

The red nucleus (RN) in the cat receives somaesthetic messages through a medial subdivision of the spinothalamic system which gives off collateral fibers to the RN (Padel, Y. et al., Behav. Brain Res., 28:139-151, 1988). It is a common knowledge that nociceptive messages are conveyed to the upper nervous system through the spinothalamic system. The aim of the present experimental series was to check whether nociceptive information is able to modify the rubral activity when the RN is isolated from cerebellar and cortical influences.

Acute experiments were carried out on cats decerebrated at the level of the caudal thalamus and in which the brachium conjunctivum was sectioned stereotaxically. The RN cell activity was recorded while noxious radiant heat was locally applied to the skin.

In this cat preparation it was confirmed that the tonic activities of the red nucleus cells are modulated when the cutaneous temperature reaches the nociceptive threshold. When the stimulation is applied inside the somaesthetic peripheral field of the cell under study, it induces progressive acceleration or deceleration of spike frequency.

This result demonstrates that the nociceptive information participates in the sensori-motor regulation through the spino-rubro-spinal loop.

NERVE GROWTH FACTORS I

70.1

NERVE GROWTH FACTOR RECEPTOR IMMUNOREACTIVITY IN HYPOTHALAMO-PITUITARY AXIS, PINEAL GLAND AND CEREBELLUM IN HUMANS AND MONKEYS. E.J. Mufson, S.D. Styren, Inst. for Biogerontology Research., Sun City, AZ 85351, G.A. Higgins, NIA, Bethesda, MD and J. H. Kordower, Univ. Illinois Med. Sch., Chicago, IL.

The degree to which nerve growth factor (NGF) may be involved with non-cholinergic systems remains unclear. Using a monoclonal antibody (NGFR5), nerve growth factor receptor (NGFR) immunoreactive (IR) profiles were observed in the hypothalamo-pituitary axis, pineal gland and cerebellum in postmortem paraformaldehyde fixed tissue from aged normal humans and adult New World monkeys (Cebus apella).

The human hypothalamic tuberoinfundibular region contained NGFR-IR small bipolar type cells and fibers of varying thickness as seen with light and electronmicroscopy (EM). Thin IR fibers appeared beaded with swellings indicative of Herring bodies. The infundibulum (median eminence) exhibited a dense matrix of NGFR immunoreactivity including vascular, cellular and fiber staining in both species. Human pituitary reacted for NGFR and viewed with light and EM revealed IR fibers, cells as well as vascular staining within the pars distalis and pars nervosa.

Pineal gland processed for NGFR-IR and analyzed at the light and EM levels revealed IR cells in the human.

Cerebellum reacted for NGFR-IR revealed periodic columns of immunoreactivity extending from the Purkinje cell layer throughout the molecular layer within the lateral cerebellar (neocerebellum) and floccular nodular (archicerebellum) lobes. Receptor immunoreactivity was present in a large percentage of Purkinje cells and dendrites. Regional differences in columnar and Purkinje cell staining occurred with the most intense NGFR immunoreactivity in the floccular nodular lobe. mRNA NGFR in situ hybridization also revealed positive Purkinje cells.

These findings suggest NGF may play a key role in primate neuroendocrine and motor function. Support: AHAF, NS 26146 NS 25655 and NS 26845.

70.2

TEMPORAL EXPRESSION, *IN VIVO*, OF LOW AND HIGH AFFINITY NERVE GROWTH FACTOR RECEPTORS IN QUAIL EMBRYOS J. Speight and P. Bernd, Dept. of Anatomy and Cell Biology, SUNY Health Science Center, Brooklyn, NY 11203.

Since nerve growth factor (NGF) receptors, as detected by ^{125}I-NGF binding, are not demonstrable in neural crest (NC) cultures until 3 days past explantation (comparable to E5 *in vivo*), premigratory and early migratory NC cells probably do not require NGF for initial differentiation and migration. We examined quail embryos *in vivo* at stages 19 to 24 (E3 to E4) for both low and high affinity NGF receptors. Serial cryostat sections (10μm) were exposed to either high (20ng/ml; 800pM) or low (2ng/ml; 80pM) concentrations of ^{125}I-NGF (90 min, 37°C); nonspecific binding was estimated with an excess of nonradioactive NGF (1μg/ml). Dorsal root ganglia (DRG) in stage 35 (E8) embryos were used as positive controls. NGF receptors were localized with radioautography, while migrating NC cells were identified in adjacent sections with the monoclonal antibody, HNK-1. At the higher concentration, specific ^{125}I-NGF binding was first seen at stage 21 (E3.5) to the lateral motor columns (LMC) and to HNK-1 positive cells beneath the ectoderm. The NGF receptors on the LMC and NC cells appear to be only of the low affinity subtype, because of the absence of ^{125}I-NGF binding at the low concentration. At the lower concentration, specific ^{125}I-NGF binding, characteristic of the high affinity receptor subtype, was first seen at stage 23 (E3.5 to 4) to DRG. These studies have shown the exact stage at which NGF receptors appear, and for the first time, differentiated between the appearance of the low and high affinity receptor subtypes. Supported by grants from the March of Dimes (#1-1090) and NSF (BNS-8896101).

70.3

INTRACEREBROVENTRICULAR (ICV) ADMINISTRATION OF NGF TO NEWBORN RATS ULTIMATELY INDUCES A PERMANENT DEFICIT OF CHOLINERGIC MARKERS IN THE HIPPOCAMPUS (THE NGF PARADOX). G. Vantini, M.A. Tria*, N. Schiavo*, M. Fusco, A. Zanotti* and A. Leon. Fidia Research Laboratories, 35031 Abano Terme, Italy

Septohippocampal cholinergic neurons respond in vivo to i.c.v. administration of NGF with a prominent increase of choline acetyltransferase (ChAT) activity. In both developing and adult animals the increase of ChAT activity returns to control levels upon discontinuation of NGF treatment. In this study we have further analyzed the time-course of the response of ChAT activity to a number of NGF treatment schedules (all injections were performed in the first postnatal week) in the hippocampus of newborn animals. Results indicate that i) all NGF treatments ultimately cause a decrease of ChAT activity in the hippocampus; ii) such a decrease is paralleled by a concomitant reduction of acetylcholinesterase and iii) these cholinergic alterations are still present at postnatal days 28 and 120. All together these data suggest that administration of NGF to newborn rats can paradoxically produce a selective cholinergic denervation in the hippocampus. Since similar alterations have not been detected in rats treated with NGF in adulthood, we suggest that developing septal cholinergic neurons might undergo to a "tropic" influence by exogenous NGF, thus disturbing proper hippocampal innervation.

70.4

THE PRESENCE OF NGF OR ITS MEMORY ARE NECESSARY FOR THE INDUCTION OF C-FOS PROTEIN BY SERUM, DEPOLARIZATION, CYCLIC AMP OR TRAUMA IN CULTURED RAT SYMPATHETIC NEURONS. A.M. Tolkovsky* and A. E. Buckmaster* (SPON: Brain Res. Assoc.) Dept. Human Anatomy, Oxford University, UK

We have defined the conditions that are required to obtain c-FOS (protein) expression in cultured rat sympathetic neurons as part of a program aimed at defining the cellular mechanisms that mediate the actions of NGF.

Using an antibody raised against the N-terminal portion of c-FOS by Gerard Evan (ICRF, St. Barts), we show that NGF induces c-FOS independently of any other stimulus both in established cultures when NGF is added after its deprivation and in newly isolated neurons. In contrast, induction of c-FOS by brief depolarization, by cutting neurites or spiking cell bodies with a needle, by cyclic AMP or by serum requires the presence of NGF or its memory. In established cultures, NGF must not have been withdrawn for more than 8-12 hr to enable induction of c-FOS, while in newly excised neurons the trauma of preparation, depolarization or serum are not able to induce c-FOS at all. Cyclic AMP can still induce c-FOS within 3-4 hr of neruonal isolation but not later. At the point when no c-FOS is induced by these stimuli, the rates of protein and RNA synthesis are still ~80% of the rates obtained in the presence of NGF.

These results show that NGF is unique among several stimuli in its ability to induce c-FOS in these neurons. Furthermore, the results suggest that c-FOS may be a useful indicator for the memory of the presence of NGF, a memory which clearly exists for a very limited period after removal of neurons from the animal.

(We are grateful for the support of the MRC and the Wellcome Trust)

70.5

IN RATS THE RESERPINE INDUCED INCREASE OF BRAIN NGF BIOSYNTHESIS IS MEDIATED BY CORTICOSTEROIDS. M.Fabrazzo*, E.Costa and I.Mocchetti§. FGIN and §Department of Anatomy and Cell Biology, Georgetown Univ., Washington, DC 20007.

Nerve Growth Factor (NGF) has been suggested to play an important role as a modulatory molecule on neuro-endocrine-immune functions (Montalcini et al., 1990). This consideration has prompted us to investigate the possible mechanism(s) regulating NGF biosynthesis during stress. We used reserpine, a drug known to activate the pituitary-adrenocortical system, thereby producing a persistent hypersecretion of ACTH and a consequent increase in blood corticosterone levels.

In 21 day old rats, a single injection of reserpine (0.125-2 mg/kg, s.c.) induced a time and dose dependent increase in plasma corticosterone which is followed by an increase in NGF-mRNA (3-4 fold) and NGF-protein (50%). The increase in NGF biosynthesis is specifically localized in the cerebral cortex; in fact the content of NGF failed to change in other brain structures. In 3 month old rats the response to reserpine is still present but the doses of the drug required are greater than in 21 day old rats. To further characterize the role of glucocorticoids in mediating the reserpine induced increase in NGF expression, we performed studies in adrenalectomized (ADX) animals. When an effective dose of reserpine was given to ADX rats, the alkaloid failed to increase NGF biosynthesis in cerebral cortex.

These results confirm that adrenal hormones play a crucial role in the regulation of NGF during stress and support the current hypothesis that the increase in NGF biosynthesis may establish a link between endocrine and central neuronal function and might be operative in modulating individual immunoreactivity.

70.6

Secretion of Nerve Growth Factor (NGF) by glial cells in vitro.

I. Neveu, R. Houlgatte, D. Wion and P. Brachet. INSERM U298, CHRU, F-49033 Angers, France.

The ability of glial cells to secrete NGF in their culture medium was investigated during the first week following their transfer in vitro. Use of a double-site ELISA assay indicated that the production of NGF remained undetectable during at least one week, when cells were cultured in a serum-free medium. In low serum (0.05%) containing medium, induction of cell division by growth factors such as EGF or FGF had no effect on NGF production. In contrast, a release of NGF was observed in high serum (5%) containing medium, and was more elevated in exponentially growing cultures that in confluent cultures. This effect was depressed in the presence of dexamethasone. Promotion of NGF production by serum appears more important when cells have been cultured for several days in a serum-free medium. This suggests that NGF synthesis in response to serum depends on some permissive event which is triggered in vitro. Phorbol esters, unlike cyclic nucleotide analogs, mimick the effect of serum. Therefore, a protein kinase C appears involved in the up regulation of NGF synthesis.

70.7

INVOLVEMENT OF SECOND MESSENGER SYSTEMS IN THE INTERLEUKIN-1 STIMULATION OF NGF SECRETION FROM CORTICAL ASTROCYTES. M. Carman-Krzan and B.C. Wise. FGIN, Georgetown University, Washington, D.C. 20007.

Our previous studies demonstrated that interleukin-1 (IL-1) stimulates NGF secretion from rat neonatal cortical astrocytes in primary culture by a mechanism independent of cell growth. In the present study, we investigated the possible intracellular second messenger mechanisms involved in the IL-1 induced NGF secretion. The IL-1 stimulation of NGF secretion was Ca^{2+} dependent since IL-1 (10 U/ml) did not increase NGF secretion from astrocytes maintained in Ca^{2+}-free media for 24 hours. Treatment of cells with the phospholipase A_2 inhibitor mepacrine (1-30 μM) inhibited the IL-1 induced NGF secretion at the highest dose used (30 μM). Indomethacin (10 μM), a cyclo-oxygenase inhibitor, produced a slight increase in basal NGF secretion, but failed to inhibit NGF secretion stimulated by IL-1. In contrast, treatment of cells with nordihydroguaiaretic acid (10 μM), a lipoxygenase inhibitor, inhibited NGF secretion by IL-1. We also tested whether the second messenger cAMP would affect NGF secretion from astrocytes. Treatment of cells with the membrane-permeable cAMP analog 8-(4-chlorphenylthio) adenosine-3':5'-monophosphate (100 μM) had no significant effect on NGF secretion during a 24 hr period. The results suggest that one of the mechanisms of the IL-1 stimulated NGF secretion from astrocytes is activation of the phospholipase A_2-lipoxygenase pathway.

70.8

NGF regulates the differentiation of neuronal precursor cells. Elena Cattaneo and Ron McKay, E25-435, MIT, Cambridge, MA 02139

Recent studies have shown that NGF and its receptor are distributed in many regions of the developing brain. However until now the only population of CNS cells demonstrated to be NGF responsive are the cholinergic neurons of the basal forebrain and striatum. In the present study we suggest that , during development, NGF may have an earlier and distinct function consistent with the wide distribution of its mRNA and receptor. In E13.5-14.5 rat striatal primary culture we show that NGF (150ng/ml) after 2 days of bFGF priming (5ng/ml) , increases the number of nestin+ precursor cells 17.7 times compared to the control in 9 days treatment. At this time, following NGF withdrawal , these proliferating precursor cells are capable of differentiating morphologically and immunohistochemically into neurons. The specificity of the NGF effect has been confirmed incubating the culture with other combination of growth factors and with an antibody against NGF. Therefore during development NGF , in combination with other growth factors may have a role in the control of neuronal cell number. Critical fate choices are made at the transition from precursor to post-mitotic neuron, the serum free in vitro system of dissociated precursor cells defined in this study, can be used to determine the signalling mechanisms regulating neuronal number and type in the CNS.

70.9

SYSTEMIC NERVE GROWTH FACTOR REGULATES THE PHENOTYPE OF DEVELOPING SYMPATHETIC AND SENSORY NEURONS AT THE GENETIC LEVEL. F.D. Miller, T.C. Mathew* and J. Toma. Dept. of Anatomy and Cell Biology, Univ. of Alberta, Edmonton, Alberta.

We have examined, using Northern blot and in situ hybridization analyses, changes in gene expression in neonatal rat superior cervical and dorsal root ganglia following systemic treatment with nerve growth factor (NGF). Daily administration of 5 mg/kg 2.5S NGF from postnatal days 2 to 11 induced increases in mRNAs encoding NGF receptor and Tal a-tubulin, the major growth-associated a-tubulin, in both sympathetic and sensory neurons. In addition, systemic NGF differentially regulated genes associated with the transmitter phenotype of sympathetic neurons: tyrosine hydroxylase mRNA increased, while neuropeptide Y mRNA was not affected. Interestingly, the NGF-mediated increase in NGF receptor mRNA was specific to developing neurons, and was not observed in nonneuronal cells of the sciatic nerve, which also express NGF receptor mRNA. A similar NGF-mediated up-regulation of NGF receptor and Tal mRNAs occurred in NGF-treated PC12 cells and was mediated at the transcriptional level. Together, these data demonstrate that systemic NGF induces tyrosine hydroxylase, Tal a-tubulin, and NGF receptor gene expression in developing peripheral neurons. Thus, endogenous, target-derived NGF may not only promote neuronal survival, but may also provide essential protein for terminal arborization, and may play a role in maturation of neuronal phenotype. Increases in NGF receptor levels could also provide a cellular mechanism for potentiating the effects of NGF on NGF-responsive neurons during the period of neuronal competition and cell death.

70.10

DEPOLARIZING INFLUENCES REGULATE NERVE GROWTH FACTOR (NGF) mRNA EXPRESSION IN CULTURED HIPPOCAMPAL NEURONS. **B. Lu, M. Yokoyama, C. F. Dreyfus, and J. B. Black. Div. Devel. Neurol., Cornell Univ. Med. Coll., New York, NY, 10021, and Dept. Neurosci. & Cell Biol, UMDNJ/Robert Wood Johnson Med. School., Piscataway, NJ, 08854**

Both trophic influences and impulse activity have been invoked in environmental regulation of neuronal plasticity. However, the relationship between neuronal activity and the trophic effects in neural development remains unclear. Using cultured hippocampus as a model system, we studied the effect of neuronal depolarization on NGF mRNA expression. Depolarizing stimuli, such as 35 mM K^+ or the Na^+ channel agonist, veratridine, elicited a 4-fold increase in NGF mRNA in cultured hippocampal neurons. Interestingly, the levels of NGF message increased with time in vivo and in vitro, paralleling the well known profile for synaptogenesis in the hippocampus. Tetrodotoxin, which antagonizes depolarization by blocking voltage-dependent Na^+ channels, prevented the developmental rise of NGF mRNA. In contrast, disruption of endogeneous inhibitory GABA activity in culture with the antagonist, picrotoxin, increased NGF gene expression two-fold. These results suggest that NGF gene expression is enhanced by depolarizing influences, and raised possibility that impulse activity and trophic gene expression may be causally linked. (Supported by NIH grants HD23315, NS10259)

70.11

MOLECULAR CLONING, EXPRESSION AND BIOLOGICAL ACTIVITIES OF A NOVEL NEUROTROPHIC FACTOR WITH STRUCTURAL SIMILARITIES TO NGF H. Persson, P Ernfors, CF. Ibáñez* T Ebendal-*Department of Medical Chemistry, Laboratory of Molecular Neurobiology, Karolinska Institute, Box 60400, S-104 01 Stockholm, Sweden; and †Department of Developmental Biology, Biomedical-Center, Uppsala University, S-751 23 Uppsala, Sweden.*

Recently, a genomic clone for brain derived neurotrophic factor (BDNF) was isolated and nucleotide sequence analysis of this clone showed that BDNF is structurally similar to NGF. We have used a pool of degenerate oligonucleotides representing all possible codons in regions of homology between BDNF and NGF to prime rat hippocampal cDNAs in the polymerase chaine reaction. The amplified DNA included a product with significant similarity to NGF and BDNF which was used to isolate a 1020-nucleotide-long cDNA from a rat hippocampal library. From the nucleotide sequence, a 282-amino-acid-long protein with approximately 45% amino acid similarity to both pig BDNF and rat NGF was deduced. In the adult brain the mRNA for this protein was predominantly expressed in the hippocampus. The developmental expression of the novel factor in brain showed a clear peak of mRNA shortly after birth, one and two weeks earlier than maximal expression of BDNF and NGF, respectively. It was also expressed in several peripheral tissues with the highest level in kidney. The protein, transiently expressed in COS-cells, was tested on chicken embryonic neurons and readily stimulated fiber outgrowth from explanted Remak's ganglion and to a lower extent the nodose ganglion. A weak, but consistent fiber outgrowth response was also seen in the ciliary ganglion and in paravertebral sympathetic ganglia. Moreover, the protein displaced binding of NGF to its receptor, suggesting that it can interact with the NGF-receptor. Thus, although structurally and functionally related to NGF and BDNF, this novel factor is expressed in a temporal and spatial arrangement different from both NGF and BDNF, and has unique biological activities, suggesting that this protein is a member of a family of neurotrophic factors that may cooperate to support the development and maintenance of the vertebrate nervous system. Due to its restricted expression in the brain, being mostly confined to the hippocampus, we have named this protein hippocampus-derived neurotrophic factor.

70.12

A DEVELOPMENTAL CHANGE IN CHOLINE ACETYLTRANSFERASE GENE TRANSCRIPTION: A POTENTIAL ROLE FOR NERVE GROWTH FACTOR? M.V. Lorenzi, F. Hefti, B.Knusel and W.L. Strauss. Dept. of Pharmacology, Univ. of Miami, Miami, FL 33101 and Andrus Gerontology Center, USC, Los Angeles, CA 90089.

Choline acetyltransferase (ChAT) enzyme activity in the mammalian CNS increases both during development and in the presence of trophic substances such as nerve growth factor (NGF). An 800 bp restriction fragment of the human ChAT gene (pCHAT1.2) detects 2 species of ChAT mRNA in human cholinergic neurons (Strauss *et al.*, this volume). To determine whether the abundances of either of these mRNAs was changed during development or in the presence of NGF, pChAT1.2 was hybridized to Northern blots prepared from rat brain RNA. In adult rat brain regions, pChAT1.2 detects a single 3700 nt mRNA in agreement with the results of Berrard *et al.* (PNAS, 84:9280, 1987). The abundance of this transcript parallels that of cholinergic neurons in different regions of the rat brain (septum > basal forebrain > striatum > cortex). In contrast, pChAT1.2 detects 2 species of mRNA in samples isolated from embryonic rat brain. Both the 3700 and 2300 nt transcripts detected by pChAT1.2 in embryonic brain also were observed in mRNA isolated from cultures prepared from E17 rat medial septum. Scanning densitometry revealed that these mRNAs were approximately equally abundant in 10 day old cultures (ratio 3700 nt:2300 nt = 1.4:1). Septal neurons grown in the presence of NGF (100 ng/ml) for an equal period showed an increase in the abundance of the 3700 nt species and a reduction in the 2300 nt species (ratio 3700 nt: 2300 nt = 7.3:1). These data suggest a developmentally regulated switch in ChAT gene transcription which may be, in part, due to the effects of nerve growth factor.

GENE STRUCTURE AND FUNCTION I

71.1

CHARACTERIZATION AND DEVELOPMENTAL ACTIVITY OF A RETINAL PROTEIN THAT BINDS TO A REGION OF THE RAT OPSIN PROMOTER M.A. Morabito and C.J. Barnstable. Yale Univ. Sch. of Medicine, Dept. of Ophth. & Visual Science, New Haven CT 06510.

The developmental expression of the rat rod opsin gene is under transcriptional regulation. Transcripts can first be detected by nuclear run-off assays at PN1 and there is a 30 fold increase in the rate of transcription to reach the adult level. We have previously shown by a combination of DNase I protection and gel retardation assays that the region 5' to the opsin gene contains a number of sites that interact with nuclear proteins from adult retina but not from other tissues. One of these sites (region B) includes a CTAAT motif and is recognized specifically by retinal proteins from both rat and bovine tissues. Quantitative inhibition studies have shown that the proteins recognizing region B are different from those binding to other retina-specific sites. The importance of this region in the developmental regulation of the opsin gene is suggested by the increase in binding activity found in nuclear extracts from retinas of different ages. Between PN1 and adult there was over 70 fold increase in the binding activity as normalized to the ubiquitous binding to an OCT 1 consensus sequence.

A bovine retinal extract was fractionated by PAGE, proteins were eluted from gel slices and tested in a gel retardation assay for binding to an oligonucleotide derived from region B. Activity corresponding to the retina-specific complex was detected in a protein fraction of approximate molecular weight 24 kD. These results indicate that the retina-specific factor binding to region B is a single protein, or oligomer of similar sized proteins. *Supported by NIH grants NS20483, EY05206, EY00785 and by Research to Prevent Blindness, Inc.*

71.2

THE HUMAN CHOLINE ACETYLTRANSFERASE GENE CONTAINS A NERVE GROWTH FACTOR RESPONSIVE ELEMENT. Gabriele Mues*, E. Edward Baetge, Craig M. Sampson*, Chuang Fong Kong*, and Louis B. Hersh. Depart. of Biochem., Univ. of Texas Southwestern Medical Center, Dallas, TX 75235 and Molecular Neurobiology, Bristol-Myers-Squibb, Wallingford, CT. 06492

An approximately 10 Kb genomic clone containing the 5' flanking region of the human choline acetyltransferase (ChAT) gene has been isolated. Identification of the 5' flanking region of this gene was made, in part, on the basis of sequence homology with the porcine ChAT gene. An expression vector was constructed which included 3 Kb of the 5' flanking region of the human ChAT gene behind a promotorless vector containing the luciferase gene. This vector was transfected into PC12 cells and its promoter activity assessed by measuring luciferase activity. The human ChAT gene sequences were found to promote basal expression of the luciferase gene. The addition of NGF or FGF to the cells increased expression of the luciferase reporter gene some 3 to 6 fold. These studies demonstrate the presence of a nerve growth factor responsive element in the human gene. Studies are currently in progress to define this element.

71.3

IDENTIFICATION OF REGULATORY ELEMENTS IN THE RAT DOPAMINE BETA-HYDROXYASE GENE. E.J. Lewis and J. Shaskus*, Dept. Biochemistry and Molecular Biology, Oregon Health Sciences University, Portland, OR 97201

Dopamine beta-hydroxylase (DBH) catalyzes the conversion of dopamine to norepinephrine in the catecholamine biosynthetic pathway, and is synthesized primarily in noradrenergic and adrenergic tissue. We wish to gain understanding into the mechanisms which govern the expression of the DBH gene, and have isolated a DNA clone for DBH from a rat genomic library. The genomic clone contains the start site of transcription of the DBH gene, as determined by RNAse protection assay and comparison of the DNA sequence of the genomic clone with the sequence of the rat cDNA (McMahon et al., J. Neurosci Res 25:395,1990). The results of the RNAse protection assay indicate that the 5'-terminal 200 bases of the DBH RNA transcript is the same in rat adrenal, superior cervical ganglia and locus ceruleus. Comparison of bovine, human and rat cDNA and genomic clone sequences predicts that the signal sequences for the proteins will differ in length by up to17 amino acids between species.

The 5'-flanking sequence of the DBH gene contains putative genetic regulatory elements for response to cyclic AMP and phorbol ester. We have fused the DBH genomic sequences from -406 to +13 to the bacterial chloramphenicol acetyltransferase gene. The recombinant 5'DBH-CAT construct has been transfected into tissue culture cells and is expressed in both rat pheochromocytoma PC12 and human neuroblastoma SHSY5Y cells. In PC12 cells, CAT activity is induced approximately 4-fold with forskolin or 8-(p-chlorophenylthio)-cyclic AMP, indicating the presence of a functional cyclic AMP response element. CAT activity is induced approximately 2 fold by either phorbol ester or forskolin in SHSY5Y cultures. We conclude that the genetic information for both basal and regulated expression of the DBH gene are present in these 400 bases of the 5'-flanking DNA.

71.4

MAPPING OF TRANSCRIPTIONAL CONTROL ELEMENTS MEDIATING CELL-SPECIFIC AND ESTROGEN-INDUCED EXPRESSION OF THE HUMAN OXYTOCIN GENE. S.Richard*, S.J.G.Richard* and H.H.Zingg. Laboratory of Molecular Endocrinology, Royal Victoria Hospital, Montreal, P.Q., Canada. H3A 1A1.

To investigate basal and hormone-induced expression of the human oxytocin (OT) gene, parts of the 5'flanking region were linked to the CAT gene and transfected into neural and non-neural cell lines. Using Neuro2a cells co-transfected with an estrogen-receptor expression plasmid, we identified an estrogen response element (ERE) at -164, that mediates a 12-fold transcriptional activation in response to estradiol (E_2) (JBC 265:6098, 1990). We have now identified a region R49 (-49 to +36) that mediates cell-specific expression: R49 is transcriptionally active in Neuro2a cells but virtually silent in C6 glioma or placental JEG-3 cells. Deletions within R49 which left the TATA box intact (-49→-30; -11→-6; +8→+36) reduced its activity to levels observed in C6 and JEG-3 cells.

To investigate to what extent the function of the ERE depends on a synergistic interaction with the R49 region, the ERE was inserted at its original position upstream of an intact or mutated R49 region. Independent of the basal expression levels, the relative responses to E_2 remained unaltered in all constructs. We conclude that transcriptional activation by the ERE and by the R49 region represent two separate mechanisms that operate independently of each other.

71.5

TISSUE- AND STIMULUS-SPECIFIC EXPRESSION OF THE RAT OXYTOCIN GENE IN TRANSGENIC MICE. W. Scott Young, K. Reynolds* and E. A. Shepard* Laboratory of Cell Biology, N.I.M.H., Bethesda, MD 20892

The oxytocin (OT) gene codes for a nonapeptide that participates in the regulation of parturition and lactation. Transcripts encoding the OT preprohormone are made in magnocellular neurons of the supraoptic (SON) and paraventricular (PVN) nuclei of the hypothalamus. The preprohormone is processed into OT and an associated neurophysin during axonal transport to the posterior pituitary where the products are secreted into the circulation. OT mRNA and serum peptide levels increase with parturition, lactation, and hyperosmolality.

Little is known about factors that trans-activate the OT gene or cis-acting sequences that allow control of expression. An estrogen responsive element has been described in the OT 5'-flanking region by Richard and Zingg and by Burbach et al.

Interactions between trans-acting factors and response elements found *in vitro* need to be validated *in vivo*. In order to study the elements of the OT gene that are responsible for its tissue specific expression and regulation we inserted a 5.5kbp rat DNA fragment containing 1.7kbp of OT gene sequence with 0.36kbp of 5'-flanking sequence and 3.8kbp of vasopressin (VP) gene sequence with 1.4kbp of 5'-flanking sequence into CD1 mice. Three of four lines of transgenic mice expressed rat OT mRNA in the PVN and SON. One line that exhibited no ectopic expression was examined for its response to dehydration. Levels of rat OT mRNA were increased, as were levels of mouse OT mRNA in control mice. Our results provide a starting point for studies of OT gene regulation and put an upper limit on the size of the DNA sequence to be studied. We are currently examining the role of orientation of the rat OT and VP genes with respect to each other as well as the expression of each gene alone.

71.6

MOLECULAR ANALYSIS OF NGF GENE STIMULATION BY PHORBOL ESTER. S.R. D'Mello and G. Heinrich. Dept of Med, University Hospital and BUSM, Boston, MA 02118.

The phorbol ester TPA stimulates NGF mRNA 10-15 fold in L929 and kidney fibroblasts but not in dispersed salivary cells. Induction is delayed for 2 hours, maximal at 4 hours, and followed by increased NGF secretion. Present evidence suggests that the induction is transcriptionally mediated via an AP-1 element at the exon 1/intron 1 junction. The induction is abolished by cycloheximide suggesting a role for immediate early gene products. Since the AP-1 element binds fos, jun, and related proteins we measured levels of the corresponding mRNAs. C-fos and jun B mRNA inductions precede NGF mRNA stimulation by TPA. C-jun mRNA is undetectable before and after TPA treatment. Gel retardation assays using L929 cell nuclear extracts and an AP-1 oligonucleotide revealed 3 protein/DNA complexes. The largest complex increased 3-5 fold upon TPA treatment. Preincubation of nuclear extracts with fos and jun antisera abolished DNA-binding confirming the presence of fos and jun-related proteins in the AP-1 complex. Serum and forskolin robustly increased c-fos and jun B but not NGF mRNA. Thus, the TPA response may be mediated by modified forms of c-fos, jun B, or both. Other proteins related or unrelated to fos and jun may also be involved.

71.7

MULTIPLE SIGNALING PATHWAYS INTERACT TO REGULATE NGF GENE TRANSCRIPTION G. Heinrich and Santosh R. D'Mello. Department of Medicine, University Hospital and Department of Biochemistry, BUSM, Boston, MA 02118.

The combined effects of agents that individually regulate NGF mRNA levels were studied in L929 fibroblasts. The phorbol ester TPA raises, corticosterone (C) rapidly lowers, and forskolin (F) has no effect on NGF mRNA levels. F inhibited the TPA-induced stimulation of NGF mRNA without effect on basal levels. C rapidly reduced NGF mRNA levels despite the presence of TPA. We recently showed that the effects of TPA are mediated transcriptionally via AP-1 complexes that bind to an AP-1 element of the NGF gene, and that the AP-1 complexes contain fos and jun antigens. We therefore measured the effects of F and C on the TPA-induced levels of these mRNAs. C failed to affect the transient induction of c-fos and jun B mRNAs by TPA whereas F had synergistic effects. Jun C mRNA was not detectable before or after treatments. Gel-shift analyses using an AP-1 oligonucleotide revealed a minute change in the migration of the largest one of three DNA/protein complexes in TPA/F treated but not in TPA/C treated L929 cells compared with TPA treatment alone. These results suggest that multiple signaling pathways affect AP-1-mediated transcription by distinct mechanisms, and that the AP-1 site serves to integrate their effects.

71.8

REGULATION OF C-FOS-RELATED PROTEINS IN ADRENAL MEDULLA (AM). A.Goc, E.K. Stachowiak*, J.S. Hong+, M.K. Stachowiak, Div. Of Neurobiol. Barrow Neurol. Inst., Phoenix, AZ 85013, +NIEHS/NIH, Research Triangle Park, NC 27709.

Stress increases expression of c-fos gene in AM cells. This report characterizes c-Fos-related proteins expressed in cultured bovine AM cells and describes their regulation by nicotine (Nic) and angiotensin (Ang). Western analyses of total cell proteins with c-Fos antibody and god-conjugated IgG revealed presence 54 Kd c-Fos protein and at least 19 additional Fos-related-antigens (FRAs; 24-68 Kd). Nic and Ang produced approximately 2 fold increases in p54 c-Fos and 10-20 fold increases in c-fos mRNA, suggesting that induced c-fos mRNA was only partially translated. While c-fos mRNA levels returned to control after 3 hours, p54 remained elevated during entire 20 hours of stimulation. The abundance of 15 and 9 FRAs was changed by Nic and Ang respectively. Nic-affected FRAs exhibited the following patterns of changes: (I) stable increases which occurred at different rate and remained elevated for at least 20 hours; (II) transient increases; and (III) decreases. Similar patterns were produced by Ang although different FRAs appeared to participate. In Ang-treated cells a group of FRA exhibited increases or decreases with a delayed onset (approx. 3 hours), whereas effects of Nic were always detected within 45 min. Most of FRA were present in nuclear fraction. p68 which may represent phosphorylated product(s) of the c-Fos was predominantly detected in cytoplasm. Expression of multiple differentially regulated FRAs in AM cells suggests that they may mediate diverse effects of hormonal and trans-synaptic stimulation of AM cells.

71.9

REGULATION OF C-JUN-RELATED PROTEINS IN ADRENAL MEDULLA. M.K. Stachowiak, E.K. Stachowiak*, J.S. Hong+, A. Goc, Div. of Neurobiol. Barrow Neurol. Inst., Phoenix, AZ 85013, +NIEHS/NIH, Research Triangle Park, NC.

Nicotine (Nic) or angiotensin (Ang) receptors increase activities of several neurotransmitter genes in cultured bovine adrenal medullary (BAM) cells. The effects of Ang and Nic are produced through partially independent pathways, and could be mediated through induction/activation of AP-1-related transcriptional factors. Western analyses of BAM cell proteins with c-Jun/AP1 antibody and gold-conjugated IgG revealed the presence of 5 distinct c-Jun-related antigens (31,34,36,38 and 71 Kd) which were detected in nuclei but not in the cytosol. The most prominent were p36 and p38 which may represent postranslational forms of c-Jun. Incubation of BAM cells with Ang resulted in a 3-6 fold increases in the p38 and p36 levels within 45 min-20 hour period. Small and delayed increases in the p36 and p38 were observed in Nic-treated cells. Northern analysis of c-jun MRNA indicated that the changes in the steady-state levels of p36-38 reflect their enhanced synthesis. The largest p71 antigen is likely to be a product of a novel c-jun-related gene. Both Ang and Nic increased its levels, however changes produced by Ang developed more rapidly. p31 and p34 antigens were not affected by Ang but appeared to be slightly increased by Nic. These results suggest that different Jun-related proteins participate in trans-synaptic and in hormonal regulation of adrenal medullary cells.

71.10

REGULATION OF PREPROENKEPHALIN DNA BINDING PROTEINS IN THE RAT STRIATUM. E.F. La Gamma, G. Weisinger, and J.D. DeCristofaro, Pediatrics & Neurobiology, SUNY at Stony Brook, NY 11794-8111.

Nuclear proteins interacting with cis-acting DNA elements form the final step in the biochemical cascade linking cellular signal-transduction pathways to gene regulation. Our previous gel retention studies identified SP1-, cfos-, and cjun- like binding activity on a 166 bp fragment of the 5′ region of the rat gene (-249 to -83) using oligo or antibody competitors. We now observe differences in banding between basal and cholingergic induced states which are revealed by competition studies. Methylation protection footprinting confirmed these observations shoving fewer SP1 protected sites, and weaker footprints over the cAMP/phorbol responsive element of Comb et al (Nature 323, 353-356, 1986) when compared to the induced state. Other intensification features were also unique to each state. Moreover, although shifted bands migrated to the same position, footprint analysis revealed a complex pattern for each gel shifted band, characteristic of each state. These data suggest that the transcription complex in basal and induced states causes a characteristic combination of protein binding at protected regions of the rat promoter in native cells. Deletion/transfection functional assays are being performed to further evaluate this speculation using cell lines and in vitro transcription assays. Supported by the National Science Foundation Grant #BN8719872.

71.11

OLFACTORY MARKER PROTEIN GENE FLANKING SEQUENCES INVOLVED IN DNA-PROTEIN INTERACTIONS. C. Stein-Izsak, M. Grillo* and F.L. Margolis. Dept. of Neurosci., Roche Inst. of Molec. Biol., Nutley, NJ 07110.

The olfactory marker protein (OMP) gene is a neuronal gene expressed in a tissue specific and developmentally-regulated manner. In a previous study with a chimeric construct using presumed upstream promoter elements of the rat OMP gene to drive the Thy 1.1 coding region, we showed that expression of this reporter gene is confined to olfactory receptor neurons in the CNS of transgenic mice (*PNAS*, 86:8565-8569, 1989). Peripherally, expression of the transgene is seen only in thymus due to the presence of Thy 1.1 intron 3 in the construct. Although sequence analysis of the OMP 5' flanking region reveals areas of high homology to sequence motifs known to influence the transcription of other genes, such specific elements have not been characterized for OMP. The present work studies the transcriptional regulation of the OMP gene by identifying specific DNA regulatory sequences and characterizing nuclear proteins that interact with these elements. We use the electrophoretic mobility shift assay to identify putative regulatory sequences in upstream (as well as downstream) OMP flanking regions. We examine DNA segments just 5' from the coding sequence as well as sequences several hundred to several thousand nucleotides upstream (and downstream). Protein extracts from rat olfactory mucosa are compared with extracts of brain, liver, thymus and lung. Preliminary evidence indicates tissue specific interaction, possibly due to differential presence of DNA binding factors or regulatory complexes. Interestingly, tissue specificity varied for different gene fragments. Extracts from neural-lesioned animals may facilitate more direct interpretation of these data. Likewise, DNase footprinting to identify specific sequences will be useful in isolation of the relevant proteins that may function in cell-specific transcriptional activation or repression of this intronless, TATA-less gene.

71.12

TRANSCRIPTIONAL PAUSING AND THE RAT PREPROENKEPHALIN GENE. G. Weisinger, J.D. DeCristofaro and E.F. La Gamma, Peds & Neurobio, SUNY at Stony Brook, NY 11794-8111.

The level of cholinergic induction of striatal preproenkephalin (ppENK) RNA is 10-15 fold greater when measured at the level of RNA initiation (S1 or primer extension assays) compared with steady state mRNA levels. This was supported by experiments probing the same northern blots with different oligomeric DNA fragments that hybridize to ppENK exons 1 and 3. These data suggest a down regulating transcriptional or post-transcriptional event between the end of exon 1 (<17 bp) and the beginning of exon 3. One possible mechanism that could result in these observations is transcriptional pausing or attenuation. To address this possibility, S1 assays were performed using uniformly labeled single stranded S1 probes that overlap the whole of ppENK exon 1 as well as a portion of intron A (229 bp). Probes were hybridized to rat striatal total RNA from basal and induced animals. A 10 fold reduction of the appropriately spliced exon 1 band (152 bp) was observed in the induced compared to the basal state. There was no co-ordinate increase in unspliced exon 1 (ie. 229 bp), but the appearance of at least 2 smaller molecular weight species was noted. These data are consistent with a blockage of transcriptional elongation occurring towards the end of the first untranslated exon of the rat ppENK gene. Additional experiments including transcriptional run-on and other S1 studies are in progress. Supported by NSF #BNS8719872.

SENSORY SYSTEMS—SUBCORTICAL VISUAL PATHWAYS: LGN

72.1

DIRECTION SENSITIVE RELAY CELLS IN THE LGNd OF CATS AND MONKEYS. Kirk G. Thompson, Yifeng Zhou, Steven J. Ault, and Audie G. Leventhal, Anat. Dept., Univ. of Utah, Sch. of Med., Salt Lake City, UT 84132

Direction sensitivity is a well known receptive field property of neurons in mammalian visual cortex. This property is generally assumed to be generated intracortically. We now report that about 30% of X and Y type relay cells in the A laminae of the LGNd of normal cats as well as of cats in which visual cortex has been inactivated exhibit some degree of direction sensitivity. Direction sensitive cells were also found in the magnocellular and parvocellular layers of the monkey's LGNd. While all LGNd cells studied responded to all directions of stimulus motion, some cells responded up to four times more strongly to stimuli moving in the preferred than non-preferred direction. In general, direction sensitivity was most pronounced when relay cells were tested with low spatial frequency sinusoidal gratings. However, some cells were direction sensitive over a wide range of spatial frequencies. Many of the direction sensitive relay cells studied were also sensitive to the direction of moving spots and bars. Some cells were orientation sensitive as well as direction sensitive. Finally, direction sensitive cells were distributed nonrandomly and were encountered in clusters. The presence of relay cells with direction sensitive responses similar to those of cortical cells in normal cats, decorticate cats, and monkeys indicates that direction sensitivity may originate in the subcortical visual pathways. Supported by EY04951.

72.2

ORIENTATION AND DIRECTION SENSITIVITY OF LGND AND AREA 17 CELLS IN DARK REARED CATS. Audie G. Leventhal, Yifeng Zhou, Kirk G. Thompson, and Steven J. Ault, Anat. Dept., Univ. of Utah Sch. of Med., Salt Lake City, UT 84132

We have studied over 500 LGNd and visual cortical cells in six month old cats reared in total darkness from birth. Relay cells in dark reared cats responded well to visual stimulation and their orientation sensitivity (85% of cells) and direction sensitivity (30% of cells) did not differ from normal. As in normal cats, there were relative overrepresentations of cells preferring stimuli oriented radially and tangentially. Direction selective cells as well as cells preferring similar orientations were clustered normally.

As reported previously, most striate cortical cells in dark reared animals were only weakly orientation biased and relatively few were direction biased. The magnitude of the orientation biases of cortical cells in dark reared cats did not differ from those of LGNd relay cells. In fact, the most strongly selective cortical cells exhibited the same degree of bias as did the most strongly selective LGNd cells. Moreover, the orientation biases of area 17 cells tended to be clearest when the stimuli employed were drifting sinusoidal gratings not drifting bars. Our results indicate that ontogenetic mechanisms mediate the development of the orientation and direction sensitivity of LGNd relay cells. We suggest that the intrinsic orientation and direction sensitivity of LGNd relay cells contributes to the development of these properties in visual cortex. Supported by EY04951.

72.3

LOW THRESHOLD CALCIUM SPIKES IN LGN CELLS DURING RESPONSES TO VISUAL STIMULI. S.-M. Lu, W. Guido, and S.M. Sherman. Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

Thalamic cells display a low threshold (LT) spike, which is a large depolarization due to an increased Ca^{2+} conductance, and typically riding its crest is a burst of 2-7 action potentials. The LT spike is voltage dependent: if the cell's membrane voltage is more depolarized than roughly -65mV, the spike is *inactivated*; if more hyperpolarized, the spike is *de-inactivated* and can be *activated* by a small depolarization, such as a retinal EPSP. Prior studies suggest that, once initiated, LT spiking continues spontaneously at roughly 10Hz, and while in this *burst mode*, the cell's activity relayed to cortex does not reflect retinal input. This suggests a dramatic interruption of retinogeniculate transmission compared to that seen while the cell responds in the *relay mode*, during which LT spiking is inactivated and more tonic firing occurs. To test this hypothesis, we recorded intra- and extracellular responses to drifting sinusoidal gratings from LGN cells in cats. Our results indicate that bursts of action potentials occur only during LT spiking, so we could recognize these events extracellularly. X and Y cells displayed similar LT spiking. LT spikes were activated reliably via visual stimulation and seemed identical to those activated by direct current injection or optic chiasm shock; likewise, activation of inputs from the parabrachial region of the midbrain blocked LT spiking activated from either visual or chiasm stimulation. When the cell's membrane was relatively depolarized, it responded tonically to drifting gratings. With further hyperpolarization, tonic discharges decreased and LT spiking began; however, the LT spike occurred at the beginning of the response cycle and was often followed by more tonic firing. We observed similar duality in extracellular recording: an initial burst followed by tonic firing, or simply tonic firing. The fact that these LT spikes and associated bursting occur in phase with the visual stimulus indicates that the burst response mode does not block retinogeniculate transmission. Instead, our data suggest the possibility that LT spikes can amplify signals that would otherwise not be relayed to cortex while the LGN cell is somewhat hyperpolarized.

72.4

N-METHYL-D-ASPARTATE (NMDA) AND NON-NMDA RECEPTORS PARTICIPATE IN EPSPS OF CAT LATERAL GENICULATE NEURONS RECORDED IN THALAMIC SLICES. S.M. Sherman, H.E. Scharfman, S.M. Lu, W. Guido, and P.R. Adams. Dept. of Neurobiology and the Howard Hughes Medical Institute, SUNY at Stony Brook, Stony Brook, N.Y. 11794-5230.

Intracellular recordings from coronal slices (400 um thick) of thalamus were used to examine the possible NMDA and non-NMDA receptor mediated components of EPSPs evoked by stimulating optic tract and optic radiations. Stimulation of either input evoked EPSPs with two components; one peaked less than 10 ms after stimulation ("early") and one peaked more than 10 ms after stimulation ("late"). The late component was reversibly blocked by the NMDA receptor antagonist APV (drop or bath application, 25-50 uM), whereas the early component was unaffected. The APV-sensitive component was decreased in amplitude and duration by hyperpolarization, while the early component increased with hyperpolarization. At hyperpolarized membrane potentials synaptic stimulation of either input often evoked a low threshold spike (LTS) that was blocked by APV; there was no effect on the LTS elicited by intracellular current injection. Morphological identification by intracellular dye injection (Lucifer yellow or Neurobiotin) revealed that the recorded neurons had diverse morphology. The presence of functional NMDA receptors in the retinogeniculate and corticogeniculate pathways may allow for geniculate neurons to act as more than simple relays of visual information.

72.5

CORTICOGENICULATE FEEDBACK GATES RETINOGENICULATE TRANSMISSION BY ACTIVATING NMDA RECEPTORS. M. Esguerra and M. Sur. Department of Brain and Cognitive Sciences, MIT, Cambridge, MA 02142.

Physiological and pharmacological evidence indicates that the NMDA subtype of glutamate receptor is present in the mammalian retinogeniculate projection (Sillito et al., *J. Neurophys. 63*: 347, 1990; Esguerra et al., *Neurosci. Abstr. 15*: 175, 1989). We wished to determine whether cortical projections to LGN cells elicit sufficient depolarization to activate NMDA receptor-mediated components of the retinogeniculate e.p.s.p.

Intracellular recordings were made in the A-laminae of LGN slices prepared from 10 ferrets 8 to 12 weeks of age. Stimulation electrodes placed on the optic tract (OT) and the optic radiations (OR) elicited postsynaptic responses from retinal and corticofugal afferents respectively. In particular, at membrane potentials between -55 and -35 mV, the OT-evoked e.p.s.p. included both early and late components that were reduced by the NMDA receptor antagonist d-APV (20 μM; n=9).

Paired stimulation of the OR and OT also activated the NMDA-receptor mediated component of the OT e.p.s.p. (n=6). The effect was manifested as a nonlinear increase in response amplitude, i.e. an OT e.p.s.p. that was larger than the algebraic sum of the single OR and OT e.p.s.p.'s, as well as a lengthening of e.p.s.p. duration. This response showed the same voltage-sensitivity and d-APV sensitivity as the NMDA-receptor mediated component of the OT e.p.s.p. in the absence of OR stimuli. The response enhancement was most pronounced when OR stimulation preceded OT stimulation by 1 - 6 ms.

These results indicate that activity in the corticofugal feedback projection to the LGN can lead to a depolarization sufficient to activate NMDA receptors at the retinogeniculate synapse. We propose that a possible role of corticogeniculate feedback is to modulate the gain of retinogeniculate transmission by increasing e.p.s.p. amplitude and duration at membrane potentials at or near the action potential threshold.

Supported by EY07023 (M.S.) and the Whitaker Health Sciences Fund (M.E.).

72.6

POSSIBLE IONIC BASIS FOR LAGGED VISUAL RESPONSES IN CAT LGNd RELAY NEURONS David A. McCormick. Neuroanatomy, Yale School of Medicine

X-cells in cat LGNd can exhibit *in vivo* two types of visual response: lagged and non-lagged. Lagged X-cells are characterized by a delay and slow rise (10's of msec) in response to the presentation of a visual stimulus (Mastronarde, J. Neurophys. 57: 357; Humphrey & Weller, JCN 68: 429). Although lagged and non-lagged responses have been proposed to define different cell groups, recent results suggest that they are actually different functional states of the same class of neuron (Uhlrich et al., PNAS 87: 2560). Here we investigate the possible ionic basis of lagged responses using intracellular recording techniques in cat and guinea pig LGNd relay neurons *in vitro*.

Intracellular injection of a depolarizing current pulse into all cat (n=12) and guinea pig (n=22) LGNd relay neurons resulted in a train of action potentials after a variable delay. The duration of the delay was determined largely by two factors: the value of the membrane potential prior to injection of the current pulse, and the amplitude of the current pulse. Injection of a strong depolarizing current pulse (> 1 nA) at resting membrane potential (e.g. -65 mV) resulted in a train of action potentials after a delay of approximately 50-150 msec, while injection of the same, or even a much smaller (e.g. 0.1 nA), current pulse when the membrane potential was depolarized with d.c. to near firing threshold (-55 mV) resulted in a delay of < 5 msec. In contrast, injection of smaller current pulses (< 1 nA) at resting membrane potential could lead to delays of up to 5-10 sec. Voltage clamp and pharmacological analysis reveal that this delay to firing is mediated by two transient potassium currents which inactivate with different time constants, I_{Af} (≈ 100 msec) and I_{As} (≈ 1 sec). Sudden depolarization of relay neurons from resting membrane potential results in the activation of I_{Af} and I_{As}, which subsequently delay the onset of firing. In contrast, tonic depolarization of the neuron to near firing threshold inactivates these currents, and therefore abolishes this initial delay. We suggest that LGNd relay neurons in the cat are capable of both "lagged" and "non-lagged" responses depending in large part upon the state of the membrane potential of the neuron, which itself depends upon the status of ascending neuromodulatory transmitters from the brainstem (McCormick, TINS 12: 215).

Supported by NINCDS and the Klingenstein Fund.

72.7

BRAINSTEM MODULATION OF LAGGED AND NONLAGGED CELLS IN THE CAT LATERAL GENICULATE NUCLEUS. E.Hartveit* and P.Heggelund. Institute of Neurophysiology, University of Oslo, Oslo, Norway.

A major distinguishing characteristic of lagged and nonlagged cells is their markedly different temporal response properties (Mastronarde, J.Neurophysiol., 57:357, 1987). However, the visual response of LGN cells is strongly influenced by input from the midbrain peribrachial region (PBR). This raises the problem whether the distinction between lagged and nonlagged cells is maintained during PBR activation. We have therefore compared the effects of electrical stimulation of the PBR on the visual response properties of lagged and nonlagged cells. The response of single cells to a stationary light spot was recorded before, during and after PBR stimulation.

The visual response of both lagged and nonlagged cells was enhanced by PBR stimulation. The mean response of nonlagged cells increased more than that of lagged cells. The latency to onset of the visual response did not change significantly for the nonlagged cells. Lagged cells with long latencies in the control recordings had reduced latencies during PBR stimulation, but no lagged cells had their latency reduced to values for nonlagged cells. We conclude that the visual response of both lagged and nonlagged cells can be enhanced by PBR stimulation, but that the division of geniculate cells into lagged and nonlagged cells is maintained during PBR stimulation.

72.8

A PROJECTION FROM THE THALAMIC RETICULAR NUCLEUS TO THE DORSAL LATERAL GENICULATE NUCLEUS IN THE CAT: A COMPARISON WITH THE PERIGENICULATE PROJECTION. J.B. Cucchiaro, D.J. Uhlrich, and S.M. Sherman. Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

In mammals, the visual portion of the thalamic reticular nucleus (*TRN*) is the major extrinsic source of inhibitory, GABAergic innervation to the lateral geniculate nucleus (*LGN*). In cats, the most widely recognized source of extrageniculate, GABAergic innervation is the perigeniculate nucleus (*PGN*), which may be part of the TRN. However, the PGN innervation is virtually confined to the LGN A-laminae. The cat's TRN may be the source of innervation to regions of the LGN not innervated by the PGN. We tested this with pathway tracing methods. First, we identified the part of the TRN that projects to the LGN by retrograde transport of WGA-HRP injected into the LGN A- and C-laminae (3 cats). We combined this with anti-GABA staining (1 of the 3). We found many retrogradely labeled TRN cells and, of these, 75% were double labeled for GABA. Second, we made small iontophoretic injections of an anterograde tracer, PHA-L, into the TRN (3 experiments). We found labeled axons in all LGN laminae. The NRT axons labeled with PHA-L were beaded and formed a dense column that extended along projection lines through all the A- and C-laminae. Beaded axons were also labeled in the medial interlaminar nucleus (*MIN*), the geniculate wing (*GW*), and the lateral division of the lateral posterior complex (*LP*). Because we cannot exclude the possibility that we labeled some PGN cells in our injections to the TRN, we cannot address the question of whether the NRT provides an innervation to the A laminae separate from that provided by the PGN. However, after our injections into the TRN, we found a projection that is much more extensive than that seen with injections limited to the PGN. Therefore, we conclude that the TRN, rather than the PGN, is the major extrinsic source of GABAergic innervation to the C-laminae, MIN, GW, as well as to visual thalamic regions not in receipt of direct retinal input, such as lamina C3 and LP.

72.9

A GABAERGIC PRETECTAL PROJECTION TO THE LATERAL GENICULATE NUCLEUS IN THE CAT. M.E. Bickford, J.B. Cucchiaro, and S.M. Sherman. Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

The pretectum contains a high proportion of cells that use the neurotransmitter, γ-aminobutyric acid (GABA). The observation that many large GABAergic cells reside in the pretectum, particularly in the nucleus of the optic tract (NOT), plus our previous observation that pretectal terminals in the lateral geniculate nucleus (LGN) have morphology similar to that defined for GABAergic terminals (Cucchiaro et al., *Soc. Neurosci. Abstr.* 15:1392, 1989), suggested the possibility that some of the pretectal cells projecting to the LGN are GABAergic. To test this possibility, we identified the projection cells by a retrogradely transported label and then determined which of these also stained positively for GABA. We used as our retrograde label wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP), which was unilaterally injected into an LGN in each of 6 cats, and revealed the transported WGA-HRP in pretectal cells by stabilized tetramethyl benzidine histochemistry. With larger pressure injections (3 cats), WGA-HRP extended into tissue surrounding the LGN, and these led to retrograde labeling in the NOT, the posterior pretectal nucleus, and the pretectal olivary nucleus; with smaller iontophoretic injections (3 cats), WGA-HRP was confined to the A- and C-laminae of the LGN, and these led to retrograde labeling primarily within the NOT. A large fraction (40%) of the retrogradely labeled pretectal cells were also stained with an antibody directed against GABA. Among the retrogradely labeled cells, the GABA-positive and GABA-negative populations were similar in soma size (mean: 350μm^2 and 331μm^2, respectively). However, GABA-positive cells not labeled retrogradely were smaller (mean: 246μm^2) than either group of retrogradely labeled cells. Thus the GABAergic cells projecting to the LGN differ from those not projecting (i.e., putative interneurons) on the basis of soma size. We also conclude that a substantial fraction of the pretectal cells projecting to the LGN, particularly those from the NOT, are GABAergic and thus may provide a non-thalamic source of inhibition to LGN cells.

72.10

GABAERGIC MODULATION OF THE TRANSFER RATIO IN CAT LATERAL GENICULATE NUCLEUS (LGN). **Dwayne W. Godwin and Thomas T. Norton. Dept. of Psychology and Dept. of Physiological Optics, Univ. of Alabama at Birmingham, Birmingham, AL 35294.**

Previous studies (Holdefer et al. & Norton et al., Brain Res. 488, 341-352, 1989) have found that GABAergic inhibition affects visual signal detectability and receptive-field sensitivity in LGN cells. To determine the mechanisms underlying these effects, we examined the transfer ratio (TR) from retinal input to LGN output by recording S-potential/LGN action potential pairs. Changes in the TR appear to produce the normal response variability of the LGN cells. In addition, when the TR was compared during maintained and during visually-driven activity, it was found that cells with a high TR during the maintained discharge tended to have a reduced TR during visual driving ($r = -.59$, $p < .05$). This reduction may be due to activity-dependent inhibition. Local microiontophoretic application of GABA decreased the TR in 6 of 7 cells including both X and Y and On and Off types. Application of the GABAa receptor antagonist bicuculline reversed the effect of GABA on the TR and increased visual signal detectability (2 of 2 cells). We conclude that GABAergic inhibition in the LGN reduces the TR, particularly during visual driving, thereby reducing both the visual signal detectability and receptive-field sensitivity. Brainstem control of this inhibition may account for changes in information flow in the LGN associated with changes in the state of arousal. Supported by F31 MH-09693, RO1 EY02909, EY-03039 (CORE), BRSG grant RR-05807, T32 EY-07033.

72.11

BINOCULAR INTERACTIONS IN THE CAT'S DORSAL LATERAL GENICULATE NUCLEUS. III. ORIENTATION AND DIRECTION SENSITIVITY OF NONDOMINANT-EYE INFLUENCES. R.J. Moore, P.D. Spear, J.-T. Xue*, and C.B.Y. Kim*. Dept. of Psychology and Center for Neuroscience, Univ. of Wisconsin, Madison, WI 53706.

Although LGN neurons give brisk excitatory responses to only one eye (the dominant eye), most also are influenced by stimulation of the other (nondominant) eye. A possible source of the nondominant-eye influence is the cortico-geniculate pathway, which arises from orientation- and direction-selective neurons. To assess the role of this pathway, we examined whether the nondominant-eye influence on LGN cells is orientation or direction selective. In experiment I, 1-sec duration 3-Hz sine-wave gratings were presented at 4-7 spatial frequencies drifting in 8 directions at 4 orientations. All stimuli were interleaved, and responses were measured against activity during interleaved "blank" trials with no grating. In experiment II, we used binocular stimulation to examine the orientation and direction selectivity of nondominant-eye influences on the responses of LGN cells to stimulation of the dominant eye at its optimal spatial frequency. In addition, 3-sec trials were used to allow for the possibility that orientation-selective nondominant-eye influences take up to 1 sec to appear (Varela & Singer, 1987). In both experiments, about 50% of the 72 cells tested were significantly influenced by nondominant-eye stimulation. Only about 10% of the responsive cells showed any evidence of orientation or direction sensitivity, and no cells showed clear selectivity. These results suggest either that cortico-geniculate pathways play little role in nondominant-eye influences on LGN cells or that LGN cells receive converging inputs from cortical cells with different preferred orientations and directions of movement.

PAIN MODULATION: ANATOMY AND PHYSIOLOGY III

73.1

EFFECTS OF NEONATAL CAPSAICIN ON INFLAMMATION-INDUCED HYPERALGESIA AND SPINAL FOS ACTIVATION. J.L.K. Hylden, B. Allen*, E. Humphrey*, K. Noguchi, A. Steinberg* and M.A. Ruda, Neurobiology & Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Neonatal capsaicin (Cap) treatment destroys a subpopulation of peptide-containing small diameter primary afferents and decreases sensitivity to noxious thermal stimuli. This study examined the effect of neonatal Cap on the production of inflammation-induced hyperalgesia and on nociceptive activation of spinal Fos protein. Rats were treated with Cap (50 mg/kg) or vehicle (Veh) on day 2 of life. Paw withdrawal latency (PWL) was measured at 5,6,7 and 8 wk of age. Inflammation was induced by s.c. administration of Complete Freund's Adjuvant (CFA) into the left hindpaw at 8 wk of age. PWL was again measured at 6, 24 & 72 h after CFA. Fos protein was localized to lumbar spinal cord using standard PAP methodology.

At 5-8 wk of age, Cap-treated rats had longer PWLs as compared to Veh-treated littermates ($\bar{x}$=12.0 vs. 9.6 s, $p<0.05$, $n=32$). At 6, 24 & 72 h after CFA, inflamed paws in Cap-treated rats had longer PWLs than inflamed paws in Veh-treated rats ($\bar{x}$=5.4 vs. 3.5 s, $p<0.01$). However, the magnitude of inflammation-induced hyperalgesia (net change from pre-inflammation baseline) was similar in the 2 groups of rats. At 48 h an increase in the number of Fos-labeled cells was observed primarily in medial laminae I-II of L5 in Veh-treated rats (mean left-right difference = 21.0 cells/30 µm section, $p<0.01$). Cap-treated animals also exhibited an increase in the number of Fos-labeled cells (6.3 cells/section, $p<0.01$). Fos activation in Cap-treated rats was significantly less than in control rats ($p<0.01$). These data show that Cap-treated rats demonstrated inflammation-induced hyperalgesia and Fos activation; however, Fos activity was significantly attenuated. Thus, inflammation-induced hyperalgesia is minimally affected by Cap treatment and likely involves activity in primary afferent fibers that are not Cap sensitive. In contrast, activation of Fos in the CFA model of inflammation appears to depend to a large extent on input from a population of Cap sensitive small diameter primary afferents.

73.2

MORPHOLOGICAL EFFECTS OF ELECTRICAL TRIGEMINAL GANGLION STIMULATION (ETG) ON INTRA- AND EXTRACRANIAL VESSELS. M.G. Buzzi,*†, V. Dimitriadou†, T.C. Theoharides†, and M.A. Moskowitz. Depts. of Neurosurgery and Neurology, Massachusetts General Hospital, Harvard Medical School, Boston, MA 02114 and †Department of Pharmacology, Tufts University School of Medicine, Boston, MA 02111.

Electrical trigeminal ganglion stimulation (1 mA, 5 msec, 5 Hz, for 5 min) provokes vasodilation and plasma extravasation, both in innervated intracranial and extracranial blood vessels. Here, we report the ultrastructural effects of unilateral ETG (n=14). Unilateral ETG was followed by intracardiac perfusion of 2.5% glutaraldehyde and preparation for electron microscopy. Ultrastructural examination revealed the following within postcapillary venules exclusively on the stimulated side: erythrocyte diapedesis, occasional endothelial damage, platelet adhesion and degranulation. Ganglion-stimulated adult rats treated as neonates with capsaicin (n=6) did not show these changes. Pretreatment with the antimigraine drug dihydroergotamine (50 µg/kg, I.V.) (n=6) significantly reduced plasma extravasation and vascular changes within the dura mater, but not within the tongue.

These preliminary findings suggest a novel mechanism for the action of antimigraine drugs and explain, in part, the platelet and serotonin involvement in vascular headaches.

73.3

IN VIVO AND *IN VITRO* ULTRASTRUCTURAL EVIDENCE FOR STIMULATION OF DURA MAST CELLS BY NEUROPEPTIDES. V. Dimitriadou, M.G. Buzzi,** M. Lambracht-Hall,* M.A. Moskowitz* and T.C. Theoharides. Dept. of Pharmacology, Tufts Univ. and *Dept. of Neurosurg. & Neurology, MGH, Boston, MA.

Mast cells are known to participate in allergic and inflammatory conditions. Here we investigated the effects of neuropeptides on dura mast cell secretion *in vivo* and *in vitro*. One trigeminal ganglion was stimulated electrically (0.1 or 1 mA, 5 msec, 5HZ, for 5 min), which provokes vasodilation and plasma extravasation, in male adult rats (n=14), which were then fixed intracardially with 2.5% glutaraldehyde. Ultrastructural examination of the dura mater showed that whereas mast cell granules in the contralateral side were intact, with homogenous and electron dense core, those in the ipsilateral (stimulated) side showed distinct changes ranging from slight alterations of the granular core to its complete intracellular dissolution. As previously reported, extrusion of metachromatic granules was not found.[1] There was also platelet aggregation and degranulation, as well as erythrocyte diapidesis within postcapillary venules. Similar changes were not observed in adult rats treated as neonates with capsaicin to destroy perivascular sensory fibers (n=6). *In vitro*, 30 min incubation of the dura with SP and CGRP (10^{-5} M) led to similar morphological changes of dura mater mast cells, and also to significant histamine release (n=4). In conclusion, sensory nerve terminals could trigger mast cell secretion without obvious degranulation, very likely in response to SP and/or to CGRP, leading to neurogenic inflammation.

[1] Markowitz, S. *et al., Brain Res.* 477:157-165, 1989.

73.4

RELEASE OF SUBSTANCE P IN THE DORSAL LUMBAR SPINAL CORD OF THE CONSCIOUS, FREELY MOVING RAT: EFFECTS OF KAINIC ACID, CAPSAICIN AND SUBSTANCE P(1-7). C.W. Murray, S.R. Skilling*, D.H. Smullin and A.A. Larson. Dept. of Veterinary Biology, University of Minnesota, St. Paul, MN 55108.

We have previously reported that NMDA and substance P (SP) antagonists are analgesic, that excitatory amino acids (EAAs) are released in the dorsal horn in response to SP C-terminal fragments and formalin, and that SP(1-7), an endogenously-generated metabolic fragment, inhibits spontaneous EAA release. In the present study we examined the interactions between EAAs and SP to test the hypotheses that EAAs can alter SP release and that SP(1-7) also decreases the release of SP from primary afferents. Rats were anesthetized with pentobarbital and implanted with transverse dorsal 50 kd MW cutoff microdialysis fibers at ca. L4-5 and chronic intrathecal (i.t.) catheters injecting rostrally at L4-5. After overnight recovery, Ringer's with 0.2% BSA and 1.2 mM Ca^{++} was infused at 6 µL/min and fractions were collected at 20 min intervals. Animals were equilibrated for 4 hr before collection of basal fractions. SP-like immunoreactivity (SPLI) was measured by commercial RIA. Data are expressed as mean % change from each subject's basal [SPLI]. Intradialysate infusions of 10 mM of the EAA agonist, kainic acid, for 5 or 10 min caused respective increases in SP release of 98 ±41 % ($p<0.05$) or 624 ±142 % ($p<0.001$). A 10 min infusion caused no neuronal damage. Intradialysate infusion with 200 µM capsaicin for 20 min, to cause selective depolarization of primary afferents, induced a 160% ($p<0.05$) increase in SP release. *The capsaicin effect was inhibited ($p<0.05$) by i.t. pretreatment with 1 nmole of SP(1-7).* These data suggest that reciprocal interactions between neurokinin and EAA systems are important in pain transmission and further support our hypothesis that **SP(1-7) acts as an endogenous regulator of nociceptive transmitter release.** [USPHS Grants DA04090, DA04190, DA00124, DA07234 and CA01342.]

73.5

LONG-LASTING ENHANCEMENT OF RESPONSES OF SPINOTHALAMIC TRACT (STT) NEURONS TO EXCITATORY AMINO ACID (EAA'S) BY COMBINED IONTOPHORETIC APPLICATION WITH SUBSTANCE P (SP). P.M.Dougherty and W.D.Willis. Mar. Biomed. Inst. & Dept. of Anat. & Neurosci., UTMB, Galveston, TX 77550.

EAA's and SP are co-localized in afferent fibers and when released may activate and sensitize nociceptive neurons, leading to secondary hyperalgesia. To test this, we studied the effects of iontophoretically released EAA's and SP, alone or in combination, on the responses of 42 STT cells in 25 anesthetized monkeys (M. fascicularis). Repeated applications of the same EAA produced either a similar or a decreased excitatory action. SP at the dosage used (15 to 50nA) produced no consistent change in background discharge. However, the co-application of an EAA and SP generally resulted in a potentiation of the excitation produced by EAA's. In about 1/3 of the cells tested, the potentiation of the NMDA responses outlasted the SP application by 15 min to as long as 4 h. The responses to ASP were enhanced in parallel to those of NMDA. In 4 cases, the QUIS response was potentiated for 25 min to 3-1/2 h. The response to GLUT paralleled that of QUIS. We have not found any cases of potentiation of the AMPA response by SP to date. We conclude that potentiation of STT cell responses to NMDA and non-NMDA may contribute to the central sensitization underlying secondary hyperalgesia. (Supported by NIH grants NS08860 (P.D.), NS09743 and NS11255.)

73.6

LONG-LASTING ENHANCEMENT OF RESPONSES OF SPINOTHALAMIC TRACT (STT) CELLS TO MECHANICAL STIMULATION OF SKIN DURING POTENTIATION OF AMINO ACID (EAA'S) RESPONSES BY SUBSTANCE P (SP). W.D.Willis and P.M.Dougherty. Mar. Biomed. Inst. & Dept. of Anat. & Neurosci., UTMB, Galveston, TX 77550.

Secondary hyperalgesia may result from sensitization of central nociceptive neurons. Since iontophoretic application of EAA's and SP sensitize STT cells, we tested for changes in responses to mechanical stimuli during this process. The activity of STT cells from 25 monkeys (M. fascicularis) were recorded in response to the following mechanical stimuli: brushing the skin with a soft-bristled brush (BRUSH); pressure (PRESS) on the skin applied with an arterial clip (near pain threshold in humans); pinching the skin (PINCH) with a different arterial clip with a firmer grip (well above pain threshold). The responses of STT cells were usually increased during application of EAA's and decreased by AP7. SP alone had little or no effect on background activity or responses to mechanical stimuli. However, in cells that showed enhancement of responses to EAA's after co-application of SP, the responses to mechanical stimulation showed a parallel increase. We conclude that the enhanced responses of STT cells caused by combined application of EAA's and SP may contribute to hyperalgesia. (Supported by NIH grants NS08860 (P.D.), NS09743 & NS11255.)

73.7

VAGAL AFFERENT STIMULATION (VAS) MODULATES TRIGEMINAL NEURONAL RESPONSES TO NOXIOUS STIMULI. D.B. Bossut and W. Maixner, Dental Research Ctr., University of North Carolina at Chapel Hill.

Previous experiments in cats have established that electrical stimulation of cardiopulmonary vagal afferents inhibits the digastric reflex elicited by tooth pulp stimulation. This study assessed VAS effects on the responses of trigeminal sensory neurons to noxious heat and electrical pulses (EP).

Continuous VAS (5 Hz, 1 mA) inhibited the responses of 23/32 wide dynamic range neurons in n. caudalis to a 5 sec 50°C contact heat pulse; 8/14 of these cells projected to the thalamus VPM area. VAS facilitated the heat evoked response of 5/32 and had no effect on 4/32 neurons. All cells received A-δ input and 13 received additional C-fiber input. VAS (7 pulses, 333 Hz, 5 mA) delivered 200 msec prior to EP diminished A-δ and C-fibers input. VAS also decreased the tooth pulp-evoked responses of 6 trigeminothalamic neurons in n. oralis.

These results suggest cardiopulmonary VAS impairs nociceptive reflexes by modulating sensory transmission and VAS may play a role in modulating pain.

Supported NIDR grant DE08013 and DE0750901 (W.M.).

73.8

EFFECTS OF CAGE BEDDING CHANGE AND TAIL TEMPERATURE MEASUREMENTS ON NOCICEPTION IN RATS. J Hulse Neufeld*, Brenda Hall* and Matthew B. Weinger. Department of Anesthesiology, VA Medical Center, San Diego, CA 92161.

As a prelude to measuring the behavior of chronically stressed rats with a tail dip assay, methods were developed to differentiate groups of male Wistar control rats from rats subjected to the stress of changing the cage bedding and to the stress of measuring the tail temperature. Three sequential tail dips at 10-min intervals into 52.5°C water were used with the rats only briefly (less than 15 sec) restrained at the base of the tail. The data from individual rats were subjected to linear regression analysis and a mean slope (SLP) and y-intercept (INT) were calculated. The mean SLP and INT values of treatment groups were compared for significance of difference using ANOVA. Changing the cage bedding 6 hr before latency determinations increased SLP. Measuring the tail temperature 6 hr before latency determination increased INT and lowered SLP. The changes in the parameters SLP and INT were not related to changes in tail temperature when the tail temperature was measured after tail dips. Tail temperature could be shown to correlate only in control groups and only with SLP and INT. SLP and INT are useful parameters with which to differentiate rats stressed by experimental manipulations.

73.9

METABOLIC ALTERATIONS ASSOCIATED WITH ISCHEMIA ACTIVATE Aδ AND C FIBER NOCICEPTORS. D.L. Tanelian and M.B. MacIver. Dept. of Anesthesia, Stanford Univ. Sch. of Med., CA 94305.

Mechanisms of pain following ischemia remain unknown, but are thought to involve hypoxia, glucose depletion and/or an increase in lactic acid, resulting in excitation of nociceptor nerve endings. This study investigated the effects of hypoxia, hypoglycemia and lactic acid on the discharge activity of Aδ and C fiber nociceptors using an *in vitro* preparation of rabbit cornea. Corneas were maintained at 35 °C and perfused from behind with artificial aqueous humor solution (AQH) to maintain a normal 'intraocular' pressure of 18 mmHg. Glass suction electrodes were used to record single and multi-unit action potential discharge from corneal nerve bundles. Substituting N_2/CO_2 for O_2/CO_2 increased discharge frequencies to 213 +/- 3.4 % of control (+/- SD; $p < 0.001$ ANOVA, n=12). Substitution of L-glucose for D-glucose produced a significantly greater increase in discharge frequency (653+/- 28%; n=8, $p<0.001$ compared to N_2 data). Increased discharge occurred 20 to 30 min after substitution of N_2 or L-glucose and was followed by depression. A brief period of increased discharge was observed during recovery. Lactic acid at concentrations up to 500 µg/ml did not alter discharge activity. Combining N_2 and L-glucose substitution did not produce a significant increase in discharge, compared with L-glucose alone (671+/-14%, $p > 0.05$, n=6). In conclusion, depletion of glucose appears to be the major factor which contributes to nociceptor activation following ischemia.

Supported by the Parker B. Francis Foundation and NIH.

73.10

CHARACTERISTICS OF PAIN PROCESSING IN THE FLUOROCARBON PERFUSED RAT. C.L. Cleland and G.F. Gebhart. Department of Pharmacology, University of Iowa, Iowa City, IA 52242

The processing of pain by the central nervous system depends on network, synaptic and cellular properties. Although network and synaptic properties have been extensively investigated, research into the role of cellular properties has been limited by the difficulties of obtaining stable intracellular recordings using *in vivo*, whole animal preparations, and the problems of identification and functional interpretation using *in vitro* slice and culture preparations. In order to surmount these difficulties, we have developed a whole animal, perfused preparation which should permit stable intracellular recording and control over the composition of the perfusate while retaining the connectivity and behavior of the intact animal.

Adult rats were perfused through the ascending aorta using non-pulsatile flow of a fluorocarbon-based artificial blood containing pentobarbital and maintained at 37° C. The somatic pain system was assessed using mechanical and thermal noxious stimuli. Visceral pain processing was tested using colorectal distension.

We found that appropriate withdrawal and pressor responses to noxious somatic and visceral stimuli were maintained for up to 6 hours. Respiration, EEG and arterial pressure remained stable and responsive to changes in O_2 concentration, afferent input and pharmacological manipulation for 6-8 hours. Thus, the perfused preparation provides the opportunity to investigate pain processing using intracellular recording, manipulation of the neuronal environment, assessment of the contributions of circulating factors to pain transmission, and independent perfusion of different systems, paradigms difficult to pursue in alternative *in vivo* preparations.

Funded by NIH Grant NS19912.

73.11

INHIBITION OF RUBRAL NEURONS BY NOXIOUS AND NON-NOXIOUS PRESSURE. R.R. Matsumoto and J.M. Walker. Department of Psychology, Brown University, Providence, RI 02912.

Single unit, extracellular recordings were conducted in halothane-anesthetized rats. The responses of 170 neurons in and around the red nucleus (RN) to a 2.8 kg/cm^2 (1s) pinch to the hindpaws and tail were assessed. Cells in both the magnocellular (RNm) and parvocellular (RNp) divisions of the RN were most sensitive to pinch to the contralateral hindpaw. Furthermore, cells in the RNm were more likely to respond to pinch than cells in the RNp. The percentage of cells that responded to pinch in each of the divisions of the RN is summarized as follows: RNm- contralateral hindpaw (73%), ipsilateral hindpaw (34%), tail (37%); RNp- contralateral hindpaw (37%), ipsilateral hindpaw (15%), tail (23%). Cells in the surrounding reticular formation did not show a lateralized response: contralateral hindpaw (39%), ipsilateral hindpaw (25%), tail (36%). Some cells in the RN were also tested for their responses to a graded pressure stimulus to the contralateral hindpaw (0-2.8 kg/cm^2 in 4s): 26% exhibited no response, 32% responded only during the non-noxious part of the stimulus, 21% responded to the noxious part, and another 21% showed two separate responses, one during the non-noxious part of the stimulus and one during the noxious part. These data suggest that the RN may be involved in the processing of noxious as well as non-noxious tactile stimuli.

REGENERATION: GENERAL STUDIES AND MOLECULAR CORRELATES

74.1

THE ULTRASTRUCTURE AND CURRENT DENSITY OF GROWTH CONES IN REGENERATING NERVE. J.M. Kerns and J.A. Freeman. Dept. of Anatomy, Rush Medical College, Chicago, IL 60612 and Dept. of Cell Biology, Vanderbilt University, Nashville, TN 37232.

Previous studies have suggested that peaks of current density (J) in the regenerating nerve detected with the vibrating probe are associated with underlying growth cones. The present study is a morphometric analysis of this correlation. Four sciatic nerves from adult rats were lesioned under nembutal anesthesia. After 7 days the J was measured along the distal segment with a circularly vibrating probe followed by perfusion fixation. Profile counts were made on electron micrographs (n=10) at 3500x from selected regions. The mean density of growth cones profiles per .01mm^2 was higher ($p<.001$) at the peak (28.1 +/- 1.4) than before (16.3 +/-1.1) or after (11.2 +/- 0.8). Unmyelinated axons were reduced after the peak ($p<.001$), while Schwann cell nuclei were uniform. The baseline J (µA/cm^2) before (0.7 +/- 0.2) and after (0.6 + 0.2) was higher at the peak (2.7 +/-0.8) and correlated ($r = 0.72$, $p<.01$) with the growth cone density. In conclusion, the vibrating probe is a sensitive in vivo detector of growth cones in a regenerating nerve.

Supported by the Enelow Foundation and NIH grants NS-19769, NEI-EY01117 and NSI8103.

74.2

OLIGODENDROCYTE RESPONSE TO AXONAL INJURY: IMPLICATIONS TO REGENERATION. T.Sivron*,A.Cohen* and M.Schwartz. Dept. of Neurobiology, The Weizmann Inst. of Science, Rehovot, Israel.

Crushed axons of fish optic nerves readily regenerate, while similarly injured axons of rat optic nerves do not. The reasons for the difference in the regenerative ability of these axons may lie in differences in the number of surrounding oligodendrocytes, known to be inhibitory to axonal growth. We have cultured nonneuronal cells from previously crushed optic nerves of fish and rat, and examined them using indirect immunofluorescence. In the rat cultures, mature, GalC-positive oligodendrocytes were abundant, while in the fish cultures oligodendrocytes, marked by the 6D2 antibody, were rare. In order to check whether the control of the oligodendrocyte response to injury is intrinsic to the optic nerve we have isolated the optic nerve immediately after the injury and kept it in organ culture, after which it was dissociated. In the rat organ cultures the number of oligodendrocytes was similar to that of optic nerves crushed *in vivo*. In the fish organ cultures, however, about 15 times more oligodendrocytes developed than in *in vivo* crushed optic nerves. In addition, macrophage-like cells which were abundant in the cultures of *in vivo* crushed fish nerves, were absent from the fish organ cultures. This suggests that the factor/s which regulates the oligodendrocyte response originates from blood-borne cells. Finally, medium conditioned by regenerating fish optic nerves (CM), which was shown to have a cytotoxic effect on rat oligodendrocytes, was found in this work to reduce the number of oligodendrocytes in organ cultures of both rat and fish (by factors of 6 and 2.5, respectively). The demonstrated difference in the response of oligodendrocytes to optic nerve injury may be responsible, in part, for the different regenerative ability of fish and rat optic nerves.

74.3

AXOTOMY-INDUCED REMODELLING IN THE DENDRITES OF ADULT RAT GANGLION CELLS. Solon Thanos and Jens Vanselow*, Dep. of Ophthalmology, Univ. Tübingen and Max-Planck-Inst. for Dev. Biology, Tübingen, FRG.

We investigated the response of ganglion cells and their dendrites to optic nerve injury. The ganglion cells were labeled post-mortem with DiI and examined after optic nerve crush in situ (group A), after axotomy and reconnection of the retina via transplanted peripheral nerve (PN) pieces with fetal targets (group B) and after axomoty and subsequent explantation of the retina in vitro (group C). In group A animals, in which cut axons can not regrow, many ganglion cells which survived the axotomy responded with dendritic growth. In group B animals, axons grew through the PN grafts andreached co-grafted fetal targes where they formed axonal terminals. The dendrites of these retinal cells were not enlarged compared to the normal ganglion cells in the retina. Explantation of the axotomized retina (group C) was accompanied with both dentritic and axonal growth. The results suggest that in the adult ganglion cell, transection of the axon can result in dendritic growth when regrowth of the cut axon is not permitted because of the inhibiting optic nerve environment.

74.4

Abortive Regeneration of Cortical Projection Axons. P.S. Fishman and D.A. Farrand*. V.A. Res. Labs., Dept. of Neurology, Univ. of Md. Sch. of Med. Baltimore, Md. 21201.

Corticospinal axons cut in the spinal cord or medullary pyramid show a primarily degenerative response in adult mammals. It has been proposed that this lack of an initial regenerative response may relate to the remaining axon collaterals and their synaptic contacts. We wished to determine the response of cortical projection axons to lesions in the subcortical white matter close to their cell bodies of origin. Using anterograde labeling with cholera B chain conjugated to horseradish peroxidase, axons projecting from motor cortex were traced into the area of injury during a three week period after a stab wound through the neighboring cortex and subcortical white matter in adult mice. During that period the majority of labeled axons project directly into the area of injury, with a variety of axon morphologies including terminal bulbs. Ultrastructural examination of the region of axonal termination was also performed. During the first 10-14 days after injury the site contains not only degenerating axon terminals, but a large number of small processes of neuronal, glial and macrophage origin. But by three weeks after injury the area of injury contains predominantly astrocytic processes. Cortical projection neurons can maintain a viable axon after close axotomy, and may show a more pronounced initial regenerative attempt than to more distant lesions in the medulla or spinal cord. (Supported by the DVA)

74.5

THE DEVELOPMENT OF RUBROSPINAL LATERALITY IN THE NORTH AMERICAN OPOSSUM. X.M. Xu and G.F. Martin, Department of Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

Rubral axons can grow around a lesion of their spinal pathway during specific stages of development in the opossum (Martin and Xu, Dev. Brain Res. 39:303-308) and such plasticity results primarily from the growth of new axons. There is evidence, however, that true regeneration of cut axons may also occur (Xu et al., Soc. Neurosci. Abstr. 15:870). In order to interpret the results of experiments designed to determine if true regeneration contributes to rubrospinal plasticity, it became necessary to determine the degree to which the red nucleus projects ipsilaterally to the spinal cord during the critical period for that plasticity. Although rubrospinal axons are primarily crossed in adult opossums, an ipsilateral projection exists and it is possible that it is particularly robust during development. Pouch-young opossums of the appropriate ages were anesthetized so that the rubrospinal tract could be cut at the T8-T9 level on the right side of the spinal cord. Immediately after the lesion was made, Fast Blue was injected into the lumbar enlargement on the opposite side. Seven days later, the animals were sacrificed and the tissues prepared for fluorescence microscopy. Although labeled neurons were numerous in the red nucleus contralateral to the injection, they were sparse on the ipsilateral side. Counts of labeled neurons on the two sides suggested that only 1% of the rubrospinal neurons projecting to the lumbar cord did so ipsilaterally. In a set of similar experiments on older pouch-young and adult opossums, the results were comparable. At all ages, the distribution of the ipsilaterally projecting neurons varied from case to case. Our results suggest that during the critical period for rubrospinal plasticity, as well as during later stages of development, the rubrospinal tract is almost entirely crossed. (Supported by NS-25095).

74.6

TRANSFERRIN RECEPTOR EXPRESSION IN THE INJURED AND REGENERATING RAT SCIATIC NERVE. G.Raivich, M.B.Graeber*, J.Gehrmann* and G.W.Kreutzberg. Department of Neuromorphology, Max-Planck Institute for Psychiatry, D-8033 Martinsried, F.R.Germany

Iron-saturated transferrin is an ubiquitous growth factor which plays a critical role in the cellular iron uptake[1]. Here we have studied the expression and the distribution of the cellular receptors to this protein following peripheral nerve injury and during regeneration.

Axotomy led to a massive but transient increase (days 1-9, maximum day 4) in specific [^{125}I]-transferrin binding at the site of the injury and in the distal, denervated part of the crushed or resected sciatic nerve, coinciding with the pattern of cellular proliferation. Immunocytochemistry using Ox-26 monoclonal antibody revealed strong and synchronous expression of the transferrin receptor protein on two different cell types: a subpopulation of blood-borne macrophages invading the injured peripheral nerve as well as on the denervated Schwann cells. Finally, studies using intravenously injected $^{55}Fe^{3+}$ (0.2 mCi) showed an aproximately 20-fold increase in the endoneural iron uptake confined to the directly injured and the distal part of the axotomised sciatic nerve with the same timecourse as observed for the transferrin receptor expression.

In summary, there is a dramatic increase in transferrin receptor immunoreactivity, specific transferrin binding and iron uptake in the injured and regenerating endoneurium of the sciatic nerve. Since iron is an essential cofactor of several enzymes needed for energy metabolism and DNA synthesis, these data suggest that the induction of transferrin receptor expression plays an important role in the regulation of cellular growth and proliferation during nerve regeneration.

[1]Raivich, G., R. Hellweg, M.B. Graeber, and G.W.Kreutzberg, *Restor. Neurol. Neurosci*. 1:217-223, 1990

74.7

REAPPEARANCE OF J1/TENASCIN AND THE 473 PROTEOGLYCAN FOLLOWING DISCRETE LESIONS IN THE ADULT MOUSE CEREBRAL AND CEREBELLAR CORTICES. E. Laywell*, A. Faissner, M. Schachner, and D.A. Steindler, Dept. of Anatomy & Neurobiol., Univ. of Tenn., Memphis; Dept. of Neurobiol., University of Heidelberg; Swiss Federal Inst. of Tech., Zurich.

Our previous studies (Laywell & Steindler, Neurosci. Abs. 15:590, 1989) have shown that lesions of the somatosensory cortex produce characteristic cellular and molecular changes around the wound. J1/tenascin and a chondroitin sulfate containing proteoglycan termed the 473 antigen, two constituents of the extracellular matrix, are prevalent in boundaries around developing brain structures such as cortical whisker barrels, the neostriatal mosaic, and nuclei throughout the neuraxis. After the second postnatal week, these boundaries are no longer apparent. Because of their location and transient expression during plastic developmental events, J1/tenascin and the 473 proteoglycan likely have a role in segregating developing neural elements. Furthermore the scarcity of these molecules in the mature brain may reflect a suppression of inhibitory factors when pattern formation is completed and the brain is anatomically stabilized. Do lesions in the adult brain provoke a "re-expression" of such developmentally regulated molecules?

Stab lesions were made in the cerebral or cerebellar cortex of adult B6C3H mice. Following various survival times of up to 21 days, the brains were processed for immunocytochemistry of J1/tenacin, 473 proteoglycan, N-CAM, GFAP, vimentin, AMOG, and a variety of other neural antigens. Many of these antigens are visible only in the vicinity of the wound, perhaps in a time-dependent gradient fashion, and are associated with reactive glia. For example, discrete clusters of vimentin-positive Bergmann glia also exhibit increased immunostaining for J1/tenascin, the 473 proteoglycan, and N-CAM 4-6 days after small cerebellar lesions. In situ hybridization experiments, using e.g. cDNA probes to J1/tenascin, will determine the cellular sources of these novel molecular expressions. J1/tenascin and the 473 proteoglycan are but two constituents common to both developmental boundaries and glial scars. The re-expression of such molecules in brain wounds may present an inhibitory substrate to regenerating neurites. Supported by the NIH, NSF, & DFG.

74.8

EXPRESSION OF INHIBITORY PROTEOGLYCANS IN A MODEL OF ASTROGLIAL SCARRING FOLLOWING LESIONS IN THE CNS R. J. McKeon, R. C. Schreiber*, J. S. Rudge and J. Silver. Dept. of Neuroscience, Case Western Reserve University, Cleveland, OH 44106

The failure of neurons to regenerate following CNS injury has been attributed to the formation of an astroglial scar which may create a mechanical barrier for axons, thereby limiting the amount of regeneration. Alternatively, cells which make up the astroglial scar may be a poor substrate for growing axons or produce inhibitory molecules which prevent regeneration. In order to examine whether, following lesions, inhibitory molecules exist in CNS regions that lack oligodendrocytes, Millipore filters were implanted in the gray matter of the cortex of either newborn or adult Sprague-Dawley rats. Newborn animals were sacrificed at 6 days or 30 days while adult animals were sacrificed 10 days or 30 days following implantation. Sections through the filter were stained with antibodies to either GFAP, NCAM, laminin, fibronectin, collagen type IV, to the putative inhibitory molecule chondrotin-6-sulfate (CS) or its ECM ligand, cytotactin (CT). GFAP staining was increased in a large area surrounding the implant but was most intense within and immediately adjacent to the implant in both neonatal and adult animals. Laminin, fibronectin, and collagen exhibited a more diffuse staining pattern in and around the implant. In contrast, CS and CT staining only occurred within and around the implants of adult animals and in the 30 day post- implanted neonates. The expression of CS and CT was coincident with the distribution of GFAP-positive cells close to the implant, suggesting that, in addition to growth promoting molecules, reactive astrocytes in the immediate vicinity of a gray matter lesion produce inhibitory molecules following injury. The production of these inhibitory molecules following cortical lesions may act to limit regeneration in the CNS. Supported by NS 25713 and the Daniel Heumann Fund for Spinal Cord Research.

74.9

THE MOLECULAR ENVIRONMENT OF THE DORSAL ROOT ENTRY ZONE DURING DEVELOPMENT AND AFTER ROOT CRUSHES. R.B. Pindzola and J.Silver. Department of Neuroscience, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

The glial environment of the spinal cord dorsal root entry zone (DREZ) was analyzed to determine if purported axon inhibitory molecules increase on the CNS side after the critical period (PD2-3, the age when afferents no longer regenerate into the cord). We studied normal rats from E14 to PD37 and rats that sustained crush injuries to dorsal roots on PD1-2 and PD23 (2 wk. survival). We used immunohistochemistry on sections through the DREZ to visualize the presence of the potentially inhibitory molecules chondroitin-6 sulfate proteoglycan (CS-PG) and its extracellular ligand, cytotactin (CT). The growth promoting molecules, NCAM and laminin, were also studied. GFAP antibodies were used to visualize astroglia.

CS-PG first appears in the dorsal columns of normal PD1 animals in two narrow bands on either side of the roof plate. Staining for CS-PG spreads laterally to the DREZ and ventrally to the region above the corticospinal tract during the next few days. The spatio-temporal pattern of staining for CT mimics that of CS-PG. In animals with root crushes at PD23 there is an increase in the staining intensity only on the lesion side of both CT and CS-PG that mimics the pattern of reactive GFAP-positive astrocytes in the DREZ and the dorsal columns. In neonates with root crushes the pattern and intensity of CT, CS-PG and GFAP staining is comparable on the two sides of the cord. CT and CS-PG are not found on Schwann cells but CS-PG is found in the endoneurium in normal and crush lesioned animals. Our results suggest that the concentration of putative neurite inhibitory molecules on reactive astrocytes, after the critical period, may help cause regenerative failure of sensory afferents into the spinal cord.

74.10

THE EFFECTS OF PHYSIOLOGICAL STOP PATHWAY ACTIVATION AT THE DORSAL ROOT TRANSITIONAL ZONE ON NF GENE EXPRESSION IN DRG NEURONS. F.J. Liuzzi and B.Tedeschi*. Dept. of Anatomy and Neurobiology, Eastern Virginia Medical School, Norfolk, VA 23501.

After dorsal root crush in adult mammals, many regenerating axons stop among dorsal root transitional zone (DRTZ) astrocytes. NFs are absent in DRTZ axo-glial endings, but accumulate and swell physically blocked axon endings within ligation neuromas. Based on these observations, Liuzzi and Lasek (Science, 237:642, 1987) proposed that astrocytes in the DRTZ stop axonal growth by activating the physiological stop pathway, part of which is the protease-dependent breakdown of NFs.

This study uses 2-D gel electrophoresis to examine NF synthesis in DRG neurons after root crush and regeneration into the DRTZ or into a physical barrier formed by tightly ligating the root. By 3 days post-crush, 68KDa NF protein synthesis is decreased compared to normal control levels. Yet by 10 days post-crush, the time that the axons reach the DRTZ, 68KDa NF protein synthesis returns to control values. By contrast, at 10 days post-crush, 68KDa NF protein synthesis in DRGs with physically blocked axons remains depressed.

Supported by a Grant (NS24309) from the NIH (FJL) and an award from the Jeffress Foundation (BT and FJL).

74.11

ACCUMULATION OF APOLIPOPROTEIN A-I AND E IN THE RABBIT FACIAL NERVE FOLLOWING TRANSECTION AND NERVE REPAIR. L.T. Wang-Bennett, D.P. Slaughter*, D.J. Liebl* and S. Moore*. Dept. of Otorhinolaryngology and Communicative Sci., Baylor College of Med., Houston, TX 77030.

Studies have shown increased synthesis of apolipoprotein (Apo) E and A-I in degenerating distal stumps of peripheral nerve and discussed their role in a local lipid transfer system needed for regeneration. We describe the identification and relative quantification of Apo E and A-I in the case of transection without repair and for two surgical repair models of rabbit facial nerve.

Nerve extract proteins were separated by SDS-PAGE and transferred onto Immobilon membrane. Apo's were detected by specific primary antibodies which were recognized by alkaline phosphatase-conjugated second antibodies. Quantitative data was derived from densitometric measurement of the blots.

A similar time course for Apo E and Apo A-I was found for stumps that underwent simple transection. The time course showed two peaks; one during the first wk and a second during wk 2 to 4. There were higher amounts of Apo E than Apo A-I in the chamber- and cable-repaired nerve, and Apo E was elevated at wk 7 while the Apo A-I declined to normal. A greater accumulation of both Apo's occurred in the chamber model versus the cable model. The results demonstrate the presence of Apo E and Apo A-I in regenerating rabbit facial nerve and support their cooperative role in nerve regeneration. A different pattern of accumulation was observed than has been reported for crushed rat sciatic nerve. (Supported by the Texas Advanced Technology Program and by Coker Memorial Research Foundation.)

74.12

TRIGEMINAL NEURONS RESPOND TO INJURY OF THEIR SENSORY TERMINALS IN CORNEA BY INCREASING LEVELS OF GAP-43 (GROWTH ASSOCIATED PROTEIN-43) PROTEIN AND mRNA. H.E.P. Bazan, R.E. Martin,* Y. Tao,* and N.G. Bazan, LSU Eye Center and Neuroscience Center, New Orleans, LA 70112.

The role of GAP-43 in neuronal signal transduction is an area of intense investigation. This phosphoprotein was previously implicated in the resurgence of neurons after ischemic insult, but the role of GAP-43 in neuronal wound healing is only beginning to be elucidated. We have colocalized GAP-43 and neuronal cell adhesion molecule (N-CAM) in the sensory nerve fibers of adult rabbit cornea. Immunocytochemical data indicate that these antigens are present in deep stromal fiber bundles and fibers of the subepithelial plexus. An immunoassay using mouse anti-GAP-43 and [^{125}I]-protein A quantitated relative abundance of GAP-43 in various tissues and subcellular fractions and Northern blot analysis was used to probe for GAP-43 mRNA production in the trigeminal ganglia. We have demonstrated that 48 h after mild alkali injury GAP-43 levels increased in the cornea and 72 h after injury, GAP-43 mRNA increased in the ganglia. These temporal differences suggest the initial transport of endogenous GAP-43 to the cornea and subsequent synthesis of GAP-43 mRNA in the ganglia. The epithelial and stromal tissue layers were assayed for relative abundance of membrane-associated and cytosolic GAP-43. More GAP-43 was membrane-associated than soluble, and some GAP-43 was retained by the membrane fraction after detergent solubilization. Collectively these data indicate that GAP-43 may play a role in corneal wound healing and that corneal trigeminal sensory neurons provide a good model for study of GAP-43 *in vivo*. GAP-43 may also be a useful marker of nerve repair during wound healing. Supported by NIH grant EY06635.

MONOAMINES AND BEHAVIOR II

75.1

STRESS IMPAIRS PREFRONTAL CORTEX COGNITIVE FUNCTION IN MONKEYS: ROLE OF DOPAMINE. A.F.T. Arnsten and P.S. Goldman-Rakic. Sect. of Neuroanatomy, Yale Medical School, New Haven, CT 06510.

Humans exposed to mild stress (white noise ≥ 95dB) show cognitive deficits similar to those produced by lesions to the prefrontal cortex (PFC). These deficits may arise from excessive dopamine (DA) activity in the PFC, as mild stress selectively activates the meso-prefrontal cortical DA system in rats. The present study explored this hypothesis in monkeys by determining 1) whether exposure to mild stress would induce a pattern of cognitive deficits consistent with PFC dysfunction, and 2) if stress-induced deficits could be blocked by treatments which decrease DA stimulation in the PFC. Monkeys were tested on either the delayed response task, a spatial working memory test dependent on the PFC, or a visual pattern discrimination task which does not rely on the PFC. Exposure to 100 dB white noise for 30 min prior to testing significantly impaired delayed response performance but had little effect on visual discrimination performance, consistent with PFC dysfunction. Pretreatment with haloperidol (0.005 mg/kg, I.M. 60 min before testing) impaired delayed response performance under control conditions, but improved performance when given prior to stress. Higher haloperidol doses which caused motor impairment were ineffective. The alpha-2 adrenergic agonist clonidine, which decreases stress-induced DA turnover in the PFC of rats, also ameliorated the cognitive deficits produced by stress. These results suggest that there is an optimal range of DA stimulation in the PFC and that either too little (haloperidol alone) or too much (stress) results in diminished PFC function. Supported by PHS grant #MH44866.

75.2

PHYSIOLOGICAL CORRELATES OF ADAPTIVE BEHAVIOR IN A VISUAL DISCRIMINATION TASK IN MONKEYS. T. Alexinsky[1], G. Aston-Jones, J. Rajkowski and R.S. Revay, Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann Univ., Philadelphia, PA, USA, and [1]U. René Descartes and LPN2 CNRS, 91198, Gif-sur-Yvette, France.

Previous studies have implicated the rostral pontine nucleus locus coeruleus (LC) in vigilance and adaptive sensory-behavioral responding. Here we have examined this framework by recording neurons in the LC area of cynomolgus monkeys performing an "oddball" visual discrimination-vigilance task. Monkeys were trained with colored lights serving as S+ or S-. Four bundles of 6 micro-wires (25-µm) were implanted bilaterally in the LC area for recording neuronal impulse activity. Event-related potentials (ERPs) were recorded from skull screws. Stimulus duration, time to respond, interstimulus interval, S+/S- ratio, and session duration were systematically varied to alter task difficulty and attentiveness. Monkeys were also subjected to reversal training.

Histologic reconstruction of all recording sites is not completed, but certain classes of neuronal responses in the rostral pons are apparent. Apart from cells that could not be driven by any aspect of the task (25%), many neurons were classified as sensory (23%), motor (14%) or reward cells (2%). However, a large population of cells in the LC area (36%) exhibited activity that was specifically related to meaningful stimuli (i.e., driven by S+ but not S-). These cells altered their responsiveness to be activated by the new S+ during reversal training in close correlation with behavioral performance. ERPs were also specifically evoked by the S+, whether overlearned or during stimulus reversal (correlation between ERP amplitudes and behavioral performance during reversal = 0.89). Thus, strong relationships exist among activity of certain cells in the LC area, cortical ERPs and adaptive behavior in a task requiring sustained attention. These relationships are being examined in more detail by monitoring attention via eye position and autonomic activity via pupillary diameter in a more sophisticated discrimination task. Supported by AFOSR grant 90-0147, and ONR contract N00014-86-K-0493.

75.3

NORADRENERGIC INNERVATION OF THE THALAMIC NUCLEUS VENTRALIS POSTEROLATERALIS: A MORPHOLOGICAL BASIS TO ACCOUNT FOR THE CONTROL OF CAT ATTENTIVE BEHAVIOR. P. Delagrange*, J.J. Bouyer, M. Conrath*, C. Durand*, M. Geffard* and A. Rougeul*. *Institut des Neurosciences, Département de Neurophysiologie comparée, CNRS-Université P. & M. Curie, 9 quai St Bernard, F-75005 Paris.*

We could previously show that acting upon the central noradrenergic system (pharmacologically or through lesioning locus coeruleus) markedly priviledged a particular pattern of attentive behavior in cat, that of expecting a non-visible target (while it impaired the animal's focused attention on a visible one). Expectancy was always accompanied by the development of 14 Hz ("mu") rhythms localized on the somatosensory SI cortex, these rhythms being commanded by a small portion of the thalamic nucleus ventralis posterior (VP) (anterior part of its posterior third). Moreover a retrograde HRP study has shown that this thalamic area receives afferents from the locus coeruleus. These various data prompted a more thorough investigation of the noradrenergic innervation of VP. This has now been carried on using an anti-conjugated NA antibody. This antibody being employed for the first time in the cat, several controls were performed, such as preincubation of the antibodies with NA, Dopamine or GABA before slices incubation and control labelings of nuclei and fibers known to contain NA.

We could then notice, in the VP zone that we knew to be the control site for mu rhythms, numerous NAergic axonal endings, mostly located around non-labeled cell bodies and proximal dendrites, and only a few labeled cell bodies. On EM pictures, the NAergic endings appeared as either axo-somatic or axo-dendritic without visible synaptic differentiation and the labeled pericarya did not show characteristics of intracellular NA synthesis.

The lack of synaptic differentiation could indicate that NA release is non-synaptic, with receptors being remote from the endings as was previously shown in other structures (Descarries et al 1977). After its release in the intercellular space, NA could increase the signal/noise ratio in the thalamic transfer of sensory messages to the cortex. A thalamic modulation of the afferent information may thus be one of the mechanisms involved in the regulation of the various types of attentive behaviors.

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75.4

THE BINDING OF ^{125}I-IODOPINDOLOL (^{125}I-IPIN) TO SUBTYPES OF ß ADRENOCEPTORS (BARs) IN THE BRAINS OF H & L RATS. **P. Areso[1], M.A. Cierpial[2], J.M. Weiss [2], K. Williams[1], A. Frazer[1], [1]Univ. of Pa & Dept. Vet. Affairs Med. Ctr., Phila., PA 19104; [2]Duke Univ. Med. Ctr., Durham, NC 27710.**

Weiss and his colleagues have successfully bred rats which display either high (H rats) or low (L rats) motor activity in a 15 min swim test. They have found that H rats exhibit higher dorsal bundle activity than L rats (see abstract by Cierpial et al.). As central BARs are regulated by their exposure to norepinephrine (NE) released from noradrenergic neurons, we measured BARs in the brains of H and L rats. Subtypes of BARs were measured by quantitative autoradiography using ^{125}I-IPIN as the radioligand (*Proc. Natl. Acad. Sci.*, 81:1585, 1984). Also, these rats were given a 15 min swim test to determine if this mild stress altered their BARs. In several regions of brain, the H rats had higher binding of ^{125}I-IPIN to both $ß_1$ and $ß_2$ adrenoceptors than that found in the brains of L rats. This was particu-larly evident in the dentate gyrus (granular layer) of the hippocampus and caudate-putamen. Such an effect was not found in amygdaloid or hypothalamic nuclei. Most striking were relatively large (about 40%) swim stress-induced increases in ^{125}I-IPIN binding to $ß_2$ but not $ß_1$ adrenoceptors in the H rats. This effect occurred in hippocampal, cortical, and hypothalamic areas. By contrast, swim stress did not change ^{125}I-IPIN binding to $ß_2$ adre-noceptors in the L rats. Although these changes in BARs may reflect differences in activity of noradrenergic neurons in the H and L rats, it is equally plausible that other, perhaps hormonal, factors are responsible for the differences observed. **(Supported by Research Funds from the Dept. of Vet. Affairs & USPHS grant MH 42637).**

75.5

ELEVATED DORSAL NORADRENERGIC BUNDLE TURNOVER AND PLASMA CORTICOSTERONE RESPONSES TO STRESS IN RATS SELECTIVELY BRED FOR HIGH SWIM TEST ACTIVITY. M.A. Cierpial, A.C. Grobin*, E.F. Hargrove*, K.P. McLean*, J.C. Ritchie, C.D. Kilts & J.M. Weiss. Department of Psychiatry, Duke University Medical Center, Durham, NC 27710.

We previously reported on the development and behavioral characterization of rats selectively bred for high or low motor activity in a swim test (Cierpial et al., Neurosci Abst, 1989). In the present studies, we have examined regional brain norepinephrine (NE) turnover and plasma corticosterone (CORT) responses to stress in 6th generation male High and Low rats.

High and Low rats were exposed to one of three stressors: a 15 minute swim test (swim stress), 5 mild electric tail shocks delivered over a 30 minute period (mild shock stress), or 90 minutes of intermittent tail shock. Animals were sacrificed immediately following the stress procedure. Trunk blood was collected for assay of CORT and brains were dissected for assay of NE and its two major metabolites, MHPG and DHPG in various regions. Nonstressed, home-cage animals served as controls.

High rats responded to swim stress and mild shock stress with greater increases in plasma CORT than did Lows. Following 90-min intermittent shock stress, plasma CORT levels were highly elevated and did not differ between Highs and Lows.

NE, MHPG and DHPG levels were similar in home-cage Highs and Lows; however, High animals responded to each stressor with greater increases in NE turnover, primarily in dorsal bundle projection regions. For example, following mild shock stress High rats displayed greater increases in MHPG in the hypothalamus (HY), frontal cortex (FC), and dorsal hippocampus (DH) with the largest differences observed in the FC and DH (dorsal bundle projection areas).

These data indicate that rats bred for high activity in the swim test display an exaggerated adrenocortical response to mild stressors and heightened dorsal bundle activity to stress compared with animals bred for low activity.

75.6

THE SAME MANIPULATION OF LOCUS COERULEUS (LC) ACTIVITY PRODUCES DIFFERENT BEHAVIORAL AND NEUROENDOCRINE RESPONSES IN RATS SELECTIVELY BRED FOR HIGH AND LOW SWIM TEST ACTIVITY. J.C. Stout, M.A. Cierpial, A.C. Grobin, K.P. McLean, P.A. Scott, J.C. Ritchie, C.D. Kilts, & J.M. Weiss. Depts. of Psychology and Psychiatry, Duke University, Durham, NC 27706.

Studies of a model of behavioral depression in rats have shown that behavioral inactivity in the swim test is associated with depletion of norepinephrine (NE) in the LC (Weiss et al.,[1981] Br.Res.Rev.:3, 167). Animals selectively bred for behavioral differences in the swim test also show marked differences in NE-LC function (Cierpial et al., see adjacent abstract). To study the role of the LC in behavioral activity, microquantities of drugs that affect LC firing were infused into the LC. Manipulation of LC activity (by infusion of the α-2 antagonist idazoxan to stimulate LC activity, or desiprimine to decrease LC activity) over 30 minutes before and during the swim test had no significant effects in normal (i.e. non-selectively bred) animals or in animals bred to show low activity in the swim test. However, in animals bred for high activity in the swim test, stimulation of the LC significantly decreased swim-test activity. Neurochemical measurement of NE and its major metabolites (DHPG and MHPG) in LC tissue collected immediately following the swim test confirmed that the drugs infused had the intended effects of increasing or decreasing LC activity.

Measurements of plasma corticosterone (CORT) also indicated that idazoxan produced divergent effects on the selectively-bred animals. Idazoxan-induced stimulation of LC firing elevated CORT in low-active animals but reduced CORT in high-active animals.

The results of these studies demonstrate that direct pharmacological manipulation of LC firing produces different behavioral and neuroendocrine responses in animals that possess particular behavioral and genetic characteristics.

75.7

WITHDRAWN

75.8

EFFECTS OF PUNISHMENT ON NEUROTRANSMITTER TURNOVER. S.I. Dworkin, S. Izenwasser, C. Co*, R. Kaltenback* and D. Yoshishige*. Dept. of Physiol/Pharmacal, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27104.

This study was a replication and extension of our previous work on the neurochemical effects of punishment. Ten littermate triads, of adult male F-344 rats, were studied using a procedure which allows for the determination of the specific neurochemical effects of punishment. Similar rates of punished and nonpunished responding, maintained with equated rates of reinforcement, were established in two rats of each triad. The third rat of each triad was exposed to noncontingent food and shock presentation yoked to the littermate on the punishment procedure. Thus, the specific effects of punishment could be determined from comparisons of the three conditions. When responding was stable all rats were implanted with jugular catheters for pulse labelling of neurotransmitters. The rats were pulsed labelled with [^{3}H]-tyrosine, [^{3}H]-tryptophan and [^{14}C]-glucose and sacrificed after either 60 or 90 minutes exposure to the schedule contingencies. The turnover rates of dopamine, norepinephrine, serotonin, aspartate, glutamate, glutamine and GABA were determined in several brain regions. Punishment specific changes in turnover of the biogenic amines included NE in the frontal cortex and caudate putamen, dopamine in the caudate putamen and pyriform cortex and 5HT in the pyriform cortex and nucleus accumbens. Punishment specific changes in GABA turnover were observed in the frontal cortex, pyriform cortex and nucleus accumbens. The turnover of aspartate, glutamate and glutamine was increased in several regions in the two groups exposed to electric shock.

(Supported in part by USPHS DA-01999)

75.9

CEREBRAL DOPA DECARBOXYLASE ACTIVITY IN SCHIZOPHRENIA MEASURED BY POSITRON EMISSION TOMOGRAPHY. J. Reith, C. Benkelfat, H. Kuwabara, G. Chouinard, P. Etienne, S. Bachneff and A. Gjedde (Positron Imaging Laboratories, McConnell Brain Imaging Center, Montreal Neurological Inst. and Dept. of Psychiatry, McGill Univ., Canada).

To test the hypothesis that positive symptoms in schizophrenia are associated with increased dopaminergic neurotransmission in brain, regional cerebral DOPA decarboxylase activity was determined in male schizophrenic patients and healthy volunteers by [^{18}F]fluoro-L-DOPA and positron emission tomography. Evaluation of patients was made independently by two psychiatrists using the semi-structured clinical interview (SCID). Patients met the DSM-IIIR criteria for schizophrenia, with a mean age of 37 y and mean duration of illness of 15 y. All were drug-free for more than 1 year. The enzyme activity was calculated on the basis of a kinetic model, that enabled the estimation of the rate constant (k_3) for the conversion of [^{18}F]fluoro-L-DOPA to [^{18}F]fluoro-L-dopamine. Patients were scanned by a Scanditronix PC2048 15 plane PET scanner for 90 min, after i.v. injection of 2.5-5 mCi [^{18}F]fluoro-L-DOPA. Serial arterial blood samples were obtained and counted, and plasma samples were assayed immediately by HPLC. PET images were correlated by MRI. The regional values of caudate DOPA decarboxylase activity (k_3) in units of min^{-1} in schizophrenic (n=3) and control (n=5) groups respectively were: 0.088±0.011 and 0.068±0.011. This increase was not significant.

75.10

CSF 5-HIAA AND HVA CORRELATE WITH THE MMPI PSYCHOPATHIC DEVIATE SCALE IN DEPRESSION BUT NOT SCHIZOPHRENIA W.O. Faustman, R.J. King*, K.F. Faull, J.A. Moses, Jr.*, K.L. Benson*, V.P. Zarcone*, and J.G. Csernansky. Stanford/VA Mental Health Clinical Research Center, 4C2, VA Med Center, Palo Alto, CA, 94304.

Brown et al. (Am J Psychiatry, 139:741-746,1982) found a correlation between the Minnesota Multiphasic Personality Inventory psychopathic deviate scale (Pd, Scale 4) and CSF 5-hydoxyindoleacetic acid (5-HIAA) in personality disordered men. The Pd scale is widely thought to reflect impulsivity and risk taking. We found that the CSF 5-HIAA/Pd relationship extends (r_S = -.38, P < .05) to a sample of 21 unmedicated males meeting RDC criteria for depression. A trend (P < .1) was found between HVA and Pd in depression. There was also a significant correlation between the MMPI Depression Scale (D, scale 2) and HVA (r_S = -.46), but the D scale was not correlated with 5-HIAA. The Pd and D scales were not intercorrelated in this sample. No relationship was noted between CSF metabolites and the MMPI scales in 24 RDC-diagnosed schizophrenics. Though our diagnostic methods could not fully assess for the presence of concurrent personality disorders defined by more recently developed diagnostic systems, our results suggest the Pd/5-HIAA relationship extends to patients with a primary diagnosis of a mood disorder. In addition, self-report measures of overall depression appear to relate to CSF monoamine metabolites.

Supported by MH 30854 from the NIMH.

PRESYNAPTIC MECHANISMS I

76.1

SELECTIVE INHIBITION OF EXCITATORY SYNAPTIC CURRENT BY ADENOSINE. K.-W. Yoon and S.M. Rothman
Departments of Pediatrics and Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, MO 63310

We examined the effects of adenosine and baclofen on inhibitory (IPSC) and excitatory (EPSC) synaptic currents in dissociated rat hippocampal neurons with the tight seal whole cell voltage clamp method. Adenosine (100 μM) reduced monosynapatic evoked EPSC's to 21.9±4.2% (n=7) of control but had no significant effect on the evoked IPSC's (n=9). In separate experiments adenosine (50 μM) diminished the peak EPSC to 19.6±4.0% of control (n=3) and this effect was attenuated by coapplication of theophylline (0.5 mM) to 38.1±4.3% of control (p<.01). Baclofen (100 μM) reduced EPSC's to 21.9±2.4% of control (n=6) and IPSC's to 29.1±5.3% of control (n=5). We were unable to elicit any significant changes in resting membrane potential or membrane conductance by bath application of either adenosine or baclofen. Our findings indicate that adenosine and baclofen have different presynaptic effects and that excitatory and inhibitory neurons have different presynaptic pharmacology. By its selective action on excitatory transmission, adenosine may be an attractive agent for controlling abnormal neuronal excitation in epilepsy. Supported by NIH NS14834 and NS19988 and the McDonnell Center for Cellular and Molecular Neurobiology.

76.2

EXOCYTOSIS OF LARGE DENSE-CORE VESICLES FROM CULTURED HIPPOCAMPAL PYRAMIDAL CELLS MONITORED BY REVERSE HEMOLYTIC PLAQUE ASSAY. A. Malgaroli & R.W. Tsien, Dept. Molecular and Cell. Physiology, School of medicine, Stanford University, Stanford CA 94305.

Like many other neurons, hippocampal pyramidal cells express large dense core vesicles (LDCV) containing peptides (e.g. substance P, NPY) as well as small synaptic vesicles (SSV). Little is known about the role of these peptides or the factors governing their release during normal or enhanced synaptic transmission. Differential control of the exocytosis of these vesicle types has been found in organ systems but not in individual central neurons. We have been studying the release of LDCV from single rat hippocampal pyramidal neurons in cell culture (6-15 days). As a general marker of LDCV, we used chromogranin A (ChrA), a secretory protein found in LDCV but not in SSV. A reverse hemolytic plaque assay (RHPA) was carried out using a polyclonal antibody against ChrA, which detected a clear immunoreactivity in our cultured neurons. Action potentials were blocked by 1 μM TTX. ChrA release was detected by complement-induced lysis of sheep red blood cells coated with protein A to bind the antibody. Exocytosis was evoked by 2-4 hours incubation with various stimulating agents (e.g. 20-30 mM KCl, 50-100 μM glutamate): about 40-50% of the neurons were associated with clear evidence of haemolysis (10 experiments). In parallel controls, plaques were not seen if either the stimulant or complement was omitted or if preimmune serum replaced the ChrA antiserum. The spatial pattern of hemolysis suggests that LDCV are released from the larger dendritic-like ramifications, and in some cases, the cell body as well. Thus, RHPA can be applied to measure peptide release from single CNS neurons.

As a preliminary step toward comparing release of LDCV and SSV, we have monitored glutamate release by recording miniature EPSCs in postsynaptic neurons under whole cell voltage clamp (also with TTX present). The frequency of minis was greatly increased by α-latrotoxin.

76.3

ARACHIDONIC ACID-INDUCED CALCIUM MOBILIZATION IN HIPPOCAMPAL MOSSY FIBER SYNAPTOSOMES. D.S. Damron*, E.J. Freeman, D.M. Terrian and R.V. Dorman. Dept. of Biological Sciences, Kent State Univ., Kent, OH 44242

Exogenous arachidonic acid, melittin and KCl stimulate dose-dependent increases in intraterminal free Ca^{2+} ($[Ca^{2+}]_i$). However, it is unclear to what degree these increases can be attributed to the influx of external Ca^{2+} or its mobilization from intraterminal stores. Therefore, we incubated Fura-2/AM loaded hippocampal mossy fiber synaptosomes with and without external Ca^{2+} and a mixture of voltage-sensitive calcium channel blockers (VSCCB). The increase of $[Ca^{2+}]_i$ induced by 45 mM KCl was reduced by 65% when Ca^{2+} was omitted, while the addition of the VSCCB to the Ca^{2+}-free incubations caused a further reduction to 93% of the stimulated control. The increase in $[Ca^{2+}]_i$ induced by 35 uM arachidonate was reduced 53% in the absence of external Ca^{2+}, while the addition of the VSCCB caused a 63% reduction. The increased $[Ca^{2+}]_i$ stimulated by the phospholipase A_2 activator melittin (0.88 uM) was reduced 45% in the absence of Ca^{2+}, while the VSCCB caused a further reduction to 69% of the stimulated control. It appears, therefore, that the increased $[Ca^{2+}]_i$ induced by membrane depolarization with K^+ is due primarily to Ca^{2+} influx. In contrast, approximately 30% of the increases in $[Ca^{2+}]_i$ observed in the presence of arachidonate or melittin are due to Ca^{2+} mobilization from intraterminal stores. Supported by AFOSR 89-2045.

76.4

CHARACTERISTICS OF ACTION POTENTIAL INDEPENDENT SPONTANEOUS INHIBITORY CURRENTS. T.S. Otis, K. J. Staley and I. Mody, Dept. of Neurology & Neurol. Sci., Stanford Univ. Sch. of Med., Stanford, CA.

Spontaneous inhibitory postsynaptic currents (sIPSCs) were recorded in granule cells using the whole-cell slice patch technique, in 400 μm rat hippocampal slices maintained at 34±1° C. These $GABA_A$ receptor mediated currents persist in 1 μM TTX, suggesting that they are independent of action potential generation in the GABAergic interneurons. This is an ideal preparation for studying both presynaptic and postsynaptic functioning of the interneuron-granule cell synapse. With this in mind, all of the described experiments were carried out in 1-1.5 μM TTX, in symmetrical Cl^- concentrations.

At -70mV, an ensemble average of hundreds of sIPSCs shows a monoexponential decay with a time constant (T_{DECAY}) of 5 to 6 ms. Amplitude histograms of events sampled over two minutes were best fitted by Gaussian distributions, with mean values of 35 pA. Inter-event intervals recorded during the same time periods were plotted as exponential probability density functions, with time constants (T_{FREQ}) corresponding to mean inter-event intervals, ranging from 50 to 450 ms. While these values show wide cell to cell variation, they are extremely stable in individual cells. Increasing $[K]_o$ from 2.5 to 18mM reversibly decreased T_{FREQ} by 55%. Conversely, 50 μM baclofen reversibly doubled T_{FREQ}. The GABA uptake inhibitor nipecotic acid (500-1000 μM) also increased T_{FREQ} in a concentration dependent manner. These results present a method for quantifiying the amount of presynaptic inhibition at GABAergic terminals, as well as the transmitter pool available for release.

Supported by the NIH. T.S. Otis is a Howard Hughes Predoctoral Fellow; K.J. Staley is a Dana Postdoctoral Fellow.

76.5

ACTION POTENTIAL INSENSITIVE SPONTANEOUS EXCITATORY POSTSYNAPTIC CURRENTS IN DENTATE GYRUS GRANULE CELLS. K.J. Staley, T.S. Otis and I. Mody, Dept. of Neurology & Neurol. Sci., Stanford Univ. Sch. of Med., Stanford, CA.

TTX-resistant spontaneous postsynaptic currents (PSCs) were recorded in dentate gyrus (DG) granule cells using the whole-cell patch technique in 400 μm thick half-brain slices maintained at 34±1°C. Slices were cut either in the coronal plane, with disruption of perforant path (PP) fibres from the entorhinal cortex (EC), or in the horizontal plane, with preserved connections between the EC and DG (demonstrated by EC antidromic responses evoked by 100 Hz DG stimulation). Both bicuculline(BMI)-sensitive IPSCs and BMI-insensitive EPSCs could be recorded in the DG. IPSCs reversed near -55 mV (intracellular solution: Cs-gluconate, 135 mM; $MgCl_2$, 2 mM; HEPES 10 mM; pH 7.2). The average IPSC frequency measured at +10 mV was 112 events/minute in the coronal slices and 104 events/minute in the horizontal slices. The spontaneous EPSCs reversed near 0 mV and were blocked by the excitatory amino acid antagonists kynurenic acid or CNQX/APV. The average EPSC frequency measured at -75 mV in BMI was 61 events/minute in horizontal slices (EC afferents intact) and 26 events/minute in dorsal coronal slices.

Cutting slices in the coronal plane disrupted the PP and reduced EPSC, but not IPSC frequency. Since IPSC's are generated by local interneurons which synapse on or near the granule cell soma, our results suggest either that cutting in the plane of the PP preserves more excitatory PP synapses on the distal granule cell dentritic tree, or that that presynaptic neuronal integrity influences spontaneous action potential-independant synaptic transmission. Experiments addressing these possibilities will also be reported.

Supported by the NIH. K.J. Staley is a Dana Postdoctoral Fellow; T.S. Otis is a Howard Hughes Predoctoral Fellow.

76.6

PROTEIN KINASE C ACTIVATORS SELECTIVELY ENHANCE SYNAPTIC TRANSMISSION IN THE CEREBRAL GANGLION OF *APLYSIA*. S.M. Fredman. Dept. of Physiology, Meharry Medical College, Nashville, TN 37208.

The synaptic connections between the A and B cluster neurons in the cerebral ganglion of *Aplysia* exhibit a novel form of enhanced synaptic transmission, slow developing potentiation (SDP, Fredman, 1988; Neurosci. Abst. 14: 1207). A single 2 sec, 20 Hz train in an A neuron causes a long-lasting, synapse specific increase in the amplitude of subsequent EPSPs in B neurons tested at 3 min intervals. The slow rise (5 min to peak) and decay (τ = 25 min) of SDP suggests that it may be in part mediated by a second messenger. Evidence now indicates that the diacylglycerol (DAG)/protein kinase C (PKC) second messenger system may be involved. Phorbol esters are potent activators of PKC. Bath application of 2 μM phorbol 12,13 diacetate (PDA) selectively increased SDP mean EPSP amplitude 49% from 7.5±1.4 to 11.2±2.0 mV. There were no short-term changes in test EPSPs prior to the tetanizing train [4.2 ±0.6 v 4.0 ±0.1 mV with PDA]. Adding 30 μM OAG (oleoylacetylglycerol, a DAG analog), caused a significant increase in potentiated EPSPs [from 143% to 214% of initial EPSP amplitude], but had no effect in the absence of a tetanizing train. Injecting PDA directly into presynaptic A neurons also caused a selective increase in EPSP amplitudes of comparable magnitude (14.6 ±2.8 mV) following the tetanizing train. A neurons injected with 4-α-phorbol did not exhibit increased SDP. The actions of the PKC inhibitor H7 were also selective. Bath application of 40 μM H7 had no effect on synaptic transmission and did not reduce test EPSPs, but completely blocked SDP. These findings suggest that activation of PKC may play a key role in modulating synaptic transmission at the A-B neuron synapse and mediating SDP. This work was supported by NIH grant NS20846, RCMI grant RR03032 and NSF MRCE grant RII8714805.

76.7

FMRFAMIDE MODULATION OF ACTION POTENTIAL EVOKED SYNAPTIC TRANSMISSION IS MEDIATED BY A PERTUSSIS TOXIN-SENSITIVE G PROTEIN. H. J. Man-Son-Hing and P. G. Haydon, Department of Zoology, Iowa State University, Ames, IA, 50011.

Under specific culture conditions, a chemical synapse will form between cell bodies of Helisoma neurons. These large spherical somata provide a model for studies of synaptic modulation.

FMRFamide, a neuropeptide endogenous to Helisoma, reduces synaptic transmission at this giant synapse (Man-Son-Hing et al., Nature 341:237, 1989). Presynaptic action potentials evoke postsynaptic currents which are decreased in amplitude by FMRFamide. This modulation is due to 1) a reduction in the magnitude of the presynaptic calcium current and 2) a coordinate reduction in the sensitivity of the secretory machinery to internal calcium. A PTX-sensitive G protein(s) is involved in mediating the actions of FMRFamide on both ion channels and secretory machinery (Man-Son-Hing et al., Soc. Neurosci. Abst. 15:1148, 1989; Haydon et al., Soc. Neurosci. Abst., 1990). In this study, we have determined whether a PTX-sensitive G protein mediates FMRFamide's action on spike evoked transmitter release.

PTX or heat-inactivated toxin was pressure injected into the presynaptic cell 4 hours before assessing synaptic connectivity. FMRFamide (1μM) reduced the amplitude of spike-evoked postsynaptic currents in cells injected with inactivated toxin. In contrast, in synapses pretreated with active PTX, the extent of FMRFamide's modulatory effect was significantly decreased. Thus, PTX-sensitive G protein(s) pathways mediate FMRFamide's modulatory effect on action potential evoked synaptic transmission. This work was supported by NIH grant NS26650.

76.8

FMRF AMIDE MODULATION OF SECRETORY MACHINERY IS MEDIATED BY A PERTUSSIS TOXIN SENSITIVE G PROTEIN. P. G. Haydon, R. T. Doyle and H. J. Man-Son-Hing. Department of Zoology, Iowa State University, Ames IA 50011.

The neuropeptide Phe-Met-Arg-Phe NH_2 (FMRFamide) causes a presynaptic inhibition of neurotransmitter release from identified neuron B5 of *Helisoma trivolvis*. Previous studies (Man-Son-Hing et al., *Nature* 341:237, 1989) have demonstrated that FMRFamide coordinately reduces the magnitude of the voltage-sensitive-calcium current and reduces the secretory response to internal calcium. The action of FMRFamide on the calcium current is mediated through a PTX-sensitive G protein pathway (Man-Son-Hing et al., *Soc Neurosci. Abst* 15: 1148,1989). In this study we determined whether a similar G protein mediates FMRFamide's actions on secretory machinery.

Pairs of neuronal somata were cultured for three days in conditions which prevent neurite extension and allow the formation of a unidirectional chemical synapse between somata of presynaptic neuron B5 and cholinoceptive neuron B19. To monitor actions on secretory machinery, the frequency of spontaneous miniature inhibitory postsynaptic currents (MIPSCs) was recorded under conditions of basal free calcium levels in the presynaptic cell. Application of FMRFamide (≥1nM) reliably reduced MIPSC frequency without changing the resting calcium level. To test for G protein involvement in mediating this inhibitory action, the non-hydrolysable analog of GTP, GTPγS, was injected presynaptically in the absence of FMRFamide. GTPγS injection reliably reduced MIPSC frequency, while GTP injection had no significant affect. Injection of the A protomer of pertussis toxin (PTX) 20 minutes prior to FMRFamide application (1μM) prevented FMRFamide from reducing MIPSC frequency. Taken together these data demonstrate that the modulation of the sensitivity of secretory machinery to internal calcium can be mediated by a PTX sensitive G protein pathway.

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76.9

ACETYLCHOLINE RELEASE BY ISOLATED NERVE TERMINALS (SYNAPTOSOMES) IS QUANTAL. R. Girod*, M.-M. Poo+, L. Eder Colli*, J. Medilanski*, S. Ofori and Y. Dunant*. Department of Pharmacology, Centre Médical Universitaire, 1211 Geneva 4, Switzerland. + Department of Biological Sciences, Columbia University, New York NY 10027, USA.

Cholinergic synaptosomes isolated from the electric organ of *Torpedo* were laid down on a culture of *Xenopus* embryonic muscle cells. Whole cell recording in one of the myocytes revealed spontaneous synaptic currents (SSCs) that were generated soon after synaptosomes application. These SSCs very much resembled those occuring normally in *Xenopus* during synaptogenesis at early times after nerve-muscle contact. The SSCs produced by the *Torpedo* synaptosomes were blocked by tubocurarine, indicating that they were due to pulsatile release of acetylcholine (ACh). Thus, isolated nerve terminals retain *in vitro* their capacity to release ACh in a discontinuous, quantal manner.

76.10

EXTRACELLULAR CALCIUM MARKEDLY AFFECTS INTRACELLULAR CALCIUM DIRECTLY MEASURED IN MOUSE MOTOR NERVE TERMINALS. Z.-P. Fang and N. Robbins. Dept. of Neuroscience, Case Western Reserve Univ., Sch. of Med., Cleveland, OH 44106.

Intracellular $[Ca^{2+}]_i$ of motor nerve terminals critically affects transmitter release and many other cell functions, but resting values in vertebrates and effects of varying extracellular $[Ca^{2+}]_o$ have not been determined. In order to address this issue directly, a new technique was used to inject fura-2 into preterminal axons in mouse soleus muscle maintained at room temperature (Fang & Robbins, Neurosci. Abstr. 15: 484, 1989), and to determine putative $[Ca^{2+}]_i$ by ratio imaging, averaging values in three different areas of each nerve terminal. In normal Krebs solution with 2.5mM $[Ca^{2+}]_o$, computed $[Ca^{2+}]_i$ was 170 ± 50 nM, remarkably similar to values reported in crayfish nerve terminals (Delaney et al., J. Neurosci. 9: 3558, 1989). In 0.05mM $[Ca^{2+}]_o$, $[Ca^{2+}]_i$ was 45 ± 20 nM, i.e. there was a 4-fold change in $[Ca^{2+}]_i$ in response to a 50-fold extracellular change. In going from low to high $[Ca^{2+}]_o$, a steady state $[Ca^{2+}]_i$ was attained in less than 10 min, in agreement with previous physiological studies of MEPP frequency. The regulation of $[Ca^{2+}]_i$ at physiological temperatures, the relation of $[Ca^{2+}]_i$ to transmitter release, and effects of aging can now be investigated directly.

Supported by NIH grant AG08886.

76.11

EFFECTS OF CURARE ALONE OR COMBINED WITH THIOPENTAL OR HALOTHANE ON THE END-PLATE CURRENT AT THE FROG NEUROMUSCULAR JUNCTION. B. Bhattacharyya* and M. Sokoll. Department of Anesthesiology, University of Iowa College of Medicine, Iowa City, IA 52242.

The ability of potent inhalation (but not barbiturate) anesthetics to potentiate curare-like muscle relaxants is long recognized. The underlying mechanisms are not clear. Both types of anesthetic agents produce ion channel blockade. We studied the effects of curare alone and combined with either halothane or thiopental on the end-plate current (EPC) of the R. pipiens sciatic nerve-sartorius muscle preparation. End-plate currents were recorded using the two micro-electrode voltage clamp technique at stimulation rates of 0.4 and 40 Hz. After control recordings, curare was applied for 20 min and a further group of EPC's recorded. The perfusate was then changed to include either thiopental or halothane in addition to curare. A range of concentrations of all drugs were studied. **RESULTS:** Curare alone caused a significant decrease in EPC amplitude and a slight decrease in time constant of decay (tau) at 0.4 and 40 Hz. Addition of thiopental caused no significant decrease of EPC amplitude and a small decrease in tau. With halothane the EPC amplitude showed a further significant decrease. Tau was decreased to a similar extent with both halothane and thiopental. **CONCLUSIONS:** Halothane potentiates curare induced neuromuscular block by decreasing the release of acetylcholine from the motor nerve terminal.

REGENERATION: GENES, INHIBITORY FACTORS AND AXONAL TRANSPORT

77.1

ALTERED EXPRESSION OF NEURONAL CYTOSKELETAL GENES AFTER CORTICOSPINAL AXOTOMY IN THE ADULT HAMSTER. S.A. Kost-Mikucki and M.M.Oblinger. Dept of Cell Biology and Anatomy, Chicago Medical School, North Chicago, IL 60064.

Injury to the nervous system provokes a number of molecular responses. In peripheral neurons which have the ability to successfully regenerate, axotomy alters the expression of genes encoding the major cytoskeletal proteins. Tubulin and actin mRNA levels increase while neurofilament (NF) mRNA expression is downregulated after axotomy of peripheral neurons. Because neurons of the CNS generally do not successfully regenerate after injury, it was postulated that the pattern of changes in cytoskeletal gene expression after axotomy might differ from that in peripheral neurons. To examine this issue, *in situ* hybridization and immunoblotting were used to characterize changes in NF-L and tubulin mRNA and tubulin levels in axotomized corticospinal neurons in the adult male Golden hamster. The corticospinal tract was unilaterally transected in the medulla and animals were sacrificed at 2, 7 and 14 d after axotomy. Corticospinal neurons were retrogradely labeled with the dye Fast Blue at the time of axotomy. This allowed their subsequent identification and confirmed survival. Histological sections of motor cortex (where the cell bodies for corticospinal neurons are located) were hybridized with ^{35}S-labeled α-tubulin and NF-L cDNA probes and prepared for autoradiography. The results of *in situ* hybridization experiments revealed that both tubulin and NF-L mRNA levels were decreased after axotomy and remained decreased for at least 14 d. Immunoblotting experiments indicated that changes in cell body gene expression were reflected as changes in the composition of the corticospinal axons at later post-axotomy times. These results clearly show that a major aspect of the CNS neuronal response to axotomy, the tubulin gene response, differs from that in PNS neurons. This may be one of the factors contributing to regenerative failure of adult CNS neurons.

77.2

RAPID CHANGES IN NONNEURONAL CELL GENE EXPRESSION FOLLOWING SCIATIC NERVE TRANSECTION. J.G. Toma, P.A. Barker, S. Pareek*, A. Acheson, and F.D. Miller. Dept. of Anat. and Cell Biol., Univ. of Alberta, Edmonton, ALBERTA.

We have examined changes in nonneuronal cell gene expression at early timepoints following transection of the rat sciatic nerve. *In situ* hybridization analysis demonstrated changes in expression of several different mRNAs in both Schwann cells and epineurial fibroblasts as early as 3 hr postaxotomy. Histone H3.3 mRNA was dramatically increased at 3 hr in cells of the epineurium, while P_0 mRNA, a major myelin transcript, was decreased in Schwann cells of both the proximal and distal stumps. The changes in histone H3.3 and P_0 mRNAs were even more dramatic at 36 hr, while remaining localized to the same cell types. By 72 hr postaxotomy, P_0 mRNA was decreased in all of the Schwann cells of the distal segment. In contrast, epidermal growth factor receptor (EGFR) and nerve growth factor receptor (NGFR) increased in both Schwann cells and epineurial fibroblasts, as detected by *in situ* hybridization and immunocytochemistry. Three hr postaxotomy, the increase in EGFR and NGFR was limited to the epineurium, while at 36 and 72 hr, elevated levels were detectable throughout the epineurium and the body of the nerve. Interestingly, by 72 hr, receptor levels were increased in a proximal-distal gradient in the proximal segment, but uniformly throughout the distal segment. These data demonstrate that there are rapid and dramatic changes in Schwann cells and epineurial fibroblasts as early as 3 hours posttransection, and that these changes occur in spatial and temporal gradients during the first 3 days following axonal injury.

77.3

A cDNA FOR A NOVEL TYPE III INTERMEDIATE FILAMENT PROTEIN FROM GOLDFISH RETINA DURING REGENERATION. E. Glasgow*, P. Tesser*, and N. Schechter. Departments of Biochemistry and Psychiatry, SUNY at Stony Brook, NY 11794.

The goldfish visual pathway displays a remarkable capacity for development and plasticity throughout life. Furthermore, functional regeneration occurs after optic nerve injury. The intermediate filament (IF) protein composition of this pathway differs from that of higher vertebrates. Certain of these IF proteins may have structural attributes which support the growth characteristics associated with the goldfish visual pathway. A PCR technique, using synthetic oligomers to conserved IF core domains, and a 15 day post crush retinal lambda gt11 library was used to generate several cDNA clones. Sequence data from a portion of the coil 2 domain from two of these clones predicts a novel type III IF having highest homology with vimentin and a low homology with the neurorfilament-L protein. (EY 05212 to N.S.)

77.4

REGROWTH OF DORSAL ROOT AXONS INTO THE SPINAL CORD FOLLOWING IRRADIATION. T.J. Sims and S.A. Gilmore. Dept. of Anatomy, Univ. of Arkansas for Medical Sciences, Little Rock, AR 72205-7199.

This study examined the ability of injured dorsal root ganglion (DRG) axons to regrow into the irradiated spinal cord of the rat. Exposure of the lumbar spinal cord in early postnatal rats to X-rays induces a marked reduction in the population of both astrocytes and oligodendrocytes. This reduction in CNS glia is present throughout maturation of the spinal cord and continues into early adulthood. Upon this background of an altered glial environment the right L3 and L4 dorsal roots were crushed and then frozen. Dorsal root lesions were performed on three groups consisting of 13, 23 and 43 day old irradiated rats and age matched non-irradiated controls. Between 30 and 80 days following dorsal root lesion rats from each group were subjected to one of three different labeling procedures: anterograde labeling by cutting the root distal to the injury site and applying WGA-HRP to the cut end for transport into the spinal cord or retrograde labeling of nerve cell bodies in the L3 and L4 DRG's by injections of True Blue or Fluoro-Gold into the spinal cord. The number of retrogradely labeled, fluorescent neurons was counted in the DRG's on both injured and contralateral, non-injured sides. Anterograde labeling with WGA-HRP revealed a robust regrowth of DRG axons in the irradiated but not in the non-irradiated control cords. These observations were strengthened by the finding of True Blue or Fluoro-Gold labeled neurons in the DRG on the injured side, as well as the contralateral, non-injured side, in irradiated rats. Non-irradiated rats lacked labeled DRG neurons on the injured side, whereas the contralateral, non-injured side appeared to have a full complement of labeled DRG neurons. Ultrastructural observations from the 23-day-old injury group revealed that the irradiated cords did not develop a thick astrocytic scar at the dorsal root entry zone as did the non-irradiated controls. Thus, it appears that reduction of the normal complement of glia creates a permissive environment for regrowth of DRG axons into the spinal cord. Supported by Grants SCRF 745 from the PVA and NIH NS04761

77.5

ANTIBODIES AGAINST MYELIN-ASSOCIATED NEURITE GROWTH INHIBITORS COMBINED WITH TROPHIC FACTORS AND BRIDGES IMPROVE REGENERATION OF LESIONED NERVE FIBERS IN SPINAL CORD AND OPTIC NERVE. M.E. Schwab, L. Schnell and D. Cadelli. Brain Research Institute, University of Zurich, August-Forel-Str. 1, CH-8029 Zurich/ Switzerland.

Neurite growth inhibitors (NI-35, NI-250) are constituents of rat CNS myelin and inhibit neurite growth in a variety of in vitro systems. In vivo application of a neutralizing antibody (IN-1, Caroni and Schwab, 1988) leads to long-distance regeneration of lesioned cortico-spinal tract (CST) axons (Schnell and Schwab, 1990) in the spinal cord. Cyst and scar formation remains, however, a major mechanical obstacle for the sprouting CST. We now combine release of IN-1 from incapsulated hybridomas with peripheral nerve-, extracellular matrix-, or astrocyte-bridges, and trophic factor sources. E14 spinal cord tissue is a good source of neurotrophic activity provoking increased sprouting of the lesioned CST, but does not serve as a bridge since only few CST axons grow into the transplants, and end within the implant. - Intracranially lesioned optic nerve fibers of young adult rats survive and sprout under the influence of bFGF, and, in presence of antibody IN-1, re-elongate into and through the optic nerve chiasm. Interaction of regenerated axons with their target regions is under investigation.

77.6

A GLYCOPROTEIN FRACTION FROM ADULT CHICKEN GREY MATTER CAUSES COLLAPSE OF CNS AND PNS GROWTH CONES IN VITRO. Roger J. Keynes, Alan R. Johnson*, Caroline J. Picart*, Olenka M. Dunin-Borkowski* & Geoffrey M.W. Cook*. Department of Anatomy, University of Cambridge, Cambridge CB2 3DY, U.K.

A glycoprotein fraction which causes collapse of dorsal root ganglion growth cones in vitro has been isolated recently from chick somites by affinity chromatography with peanut agglutinin (PNA) (Davies et al., Neuron **4**, 11-20, 1990). Immobilised polyclonal antibodies directed against the 48K and 55K components of this fraction can be used to eliminate the collapse activity. We now find that these antibodies also cross-react with a component of M_r 48K in the adult chicken brain, and that detergent (2% CHAPS in PBS) extracts of grey matter contain potent collapsing activity for both CNS (retina) and PNS (DRG) growth cones in culture. The activity can be removed from the extracts by treatment with PNA-agarose, and can be recovered by elution of the immobilised lectin with 0.4M lactose. SDS-PAGE of the lactose eluate demonstrates that material of M_r 48K is recovered as the major component. To date, we have been unable to detect collapse activity in chicken white matter using these assay procedures. The presence of the 48K component in grey matter raises the possibility that this material could contribute to the failure of effective regeneration following injury to the adult CNS.

77.7

STIMULATION OF MAMMALIAN NEURITE OUTGROWTH BY TELEOST PNS. M.J.Anderson, T.D.Hassinger and L.R.Whalen. Dept. Anatomy & Neurobiol.,Colorado State Univ., Ft.Collins, CO 80523.

Several lines of evidence indicate that white matter in mammalian CNS possesses inhibitory properties that prevent the regrowth of axons in that environment. In order to test the limits of "white matter inhibition," we have plated postnatal rat dorsal root ganglion (DRG) neurons on sections of CNS and PNS tissue from the teleost Apteronotus albifrons. Previous results have shown that postnatal DRG neurons do not show significant growth on mammalian CNS white matter or nondegenerated mammalian PNS. In Apteronotus, regeneration of spinal cord and peripheral nerves normally does occur after injury, and it was expected that mammalian neurons would grow on these substrata. DRGs from 1-day postnatal rats were dissociated in collagenase/trypsin, and enriched for neurons by preplating. Teleost spinal cord and/or electric organ (comprised of PNS axons) were quick-frozen, and 10 um sections were thaw-mounted on glass-bottomed dishes. DRG neurons plated on sections of Apteronotus electric organ (DMEM medium, 10% CFCS, 37°C) showed exceptional neurite outgrowth. The number of cells with neurites and neurite lengths far surpassed that on other tissue substrates. In contrast, DRG neurons did not grow on sections of normal Apteronotus spinal cord. We conclude that Apteronotus electric organ lacks the inhibitory properties of mammalian white matter, and may contain neuron stimulating factor(s). Apteronotus spinal cord, although it can regenerate in response to injury, is not normally growing, and may have growth-inhibiting properties similar to those of mammalian spinal cord (but reversible in nature). Supported by NS 25951, the Spinal Cord Research Foundation, and the College of Veterinary Medicine and Biomedical Sciences, CSU.

77.8

EFFECT OF ASTROCYTIC AGE AND CONFLUENCE ON THE GROWTH OF PC12 NEURITES. D.M. Baorto and M.L. Shelanski. Dept. of Pathology, Columbia University, New York, NY 10032.

At the site of injury in the CNS, an astrocytic scar forms which is thought to impede the regrowth of nerve fibers through the area. However, tissue culture studies have shown that plating neurons onto confluent monolayers of astrocytes results in neurite outgrowth over the cells. In this study we tested neuronal decision-making between two substrates (collagen and astrocytes in low density culture), where extending neurites would reach a border between collagen and the astrocytic surface. Astrocytes that had been cultured from neonatal cortex either for 2 days or for 4-6 weeks in confluent culture were harvested and replated at low density on collagen-coated dishes. NGF-treated PC12 cells were added after attachment of the astrocytes. In cocultures with 2 day old astrocytes, we found that neurites extending along the collagen substrate grew freely over the astrocytes they encountered. In contrast, the growth of neurites from the collagen substrate onto the *in vitro* aged astrocytes was strongly inhibited. There was a marked preference for the collagen substrate, with neurite-bare areas often observed around the astrocytes. These findings suggest that astrocytes aged in confluent cultures undergo a change which results either in their becoming less suitable substrates for neurite growth or in their actively inhibiting this growth. This change is preserved after replating at low density. This may model the situation *in vivo*, and suggests that mature astrocytes may serve to impede nerve regeneration through mature CNS tissue.

77.9

ASTROCYTES MAINTAINED IN THE PRESENCE OF SOLUBLE MEDIATORS OF INFLAMMATION RELEASE INHIBITORS OF NEURONAL GROWTH FACTORS BOUND TO A LAMININ SUBSTRATUM. R.J. Riopelle, P.C. Johnson-Green*, M. Guo*, K.E. Dow. Apps Medical Research Centre, Kingston General Hospital, Queen's Univ., Kingston, Canada K7L 3N6.

Astrocyte enriched cultures release molecular species that copurify with heparan sulphate (HS) proteoglycans (PGs) and promote neurite growth following pretreatment of a laminin substrate in the absence, but not in the presence, of HS glycosaminoglycans (GAGs). Biosynthetic studies reveal that astrocytes release at least two families of HSPG, one of which may form a heparan sulphate-chondroitin sulphate hybrid proteoglycan. The titer of neurite promoting activity bound to the laminin substrate in conditioned medium (CM) of astrocyte monolayers treated with interleukin-1 (IL-1-CM) was 50% of untreated control (ACM). In the presence of lipopolysaccharide endotoxin the titer of neurite promoting activity of the conditioned medium (LPS-CM) was 1% of control. A 50-50 mix of ACM and LPS-CM had a titer of neurite promoting activity identical to that of LPS-CM suggesting the presence of inhibitors of neurite growth in LPS-CM. Excessive concentrations of free GAGs did not account for the inhibitory effects of LPS-CM.

The present studies suggest that soluble mediators of inflammation influence the functional phenotype of astrocytes directly or via astrocyte-microglia interactions and may begin to address a molecular basis for failure of the astrocyte milieu to support regenerative neurite elongation following injury to the CNS.

Supported by MRC Canada and the Rick Hansen Man in Motion Legacy Fund.

77.10

AXOTOMY ACCELERATES SLOW COMPONENT b OF AXONAL TRANSPORT. JM JACOB and IG McQUARRIE. DEPT. NEUROSCI., CASE WEST. RES. U., CLEVE., OH 44106.

Because the integrity of the axon depends on the supply of structural proteins that are synthesized in the nerve cell body, we examined the effect of axotomy on the delivery of these via slow axonal transport. We labeled newly synthesized proteins in rat motor neurons with 35S-methionine 7 d after crushing the distal sciatic nerve. The sciatic nerve system was harvested 7-21 d later for SDS-PAGE; tubulin, actin, calmodulin, and the 68 kD neurofilament protein (NF-L) were identified from fluorograms and removed for liquid scintillation counting. The peaks of labeling for SCb, the faster subcomponent of slow transport that represents the moving cytomatrix, advanced at 4.2 ± 0.2 mm/d in proximal axons, vs. 3.5 ± 0.2 mm/d after sham axotomy ($p < 0.01$). There was no change in the rate of transport for NF-L labeling that marks SCa--the moving microtubules and neurofilaments. Our findings suggest that the motors driving SCa and SCb differ, and that the SCb motor can adapt to the changing needs of the axon tip.

Supported by NINDS grant NS-18975.

77.11

ENHANCED TUBULIN DELIVERY CORRELATED WITH ALTERED MAP PHOSPHORYLATION DURING REGENERATION. Denis Larrivee, Dept. Physiology, Cornell Univ. Med. Coll., NY, NY 10021.

While investigating the role of protein phosphorylation in regenerating goldfish optic nerves, we identified a microtubule associated phosphoprotein (MAP), designated p9, that underwent a prominent pI shift during synaptogenesis. The shift in pI paralleled a redistribution of p9 from the assembled to unassembled tubulin compartment, suggesting that p9 phosphorylation regulated tubulin exchange between both compartments. To test this hypothesis tubulin labelling was monitored along the length of the axons following intraocular pulse labelling with ^{3}H-proline. At 16 and 25 hours post injection, tubulin labelling was greatly increased, but evenly distributed, suggesting that a fast transported tubulin fraction was incorporated into a more slowly moving tubulin pool. By contrast, GAP and synapsin showed well defined labelling peaks. When tubulin labelling was monitored in synaptosomal preparations during synaptogenesis, it remained elevated for many weeks. Labelling of the 2 synaptic proteins, however, was increased only transiently at the onset of synaptogenesis. TTX-induced impulse blockade from 21 to 35 days further increased tubulin labelling by 2 fold but had little effect on the synaptic proteins. These results suggest that an increase in tubulin exchange, mediated by p9 phosphorylation, may facilitate activity dependent, optic fiber reorganization during synaptogenesis.

77.12

ANTEROGRADE AXONAL TRANSPORT AND RELEASE OF THE PERMISSIVE TROPHIC FACTOR TRANSFERRIN IN REGENERATING PERIPHERAL NERVES. W. R. Kiffmeyer and A. L. Mescher. Medical Sciences Program, Indiana Univ. Sch. of Med., Bloomington, IN 47405.

Transferrin supplies iron to cells for use as a cofactor in several enzymes, including the rate limiting enzyme in DNA synthesis and those involved in respiration. Developing neurons contain relatively large amounts of this iron transport factor. We are testing the hypothesis that neural transferrin is involved in the growth-promoting effects of peripheral nerves in processes such as amphibian limb regeneration. Using a sensitive ELISA procedure for transferrin from the salamander Ambystoma mexicanum, we have examined axonal transport and release of this factor in intact and regenerating adult sciatic nerves. Bilateral effects were seen following unilateral amputation, but regenerating nerves showed higher concentrations of transferrin than controls. In the double ligature technique for determining direction and rate of transport, transferrin accumulated proximal to the first ligature at a concentration 2.5 times higher than the average value between the ligatures. The rate of transferrin transport in the fast anterograde component was approximately 3 mm per hour. There was no evidence for retrograde transport of this factor. Colchicine implanted around the lumbar plexus in a matrix of slow release polymer abolished the accumulation, indicating the increase was due to axonal transport rather than to edema or vascular blockage. In vitro studies with a 2-chambered system demonstrated release of transferrin from regenerating nerves four weeks after crush injury and this release was also blocked by colchicine. These studies suggest that the permissive trophic effect of nerves in avascular tissues may be due to axonal release of transferrin. (Supported by the US Army Med. Res. Contract DAMD17-87-C-7098).

77.13

PROTEOGLYCAN TRANSPORT IN REGENERATING GOLDFISH RETINOTECTAL PROJECTION. K.E. Dow, R. Levine*, M. Solc*, P. Merchant*, R.J. Riopelle. Queen's Univ., Depts. of Pediatrics & Medicine, Kingston, Canada K7L 3N6 & McGill Univ., Dept. of Biology, Montreal, Canada.

Retinal biosynthesis and fast axonal transport of glycoproteins and proteoglycans were examined in the regenerating retinotectal projection of the goldfish (Carassius auratus). At various times following optic nerve crush animals received intraocular injections of ^{3}H-glucosamine (^{3}H-Glc, 100 μCi) and were allowed to survive for 24 hours. Labelled glycopeptides (GP), heparan sulphate GAGs (HS) and chondroitin sulphate GAGs (CS) were separated and quantified in retina (R), optic nerve (N) and optic tectum (T) by HPLC.

^{3}H-glucosamine incorporation into GP, HS and CS in R increased dramatically between days 10/21 and day 35. The amount of ^{3}H-HS and ^{3}H-CS in N decreased from days 10/21 to day 35 while ^{3}H-GP decreased from day 10. Finally, in T, ^{3}H-GP and ^{3}H-CS followed the same general pattern seen in R while ^{3}H-HS decreased between days 10/21 and then increased to day 35. The ratio of HS/CS was approximately 1.5 at all times except at day 35 in R and T where it was increased.

These patterns of incorporation of ^{3}H-Glc into glycopeptides and GAGs do not correspond to previously reported patterns of precursor incorporation into new axon segment total protein. Furthermore, there was a differential increase in the HS/CS ratio in R and T as compared to N at day 35.

These observations suggest that the stages of regeneration in the goldfish retinotectal projection may correlate more closely with posttranslational processing of glycoprotein and proteoglycans than with gene expression of core proteins. Furthermore, in R and T, the markedly increased incorporation of ^{3}H-Glc into glycoconjugates at day 35 and the increased ratio of HS/CS at that time suggest that separate regulatory events may control posttranslational modifications during regeneration (days 10 and 21) and synaptogenesis (day 35).

NEURONAL DEATH: MECHANISMS

78.1

POLYAMINES INCREASE NERVE CELL SURVIVAL. G.M. Gilad and V.H. Gilad. Neuropsychiatry Branch, NIMH Neuroscience Center at St Elizabeths Hospital, Washington, DC, 20032.

The biochemical mechanisms that underlie the capability of neurons to survive after trauma are still unknown. Recently, an immediate early and transient elevation in polyamine biosynthesis (termed "the polyamine response")was found to be a general characteristic of peripheral and probably of CNS neurons after trauma. This finding led us: 1) to postulate that the polyamine response is an essential part of a protective molecular program mounted by neurons responding to trauma, and 2) to the rationale for the use of exogenous polyamines (PA) to enhance neuron survival after trauma. We have demonstrated that treatment with PA for a limited time interval (days) can prevent nerve cell loss during the period of programmed cell death and after axonal, neurotoxic, or ischemic injuries. Studies on the possible site(s) of polyamine action indicate that the injured neurons themselves can transport and use exogenous PA and that PA may act indirectly by increasing neurotrophic factor production.

78.2

EFFECTS OF MUSCLE INACTIVITY AND DENERVATION ON THE REGULATION OF A PUTATIVE MUSCLE-DERIVED NEUROTROPHIC AGENT: IN VIVO AND IN VITRO STUDIES. L.J. Houenou, L. Haverkamp*, R. Kunzi & R.W. Oppenheim. Dept. of Neurobiology and Anatomy, Bowman Gray Sch. of Med., Winston-Salem, N.C. 27103 and *Dept. of Neurology, Baylor College of Med., Houston, TX 77030.

Motoneuron (MN) survival in vivo can be enhanced by treatment with muscle extracts. Muscle activity and innervation have both been suggested to down regulate the availability of a putative muscle-derived neurotrophic agent. To test this notion we have examined MN survival in vivo and choline acetyltransferase (ChAT) activity in spinal cord neurons in vitro following treatment with partially purified extracts from inactive, denervated, aneural, and slow tonic and fast twitch muscles from embryonic and postnatal animals. MN survival in chick embryos treated in vivo with extracts from active and chronically inactive embryonic muscles did not differ. Extracts from fast twitch and slow tonic postnatal avian muscles are equally effective in promoting both MN survival in vivo and ChAT activity in vitro. Preliminary results indicate that denervated postnatal avian muscle extract is more effective than innervated muscle extract in promoting both MN survival in vivo and ChAT activity in vitro. Assessment of trophic agent levels in aneural avian embryonic muscle is in progress. At present, our results suggest that the availability of putative neurotrophic agent(s) may be regulated by innervation but not muscle activity.

78.3

IDENTIFICATION OF DEATH-ASSOCIATED PROTEINS SYNTHESIZED BY NGF-DEPRIVED NEURONS. D.P. Martin, R.M. Leimgruber,* and E.M. Johnson, Jr. Dept. of Pharmacology, Washington Univ. School of Medicine, Monsanto Co., St. Louis, MO 63110.

We have previously demonstrated that the death of NGF-deprived neurons requires the synthesis of RNA and protein. We hypothesize that neurons synthesize new proteins that actively mediate cell death after trophic factor deprivation. To test this hypothesis, we have asked whether new proteins are significantly induced in rat sympathetic neurons *in vitro* after NGF deprivation.

Neuronal cultures from E21 rat superior cervical ganglia were grown for one week in the presence of NGF. Triplicate cultures were acutely deprived of NGF, and at various times thereafter pulsed with [^{35}S]methionine/cysteine. Proteins were extracted and separated by 2D gel electrophoresis. Autoradiograms reproducibly resolved about 1300 spots. Different exposures from each gel were digitized and merged into a composite database, which permitted quantification of differential protein synthesis.

Several proteins were induced greater than ten-fold by NGF deprivation. The timecourse of their appearance was compared to the timecourse of commitment, the duration of NGF deprivation after which inhibitors of RNA and protein synthesis no longer prevent neuronal death. Samples were also prepared from living NGF-deprived neurons maintained in the presence of a cAMP analogue, interferon γ, or a depolarizing concentration of potassium. Some proteins were significantly decreased after NGF deprivation.

78.4

Ciliary Neurotrophic Factor (CNTF) prevents the degeneration of motoneurons after axotomy in new-born rats.

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Max-Planck-Institute for Psychiatry, D-8033 Martinsried, FRG.

The developmental period of naturally occuring motoneuronal cell death in rats is followed postnatally by a period of high sensitivity to axonal injury. After transection (on postnatal day 1) of the facial nerve, which was chosen as an experimental system, 81% of the corresponding motoneurons degenerated within 1 week. Application of 5μg purified CNTF in gelfoam to the lesion site rescued 76% of the corresponding motoneurons. Morphological changes, such as chromatolysis and eccentric location of the nuclei occuring in the facial motoneurons after lesion, were markedly reduced by CNTF. However, a significant increase in GFAP-immunoreactivity detectable in astrocytes surrounding the lesioned facial motoneurons was not eliminated by CNTF administration. CNTF, which is immunohistochemically detectable in the cytoplasm of Schwann cells in peripheral nerves, is only expressed at low levels in the first postnatal week, but then CNTF mRNA and protein increase more than 30 fold to the high adult levels, as determined by Northern blot and ELISA. As soon as these high levels of CNTF are reached, axonal lesion no longer results in neuronal degeneration. Thus, in adult rats endogenous CNTF is released by nerve lesion from Schwann cells and protects motoneurons from degeneration, whereas in new-born rats the endogenous levels of CNTF are too low to protect the axotomized neurons.

78.5

FLUNARIZINE PROTECTS SENSORY NEURONS FROM DEATH *IN VIVO* AND *IN VITRO*. K.M. Rich, J.P. Hollowell. Depts. of Neurosurgery and Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

Flunarizine, a diphenylpiperazine calcium channel blocking agent protects neurons from death both *in vitro* and *in vivo*. Rat E-15 DRG neurons cultured in the presence of NGF for 10 days consistently died 48 to 72 hours after NGF deprivation. Flunarizine (30-40 μM) added to the culture media at the time of NGF deprivation protected the neurons from death both morphologically and biochemically as revealed by phase contrast microscopy, scanning electron microscopy, and [35S] methionine protein incorporation assay. *In vivo* experiments demonstrated the protective effects of flunarizine on DRG neurons in neonatal rats after sciatic axotomy. Newborn rats (PND-1) were injected subcutaneously with flunarizine (25 mg/kg) every 12 hours. Unilateral sciatic nerve axotomy was performed under hypothermic anesthesia on PND-2, with continuation of flunarizine injections. On PND-3, 24 hours after nerve injury, the L4 and L5 DRG were removed bilaterally. Untreated rats experienced a 32% neuronal loss compared to only 14% neuronal loss in the flunarizine-treated group. Another group of rats was treated similarly, but with the twice daily dose of flunarizine increased to 50 mg/kg on PND-3 and continued until DRG removal on PND-8. These rats experienced a 35% loss in the untreated control group, compared to only a 10% loss in the flunarizine-protected group. The mechanism of this protection is not known. In cell culture, lidoflazine, another type IV calcium channel blocker appears similarly protective, while nimodipine, a dihydropyridine, did not demonstrate these protective effects. The effective dose range of flunarizine (30-40 μM) greatly exceeds that which is required for blockade of calcium channels (3-10 μM) and is the range at which flunarizine is known to inhibit calmodulin. However, calmidazolium, a potent calmodulin inhibitor does not provide the protective effects of flunarizine in tissue culture. Other intracellular mechanisms, in addition to calcium channel blockade, may explain its neuronal protective effects.

78.6

ALTERED CYTOSOLIC FREE CALCIUM, ATP AND CELL VIABILITY WITH REDUCED OXYGEN AND GLUCOSE AVAILABILITY IN PC12 CELLS. Gary Gibson and Lourdes Toral-Barza*. Cornell University Medical College at Burke Rehabilitation Center. White Plains, NY 10605

Previous studies in synaptosomes demonstrate that either hypoxia or hypoglycemia will elevate cytosolic free calcium ($[Ca^{2+}]_i$) and diminish ATP. To examine these interactions in a cellular system, the availabilty of oxygen and/or glucose to PC12 cells was manipulated under basal conditions and after stimulation with K^+. With potassium depolarization, basal $[Ca^{2+}]_i$ (92 $\pm$ 3 nM) rapidly peaked (587 $\pm$ 38 nM) and then declined to a new plateau (262 $\pm$ 4 nM). Decreasing oxygen availability with KCN or removing glucose from the media did not significantly alter $[Ca^{2+}]_i$. However, reducing the availability of both glucose and oxygen elevated $[Ca^{2+}]_i$ basal values (310 $\pm$ 6 nM), the peak after K^+ (848 $\pm$ 55 nM) and the plateau level after K^+ (724 $\pm$ 41 nM). Although reduced oxygen availability diminished ATP by only 14% and a lack of glucose was without effect on ATP, the combined treatment with or without K^+ reduced ATP by 94% within 5 minutes. Decreased oxygen availability diminished viability only 3% and lack of glucose was without effect, whereas reduced availabilty of glucose and oxygen diminished viability 10% within the 5 minutes treatment period. These studies demonstrate that $[Ca^{2+}]_i$ in these cultured cells is less sensitive to hypoxia and hypoglycemia than in isolated nerve endings. However, even within short time intervals a lack of glucose and oxygen can alter cell viability, perhaps through altered $[Ca^{2+}]_i$ and ATP.

78.7

INCOMING IMPULSE ACTIVITY DETERMINES NEURONAL DEATH IN THE DEVELOPING RAT SUPERIOR COLLICULUS. E. Fusco*, A. Gravina*, B. Margheritti* and L. Galli-Resta. Istituto di Neurofisiologia CNR, 56127 Pisa Italy.

In the developing rat visual system extensive cell death is observed in the retina and in the superior colliculus during the first postnatal days. It has been suggested that elimination of topographically incorrect projections and numerical matching of connecting structures result from cell death. Little is known of the mechanisms triggering this phenomenon. We have reasoned that electrical activity of incoming afferents could determine cell death in a developing brain structure. To test this hypothesis we have either blocked retinal ganglion cell impulse activity with intraocular injection of tetrodotoxin or temporarely increased the frequency of ganglion cell firing by threshold electrical stimulation in neonatal rats. Blocking retinal impulse activity caused a consistent decrease of cell death in the superior colliculus while increasing the firing frequency of incoming retinal fibers caused a significant increase of cell death. We conclude that incoming electrical activity can trigger the phenomenon of neuronal death during development.

78.8

CORTICOSTERONE ENHANCES KAINIC ACID-INDUCED CALCIUM MOBILIZATION IN CULTURED HIPPOCAMPAL NEURONS. E.M. Elliott, I.J. Chang * R.M. Sapolsky. Dept. Biol. Sci., Stanford Univ., Stanford, CA 94305

Corticosterone (CORT), the rat adrenal steroid secreted during stress, increases hippocampal vulnerability to excitotoxins, metabolic poisons, and hypoxia-ischemia. Energy supplementation and NMDA receptor blockers prevent this CORT effect. As calcium (Ca) regulation is energy dependent and a large Ca influx accompanies NMDA receptor activation, we investigated whether CORT exacerbates the mobilization of Ca induced by the excitotoxin Kainic Acid (KA). Primary cultures of fetal rat hippocampal neurons and glia were treated with 1 uM CORT for 24 hours. Following incubation in 6 uM fluo-3 AM (fluorescent Ca indicator), each neuron was exposed to 100 uM KA for 300 seconds.

CORT caused a 7-fold increase in the magnitude of the Ca response to KA and a 2-fold increase in recovery time. Raising the glucose concentration from 5 to 30 mM decreased the magnitude of the CORT effect by 70%. CORT decreases hippocampal neuronal glucose transport by 30%; decreasing glucose concentration 30% in control neurons (from 5.5 to 3.9 mM) mimicked the CORT effect on [Ca]. Thus, CORT increases the cytosolic free [Ca] following KA exposure in hippocampal neurons. This may be due to CORT's effects on neuronal energetics. The enhanced Ca mobilization may then explain the neuronal vulnerability and toxicity evident following CORT exposure.

78.9

THE PROTEIN SYNTHESIS INHIBITOR, ANISOMYCIN, REDUCES NEURONAL DEATH IN YOUNG ADULT RATS. L. López-Mascaraque and J.L.Price. Dept. Anat. & Neurobiol., Washington Univ., St. Louis, MO 63110.

Previous studies have shown that inhibition of protein synthesis can delay or prevent NGF-dependent neuronal death (Martin et al., *J.Cell Biol.*, 106:829, 1988). In this study, we tested whether a similar phenomenon occurs in non-NGF-dependent system, using layer IIa of the rat piriform cortex as a model. After the removal of olfactory bulb in the rat this layer undergoes consistent and very rapid transneuronal cell death (Heimer & Kalil, *J.Comp.Neurol.*, 178:559, 1978) .

Sprague-Dawley rats (50-100 g) were anesthetized with halothane and the olfactory bulb was removed. Three to six subcutaneous injections of anisomycin (100 mg/k) were given every two hours, beginning 15 to 30 minutes prior to the surgery. During a 24 h postoperative survival period, the body temperature was maintained at 35.5°-37.5°C. Brain tissue was stained with thionin and the De Olmos cupric-silver method for degenerating neurons. A control group did not receive anisomycin.

In the control group, many degenerating picnotic and/or argyrophilic cells were found in layer IIa of the piriform cortex. The group of rats with 6 anisomycin injections showed almost no neuronal death, but the mortality of the animals was very high. Most rats with lower numbers of anisomycin injections survived. In these rats, the number of degenerating cells in layer IIa of the piriform cortex, was significantly lower than in the control animals.

These results implicate protein synthesis in mechanisms of neuronal death in adult rats.

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78.10

A PUTATIVE CELL DEATH mRNA IN THE RAT CENTRAL NERVOUS SYSTEM: CONSTITUTIVE AND DEVELOPMENTAL EXPRESSION. G.A. Garden, M. Bothwell and E. W Rubel Depts. of Physiology & Biophysics and Otolaryngology, Univ. of Washington, Seattle, WA 98195

Testosterone repressed prostate message - 2 (TRPM-2) encodes clusterin, a secretory glycoprotein. Expression of this message is induced in systems of tissue regression and programmed cell death. In order to begin exploring the relationship of TRPM-2 regulation to programmed cell death in the nervous system, we examined the expression of TRPM-2 using *in situ* hybridization in E15, P8 and adult rat central nervous system.

TRPM-2 is expressed widely throughout the rat CNS. At all three ages, the highest level of expression was found in the choroid plexus and ependyma. In developing neural tissue, expression correlated with the gradient of differentiation; TRPM-2 is not detectable in the proliferative zone of the E15 animals, but levels of expression increase as cells migrate from the ventricular zone. In the adult, TRPM-2 is expressed throughout the CNS and certain distinct neuronal populations show enhanced expression.

Neuronal populations known to be undergoing peak levels of developmental neuronal death, motor neurons in the ventral horn of the spinal cord at E15 and cortical subplate neurons of P8 animals, were examined. Moderate to high levels of TRPM-2 expression are found in these two neuronal populations. Since these are also the most differentiated neurons in their respective regions, we cannot yet distinguish whether TRPM-2 expression is related to neuronal death or advanced differentiation.

We conclude that TRPM-2 is constitutively expressed in CNS tissue at these three stages. This mRNA is localized to stable adult neuronal populations as well as neuronal populations undergoing developmental cell death. It is therefore unlikely that induction of TRPM-2 in rat CNS is exclusively related to neuronal cell death. (Support: Hartford Foundation Fellowship and N.I.H. grants DC00395 and NS43323)

78.11

CHARACTERIZATION OF A PROTEIN THAT APPEARS IN THE NERVOUS SYSTEM OF THE MOTH MANDUCA SEXTA COINCIDENT WITH NEURONAL DEATH. S.E.Fahrbach, M.E.Montemayor, C.S.Giometti*, and E. J. Roy. Neurosci. Program, Univ. Illinois, Champaign, IL 61820; Bio.& Med. Res. Div., Argonne National Laboratory.

The 3 unfused abdominal ganglia of the moth Manduca sexta provide an enriched preparation for the study of neuronal death, as half of their nerve cells die shortly after adult ecdysis. Continued synthesis of RNA and protein is needed to mediate this postmetamorphic cell death, as treatment with cycloheximide and actinomycin D promotes neuronal survival (Fahrbach & Truman, 1988).

Two-dimensional gel electrophoresis followed by silver staining was used to discover potential neuronal death-related proteins in the moth. When protein patterns of ganglia taken prior to adult ecdysis (at a time when no neuronal death is occurring) were compared with protein patterns of 1-day old adults, an acidic protein of approximately 40,000 M.W. was detected in all samples from moths undergoing neuronal death. Identification of this protein and studies of its cellular distribution should yield insight into whether its expression is related to the functioning of neurons fated to die or to the continued existence of the survivors.

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78.12

IDENTIFICATION OF DYING NEURONS WITH ALZ-50: EFFECTS OF INFRAORBITAL TRANSECTION ON THE TRIGEMINAL PRINCIPAL SENSORY NUCLEUS (PSN). M.W. Miller and W.M. Al-Ghoul. Dept. Anatomy, Univ. Med. Dent. N.J.- Sch. Osteopathic Med. and R.W. Johnson Med. Sch., Piscataway, NJ 08854.

During normal development, many neurons in the rat PSN die in the period from 2 days before to 5 days after birth. We examined the expression of Alz-50 in the developing and mature PSN using two sets of subjects: (1) normal neonates and 30-day-old rats and (2) rats in which the right infraorbital nerves were transected on the day of birth or on postnatal day 30. Few immunoreactive neurons were present in the PSN of neonatal rats, but none were evident in 30-day-old rats. Three days after a neonatal lesion, a few immunoreactive neurons were scattered in sections of the left (unaffected) PSN and 5-10-fold more labeled cells were detected in the ventral portion of the right PSN (a termination site of the infraorbital nerve). Ten days after birth, no Alz-50-positive neurons were evident in the right or left PSN, but the number of Nissl-stained neurons in the right PSN was lower. Three and 10 days after transection of the right infraorbital nerves of 30-day-old pups, there were no Alz-50-immunoreactive neurons, nor was there a change in the number of neurons in the PSN. Thus, Alz-50 appears to label dying neurons when the degeneration occurs during the period of naturally occurring neuronal death. Funded by DE 07734 and AA 06916.

78.13

TESTOSTERONE FAILS TO REDUCE AXOTOMY-INDUCED NEURONAL CELL LOSS IN PREWEANING RATS. W.H.A. Yu and C.G. Cao*. Dept. of Cell Biol. & Anat.Sci.,CUNY Med. Sch., New York, NY 10031.

Motoneuron loss from axotomy in adult rats has been shown to correlate inversely with the level of testosterone (T) in circulation (Yu, W.H.A., Exp. Neurol. 102:230, 1988; J. Neurosci. 9:3908, 1989). The primary site of T action for enhancing neuronal survival appears to reside at the muscles. To further explore this view, axotomy was done in rats 10 days of age when androgen receptors were already present in muscles (Tremblay et al., Steroids, 29:185, 1977) but presumably not yet developed in motoneurons. Following unilateral transection of the facial nerve, two 5 mm-long Silastic capsules filled with T or saline were implanted subcutaneously. Neuronal cell number in the facial motor nucleus was counted in serial Paraplast-embedded sections stained with cresyl violet. Results indicate that cell loss was rapid and severe without gender difference or attenuation by T. Two weeks after axotomy, the cell number in the axotomized nucleus was reduced nearly in half. By 4 and 9 weeks after axotomy, less than 50% neuronal cell population remained. Failure of T to reduce cell loss could be due to the absence of androgen receptors in the facial motoneurons and their target muscles at young age. Alternatively, motoneurons of immature rats may be vulnerable to target deprivation, whereby muscle-derived trophic substances stimulated by T could not be brought to bear on motoneurons in a timely manner to prevent cell death.

79.1

CLONAL AND POLYCLONAL ARCHITECTURE OF THE CHIMERIC MOUSE RETINA. Dan Goldowitz and Robert W. Williams. University of Tennessee, School of Medicine, Memphis, TN 38163

Chimeric mice were made by combining *Mus musculus* and *Mus caroli* embryos. At maturity, retinas were processed to identify retinal cell genotypes using species-specific DNA probes (Rossant et al. '83). Using these in situ probes we find that all retinal cell types are labeled in a perfectly complementary pattern in alternate 4-5 μm sections.

The chimeric retina is composed of large and small blocks of cells called cohorts. Borders between cohorts are remarkably sharp. There are usually 10 to 50 cohorts in a cross section. Some are clones but many appear to be collections of clones (polyclones). The chimerism ratio in retinas of the five cases is close to that in ventral diencephalon (Goldowitz '89), suggesting that these structures arise from a common progenitor population. Differences in the size and position of cohorts in different parts of the retina do not appear to be systematic; this variation may reflect early mixing of the two sets of stem cells.

The majority of cohorts span all three layers in well-defined ontogenetic units. This pattern is consistent with the idea that many stem cells are pluripotent. However, the particular cellular composition of cohorts can differ markedly and a few cohorts are confined to a single layer. This raises the possibility that there are a variety of stem cell types in embryonic retina. An analysis of serial sections demonstrates that cohorts can be skewed across the layers of the retina at surprising angles. As a result, functional units in the mouse retina, which are oriented straight across the layers, often combine cells from both species.

Supported in part by NS23475 and EY06627.

79.2

REGULATION OF CELL NUMBER IN THE FROG RETINA. I. ABLATION STUDIES. Richard Wetts, Robert F. Quon, and Scott E. Fraser. Dept of Physiology & Biophysics and the Developmental Biology Center, Univ Calif, Irvine, 92717.

To fully understand the mechanisms that underlie the formation of the diverse cell types in the CNS, it is necessary to also understand the regulation of their numbers. Four possible mechanisms are: 1) extrinsic factors (ie growth factors), 2) intrinsic factors (ie counting divisions), 3) positional information, and 4) total population size. The latter two appear able to regulate some non-neuronal populations after experimentally removing a portion of the tissue. To determine whether either of these two factors are involved in regulating cell numbers in the *Xenopus* retina, we examined total size or clonal numbers following removal of more than half of the retina at St 30-32.

Individual optic cup cells were labeled with a lineage tracer (rhodamine dextran) at the time of the ablation. After 2 days (St 43), each precursor had formed a clone of labeled descendants in the experimental (tissue removed) and the operated-control (no tissue removed) retinas. These clones showed no significant difference in size (12.1 cells $\pm$ 2.4 vs 10.8 $\pm$ 1.8, respectively), suggesting that no regulation had occurred.

Does the ablation affect the ciliary margin cells (which proliferate at the front of the eye throughout life)? As the animals grew (2-48 d after the ablation), the size *difference* between the experimental and the contralateral (un-operated control) eyes increased, suggesting that the experimental eye did not compensate for the ablated cells but instead grew 10% slower than the control eye. Thus, the ciliary margin, like the optic cup, does not use size or position to regulate cell numbers, implying that they use either extrinsic or intrinsic information.

79.3

6-HYDROXYDOPAMINE AND PROLIFERATION IN GOLDFISH RETINA. J.E. Braisted & P.A. Raymond. Dept.Anat. & Cell Biol., Univ.Mich., Ann Arbor, MI 48109.

Negishi, *et.al* [Neurosci.Res.Suppl.,1988, 8:S43] reported that dopaminergic interplexiform cells (DAergic IPCs) in goldfish retina can be permanently destroyed by intraocular injection of 6-hydroxydopamine (6OHDA), estimated intraocular concentration .023μg/μl. However, after a higher dose of 6OHDA (.23μg/μl), DAergic IPCs are regenerated after 2 mons. One hypothesis to explain this result is that non-specific damage to the retina at high dosages triggers proliferation of rod precursors, which we have previously shown to be the source of regenerated neurons in ouabain-treated retinas. To test this hypothesis, we injected 6OHDA intraocularly (.14-.29μg/μl) and assessed loss of cells other than DAergic IPCs using various antibodies. Results thus far have not demonstrated reappearance of DAergic IPCs, and there was no evidence that other neurons were lost. To determine whether proliferation was stimulated, we injected bromodeoxyuridine (BrdU) at 6-23d after 6OHDA, and we found no increase in BrdU-labeled cells. In further experiments we will increase the dose of 6OHDA and inject BrdU to look for double-labeled, regenerated DAergic IPCs.

79.4

ROD PRECURSORS DO NOT EXPRESS OPSIN. Jennifer K. Knight and Pamela A. Raymond Neuroscience Program and Department of Anatomy & Cell Biology, University of Michigan, Ann Arbor,MI 48109.

Rod photoreceptors are generated continuously by dividing rod precursor cells located in the outer nuclear layer of the mature retina. In this study we asked when young rods commit to the rod pathway of differentiation. The possibilities are: rod precursors could be committed to the rod fate while still in mitosis, at terminal mitosis but before differentiation, or the young rods could commit during differentiation.

Goldfish were injected intraocularly with the thymidine analogue bromodeoxyuridine (BrdU) and sacrificed from 4 hours up to 1 month after injection. Cells were visualized with immunofluorescent microscopy using two antibodies, one against opsin (rho 4D2) and the other against BrdU. Our assay was to look for cells double labelled by both.

If mitotic rod precursor cells are committed, we would expect to find double-labelled cells within a few hours after BrdU injection. We found that between 4 hours and 3 days, rho 4D2 cells are not double labelled; the first double labelled cells appear at 4 days after BrdU injection. The number of double labelled cells peaks at 10 days, and then falls off, disappearing completely at 1 month. These results support previous work in our laboratory and also work by Watanabe and Raff (Neuron 4: 461, 1990). We suggest that rod precursor cells are multipotent stem cells not committed to the rod cell fate.

This work supported by NIH grants T32EY07022 and RO1EY04318.

79.5

CLONAL CELLS FROM EMBRYONIC RETINAL CELL LINES EXPRESS QUALITATIVE ELECTROPHYSIOLOGICAL DIFFERENCES. D. Lenzi, K. Radke *(1) and M. Wilson. Depts. of Zoology and Avian Sciences (1), University of California, Davis CA 95616.

We have established immortal cell lines from the embryonic retina of the quail by infecting cells with the avian retrovirus MH2 PA200 (RAV-1 pseudotype), which carries the *mil* oncogene. We have shown previously (Lenzi *et al*, 1988 Soc. Neurosci. Abstr. 14:147.13) that putative clones derived by limiting dilution are heterogeneous in their electrophysiological phenotype. We now show that at least two of these populations are true clones, and we have identified some of their membrane currents.

Viral integration site was used to assess clonality of cell populations. Genomic DNA was digested with Hind III and Southern blots were probed with a *v-mil* fragment expected to hybridize with one band per viral integration site. Each of eight populations tested revealed three to seven *v-mil*-specific bands. We therefore repeated Southern blot analysis on three subclones of each of two parent populations. For each subclone and its parent line we found the same pattern of probe hybridization with restriction fragments. We conclude that both parent lines are truly clonal, and each cell within a line contains several integrated proviruses.

We have used whole-cell patch-clamp to examine in detail the phenotype of 85 cells within one subclone. Inward rectifier potassium current was found in 97% of cells. This current was blocked by 5mM external cesium, was abolished in the absence of external potassium and was fully activated in less than 10msec. A non-inactivating delayed-rectifier-like current, blocked by 25mM external TEA, was expressed in 41% of the cells. Sodium current, blocked by 300nM TTX, was seen in 18% of the cells. Four percent of cells showed non-inactivating inward currents that were blocked by 3mM cobalt, and were probably carried by calcium ions.

These data show that clonal cells can express distinct sets of channel proteins.

79.6

CELL LINEAGE IN THE CEREBRAL CORTEX STUDIED WITH RETROVIRAL VECTORS. E.A. Grove, J. Read* and J. Price, Lab. of Embryogenesis, N.I.M.R., London NW7 1AA, U.K.

Lineage relationships in the cerebral cortex have been studied using retroviral vectors, such as BAG, to mark embryonic progenitor cells. BAG inserts the *E.coli* β-galactosidase (*lac*-Z) gene into the genome of infected cells whose progeny can then be identified histochemically. Two types of progenitor cells are revealed by the composition of clones in postnatal animals. One generates only astrocytes; the second both neurons and glia (Price & Thurlow, Dev. 104:473, 1988). Current studies address two issues: (1) Labelled cells have been classified only by light microscopy. EM and cell type-specific markers can determine the variety of neuronal subtypes present in single clones, and characterize the glial cells that appear in mixed clones. (2) Features of the BAG vector could lead to an underestimate of clonal variety. Because the endogenous retroviral promoter in BAG controls expression, a sub-population of infected cells may not express the gene, as is the case in embryonic stem cells. In another vector, PIRV (Beddington et al., Dev. 106:37, 1989), *lac*-Z expression is driven by the rat β-actin promoter. Preliminary studies indicate that labelling of clones in cortices infected with high titers of PIRV is quantitatively and qualitatively similar to that seen in BAG-infected brains. It thus appears unlikely that previous analysis of cortical cell lineage was compromised by a selective failure of some cells to express the marker enzyme.

79.7

GRANULE CELL NEUROGENESIS *IN VITRO* W.-Q. Gao and M.E. Hatten, Dept. of Pathology, Center for Neurobiology and Behavior, Columbia Univ, College of Physicians and Surgeons, New York, NY 10032

Previous studies on neurogenesis in developing brain have assumed that neuronal proliferation is controlled by a chronological sequence of cell divisions, sometimes termed a "clock". The cerebellar granule cell presents an ideal system to examine neurogenesis, because the precursor neurons can be purified in large numbers at early postnatal periods, and cultured *in vitro*. When cultured in uncoated plates at high density in serum-supplemented medium, conditions where the cells reaggregate, granule cell DNA synthesis, measured by ^{3}H-thymidine and BrdU incorporation, continues for at least 10 days. By BrdU immunocytochemistry, the labeling index was 30-50% in medium supplemented with serum. In contrast, when neurons were prevented from interacting homotypically, by dispersing them on a polylysine substrate, they ceased DNA synthesis within 24-72h. Similar results were seen with granule cells harvested from the mutant mouse *weaver*, an animal that suffers a failure of glial-guided migration. DNA synthesis was also stimulated by bFGF, EGF, and IGF-1 (5-20ng/ml), but not by NGF, PDGF and insulin (5-100ng/ml). To test whether the neuronal proliferation induced by homotypic neuron-neuron interactions can be overrided by heterotypic interaction with astroglia, we added purified astroglial cells to dividing granule cell precursors. In co-culture with glia, neuronal ^{3}H-thymidine incorporation dropped rapidly and neuronal cell division was arrested dramatically, as measured by BrdU labeling.

These results suggest that cell-cell interactions play a regulatory role in neuronal proliferation and that the *weaver* gene does not perturb granule cell neurogenesis. Supported by the Pew Memorial Trust (MEH) and NS 15429 (MEH).

79.8

CORRELATION BETWEEN MAPS OF SOMATOSENSORY RECEPTIVE FIELD AND PURKINJE CELL LINEAGE IN THE CEREBELLUM OF MOUSE CHIMERAS. H. Shojaeian Zanjani, J. Bower and K. Herrup. Devel. Neurobiology, E.K. Shriver Center, Waltham, MA and Div. Biology, Caltech, Pasadena, CA

Studies of cerebellar cortex reveal a consistent pattern that resembles a fractured mosaic. The pattern is repeated in many modalities as shown by a variety of anatomical, physiological and biochemical studies. The overlap of these systems led us to ask whether this seemingly fundamental pattern was also revealed in the spatial organization of the constituent Purkinje cell lineages.

Somatosensory projections to cerebellar cortex were used as a model system. Aggregation chimeras were made between two inbred mouse strains that differed in activity levels for the enzyme ß-glucuronidase. Multiple unit activity was recorded in the granule cell layer of Crus II (CII) of the chimera using light touch stimulation of the body surface. In this manner a receptive field map of CII was made. Subsequent ß-glucuronidase histochemistry on a set of serial 10µm sections through this region was then used to reveal the Purkinje cell lineage map. The distribution of the Purkinje cells lineages across the crown of CII was reconstructed. The results from 3 chimeras demonstrate that, in Crus II, there is a near perfect correspondence between transitions in the Purkinje cell lineage map and in the physiologically defined receptive field map.

Supported by NIH (NS18381) and the March of Dimes (#1-1175).

79.9

PURKINJE CELL LINEAGE MAP IS CONGRUENT TO THE ANTIGENIC MAP OF ZEBRIN II IN THE MOUSE CEREBELLUM. K. Herrup, N. Leclerc, D. Drinkwater and R.B. Hawkes. Devel. Neurobiol., E.K. Shriver Ctr, Waltham, MA & U. Calgary, Alberta, Canada.

A monoclonal antibody against Zebrin II (ZII) defines a series of parasagittal compartments in cerebellar cortex. ZII is expressed by a subset of Purkinje cells distributed in 7 parasagittal bands in each hemicerebellum. This pattern is particularly evident in the vermis, and the phenotype of positive and negative Purkinje cell has proven to be resistant to a variety of experimental manipulations. This suggests a cell autonomous nature to the staining pattern and led us to ask whether the Zebrin phenotype had a relationship to the lineages of the constituent Purkinje cells. Aggregation chimeras were made between two embryos that differed in activity levels of the enzyme ß-glucuronidase. The cerebellar cortices of these animals were stained for both Zebrin II and ß-glucuronidase. 3-D reconstruction of the Purkinje cells in lobule III reveals a high level of correlation between the lineage map and the ZII antigenic zonation pattern. These results are consistent with an early, lineage based establishment of the Zebrin staining phenotype.

Supported by NIH (NS 18381 & NS 20591) and the March of Dimes #1-1175.

79.10

DEVELOPMENT OF PRIMARY AND SECONDARY TYROSINE HYDROXYLASE POSITIVE CELLS IN XENOPUS. S.A. Moody and S. Huang. Department of Anatomy & Cell Biology, University of Virginia, Charlottesville, VA 22908.

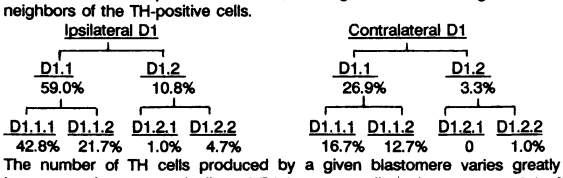
Using an antibody to tyrosine hydroxylase (TH), a rate-limiting enzyme for catecholamine synthesis, we investigated the development of TH-positive cells in Xenopus embryos. Two categories of cells, primary and secondary, were identified in earlier and later embryos, respectively. At stage 10 primary TH-positive cells were found in epidermis. By stage 20 they were detected in somitic muscle, the gut wall, notochord and neural crest. The staining was located in both the nucleus and cytoplasm of these cells. The distribution of primary TH-positive cells in the CNS was widespread. TH-positive cells were present in the neural plate as early as stage 17. At stage 20 most of the TH-positive cells were confined to the dorsal part of the spinal cord, including Rohon-Beard and commissural neurons, and the ventro-lateral part of the hindbrain, most of which were commissural neurons. TH-positive cells were first detected in the forebrain at stage 25. Through the tailbud stages more and more CNS neurons became TH-positive. These included most of the sensory neurons, motoneurons and interneurons that comprise the primary nervous system. Non-neural TH-staining began to decrease by stage 34, and primary TH-staining in the CNS began to decrease after stage 37; most primary cells were TH-negative by stage 48. The function of this transient TH-activity in the primary cells is unknown.

At stage 40 secondary TH-positive neurons, which were generally more darkly stained than the primary ones, began to appear. The secondary neurons were found only in the hypothalamus, retina, and KA neurons of the spinal cord, and are believed to be the dopaminergic neurons identified in the adult. As in the adult, TH-staining in these cells was exclusively cytoplasmic. Supported by NINDS NS23158

79.11

QUANTITATIVE CLONAL ANALYSIS OF TYROSINE HYDROXYLASE IMMUNOREACTIVE CELLS IN THE HYPOTHALAMUS-INFUNDIBULAR AREA OF XENOPUS TADPOLES. S. Huang and S.A. Moody. Department of Anatomy and Cell Biology, University of Virginia, Charlottesville, VA 22908

At stages 41-44 tyrosine hydroxylase immunoreactive cells form a discrete cluster (20-40 cells) in the Xenopus hypothalamus-infundibular area. This small number of cells allows a quantitative clonal analysis of a neurotransmitter-related phenotype. A lineage dye was injected at the 16- or 32-cell stage into certain blastomeres that are known to give rise to cells in ventral forebrain. 14µm frozen sections were immunoreacted with an antibody to tyrosine hydroxylase (TH). TH-positive cells were visualized by FITC- or AMCA-conjugated secondary antibody. TH cells descend exclusively from bilateral D1.1 and D1.2 at the 16-cell stage and their daughters (see Diagram). Other blastomeres do not produce TH cells, although some of them give rise to neighbors of the TH-positive cells.



The number of TH cells produced by a given blastomere varies greatly between embryos, e.g., ipsilateral D1.1 may contribute between 10-90% of the TH cell population. Futhermore, the clones from a particular blastomere in different embryos occupied different positions within the TH cell clusters. These results indicate that neurochemically specified phenotype is not associated with one progenitor. Supported by NINDS NS23158.

80.1

GRANULE NEURONS REGULATE ASTROGLIAL DIFFERENTIATION VIA TGFβ1. S. Vidan, D. Cooper, R. Silverman, P. Scala and M.E. Hatten.
Dept. Pathology and Ctr. for Neurobiology and Behavior, College of Physicians and Surgeons of Columbia University, New York, NY 10032

Previous studies have shown that neurons regulate the differentiation of astroglial cells by a membrane-mediated mechanism (Hatten, J Cell Biol:**104**:1353, 1987), and that neuron-glia contacts alter the dose reponse of glial cells to bFGF (Hatten et al Devl. Biol.**125**:280,1988). To test the role of growth factors in glial proliferation and differentiation, we purified astroglia from early postnatal mouse cerebellum and cultured them in the presence of EGF, PDGF, bFGF, insulin, IGF-1 and TGFβ1 (1-200ng/ml). Among these, EGF, PDGF and bFGF were glial mitogens (1-20ng/ml); none of these factors induced astroglial process extension. In the presence of a combination of TGFβ1 and either EGF, PDGF or bFGF (10ng/ml), however, glial DNA synthesis arrested and the glia extended long processes, generating glial forms which resembled forms seen in co-culture with neurons and *in vivo*. By Northern blot analysis, differentiated glial cells expressed higher levels of the glial filament protein. To determine whether the cells express TGFβ1, we carried out Western and Northern blot analysis. These experiments indicate that TGFβ1 RNA and protein are expressed in co-cultures of neurons and astroglia. We are presently using *in situ* hybridization to determine which cell, the neuron or the glial cell, provides TGFβ1. To test the function of TGFβ1 in co-cultures of neurons and glia, conditions where extensive glial differentiation is usually seen, we added antibodies to TGFβ1 and visualized glial form with immunocytochemical localization of antibodies against the glial filament protein. TGFβ1 antibodies blocked glial process extension in a dose-dependent manner.

These studies suggest that neuronal regulation of glial differentiation involves alterations in the levels of TGFβ1, a step which decreases the responsiveness of glial cells to mitogens and directs the glial cell toward a differentiation pathway. Supported by NIH grant 21907 (MEH).

80.2

GLIAL-NEURONAL INTERACTIONS IN RAT OLFACTORY BULB GLOMERULUS DEVELOPMENT. Mary S. Bailey, Michael T. Shipley, Dept. of Anatomy and Cell Biology, Univ. of Cincinnati, Cincinnati, OH 45267

Recent studies suggest that astrocytes provide a morphological and/or molecular substrate for growth and target recognition by developing axons, and for the formation of multineuronal assemblies such as the barrel fields. We previously reported that olfactory bulb (OB) radial glia exhibit a unique branching pattern just deep to the nerve layer in early embryonic development (E16-18). At this stage primary olfactory neuron (PON) axons surround the OB with many fibers penetrating to deeper layers. The organization of terminal PON axons into glomerular-like condensations correlates with increasing complexity of the radial glial plexus in the presumptive glomerular layer. We now report a transition from radial glia to astrocytes in the late embryonic and early postnatal rat OB which correlates with further maturation of glomeruli: GFAP-positive astrocytes increase with a concommittant decrease of vimentin-positive radial glia just below the nerve layer. We are directly testing whether radial glia *transform* into astroyctes in this layer. The appearence of atrocytes is coincident with increased focal condensations of OMP (olfactory marker protein) stained olfactory axons marking further maturation of glomeruli. Preliminary double label experiments show a close association of new astrocytes with the presumptive glomeruli. These results support the idea that dynamic glial-neuronal interactions are critical to the development of the OB glomerulus, and suggest that olfactory axons trigger the transformation of radial glia into astroyctes. Supported by NIDCD-DC00347.

80.3

OLIGODENDROCYTE MIGRATION IN VITRO IS DEPENDENT UPON TARGET-STIMULATED DIFFERENTIATION OF SPINAL CORD NEURONS. W.L. Muhlach and H.B. Lim*. Dept. of Zoology, Southern Illinois University, Carbondale, IL 62901

Previous culture work has demonstrated that amphibian hindlimb mesenchyme stimulates, among other things, neurite outgrowth and cholinergic maturation of spinal cord motoneurons in a stage-specific fashion (Int. J. Dev. Neurosci. 7:383, 1989). Non-neuronal cells are often found associated with the nerve fibers in these cord cultures. Immunohistochemical preparations of spinal cord cultures (R. catesbeiana tadpole) demonstrated that these cells were oligodendrocytes (galactocerebroside positive). The association of the oligodendroglia with nerve fibers was dependent upon the developmental state of the spinal cord tissue. It is important to note that target tissue effects on the development of spinal cord neurons in culture were required before the neuroglia would associate with them. The neuroglia were seldom observed with spinal cord explants from young animals, but were often observed associated with explants from older tadpoles. However, co-culture of young spinal cord with target tissue resulted in oligodendrocyte migration along nerve fibers. In addition to effects upon neurite outgrowth, neurite fasciculation, and cholinergic maturation, the target tissue also triggers a change in the interaction between oligodendrocytes and neurons which may be required before myelination proceeds.

80.4

BRAIN MACROGLIA EXHIBIT REGION-SPECIFIC REGULATION OF NEURONAL MORPHOGENESIS IN VITRO. J.Qian, M.Bull, I. Fischer* and P.Levitt, Dept. of Anatomy, Med.Coll.of Penn., Phila., PA 19129 and Dept. Biochemistry, E.K. Shriver Ctr., Waltham, Ma. 02254

Neuronal morphogenesis during development appears to be influenced by both intrinsic and extrinsic factors. A neuron-glia co-culture system was used in which neurons from fetal rat spinal cord and hippocampus were grown (without direct contact) with macroglia derived from different CNS regions of perinatal rats to investigate the role that CNS glia may play in regulation of neuronal shape and neurite outgrowth. Examination of the expression of microtubule-associated protein 2 (MAP2), a dendritic marker, and the phosphorylated form of high molecular weight neurofilament protein (NF-H), an axonal marker, revealed that: 1) spinal neurons survived equally well under all co-culture conditions and, when grown with glia from spinal cord, hippocampus, cerebellum and cerebral cortex, they expressed similar patterns of MAP2 expression. NF-H-stained neurites were more extensive in spinal neuron-cerebellar glia co-cultures than other combinations; 2) hippocampal neurons survived equally well when combined with glia from hippocampus or from cerebral cortex, but did not in an environment of spinal cord or cerebellar glia. While MAP-2 expression was similar in a conditioning environment provided by hippocampal or cerebral cortex glia, NF-H neurites were more extensive with cerebral glia. These results suggest that regional specificity of neuron-glia interactions are involved in regulating both survival and neuronal morphogenesis, with axons exhibiting a greater sensitivity to the glial environment than dendrites. Supported by NIMH grant MH45507.

80.5

A MONOCLONAL ANTIBODY THAT LABELS DEVELOPING SCHWANN CELLS AND A SUBSET OF MIGRATING NEURAL CREST CELLS. Anita Bhattacharyya, Eric Frank[1], Robert Brackenbury*, and Nancy Ratner. Dept of Anat & Cell Biol, Univ of Cinti Col Med, Cincinnati, OH 45267, and [1]Dept of Neurobiol, Anat & Cell Sci, Univ of Pitt Sch of Med, Pittsburgh, PA 15261.

Specific immunocytochemical markers are needed to study the migration and differentiation of Schwann cell precursors and their early interactions with extending axons. We have generated a monoclonal antibody, termed 1E8, that stains Schwann cells in mature chicken peripheral nerve. 1E8 reacts in Western blots with a series of proteins migrating at 23-30kD. When serial cryostat sections of chicken embryos are stained with 1E8, immunoreactivity is first detected at stage 19 in isolated cells located between the neural tube and somites. Double labelling with HNK-1 establishes that the 1E8 positive cells represent a small subset of migrating neural crest cells. At stage 21, a small population of cells in the ventral root expresses the antigen. Similarly, a population of cells in the dorsal root is stained at stage 23. 1E8 antigen continues to be expressed on the surface of cells associated with axons as they extend into the hindlimb. 1E8 immunoreactivity is also detected on a subset of HNK-1+ cells near the dorsal aortic arteries, presumed precursors of enteric glial cells, but mature enteric glia are not stained. 1E8 does not stain neurons in the CNS or PNS, muscle, or satellite cells.

Expression of the 1E8 antigen on a subset of migrating crest cells and on cells associated with extending axons suggests that the 1E8 antigen is a specific marker for early Schwann cell precursors. Supported by NIH grants NS27227 and NS24373.

80.6

DIFFERENTIAL EXPRESSION OF GLIAL MARKERS IN RADIAL GLIA DURING NEUROGENESIS IN THE RAT AND OPOSSUM BRAIN. J. Pecci Saavedra, A. Brusco, L.A. Gómez, I. Benítez and J. Affani. Instituto de Biología Celular and Biology Dept. Univ. of Bs As., ARGENTINA

We have studied the expression of the glial acidic protein (GFAP) and the S-100 protein in the developing brain of rat and sauth american opossum (Didelphis albiventris). This study was performed in 16 and 18 days rat embryos, and in 5, 14, and 23 day old opossum pseudoembryos. The animals were perfused intracardially with 4% paraformaldehyde + 0.5 % glutaraldehyde, the brains were then immersed in the same fixative solution for an additional 6 hours. Vibratome sections were processed according to the immunocytochemical PAP technique.

The ventral portion of opossum and rat developing brainstem and spinal cord showed a similar pattern of glial organization: radial glia is arranged in parallel cephalocaudal plates, and immature astrocytes appeared in the ventral zone following a ventro-dorsal gradient of maturation. The glial pattern described could only be revealed by the aid of anti-GFAP antibodies in the opossum, and with anti- S - 100 serum in the rat embryo.

These differential expression of glial markers suggests that radial glia and immature astrocytes may have acquired new roles during evolution to adapt to the particular development of brain in each species.
(work performed with grants from the CONICET, Argentina)

80.7

MOLECULAR TYPES RECOGNIZED BY MONOCLONAL ANTIBODY AB-2, WHICH REVEALS RADIAL GLIA DURING DEVELOPMENT. S.A. Tobet, R.C. Whorf *, G.A. Schwarting & T.O. Fox. Depts. of Biochemistry & Developmental Neurobiology, EK Shriver Center, Waltham, MA 02254 & Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

The expression of monoclonal antibody AB-2 immunoreactivity in radial glia of developing rat hypothalamus is age and sex-dependent and regulated by prenatal exposure to gonadal steroids (Tobet & Fox, PNAS 86:382, 1989). High molecular weight proteins were recognized by AB-2 and were distributed selectively in subcellular fractions from neonatal hypothalamus (HYP), remaining forebrain (ROB), and brainstem regions. Immunoblots revealed 6 major protein bands: a 195kD protein in the cytosolic compartment; a 250kD doublet in microsomal and crude mitochondrial membrane fractions; and a 220kD doublet in Triton-insoluble and membrane fractions, suggesting a cytoskeletal association. Finally, a 20kD protein was enriched selectively in brainstem microsomal fractions. AB-2 also recognized specific glycolipids on immunoblots following high performance thin layer chromatography. Glycolipid blots of several brain regions at different postnatal ages indicated sulfatide was the major glycolipid recognized. AB-2 immunoreactivity in hypothalamic glycolipids (sulfatide) did not correlate with the timing of AB-2 immunoreactivity in radial glia. Other lower migrating lipids were noted only in postnatal day 2 cerebellum.

The presence of AB-2 reactive protein and lipid antigens suggests that the epitope is a carbohydrate present on both types of antigens in multiple cellular compartments. There were no *qualitative* sex differences in the binding of AB-2 to the molecules recognized. Hormone influences might affect individual AB-2 antigens in particular cellular elements (i.e., radial glia) or might modify the glycosylation of multiple AB-2 antigens in selected cell types.

80.8

EVIDENCE FOR THE PRESENCE OF TWO MEMBERS OF THE THYMOSIN PEPTIDE FAMILY IN DEVELOPING RAT CEREBELLUM. B. Border, S-C. Lin*, W.S.T. Griffin, and M. Morrison-Bogorad. Depts. of Neurology and Biochemistry, University of Texas Southwestern Medical Center, Dallas, and Dept. of Pediatrics, University of Arkansas Medical School, Little Rock.

Polyclonal antisera raised in rabbits against thymosin β4 (V416) and thymosin β10 (V411) carboxy-terminal synthetic peptides were utilized to demonstrate their presence in rat cerebellum. Immunoperoxidase techniques were applied to Bouin's-fixed, paraffin-embedded rat brain ranging in age from one day (P0) to 30 days (P30) postnatal. At P0, intense V416-immunoreactivity was present within radial fibers of the molecular layer as well as a plexus of fibers and cell somata in the internal granular layer, while antiserum V411 labeled only thick fibers in the molecular layer. At P6, an increased number of V416-immunolabeled cell bodies and processes were present in the white matter and the internal granular layer, in addition to labeled radial fibers; however, little V411 immunolabeling could be discerned. Immunostaining was completely abolished by P14 in V411-treated tissue. Finally, in the adult rat, a population of cell bodies and processes located in the deep white matter near the cerebellar nuclei as well as radial glial fibers in the molecular layer were immunolabeled by V416. Therefore, it appears that antisera against thymosin β4 immunolabels a subpopulation of astrocytes or oligodendrocytes in newborn and adult cerebellum, while anti-thymosin β10 immunoreactivity becomes undetectable by P14. These findings correlate with quantification of relative levels of thymosin β4 and β10 mRNAs in the developing rat cerebellum as revealed by Northern blot analysis (Lin and Morrison-Bogorad, J. Mol. Neurosci., in press). Supported by NIH HD14886 (MM-B).

80.9

IDENTIFICATION OF GLIAL AND NEURONAL CELL MARKERS IN TISSUE CULTURE. D.S. Grega+, E. Pertile* and N. Nousek-Goebl*. R&D Division, Boehringer Mannheim Corp.,and +Indiana Univ. School of Medicine, Program in Medical Neurobiology Indianapolis, IN 46250.

Cell identification is critical to the study of cell type development in the nervous system. The presence of epitopes as cells mature can be tracked using specific antibodies and immunohistochemical (IHC) localization.

We are presenting IHC data from two culture systems: 1) oligodendrocyte (oligo) and 2) NGF-treated, mixed spinal cord/dorsal root ganglia (SC/DRG) neurons, from neonatal and fetal rat respectively. We compare anti-GalC (galactocerebroside an accepted marker for oligodendrocytes and Schwann cells) and anti-CNPase (2',3'-cyclic nucleotide 3' phosphodiesterase associated almost exclusively with myelin-producing cells), for oligos over the first 21 days in culture. Little CNPase IHC characterization has been done. The two antibodies stain the same cell type, but differ in the extent of staining of fine processes. The binding of tetanus toxin or its C fragment (TTC) has been used as a neuronal marker. Neurons in the SC/DRG cultures were identified comparing several antibodies, anti-A2B5, anti-neuron specific enolase, anti-MAP2 (microtubule associated protein-2 located in the somatodendritic compartment), and the localization of recombinant TTC (rTTC) with anti-TTC. The advantages of these different neuronal markers were determined.

80.10

GLIAL CELL DIFFERENTIATION IN THE DEVELOPING RAT CEREBELLUM. J.M.Levine, F.Stincone* and Y.S.Lee,* Dept. of Neurobiology and Behavior,SUNY at Stony Brook,Stony Brook,NY,11794

The development of glial cells in the late embryonic and early post-natal cerebellum (CBL) was studied immunohistochemically using antibodies that identify glial precursor cells and their differentiated progeny.

At embryonic day(ED) 17, glial progenitor cells, identified with antibodies against the NG2 chondroitin-sulfate proteoglycan, appeared within the cerebellar anlage. These bipolar cells were distinct from the vimentin+ radial fibers present at this early stage. By ED21, the numbers of the NG2+ cells had increased and they were distributed throughout the CBL. These cells,which had a multi-polar morphology, were only weakly labeled with monoclonal antibodies against GD3 ganglioside (LB1) that have been used to identify oligodendrocyte precursors. Between post-natal day(PND)1-3, a population of intensely LB1+ cells appeared in developing white matter and, over the next 2-3 days, GalC+ oligodendrocytes developed in the white matter. Although a small number of the NG2+ cells co-expressed GalC and an oligodendrocyte-specific antigen recognized by monoclonal antibody RIP, most of the NG2+ cells within the developing CBL failed to develop into oligodendrocytes. GFAP+ astrocytes were first observed at about PND 5 within the white matter and the distal regions of each folium. As was the case with oligodendrocytes, few of the NG2+ cells expressed GFAP antigens. Rather a population of NG2+,GFAP-,GalC- cells persisted into the adult.

These observations suggest that there may be 3 separate populations of glial precursor cells within the CBL; a vimentin+ cell which develops into an astrocyte (Bovalenta,etal.,DevBiol:102,248,1984), a LB1+ cell which develops into an oligodendrocyte (Reynolds and Wilkin,Development:102,409,1988) and the NG2+ progenitor cells which fail to develop either GFAP or GalC immunoreactivity. Although the NG2+ cells display bipotentiality *in vitro*, most of these cells fail to do so *in vivo*. These persistent NG2+ cells may represent either a third type of glial cell or an adult form of a bipotential glial progenitor cell.

80.11

CULTURING THE GLIAL PROGENITOR CELLS OF THE NERVE FIBER LAYER OF THE OLFACTORY BULB. R. Doucette. Dept. Anat., Univ. Sask., S'toon, Sask., Canada.

Some of the glial progenitor cells (GPC) of the olfactory bulb differentiate in vitro into bipolar cells that contain not only GFAP, but also the 217c and RAN-1 antigenic epitopes; the latter two antigens are normally expressed only by Schwann cells. GPCs of the olfactory nerve fiber layer (NFL) also differentiate into cells expressing phenotypic features of both astrocytes and Schwann cells. The purpose of the present study was to determine whether bipolar cells were still present when the only GPCs that were cultured were those of the NFL, obtained by peeling the NFLs off the olfactory bulbs of E14-16 mouse (Swiss) embryos. Bipolar cells, some of which contained GFAP, were always present in these cultures; these same cells never expressed neurofilament proteins making it unlikely that they were neurons. Obtaining a purified population of the GPCs of the NFL should make a significant contribution towards identifying some of the factors that control the growth of olfactory axons. (Supported by a grant from the MRC).

80.12

MICROGLIA IN THE DEVELOPING OPOSSUM SUPERIOR COLLICULUS. L.A. Cavalcante and A.Santos-Silva*, Instituto de Biofisica C. Chagas Filho , UFRJ, Rio de Janeiro, RJ 21944, Brazil.

The differentiation of astrocytes in the opossum superior colliculus (SC) follows both outside-in and medio-lateral gradients (Barradas PC et al., Glia 2: 103, 1989). In order to test whether that reflects non-neural cell influence, we have mapped macrophages and microglia in the maturing SC . Lectin binding shows macrophages only in leptomeninges, SC midline and adjoining, at first, the subventricular zone and later the ependyma. The SC upper layers acquire microglia later (by 24-31 days) than deep and intermediate layers (by 17 days) and still retain a large proportion of ameboid cells as $GFAP^+$ astrocytes appear (40 days). However, upper layer astroglia still retain augmented GFAP immunoreactivity as microglia change from ameboid to ramified shape. Our results show that sequences of acquisition of microglia neither predict or correlate with the differentiation of astrocytes in the SC. (Support: FAPERJ, CNPq, FINEP, CEPG/UFRJ)

81.1

Expression of a Tetrodotoxin-resistant Voltage-dependent Sodium Current in an Immortalized Hypothalamic Cell Line. K.D. Phelan, V. Quinones-Jenab† and H.M. Geller. Dept. of Pharmacology, UMDNJ-R.W. Johnson Medical School and †The Graduate School, Rutgers University, Piscataway, NJ 08854.

The immortalized mouse hypothalamic cell line, V1, is a multipotential precursor cell line that gives rise to glial-like (flat, $GFAP^+$) as well as neuronal-like (round, neurofilament$^+$) cells (**see Quinones-Jenab et al.**). The present study utilized whole-cell patch clamp recording techniques to characterize and compare the expression of voltage-gated currents in the "neuronal-like" V1 cells grown at the permissive (33°C) and non-permissive (39°C) temperatures of the immortalizing oncogene.

Sustained (non-inactivating) outward potassium currents were present in all cells cultured at either temperature. However, no inactivating outward potassium currents were observed in either condition. In addition, 45% (9/20) of the cells grown at 33°C exhibited a fast inward current with a peak amplitude that varied between 80 and 1900 pA (median = 240 pA). The proportion of cells expressing this inward current increased to 94% (30/32) after 9-16 days in culture at 39°C (50-2270 pA peak; median = 387 pA). This inward current displayed voltage-dependency with an activation threshold near -60 mV and a peak current activation around -20 mV. The current was reversibly reduced by ionic substitution of extracellular sodium, but was completely resistant to bath application of tetrodotoxin (1-20 μM; n=10). This inward current has been stably expressed by this cell line for one year in continuous culture.

This study demonstrates that the "neuronal-like" cells of the immortalized V1 hypothalamic cell line express a tetrodotoxin-resistant voltage-dependent inward sodium current. This cell line may be a useful model for characterizing the developmental expression of sodium currents during cellular differentiation in the mammalian CNS.

81.2

CALCIUM CHANNELS IN INSECT CENTRAL OLFACTORY NEURONS DEVELOPING *IN VITRO*. J.H. Hayashi and J.G. Hildebrand. ARL Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

Neurons derived from the antennal lobe (AL, the primary olfactory center in the brain) of the moth *Manduca sexta* thrive in our long-term culture system as solitary cells [*J. Neurosci.* 10:848 (1990)]. Inward currents are evident in cells only after they have been in culture for about a week [*Soc. Neurosci. Abstr.* 15:1145 (1989)]. We used the patch-clamp technique and specific blockers to isolate and examine voltage-gated currents that arise in these cultured neurons concomitantly with their morphological development. Neurons were dissociated from ALs at stage 6 of the 18 stages of metamorphic adult development and examined after they had been allowed to develop *in vitro* for 1-3 weeks.

The voltage-sensitive outward current comprised 3 separable components: one that could be blocked by external TEA superfusion, a TEA-insensitive outward current blocked when the patch pipette was loaded with internal Cs^+ ions in place of K^+ ions, and a TEA- and Cs^+-insensitive current that activated at about +40 mV. A fast, rapidly inactivating inward current was blocked by TTX (10^{-8} M) and presumed to be the channel that underlies the action potential observed in these cells. With these Na^+ and K^+ currents blocked, we were able to observe the slowly activating inward currents in several cell types. Ba^{++} could substitute as the permeant ion, and this current was blocked irreversibly by Cd^{++} (100 μm) and reversibly by Mg^{++}. We measured an activation potential of about -30 mV from a holding potential of -70 mV. This current inactivated with a time constant of hundreds of msec. When the holding potential was shifted to -40 mV, most of the inward current was abolished, but a residual current with an activation point of roughly -10 mV persisted. We conclude that dissociated central olfactory neurons develop Na^+ and K^+ channels and possibly two types of Ca^{++} channels in the absence of direct contact with other cells. [Supported by NS28495 & AI-23253.]

81.3

CALCIUM CHANNELS IN INSECT MOTONEURONS DURING METAMORPHOSIS. R.B.Levine and J.H.Hayashi. Division of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

During the metamorphosis of *Manduca sexta* identified thoracic leg motoneurons (MNs) persist to innervate the new adult legs. To test for possible developmental alterations in their biophysical properties, we generated primary cultures (technique adapted from Hayashi & Hildebrand, J.Neurosci.,'90) and examined leg MNs that had been retrograde labelled with di-I prior to metamorphosis (Griffin & Levine, S.N.Abs.'89). MNs also constituted a distinctive size class in the cultures.

In most experiments the cells were examined 15-24 hours after dissociation, and so lacked extensive processes. Immediately after achieving whole-cell patch clamp configuration, depolarization of the MNs in normal saline activated large inward and outward currents. Although we have evidence for K^+ and Na^+ channels, the present analysis concentrates on putative Ca^{++} channels. With CsCl (150mM) inside the patch pipette, and external solution containing TTX (10^{-8}M), TEA (30mM), and with Ca^{++} replaced with Ba^{++} (6mM), a slowly inactivating inward current was activated by depolarizing commands from -70mV in all MNs examined. This current could be blocked irreversibly with Cd^+, and reversibly by Mg^{++}, Ni^{++} and Co^{++}. We conclude that the somata of the dissociated MNs contain a voltage-sensitive Ca^{++} channel. From a holding potential of -20mV, a persistent minor current remained. Its activation point was more depolarized than that of the major current which, from a holding potential of -70mV, dominated the records. Thus, there may be two types of Ca^{++} channels in these neurons.

For MNs examined the day after dissociation, the level of Ca^{++} current in MNs from pharate adults was greater than in MNs from new pupae (P0). A similar increase was noted by comparing cells dissociated from P0 animals, and tested after different numbers of days in culture. Furthermore, the current density was greater for cells grown in the presence of physiological levels of 20-hydroxyecdysone than for cells maintained in the absence of the steroid.(NS28495 & BNS 89-11174).

81.4

DIFFERENTIATION OF THE CALCIUM SEQUESTRATION APPARATUS IN THE SARCOPLASMIC RETICULUM OF FROG MUSCLE B.E. Flucher*, M. Terasaki*, J. Drazba, S.B. Andrews* and T.S. Reese. Lab. of Neurobiology, NINDS, National Institutes of Health, Bethesda, MD 20892.

In a variety of cell types, including nerve and muscle, Ca uptake, storage and release are properties of the endoplasmic reticulum (ER). In muscle, these aspects of Ca regulation are located in specialized regions of the sarcoplasmic reticulum (SR); such regions are arranged in an orderly fashion within the sarcomere. Immunolabeling of mature frog muscle with antibodies against BIP (an ER marker protein) and against the fast Ca-ATPase resulted in similar labeling patterns, suggesting that BIP is expressed in the SR. In cultured frog myocytes that have just developed a cross-striated organization of contractile elements, anti-BIP, as well as an antibody against T-tubules (aTT), labeled in cross-striated patterns, indicating that SR and T-tubules had already become arranged in specific domains of the sarcomere. The Ca-ATPase, however, did not codistribute with the SR and T-tubules at this stage, but was restricted to a network of tubular membranes underneath the sarcolemma. The distribution of Ca-ATPase later became cross-striated, progressing from the periphery toward the center of the myocyte. In mature muscle fibers where the Ca-ATPase and BIP were co-localized in the sarcomeres, the peripheral network of Ca-ATPase-positive membranes had disappeared. These results suggest that in developing frog muscle Ca regulation resides first in a sub-sarcolemmal membrane system that possibly forms peripheral junctions with the sarcolemma, while the SR proper is still undifferentiated. The differentiation of the Ca sequestration apparatus in the sarcomeres seems to occur after the structural organization of SR is completed and ultimately supplants the peripheral Ca regulation system. The presence of BIP in undifferentiated as well as functionally differentiated SR supports the hypothesis that the SR is a derivative of the endoplasmic reticulum.

81.5

DISTRIBUTION OF MUSCLE PRECURSORS IN IMAGINAL DISCS OF WILD TYPE AND MUTANT *DROSOPHILA*. P.K. Rivlin, A. M. Schneiderman & V. Yu*. Section of Neurobiology & Behavior, Cornell University, Ithaca, NY 14853.

Our focus is on genes that control development of the thoracic motorneurons and muscles necessary for adult flight initiation in *Drosophila*. One of our goals is to characterize the postembryonic development of muscles in the jump and flight motor systems. Adepithelial cells of imaginal discs are presumed to be the precursors for at least some adult muscles (Poodry & H. Schneiderman, *Roux's Archiv.*, 166:1, 1970). We used peanut agglutinin (PNA) as a marker (Brower et al., *J. Embryol. Exp. Morph.*, 67:137, 1982) to follow the distribution and differentiation of adepithelial cells during larval and metamorphic development. In previous screens of imaginal discs PNA has been observed to bind preferentially to the precursors of photoreceptor cells in eye discs (Fristrom & Fristrom, *Dev. Biol.*, 92:418, 1982) and to cells presumed to be adepithelial in wing discs (Brower et al., 1982). We analyzed the binding patterns of peroxidase- and fluorescein-conjugated PNA in thoracic discs from wild type and mutant flies at various developmental stages. We have confirmed that PNA binding in the wing disc is restricted to cells previously described as adepithelial (Madhavan & H. Schneiderman, *Roux's Archiv.*, 183:269, 1977; Brower et al., 1982) and have found that PNA staining is substantially less in a homologous region of the haltere disc. The distribution of adepithelial cells in abx bx3 pbx / Df(3R)P2 haltere discs resembles that of wild type wing discs. We are extending our analysis to the electron microscopic level to resolve the subcellular location of PNA binding sites.

81.6

MUSCLE PHENOTYPE IS INDEPENDENT OF MOTONEURON GENOTYPE IN HEAT PULSED shibire MOSAICS OF DROSOPHILA . M.R. Hummon and W.J. Costello. Dept. Zool. Biomed. Sci./Col. Osteopathic Med., Ohio Univ., Athens, OH 45701.

Motoneurons (MNs) and muscles must connect correctly, implying specific nerve-muscle interaction. We are using $shibire^{ts1}$ (shi), a membrane recycling mutant of Drosophila, to study this interaction. After exposure to heat pulse (HP: 6h at 30C) as early pupae (age 20 h at 25C), shi adults have fused and/or deleted flight muscles (DLM; DVM); MNs of deleted DLM terminate in a neuroma. Normal DLM consists of 6 fibers, innervated by ipsi- and contralateral MNs; thus, bilateral mosaics would have a known combination of MN and muscle genotypes. Pupae from y w sn^3 shi X $In(1)w^{vc}$ (Ring X) were exposed to HP (age, 20 h); mature pupae with thoracic mosaicism were selected. Markers and the acute shi phenotype (paralysis at 30C) indicated shi ventral ganglion tissue. Serial sectioning (37 flies) permitted a census of DLM and DVM fibers, classified as wild type (+) or shi phenotype. Both muscle phenotypes occurred in 25 mosaics; in two of these, DLM/DVM on each side was all + or all shi, and thus did not reflect the genotype of the contralateral DLM MN. Both + and shi phenotypes occurred on a single side in 23 mosaics (28 sides), as well as within the 6 DLM fibers of 21 sides. Again, there was no correlation between DLM fiber phenotype and the imputed genotype of its MN. Thus, the HP induced shi muscle phenotype is independent of MN genotype, and the MN neuroma is a secondary response. NIH-NRSA (MRH).

81.7

ADAPTATIONS OF RAT DIAPHRAGM TO PRENATAL UNDERNOURISHMENT. Y.S. Prakash, M. Fournier and G.C. Sieck. Dept. of Biomed. Eng., Univ. Southern California., Los Angeles, CA 90089.

Pregnant rats were undernourished (UN) from day 2 of pregnancy, and restored to normal diets after parturition. Body weights of UN pups were lower than controls (CTL) until 2 weeks of age. Formation of secondary myotubes was inhibited or delayed in the UN embryonic diaphragm (DIA). Postnatally, the proportion of type I fibers was 75% higher in the UN DIA compared to CTL at birth, and 36% higher at 3 weeks. Cross-sectional areas of both type I and II fibers were greater in the UN DIA compared to CTL throughout the first 3 weeks. Twitch half-relaxation times were longer in the UN DIA, while contraction times were similar. The postnatal increase in specific tension (force/muscle area) in the UN DIA lagged behind that in CTL until 3 weeks, as did the decrement in UN DIA fatigue resistance. We conclude that prenatal undernourishment decreases the total number of DIA fibers, and, to compensate, fiber size increases. The inhibition of secondary myotube formation also affects the subsequent differentiation of fiber types and postnatal changes in DIA contractile properties.

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: CELL SURFACE AND MATRIX COMPONENTS

82.1

PATTERNS OF HNK-1 AND NCAM DISTRIBUTION IN THE EARLY XENOPUS SPINAL CORD. R.H.Nordlander and S-X. Liu*. Depts. Oral Biol., Anat., Ohio State Univ., Columbus,OH 43210.

The carbohydrate epitope recognized by the antibody HNK-1 is also expressed on several cell surface glycoproteins associated with cell adhesion, including the neural cell adhesion molecule (NCAM). We have compared immunoreactivity (IR) patterns of HNK-1 and anti-NCAM in wholemounts and transverse sections of the developing spinal cord at stages when a range of differentiation states can be followed along the rostrocaudal developmental gradient.

NCAM-IR appears on the surfaces of neural tube cells outlining borders of adjacent neuroepithelial cells and providing a view of changing cell configuration. This is particularly striking in wholemounts where floor plate cells, for example, are seen to elongate horizontally at the time that commissural axons first cross the cord. Anti-NCAM also outlines the surfaces of differentiating neurons and glia, but, because it stains so many elements, NCAM provides a less clear picture in wholemounts once substantial axonal outgrowth is underway. HNK-1, on the other hand, provides crisp and distinct surface marking of neurons from the beginning of their differentiation. Neither neuroepithelial cells nor developing glial cells show HNK1-IR above control levels. The comparative biochemistry of these patterns is under study.

Supported by NS 18773.

82.2

DISTRIBUTION OF ADHESION AND CELL SURFACE MOLECULES IN DEVELOPING MOUSE NEOCORTEX. W.-W. Chung, C. Lagenaur, and J.S. Lund. Dept. of Neurobiology, Anatomy and Cell Science, University of Pittsburgh, PA 15261

The differential distribution of a number of cell adhesion molecules as well as other cell surface molecules during pre- and postnatal cortical development in the mouse emphasizes a complex partitioning of these molecules in the neuropil. For instance, at E16, polyclonal L1 antibody emphasizes fiber staining in the subplate and intermediate zone; polyclonal NCAM antibody shows fiber staining in the same region but includes cell surface markers in the cortical plate, intermediate and germinal layers; 12F8, antibody to the embryonic form of NCAM, emphasizes staining of the subplate region; M6, antibody to another cell surface protein, shows staining in the subplate and intermediate zone (which is not fiber-related) with extension through the cortical plate. In addition, all these antibodies at E16 stain the marginal zone heavily. By postnatal day 0, the two NCAM antibodies stain throughout all layers, except for the germinal zone, with intense staining in the marginal zone; M6 emphasizes staining of white matter and marginal zone; L1 emphasizes staining of white matter, subplate, lower cortical plate and marginal zone. These changing patterns indicate a considerable complexity to the internal milieu of the developing cortex. (Supported by NS25543 and EY05282).

82.3

TOR 23 IS A PROMINENT BRAINSTEM AND SPINAL CORD MARKER IN THE DEVELOPING RAT CNS. P.D. Kushner, A.O. Esty*, D.A. Garrett*, S. Wright*, and D.T. Stephenson. ALS Research Center, Pacific Presbyt. Med. Ctr, San Francisco, CA 94115.

The evaluation of surface markers of subsets of neurons promises to add significantly to our understanding of neuronal relationships in the developing CNS. This study has examined the distribution the monoclonal antibody (MAb) *Tor* 23 in the rat embryo. Heads from embryonic stage 15 were serially cryosectioned (8 um thick) and stained with *Tor* 23 and for cytoarchitecture stained for Nissl substance and with MAbs to neurofilament proteins (68 and 180 kDa) and vimentin. We have determined that *Tor* 23 is an exclusive CNS marker and does not bind any peripheral structures; within the CNS *Tor* 23 stains neuronal surfaces broadly in the spinal cord and brainstem, marking the myriads of fiber tracts that are present in these regions at this stage; in contrast, forebrain surface staining exists in precisely circumscribed somatic regions, the septum and the presumptive region of the nuclei of the diagonal band and in the rostral portion of the cortex, where significant staining is associated with the top and bottom regions of the cortical plate. These data are generally consistent with the distribution in the adult rat brain (J. Neurosci 8 3035) and support the conclusion of that earlier study that *Tor* 23 is a motor system marker. The data constitute further evidence for a unique relatedness between the motor neurons and the basal forebrain cholinergic neurons

Supported by ADRDA and the ALS Research Center.

82.4

EXPRESSION OF SC1, A PUTATIVE EXTRACELLULAR MATRIX GLYCOPROTEIN, IN ADULT AND DEVELOPING RAT BRAIN. S. Shahin*, I. Johnston*, I.R. Brown and J.W. Gurd. Scarborough Campus, University of Toronto, Ontario, M1C 1A4.

Polyclonal antisera raised against synaptic junction glycoproteins were used to screen a brain lambda gt11 expression library and isolate a cDNA clone termed SC1 (Johnston et al., Neuron,2:165, 1990). DNA sequencing suggested that the SC1 protein is a secreted, calcium-binding glycoprotein, regions of which exhibit a striking sequence similarity to the extracellular matrix glycoprotein osteonectin/BM40/SPARC. In cerebellum in situ hybridization showed that the SC1 message was predominately expressed in the Purkinje cell layer and continued out into the molecular layer. Polyclonal antibodies were raised against the fusion protein encoded by SC1 cDNA. The antiserum designated E/P11 recognized a glycoprotein doublet of apparent molecular weight 116kd/120 kd. In cerebellum homogenates the SC1 doublet was present at birth and increased during the first 20 postnatal days. The immunohistochemical localization of the E/P11 epitope in rat cerebellum was determined using 16μm frozen sagittal sections and visualized with FITC-conjugated secondary antibody. In the adult strong immunofluorescence was associated with the Bergmann glia perikarya and their ascending processes. The immunoreactivity was blocked by preincubating the E/P11 serum with a bacterial lysate containing a fully overlapping fragment of the SC1 protein but was not affected following preincubation with a similar lysate lacking the SC1 fragment. The granule cell layer, deep white matter, Purkinje cell bodies and dendrites did not stain. The strong reactivity of the Bergmann glia was observed at postnatal day 10 whereas the glial processes did not stain until day 16. Supported by MRC (JWG) and NSERC (IRB).

82.5

PRESENCE OF AN INTRAVENTRICULAR MATRIX IN THE DEVELOPING RAT BRAIN. A. Nadeau, S. Woerly and R. Marchand. Lab. de Neurobiologie, Univ. Laval and Hôp. Enfant-Jésus, 1401, 18e Rue, Québec (QC) Canada, G1J 1Z4.

The present study reports the presence of a floccular matrix within the ventricular system of the developing rat brain. For the visualization of this matrix and the determination of its molecular composition, rat embryos aged from E13 to E16 were perfused (neutral buffer formol 10%), dehydrated, embedded in glycol methacrylate and stained following different histochemical methods. The ventricles were also studied with the scanning electron microscope. The results show that this matrix extends from the ependymal lining and completely fills the ventricular lumen of E15 embryos. It is composed of glycoconjugates, glycosaminoglycans and collagen. Necrotic cells and macrophages (microglia) were often trapped within the matrix.

According to its molecular composition and by analogy to the function of similar matrices in other vesicular structures, three different functions can be hypothesized about the physiological significance of this ventricular matrix: it can, 1) act as a filtration system that could entrap cells debris, 2) provide an architectural scaffold for the developing ventricle, 3) be involved in ionic exchanges and hydrodynamic function related to the cerebrospinal fluid. Other preliminary results obtained from chick embryos reveal that a similar matrix is also present in the developing ventricular system of this species. We suggest that the intraventricular matrix is present in the developing ventricules of many species and that it constitutes a key element in the normal morphogenesis of the brain. (Suported by MRC, FCAR and FRSQ).

82.6

The Role Of The Extracellular Matrix In Photoreceptor Differentiation. D.D.Hunter[1], M.D.Murphy[2*] and W.J.Brunken[2]; Dept. of Biology, Boston College[2] and Division of Neurosci., Tufts University[1], Boston, Massachusetts.

The extracellular matrix (ECM) is important for several aspects of neural development in the PNS. However, the role of the ECM in the development of the CNS is only now being elucidated. The vertebrate retina as an ideal model system for studying the role of the ECM in neural migration and differentiation. We have studied the expression of an ECM protein s-laminin, a laminin homologue that is highly concentrated at the neuromuscular junction, in the skate retina by using immunological probes.

The antiserum GP1 recognizes epitopes in the C-terminal 20 kDa of s-laminin. In the adult skate, intense GP1 immunoreactivity is seen in the interphotoreceptor matrix and at the bases of photoreceptors. During development, s-laminin-like proteins first appear after presumptive photoreceptors have migrated to their final position in the outer retina. Subsequent to this expression, synaptic development proceeds, followed by expression of rhodopsin. Thus, s-laminin-like proteins may be important differentiation signals for photoreceptors.

Protein immunobloting demonstrates two GP1-immunoreactive proteins in the skate retina: one 190 kDa protein, and another 350 - 400 kDa protein that may be a dimer of the lower M_r species. These data are consistent with the original description of s-laminin. We are currently studying the role of s-laminin(s) in a tissue culture system of the developing skate retina in order to elucidate the functional role of this extracellular protein in photoreceptor differentiation.

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82.7

DEVELOPMENTAL REGULATION OF TENASCIN IN THE CHICK EMBRYO SPINAL CORD. S. E. McKay, R. P. Tucker & R. W. Oppenheim. Department of Neurobiology and Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103

Previous studies have shown that the expression of the extracellular matrix protein tenascin is developmentally regulated in avian neural tissue. Here we describe tenascin expression in the developing spinal cord. Spinal cords from chick embryonic day (E) 5, 7, 10, 14 and 18 were homogenized and studied by western and slot blotting using a tenascin polyclonal antiserum. All three tenascin splice variants (Tn190, Tn200 and Tn230) are expressed at E5, but by E10 a high-molecular weight splice variant (Tn230) is predominant. Tenascin expression at E5 is low, but the relative amount of tenascin in the homogenates increases through E14. Immunohistochemistry reveals tenascin in ventral white matter at E5 and E10, and by E14 there is also anti-tenascin staining in dorsal white matter. In situ hybridization studies reveal tenascin is being expressed by oligodendroglia. Our results show that the expression of tenascin in the spinal cord is primarily associated with neuronal-glial interactions, and not with active neurite outgrowth.

82.8

DISTRIBUTION OF CYTOTACTIN mRNAs IN THE NERVOUS SYSTEM OF CHICKEN EMBRYOS. A.L.Prieto*, F.Jones*, K.L.Crossin*, B.A.Cunningham*, G.M.Edelman. Lab. of Developmental and Molecular Biology, The Rockefeller University, New York, NY 10021.

The localization of alternatively spliced forms of cytotactin mRNA was analyzed by *in situ* hybridization and compared to the polypeptide distribution throughout chicken embryonic development. Two probes were used, one probe (CT) corresponding to a region that is contained in all forms of cytotactin, and another (VbVc) included portions of two alternatively spliced type III repeats. In telencephalon, optic tectum, cerebellum, spinal cord and peripheral nervous system, the mRNAs were more transiently expressed and more localized than the polypeptides which were diffusely distributed within the extracellular matrix. Levels of mRNA and protein increased from E8 to E15, then gradually decreased to barely detectable levels by P3, except in the cerebellum, where the levels remained comparatively high. Cytotactin mRNA was observed in radial glial somata of the optic tectum and cerebellum, while the polypetide was found distributed along the processes. The hybridization patterns of the CT and VbVc probes overlapped in the spinal cord and cerebellum, but differed in the outermost tectal layers. In the peripheral nervous system, cytotactin mRNA was detected in large ganglia and in the plexi of Meissner and Auerbach. These results emphasize the importance of the study of factors regulating the developmental expression and function of this molecule.

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: CYTOSKELETON

83.1

MATURATION OF CYTOSKELETAL PROTEINS OF CALLOSAL AXONS. B.Riederer[1], G.M.Innocenti[1] and A.Guadano-Ferraz*[2]. [1]Inst. of Anatomy Univ.of Lausanne, CH 1005 and [2]Dpt. of Morphology Univ. of Alicante, 03080, Spain.

Only a fraction of the juvenile callosal axons are maintained in development (Berbel, P. and Innocenti, G.M., *J. Comp. Neurol.*, 276:132, 1988). In order to characterize molecular features specific to these axons, neurofilament subunit NFH and microtubule associated proteins Tau were studied with electrophoresis, Western blots and immunocytochemistry in the corpus callosum of cats sacrificed on postnatal days (P) 3, 11,19,28,39,adult. Unlike subunits NFL and NFM, which were already present at P2-3 (Figlewicz, D. et al., *Dev. Brain Res.*, 42:181, 1988), NFH could be demonstrated only from P11-19 onwards with monoclonal antibody NE14 and from P29 onwards with monoclonal antibody SMI-32. Pretreatment of tissue with alkaline phosphatase (50 U/ml of Tris-HCl buffer, pH 8.2, with 135mM NaCl, 1mM EDTA, 1mM PMSF, 10 µg/ml Leupeptin, Antipain and Pepstatin A, and 5 µg/ml E64; at 37°C for 2 or 24 h) eliminated positivity to NE14 in blots and sections indicating that this antibody recognized a phosphorylated form of NFH. The same pretreatment revealed positivity to SMI-32 at P11-19 (but not at P3), suggesting that the recognition is conditional to loss of phosphate. Therefore a partially dephosphorylated NFH may appear late in callosal axons. Tau proteins with adult molecular weights appear at P28. These changes occur at the end of the period of fast elimination of callosal axons and therefore probably only in axons which are maintained. They are synchronous with changes in distance between Fs (for NFH) and Ts (for Tau) (Innocenti, G.M. et al., *Neurosci. Abs.*, 1990).

83.2

MATURATION OF CYTOSKELETAL ULTRASTRUCTURE OF CALLOSAL AXONS. G.M.Innocenti[1], P.Berbel*[2] and R.Kraftsik*[1]. [1]Inst. of Anatomy, Univ. of Lausanne, CH 1005 and [2]Dpt. of Morphology Univ. of Alicante, 03080, Spain.

In order to characterize structural changes related to the stabilization of juvenile axons, the distance between neurofilaments (Fs) or microtubules (Ts) was studied as function of age, axon area and Fs, Ts or Fs+Ts density. Electron microscopic photographs of corpus callosum from kittens sacrificed on embryonic (E) or postnatal (P) days E53,58, P4,9,18,26,39,57,92,107,150, for a previous study (Berbel, P. and Innocenti, G.M., *J. Comp. Neurol.*, 276:132, 1988) were employed. Three computerized measurements were performed in over 5000 axons. Two of them (D1 and D2) estimated average - the third (Dmin) absolute minimal distances. With age, D1 and D2, between Ts decreased slightly, and increased slightly between Fs. Dmin between Ts showed a progressive and statistically significant decrease from (median values) 57nm at E53 to 38nm at P39 and remained stable thereafter. Dmin between Fs increased slightly between E53 (43nm) and P18 (64nm). Then it decreased to 38mn at P39 and remained unchanged thereafter. When the adult values of Dmin were reached, they became also uncorrelated with, and therefore independent of, the density of Ts, Fs or Ts+Fs. Interestingly, this was simultaneous to the appearance of adult Tau (for Ts) and NFH (for Fs) proteins (Innocenti, G.M. et al., *Neurosci Abs.*, 1990). NFH and Tau cross connect Fs and Ts, respectively; their maturation may influence the spacing of these elements and reflect or cause the stabilization of the juvenile axons.

83.3

INTERACTION OF MAP2 WITH THE NEURONAL CYTOSKELETON. B. Brugg* and A. Matus. Friedrich Miescher-Institut, P.O. Box 2543, CH-4002 Basel, Switzerland.

Microtubule-associated protein 2 (MAP2) is a 200 kDa neuronal protein that can bind to and cross-link microtubules in living cells. Little is known about how these inter actions are regulated, but phosphorylation may be involved since it is known to influence the binding of MAP2 to tubu lin polymers in vitro. We have studied this possibility by preparing purified MAP2 in different states of phosphorylation, injecting it into RAT-1 fibroblastic cells, where MAP2 is normally absent, and then following its distribution by immunofluoresecence staining. The results show that MAP2 prepared as close to its native phosphorylation state as possible (10 mole Pi/mole) bound to cellular microtubules immediately after microinjection. Enzymically dephoshorylated MAP2 (2 mole/mole) did not initially bind to cellular microtubules but its distribution slowly changed so that 15 min after injection it was bound to microtubules. However, in cells grown in serum-free medium this redistribution did not occur, and MAP2 remained spread throughout the cytoplasm. Since protein kinase activity is high in cells grown in serum and low in those grown in serum- free medium, these results are consistent with the idea that phosphorylation of MAP2 is required for its binding to microtubules. However, further in vitro phosphorylation of MAP2 to 20 mole/mole via kinase activity that co-purifies with microtubules resulted in its not binding to cellular microtubules. We conclude that MAP2 contains various phosphorylation sites that differently influence its binding to microtubules in living cells.

83.4

MECHANISM OF INSULIN AND INSULIN-LIKE GROWTH FACTOR DIRECTED NEURITE FORMATION: ROLE OF CYCLIC AMP IN NEUROFILAMENT AND TUBULIN GENE EXPRESSION. C. Wang*, Y. Li*, B. Wible*, K. Angelides*, and D.N. Ishii Physiolology Dept., Colorado State Univ., Ft. Collins, CO 80523; Physiology and Molecular Biophysics Dept., Baylor College of Medicine, Houston, TX 77030.

There is a close correlation between insulin-like growth factor (IGF) gene expression and synaptogenesis, and IGFs can enhance neurite growth *in vivo* and *in vitro*. We tested the hypothesis that cAMP is a second messenger mediating neurite outgrowth directed by insulin and IGFs in human neuroblastoma SH-SY5Y cells. Neurofilament (NF) 68 kd, NF 170 kd, α-tubulin, and β-tubulin mRNAs were selectively increased in response to insulin, IGF-I, IGF-II, dibutyryl cAMP, forskolin, or cholera toxin. Theophylline increased the response to insulin, IGF-I, or IGF-II. Like IGFs, dibutyryl cAMP or forskolin enhanced neurite outgrowth. Both 8-bromo cAMP and NGF selectively increased tubulin and NF mRNAs in PC12 cells as well. Therefore cAMP may mediate the insulin-, IGF-, and NGF-dependent increases in selective mRNAs, which encode proteins of the axonal cytoskeleton and support neurite outgrowth. (NIH grant RO1 NS24327)

83.5

LOCALIZATION OF PHOSPHATE DEPENDENT (RMO2.4) AND INDEPENDENT (RMDO9.5) EPITOPES OF THE HEAVY NEUROFILAMENT SUBUNIT IN THE HIPPOCAMPUS AND DENTATE GYRUS OF NORMAL AND MUTANT MICE. P.R. Patrylo and R.S. Nowakowski. Department of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School and Physiology/Neurobiology Program, Rutgers University, Piscataway, NJ 08854.

The immunohistological localization of phosphate dependent (RMO2.4) and independent (RMDO9.5) epitopes of the heavy (200kDa) neurofilament subunit was examined in C57BL/6J, NZB/BINJ, *Hld/Hld* (on a BALB/cByJ background), nf^{or}/nf^{or} (on a non-inbred background), and $dr^{sst\text{-}J}/dr^{sst\text{-}J}$ (on a B6C3Fe background) mice. In all mice examined the mossy fibers in the hippocampus were RMO2.4 immunopositive, but in NZB/BINJ and *Hld/Hld* only a few fibers in the infrapyramidal limb were immunoreactive. RMDO9.5 was localized in the soma and dendrites of almost all of the neurons in the pyramidal cell layer of CA2 and CA3 and in the hilus of the dentate gyrus. However, RMDO9.5 immunoreactivity was observed in only a few neurons in CA1 and in the granule cell layer of the dentate gyrus. In addition, ectopic pyramidal cells in CA3 of *Hld/Hld*, nf^{or}/nf^{or}, $dr^{sst\text{-}J}/dr^{sst\text{-}J}$ and NZB/BINJ were RMDO9.5 immunoreactive, while ectopic pyramidal cells in CA1 of nf^{or}/nf^{or} and ectopic granule cells in the dentate gyrus of $dr^{sst\text{-}J}/dr^{sst\text{-}J}$ and NZB/BINJ were not. Our data indicates that phosphate dependent and phosphate independent heavy neurofilament subunit epitopes are differentially distributed in the mouse hippocampus. Furthermore, we suggest that the expression of RMDO9.5 is a property of cell-class (e.g., CA3 pyramidal cell *vs* CA1 pyramidal cell) and not laminar position (e.g., stratum pyramidal *vs* stratum radiatum).

Supported by a grant from the Schizophrenia Research Program.

83.6

VIMENTIN AND GFAP IN THE IRRADIATED RAT SPINAL CORD. S. A. Gilmore, M. A. Pippenger*, and T. J. Sims. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205-7199.

Many previous studies by Gilmore and colleagues have demonstrated that exposure of the lumbosacral spinal cord of the immature rat to x-radiation results in a marked decrease in the glial population. The present study was undertaken to determine the pattern of intermediate filament immunoreactivity in the glial cells that do persist during the first two weeks post-irradiation (P-I).

Rat pups were irradiated with a single 4000 R dose of "soft" X-rays to a 5mm length of LS spinal cord. The animals and their non-irradiated litter-mates were deeply anesthetized and sacrificed on postnatal days 6, 9, 12, and 15. Paraffin sections of the spinal cords were stained for vimentin and/or GFAP using the PAP technique.

At the earliest age examined (3 days P-I), few GFAP-positive cell bodies and processes were noted in the lateral and ventral white matter. By 9 days of age (6 days P-I) an occasional astrocyte in these areas appeared to have become reactive, i.e. was intensely positive for GFAP, and these reactive white matter astrocytes increased in numbers in the 12- and 15-day-old animals. The gray matter, although initially GFAP negative, underwent an abrupt increase in GFAP-positive astrocytes between 9 and 12 days of age (6 and 9 days PI). Vimentin immunoreactivity was limited to only a few cells and processes and never approached the level of GFAP reactivity.

These results suggest that surviving astrocytes in the irradiated spinal cord include GFAP-positive astrocytes in the white matter, as well as a group of non-immunoreactive gray matter astrocytes which respond to radiation by becoming GFAP-positive without first passing through a vimentin-positive stage.

Supported by NIH Grant NS 04761 and Neuroscience Research Funds from the College of Medicine, UAMS.

83.7

VIMENTIN AND GFAP IN THE IMMATURE RAT SPINAL CORD. M. A. Pippenger*, S. A. Gilmore, and T. J. Sims. Department of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR 72205-7199.

Studies by others have shown that vimentin is the major intermediate filament (IF) protein in astrocytes early in development, whereas GFAP is the major astrocyte IF protein in adults. Aspects of this vimentin-GFAP transition were examined in the white matter of the normal immature rat spinal cord using immunohistochemistry.

Rat pups were deeply anesthetized and perfused with fixative on postnatal days 3, 6, 12, and 21. Paraffin sections from the cervical, thoracic, and lumbosacral regions of the spinal cords were stained for vimentin or GFAP using the PAP technique. Selected sections were double-labeled for both IF proteins using the 1-naphthol basic dye technique (Mauro *et al., Histochem.*, 86:123, 1986).

The major observations made in this study were: that vimentin was the predominant IF at all cord levels at 3 days of age, that vimentin-positive cell bodies and processes in the white matter decreased rapidly in numbers so that after 9 days of age, only an occasional vimentin-positive process was noted; that GFAP-positive cell bodies and processes increased with age, outnumbering those positive for vimentin by 6 days of age, particularly in the more rostral segments; that a rostrocaudal gradient in development of GFAP positivity occurred; that the adult pattern of GFAP immunoreactivity was reached by day 21; that during this transition only a few double-labeled cell bodies and processes were noted; and that these few double-labeled cells occurred only in younger animals.

This study demonstrates that GFAP is the predominant intermediate filament protein in astrocytes in the white matter of rat spinal cord by 6 days of age. The results suggest that, although a few astrocytes express both vimentin and GFAP simultaneously, most appear to produce GFAP without first producing vimentin.

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84.1

REMOVAL OF Na INACTIVATION ELIMINATES Na CURRENT ACTIVATION DELAYS. R. Hahin. Dept. of Biological Sciences, Northern Illinois University, DeKalb, IL 60115

Na current activation delays were obtained from voltage-clamped frog skeletal muscle fibers. Na currents elicited by depolarizing steps from hyperpolarizing holding potentials are delayed in their activation. In untreated fibers, the magnitude of the delay was maximized by using the most negative holding potentials (-150 mV), and the smallest depolarizing test pulse potentials. Delays, observed following partial inactivation removal using chloramine-T treatment, did not differ from delays observed prior to treatment. Complete removal of inactivation eliminated hyperpolarization-induced delays in activation. Steady-state slow inactivation was virtually unaffected by chloramine-T treat-treatment that removed inactivation. The results suggest that chloramine-T oxidizes methionine residues that play key roles in determining initial channel opening latencies and life-times.

84.2

NEGATIVE MODULATION OF TETRODOTOXIN-SENSITIVE, CONOTOXIN GIII A-LESS SENSITIVE SODIUM CHANNELS IN CULTURED BOVINE ADRENAL MEDULLARY CELLS. A. Wada, Y Uezono*, M. Arita*, H. Kobayashi* and F. Izumi. Dept. of Pharmac., Univ. of Occupational and Environmental Health, Sch. of Med., Kitakyushu 807, Japan.

Existence of multiple subtypes of Na channels has been noted. We previously reported that in adrenal medulla, conotoxin GIII A which blocks Na channels in skeletal muscle and adrenergic neuron but has no effect in brain, inhibits veratridine-induced ^{22}Na influx (IC_{50} 6 μM) without affecting ^{3}H-saxitoxin binding (Jap. J. Pharmac., 49:185p, 1989). In the present study, to examine the regulation of Na channels, cells were pretreated with veratridine, and the number and properties of Na channels were determined. In cells preexposed to 100 μM veratridine, veratridine-induced influx of ^{22}Na was decreased by 82% with a half-time of 2-3h. This decrease was reversible and prevented by tetrodotoxin. Potentiation of veratridine-induced ^{22}Na influx by a α-scorpion venom was 3-4 fold lower in veratridine-pretreated cells. Scatchard plot of ^{3}H-saxitoxin binding showed that 1h pretreatment with 100 μM veratridine produced 32% reduction in Bmax, but has no effect on Kd. The number of functional Na channels seems to be reduced by the increased activity of Na channels.

84.3

INTERLEUKIN-2 REDUCES VOLTAGE-ACTIVATED Na-CURRENTS IN EMBRYONIC RAT HIPPOCAMPAL NEURONES. C. ZONA[1], E. PALMA*[1], A. SANTONI*[2], F. GRASSI*[3], & F. EUSEBI*[3]. [1]Dept. Medicina Sperimentale e Scienze Biochimiche, Univ. di Roma "Tor Vergata". [2]Dept. Medicina Sperimentale, Univ. di Roma "La Sapienza". [3]Lab. di Biofisica, Ist. Regina Elena, Roma-ITALY.

Interleukin-2 (IL-2) plays a pivotal role as a polypeptide mediator of cellular immune response and is a member of the class of molecules termed cytokines that mainly regulate growth and differentiation of cells involved in immunity. In the present work, voltage-activated whole-cell currents and GABA-activated currents have been recorded in hippocampal embryonic nerve cells which, in the rat, exhibit specific IL-2 binding sites. We show here that human recombinant IL-2 (rIL-2), while does not affect neither K+, nor Ca2+ currents, nor GABA-activated currents, significantly reduces the amplitude of Na+ currents. This may explain, at least in part, the rIL-2-induced modification in the electrical activity of central nerve cells, described elsewhere.

84.4

DIFFERENTIAL PROPERTIES OF TETRODOTOXIN-SENSITIVE AND TETRODOTOXIN-RESISTANT SODIUM CHANNELS IN RAT DORSAL ROOT GANGLION NEURONS. M.-L. Roy and T. Narahashi, Dept. of Pharmacol., Northwestern Univ. Med. Sch., Chicago, IL 60611.

Tetrodotoxin (TTX)-sensitive (TTX-S, K_d~1 nM) and TTX-resistant (TTX-R, K_d~100 μM) sodium channel currents were analyzed in acutely dissociated dorsal root ganglion neurons isolated from 3-10 day old rats. Currents were recorded using the whole-cell patch clamp technique. These two types of current were found in varying proportions dependent upon the rat age. Cells from younger rats (3-7 days) expressed largely TTX-R current, whereas cells from older rats (7-10 days) expressed either a combination of TTX-S and TTX-R currents or TTX-S current only. TTX-R current showed a distinctively slow time course compared with the much faster time course of TTX-S current. These current types also differed in their steady-state inactivation, with TTX-R $V_{1/2}$ being -40 ± 4 mV (n=4) and TTX-S $V_{1/2}$ being -70 ± 3 mV (n=3). Lidocaine (100 μM) and chlorpromazine (1 μM) reduced TTX-R current by approximately 60%, and virtually abolished TTX-S current. The biophysical and pharmacological properties of these two types of sodium channels are of particular importance in the development of CNS and in the mechanism of action of drugs on the CNS neurons.
Supported by NIH grants RO1 NS14143 and F31 MH09839.

84.5

THE PHOTOACTIVE PROBE BATRACHOTOXININ-A ORTHO AZIDOBENZOATE COVALENTLY LABELS LIPID COMPONENTS OF THE BATRACHOTOXIN BINDING SITE OF VOLTAGE-SENSITIVE SODIUM CHANNELS. T.L. Casebolt and G.B. Brown, Dept. of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL. 35294.

The voltage-sensitive sodium channel has been extensively studied using at least five classes of specifically-acting neurotoxins, each of which binds to a different site on the sodium channel. Batrachotoxin (BTX), the most potent of the "lipid soluble" class of toxins binds the sodium channel, resulting in profound changes of channel function and electrophysiology. While much has been learned about the interaction of the BTX binding site with other sodium channel neurotoxin binding sites, specific information about the structural components of the BTX binding site is of interest to determine the component or components contributing to altered sodium channel function at the molecular level. To accomplish this goal, a photoreactive [3H]BTX derivative, [3H]Batrachotoxinin-A ortho azidobenzoate ([3H]BTX-OAB), was prepared by partial synthesis. The probe binds covalently to the BTX sodium channel site, allowing detailed examination of the elements within the site. After photolabeling of rat cerebral cortical synaptoneurosomes with [3H] BTX-OAB, approximately 90% of the specifically labeled material was recovered in lipid extracts. Two specifically labeled products were identified by thin layer chromatography. Additional purification by LH-20 gel filtration chromatography suggested that [3H]BTX-OAB covalently labels a small mw compound, possibly phospholipid, with an estimated mw of 600-800. To test this hypothesis, experiments are in progress to analyze the labeled lipid-like material by chemical cleavage and specific enzymatic hydrolysis using purified phospholipases. Presuming the lipid-like material is phospholipid, this approach is expected to provide information about the point of linkage of [3H]BTX-OAB to the head group or fatty acyl chains.

84.6

EFFECTS OF LIDOCAINE ON PURIFIED BATRACHOTOXIN-MODIFIED SODIUM CHANNELS FROM EEL ELECTROPLAX. D.E. Flash[a]* S.R. Levinson[b], E. Recio-Pinto[a]. [a]Depts. Anesthesiology and Physiology. Cornell Univ. Med. Coll. NY, NY 10021. [b]Dept. Physiology, Univ. of Colorado Med. Sch., Denver, CO 80262.
Supported in part by the Lois & Rose Klosk Award.

Sodium channels are an important site of local anesthetic action. The effects of Lidocaine (LI) in the single sodium channel current and steady-state activation characteristics were studied on purified eel electroplax sodium channels. Sodium channels were incorporated into planar lipid bilayers in the presence of batrachotoxin (BTX), 0.5M NaCl. Under control conditions the channels I-V relationships were linear and symmetrical with a mean slope conductance of 24 pS. LI increased the rate of channel disappearance from the bilayers, suggesting that the LI increases the rate of BTX dissociation. LI reduced the single sodium channel conductance in a dose- and voltage-dependent manner. The block with LI increased with membrane depolarization. The noise level of the open channel increased as the voltage became more positive, as if the kinetics of LI block became slower with depolarization. The maximum current at steady-state decreased due to a decrease in the number of BTX- modified channels and a decrease in the single channel current of BTX-modified channels. The steady-state activation characteristics of BTX-modified channels (midpoint potential and apparent gating charge) were not affected by LI.

84.7

EFFECTS OF BREVETOXIN ON GUINEA PIG HIPPOCAMPAL NEURONS. *J.P. Apland, M. Adler, and R.E. Sheridan.* Neurotoxicology Branch, U.S. Army Medical Research Institute of Chemical Defense, Aberdeen Proving Ground, MD 21010.

Brevetoxin (PbTx-3) depolarizes nerve and muscle membranes by shifting the activation potential of voltage-dependent Na^+ channels to more negative values. Since PbTx-3 is lipid soluble and exhibits behavioral effects believed to be of central origin it was of interest to examine its actions on CNS function.

Extracellular recordings were obtained from area CA1 of guinea pig hippocampal slices. PbTx-3 was applied by bath perfusion in a submersion chamber at 32° C. PbTx-3 produced a concentration-dependent depression of the evoked population spike with an EC50 of 82 ng/ml. Concentrations below 30 ng/ml were without effect and concentrations of 300 ng/ml or greater led to total inhibition of evoked responses. PbTx-3 did not produce spontaneous synchronous discharges but did increase single unit activity at concentrations of 100 ng/ml or higher. The toxin appeared to be equally effective in inhibiting orthodromic and antidromic responses, suggesting an action on excitation rather than on synaptic transmission. Recovery from PbTx-3 was slow, requiring up to 3 hr of wash for complete restoration of function. The toxin-induced suppression of spike generation in hippocampal slices resembles its action on skeletal muscle and is consistent with membrane depolarization.

84.8

DIFFERENTIAL ACTIONS OF BREVETOXIN ON PHRENIC NERVE AND DIAPHRAGM MUSCLE IN THE RAT. S. S. Deshpande, R. E. Sheridan and M. Adler. Neurotoxicology Branch, U.S. Army Medical Research Institute of Chemical Defense, APG, MD 21010.

The lethal actions of the dinoflagellate poison brevetoxin (PbTX-3) are thought to be mediated by depression of respiration. We have examined the mechanism of PbTX-3 inhibition of phrenic nerve-diaphragm responses *in vitro* and found that low-dose effects are mediated largely by inhibition of impulse transmission in the phrenic nerve, while depression of the muscle itself occurs at higher doses. In isolated hemidiaphragms from the rat, nerve-elicited twitches were blocked by 50 to 100 nM PbTX-3. Higher toxin concentrations, between 100 and 500 nM, were needed to block directly elicited twitches in the muscle. Intracellular recordings indicated that failure in the evoked endplate potential (EPP) occurred in an all-or-none fashion in PbTX-3, without a graded reduction in the quantal content. Higher spontaneous miniature endplate potential (MEPP) frequencies were observed, but only after EPP failure (20 min. or more) or at the higher concentrations of PbTX-3. At toxin concentrations below 100 nM, direct stimulation of single muscle fibers still elicited action potentials, however, 0.4 to 1 μM PbTX-3 blocked direct action potentials and depolarized muscle fibers (from a mean of -73 mV to a maximum level of -46 mV with an EC50 of 1.3 μM). Measurements of the compound action potential in the phrenic nerve showed reductions of 30% or more at 50 nM PbTX-3 and complete block at 100 nM, paralleling the loss of nerve-evoked twitch and loss of the EPP. Thus, we conclude that diaphragmatic failure in PbTX-3 is caused by a loss of conduction in the phrenic nerve due to a higher sensitivity of the nerve to the toxin.

84.9

INTERACTIONS BETWEEN THE PUMILIOTOXIN B (PTX-B) SITE AND OTHER SITES ON THE VOLTAGE-DEPENDENT SODIUM CHANNEL. Fabian Gusovsky, William Padgett* and John W. Daly LBC, NIDDK, NIH, Bethesda, MD 20892.

The alkaloid PTX-B "activates" voltage-dependent sodium channels in synaptoneurosomes and neuroblastoma cells (Gusovsky et al., 1989). It appears that PTX-B activates sodium channels by interacting with a site that is allosterically regulated by other sites (alkaloid site 2, scorpion toxin sites 3 and 4) on the sodium channel. In guinea pig cortical synaptoneurosomes, α-scorpion toxin and the marine toxin brevetoxin induce a dose-dependent potentiation of PTX-B-induced $^{22}Na^+$ influx (EC_{50}s: 3 nM and 70 nM respectively). In addition, ß-scorpion toxin induces a dose-dependent potentiation of PTX-B-induced $^{22}Na^+$ influx (EC_{50}: > 10 nM). The synergism with ß-scorpion toxin differentiates PTX-B from the alkaloids batrachotoxin and veratridine, which induce an activation of sodium channels that is not affected by ß-scorpion toxin. Furthermore, PTX-B did not inhibit [^{3}H]batrachotoxinin-A benzoate ([^{3}H]BTX-B) binding to sodium channels. On the other hand, aconitine, which activates sodium channels and inhibits [^{3}H]BTX-B binding, induces sodium influx that can be potentiated by α-scorpion toxin, brevetoxin and ß-scorpion toxin. The type I pyrethroid allethrin, but not the type II pyrethroid deltamethrin, inhibits PTX-B- and PTX-B/α-scorpion toxin-mediated sodium influx. Inhibition also is observed with aconitine- mediated sodium flux, but does not occur with BTX- and veratridine-mediated sodium influx. It is proposed that on the sodium channel there is an "alkaloid binding domain" at which alkaloids exert stimulatory actions. However, depending on the region on the domain on which the binding occurs, different allosteric actions with other sites can be observed. PTX-B is proposed to interact with a part of the "alkaloid binding domain" that is shared by aconitine, but not by batrachotoxin or veratridine, while aconitine interacts with a part of the domain shared by PTX-B and by batrachotoxin/veratridine. Such a model may be useful in defining sodium channel topography.

84.10

DIVALENT ION SENSITIVE SODIUM CHANNELS IN AtT20/D16-16 CELLS. R. E. Sheridan and M. Adler. Neurotoxicology Branch, U.S. Army Medical Research Institute of Chemical Defense, APG, MD 21010.

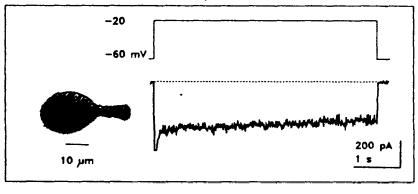
The pituitary cell line AtT20/D16-16 produces spontaneous spike activity involving both Na^+ and Ca^{++} channels. These spikes can be completely abolished by application of 1 to 10 mM $CoCl_2$ or other traditional divalent cation blockers of calcium channels, leading to suggestions that only calcium was involved in carrying the inward current. However, we have determined that the spikes are also sensitive to tetrodotoxin and involve a sodium channel current that is unusually sensitive to divalent cations.

In whole cell patch clamp experiments, application of 1 mM Co^{++} reduced the peak sodium conductance by approximately 50% and shifted the midpoint of activation for both sodium and potassium currents by +10 mV. In 10 mM Co^{++}, the sodium current was almost totally suppressed and the activation voltage for Na^+ conductance was shifted by +30 mV. In single channel experiments, 1 mM Co^{++} reduced the conductance of single Na^+ channels in a voltage-dependent manner, with approximately 50% block at -60 mV and only modest reductions in current at -20 mV. These data suggest that in the AtT20/D16-16 cell line, the sodium channel has a high affinity for cobalt at a voltage dependent binding site, presumably within the ion channel. The shifts in whole-cell sodium activation voltage seen in cobalt are likely due to a combination of the voltage dependent suppression of sodium conductance at negative potentials and a shift in the surface potential of the cells due to screening effects on fixed negative charge sites in the plasma membrane.

84.11

VOLTAGE CLAMP ANALYSIS OF A PERSISTENT TTX-SENSITIVE Na CURRENT IN CEREBELLAR PURKINJE CELLS. [1]A.R.Kay, [2]M. Sugimori and [2]R.R. Llinas. [1]AT&T Bell Labs, Biophys. Dept., NJ. 07974 and [2]Dept. Physiol. & Biophys., NYU Med. Ctr., New York, NY 10016.

A persistent Na current ($I_{Na}P$) has been shown to exist in cerebellar Purkinje cells (Llinas & Sugimori,J. Physiol. 1980). Here we have subjected this current to a detailed voltage clamp analysis, in whole-cell recordings from Purkinje cells acutely dissociated (Kay & Wong J.Neurosci. Methods. 1986) from guinea pig cerebellum. $I_{Na}P$ activated rapidly (<1ms), deactivated rapidly (< 0.2 ms) and exhibited very little inactivation during voltage clamp pulses as long as 10s (see Fig.). We suggest that $I_{Na}P$ represents a separate current from the conventional Na current on the following grounds: (a) $I_{Na}P$ activates at a lower threshold than the inactivating current. (b) $I_{Na}P$ does not arise solely from the overlap of the steady state activation and inactivation of the inactivating current (i.e. it is not a window current).



84.12

BLOCKADE OF OUTWARD CURRENT UNCOVERS A PERSISTENT INWARD CURRENT IN HEART INTERNEURONS OF THE LEECH *HIRUDO MEDICINALIS*. C.A. Opdyke and R.L. Calabrese. Dept. of Biology, Emory University, Atlanta, GA 30322.

In the medicinal leech, a neural oscillator composed of rhythmically active HN interneurons paces heartbeat. HN cell activity is characterized by bursts of spikes that are periodically interrupted by barrages of IPSP's originating from other HN cells (Thompson and Stent, 1976a and Calabrese, 1977). Blockade of competing currents has revealed a sodium dependent persistent inward current, I_P, in these cells that has been studied using the single electrode voltage clamp.

Outward current comprising a transient current similar to A-current (Conner and Stevens, 1971 a,b,c) and a delayed rectifier was blocked using a combination of 1.5M TEA-Acetate and 1.5M Cs-Acetate in the recording microelectrodes and 1mM methohexital, a short acting barbiturate, in the saline. Synaptic transmission and Ca^{++} currents were blocked by removing Ca^{++} from the saline and substituting 10mM Co^{++}, and the hyperpolarization activated inward current, I_h (Angstadt and Calabrese, 1989), was blocked by adding 4 mM Cs^+ to the saline. Under these conditions, HN neurons produce periodic plateaus that are accompanied by a conductance increase. These plateaus are associated with a non-inactivating sodium dependent inward current, I_P, that exhibits a maximal activation at -20mV, when stepped from a holding potential of -60mV.

84.13

ACTION POTENTIAL LENGTHENING INDUCED BY MEROCYANINE 450 IN SNAKE MUSCLE. S.T. Reikes and R.S. Wilkinson, Dept. of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110.

Merocyanine 450 (MC450) is a voltage-sensitive fluorescent dye which binds to living membranes. We noticed an unusual effect of MC450 on myofibers of the garter snake transversus abdominis muscle while exploring its use as a nerve terminal stain. Action potential (AP) duration increased significantly, from the normal 2-3 ms to 5-250 ms, when MC450 (30-75 μM) was applied to the bath. The MC450 AP resembled a cardiac muscle AP, with an initial peak followed by a long plateau. Occasionally, the plateau was interrupted by repeated (~15 ms) burst-like oscillations. AP lengthening persisted in a bath containing zero added Ca^{++} (1 mM EGTA) or in the presence of the calcium channel blocker $CdCl_2$ (2 mM). Similarly, the K^+ channel blocking agents 3,4-diaminopyridine (1.5 mM) and tetraethylammonium (TEA, 50 mM) had no effect, although TEA slowed the falling phase of control APs. In contrast, venom of the scorpion Leiurus quinquestriatus (0.5 μM) lengthened APs in a manner strikingly similar to MC450; this venom is known to slow Na^+ channel inactivation in other preparations. Subthreshold conductance changes in the presence of MC450 were consistent with prolonged Na^+ channel opening. We conclude that MC450 specifically affects Na^+ channel kinetics in this snake muscle. Supported by NIH grant NS24752 and the MDA.

84.14

A UNIQUE *IN VIVO* MODEL FOR EVALUATING LOCAL ANESTHETIC AGENTS. V.E. Paris*, N.A. Pahno*, D.D. Rigamonti, V.L. Jimmerson and G.F. Seng*. Dept. Chemistry, Walter Reed Army Medical Center, Washington, DC 20307-5100.

Studies of local anesthetics performed on rat sciatic nerve *in vitro*, using a standard nerve recording chamber, initially yielded inconsistent results (e.g. lack of long term nerve viability). The present study used an *in vivo* model to characterize local anesthetics in the PNS. The sciatic nerve of anesthetized rats was stimulated with a pair of electrodes attached proximally at the greater sciatic notch and recorded with a distal pair of electrodes in the tibial division near the ankle. Both pairs were embedded in wax to isolate them from surrounding tissue to avoid signal interference and ensure that the electrical signal traversed only the nerve. The elicited compound action potential was amplified, visualized and averaged (MI^2). Local anesthetics and saline ($\leq$ 30 μl) were injected directly into the nerve distal to the stimulating electrodes via a glass micropipette. Results from the *in vivo* model were consistent with previous reports using the *in vitro* chamber. Unmyelinated C fibers were blocked first, followed by A-δ fibers, and finally the A-α fractions. EMG response and the conduction in the fiber fractions typically returned in reverse order. These results demonstrate the utility of the model for evaluating local anesthetics.

84.15

ROOM TEMPERATURE CULTURE EXTENDS THE USEFUL LIFE OF ADULT NEURONS FOR VOLTAGE-CLAMP EXPERIMENTS. J.C. Magee and G.G. Schofield, Dept. of Physiology, Tulane Univ. Sch. Med., New Orleans, LA 70112.

The detailed investigation of membrane currents has been greatly advanced in recent years through the use of the patch-clamp technique. The utility of acutely isolated adult mammalian neuronal populations in such investigations has been established. In an effort to increase the utility of these preparations, adult SCG neurons were maintained for four days in a room temperature (22° C) culture (22° C neurons). Under these conditions the neurons retained the advantages of the acutely isolated cells, including the lack of processes. Properties of the voltage-dependent sodium current (I_{Na}) in these neurons were compared with those of neurons of the same cell type maintained in a 37° C culture for the same period of time (37° C neurons) and with neurons used on the day of isolation (acute neurons). The 37° C neurons displayed a hyperpolarized half activation voltage and a smaller inactivation slope factor (K) than the acute neurons. No differences were found between 22° C neurons and acute neurons for these parameters. However, maximal conductance was found to be significantly higher in the 22° C neurons than the other two groups. The observed alterations in the I_{Na} kinetics of the 37° C neurons are most likely indicative of poor spatial voltage control. The 22° C neurons did not show any alterations in I_{Na} kinetics following culture. Thus, short term room temperature culture extended the useful life of the acutely isolated neurons for most voltage-clamp experiments. Supported by the American Heart Assoc., Louisiana affiliate.

SODIUM CHANNELS II

85.1

FUNCTIONAL ANALYSIS OF SODIUM CHANNEL PHOSPHORYLATION. R.D. Smith and A.L. Goldin. Dept of Microbiology & Molecular Genetics, U. California, Irvine, CA 92717.

The effects of phosphorylation on rat brain Na^+ channel function have been investigated using *Xenopus* oocytes as an expression system. Phosphorylation at protein kinase C (PKC) sites of the Na^+ channel appears to decrease the amplitude of whole-cell Na^+ currents, as determined by voltage-clamping. This is suggested by a significant decrease in measured Na^+ currents when oocytes injected with Na^+ channel α subunit mRNA are perfused with the phorbol ester PMA. In contrast, channel phosphorylation at protein kinase A (PKA) sites appears to increase the Na^+ current amplitude, as evidenced by the fact that co-injection of an inhibitor of protein kinase A results in decreased current amplitudes. Co-injection of phosphatase leads to a significant decrease in Na^+ currents, suggesting that phosphorylation at PKA sites is functionally more prominent than PKC phosphorylation. In addition to effects on current amplitude, phosphorylation appears to affect the voltage-dependent properties of the channel. PKC phosphorylation shifts voltage-dependent activation and inactivation in the hyperpolarizing direction, whereas PKA phosphorylation shifts only inactivation in the same direction and has no effect on the voltage-dependence of activation. The effects we are seeing may be due to phosphorylation of either the Na^+ channel α subunit or accessory proteins of the channel. To determine which is the case, we have mutated five consensus PKA sites in the cytoplasmic linker between domains I and II and one consensus PKC site in the same linker region. The effects of these mutations on Na^+ channel function will be presented.

85.2

IDENTIFICATION OF AMINO- AND CARBOXYL- TERMINAL SERINE RESIDUES ON THE VOLTAGE-SENSITIVE SODIUM CHANNEL FROM *E. electricus* PHOSPHORYLATED BY ENDOGENOUS KINASE. Mark C. Emerick* and William S. Agnew. Dept. of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06510.

In a previous study we have identified four amino- and carboxyl-terminal sites on the electroplax sodium channel high-molecular weight glycopeptide that serve as highly active substrates for cyclic-AMP-dependent protien kinase (PKA). The present study reveals two additional sites on the molecule that are susceptible to phosphorylation by PKA, and further demonstrates, by means of back-phosphorylation of purified sodium channels following phosphatase treatment, that four of the identified PKA phosphorylation sites are phosphorylated *in vivo* by cellular kinases. These sites of endogenous phosphorylation are all on serine residues: 6 or 7, 440, 1680, and 1776. All are on amino- and carboxyl-terminal domains. Threonine-17, while highly susceptible to phosphorylation by PKA *in vitro*, was not shown by this method to be phosphorylated *in vivo*. The location of phosphorylated residues on the terminal domains parallels the distribution of PKA consensus sequences in the primary structure of this channel, in contrast to the *in situ* phosphorylation of mammalian neuronal sodium channel alpha-subunits on non-terminal domains, and is consistent with the possibility that these different sodium channel types are regulated in different ways (*c.f.* Emerick and Agnew *Biochemistry*, 1989, *28*, 8367).

85.3

SEQUENCE-SPECIFIC ANTI-PEPTIDE AND ANTI-FUSION PROTEIN ANTIBODIES FOR CHARACTERIZATION OF MUSCLE SODIUM CHANNEL ISOFORMS IN NATIVE AND TRANSFECTED CELL LINES. C.Ukomadu, J.S.Trimmer and W.S.Agnew. Department of Cellular and Molecular Physiology, Yale University, New Haven, CT 06510.

We have previously cloned cDNAs encoding a TTX-sensitive voltage-sensitive sodium channel expressed in adult rat skeletal muscle, designated μ1. We have generated a panel of antibodies against the μ1 protein. Antibodies were raised against (1) synthetic peptides constructed from the μ1 amino acid sequence, and (2) fusion proteins derived by subcloning μ1 cDNA sequences downstream of the tryptophan E gene of *E. coli*. These antibodies recognize antigenic sites in rat skeletal muscle membranes (demonstrated by ELISA) and specifically label a glycopeptide of 240,000-260,000 Da on Western blots of the same membranes. Immunocytochemical experiments have revealed one antibody that binds to an extracellular site of the protein. Immunoprecipitation and immunocytochemical experiments indicate that this antibody cross-reacts with a second sodium channel isoform present in L6 cells. This cell line does not express μ1 mRNA or TTX-sensitive sodium currents, but does express a different transcript (μ2, or SkmII; Kallen et al: Neuron, 4:233,1990. Trimmer et al., Dev.Bio., submitted), and TTX-resistant sodium currents. These antibodies have been used to investigate redistribution of sodium channels accompanying formation of L6 myotubes, and in preliminary characterization of sodium channels in rat fibroblasts stably transformed with μ1 cDNA.

85.4

EXPRESSION OF SODIUM CHANNEL mRNA IN THE ADULT RAT SKELETAL MUSCLE FOLLOWING SURGICAL AND CHEMICAL DENERVATION. J. Yang, J.T. Sladky, and R.L. Barchi. Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

We have recently cloned a second sodium channel characteristic of denervated skeletal muscle (SkM-II). The tissue and developmental expression of SkM-II mRNA correspond to that of a TTX-resistant muscle sodium channel (Neuron 4, 233-42, '90). We are now examining factors controlling expression of this message and of SkM-I mRNA. Rat sciatic nerve was cut in the mid-thigh region and lower leg muscle was sampled at 6hr intervals between 24 and 48 hrs and at longer intervals until 17 days post-section. SkM-II mRNA was first detectable at 30 hr, reached a maximal level by 48 hr, and declined slowly to 50% of maximum by 17 days. Over the same period, SkM-I mRNA exhibited a small but variable increase over control values. The ratio of SkM-I : SkM-II mRNA was 2:1 and the absolute level of SkM-II mRNA ranged between 5 and 10 fmol/mg total RNA. Axotomy close to the innervated muscle resulted in 2 hr left shift in the early increase of SkM-II mRNA compared to section near the hip. 48 hr following intramuscular injection of botulinum toxin, a selective inhibitor of impulse-induced quantal acetylcholine release, a very low but detectable level of SkM-II mRNA was seen. By 5 days post-injection, however, the SkM-II message level was 4 times greater than that seen 5 days after axotomy. The left-shift in the time course of SkM-II expression and the shortening in the latency of induced expression with distal vs. proximal axotomy, as well as the low level SkM-II expression early in botulinum-treated muscle, suggest that both non-quantal factors and impulse-induced cholinergic vesicle release may be involved in the suppression of SkM-II mRNA. Supported in part by NIH NS-08075 and by the MDA.

85.5

MOLECULAR CLONING AND CHROMOSOMAL LOCATION OF TWO HUMAN MUSCLE VOLTAGE-GATED SODIUM CHANNELS A.L. George, M.E. Gellens, R.G. Kallen, and R.L. Barchi, *Mahoney Institute of Neurological Sciences,* University of Pennsylvania School of Medicine, Philadelphia PA 19104.

Multiple isoforms of voltage-gated sodium channels have been identified in skeletal muscle, brain, and heart. In rat skeletal muscle, two isoforms (SkM1, SkM2) have been cloned; SkM1 is the predominant tetrodotoxin (TTX)-sensitive sodium channel present in adult muscle, whereas SkM2 is expressed only during early development and following surgical denervation. SkM2 also appears to be identical to the predominant rat cardiac sodium channel, RH1, a putative TTX-resistant isoform. Using cDNA probes derived from rat SkM1 and SkM2, we have now cloned cDNAs representing homologous human muscle sodium channels by screening human skeletal muscle and cardiac cDNA libraries. From the skeletal muscle library we have isolated and sequenced a 3.6 kb cDNA clone which includes a region highly homologous with domain IV and C-terminus of rat SkM1. From the human cardiac library, we have isolated and partially sequenced three overlapping cDNA clones (3.6, 4.3, 4.5 kb) which have ~ 85% nucleotide sequence identity with rat RH1 and SkM2, and encompass the entire coding sequence. Both human SkM1 (HSkM1) and human RH1 (HH1) homologs have extensive 3' noncoding regions (> 2 kb) which have distinct subtype specific nucleotide sequences. Using subtype specific probes and Southern blots prepared from BamHI-digested DNA from a panel of hamster/human somatic cell hybrids (BIOS, Corp.) we have assigned the HSkM1 gene to chromosome 17 and the HH1 gene to chromosome 3. *In situ* chromosome hybridization experiments are underway to confirm and refine these findings.

85.6

Cloning and production of site specific fusion proteins to human skeletal muscle sodium channel

Tejvir S. Khurana and Eric P. Hoffman* - Program in Neuroscience & *Dept. of Pediatrics, Harvard Medical School and The Children's Hospital, Boston, MA 02115.

We have used Trans-species Polymerase chain reaction mediated cloning to isolate cDNA's from the human homologue of the recently described vertebrate skeletal muscle sodium channels. Using this technique we targeted two predicted cytoplasmic domains of the channel for analysis. The Na 2 and Na 3 cDNA's that we have cloned are representative of regions similar to rat sequences that span the predicted cytoplasmic interdomain II-III and carboxyl tail domains, respectively. Na 2 and Na 3 clones were sequenced and the human sequence found to be extremely homologous to the rat sequence.These clones were spliced into pATH protein expression vectors, and 25 mg of homogeneous pure trpE fusion proteins expressed/ 100mL of bacterial culture. Antisera have been raised to these fusion proteins and seem to be quite sensitive, detecting as little as 10 ng of fusion proteins on western blots. The antisera also detect a large, ~250 kDa protein species from human muscle upon immunobloting.

85.7

EFFECTS OF THE SEA ANEMONE TOXIN, ATX II, ON RAT BRAIN Na CURRENTS EXPRESSED IN XENOPUS OOCYTES. D.S. Krafte, K. Dillon , W.A. Volberg , and A.M. Ezrin . Dept. of Cardiovascular Pharmacology, Sterling Research Group, Rensselaer, NY 12144.

We have utilized the Xenopus oocyte expression system to study the interaction of Anemonia sulcata toxin II (ATX II) with neuronal Na channels expressed from poly(A+) RNA as well as in vitro transcripts of a plasmid (pM860) encoding a Na channel alpha subunit. Poly(A+) RNA was extracted from the whole brains of two week old rats using a LiCl/Urea procedure while M860 RNA was in vitro transcribed using T7 polymerase. We injected 50-70 ng of poly(A+) or 5-7 ng of M860 RNA and voltage-clamp recording was done 2-5 days later. ATX II (50 nM) decreased rates of inactivation and increased peak current amplitude for channels expressed from poly(A+) RNA. Time constants at -10 mV increased by 19.3% and peak currents increased by 91%. In contrast, ATX II (50 nM) had less of an effect upon Na channels expressed from M860 RNA. The mean time constant increase was 5.1% while peak current increased 10%. These data suggest that ATX II has a reduced effect upon the Na channel alpha subunit alone compared to its effects on channels expressed from poly(A+) RNA.

85.8

POINT MUTATIONS IN A CYTOPLASMIC REGION WHICH MODIFY ACTIVATION AND INACTIVATION GATING OF SODIUM CHANNELS. R.H. Joho, J.R. Moorman*, G.E. Kirsch, A.M. Brown. Dept. of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX; *Dept. of Medicine, University of Texas Medical Branch, Galveston, TX.

We studied single channel gating kinetics of Xenopus oocyte-expressed type III Na^+ channels with mutations of the putative cytoplasmic segment linking domains III and IV. In cell-attached patches at test potential 0 mV, type III channels had fast activation (mean first latency 1.5 msec), slow inactivation (mean burst duration 11.4 msec), and quickly peaking, slowly decaying average single channel currents. Mutation KKK1453/4/7NNN (net charge change Δ-3) resulted in channels with short first latency (1.5 msec), short bursts (3.2 msec), and quickly peaking, quickly decaying average currents. Mutation R1461E (Δ-2) resulted in channels with long first latency (10.1 msec) and short bursts (2.3 msec). The gating changes were offsetting for this mutation, and average currents were not different from control. Mutation S1452A (Δ0) channels had long first latency (8.2 msec), short burst duration (4.8 msec), and slowly peaking, slowly decaying average currents. We conclude that the segment linking domains III and IV of voltage-dependent sodium channels modulates both activation and inactivation gating. Supported by NIH, Advanced Technology Program of the State of Texas, Kempner and Sealy Foundations, and AHA (Texas Affiliate).

85.9

ANALYSIS OF SODIUM CHANNEL INACTIVATION AS EXPRESSED IN XENOPUS OOCYTES FROM A SINGLE MRNA. V. V. Patel, S. R. Levinson and A. R. Martin. Dept. of Physiology. Univ. of Colo. Sch. of Med., Denver, CO 80262.

Sodium channels expressed in the *Xenopus* oocyte from a cRNA encoding just the α subunit often display slow inactivation kinetics (Auld *et al.*, Neuron, 1:449, 1988). In order to determine the biophysical characteristics of this slow inactivation, we injected *Xenopus* oocytes with RNA transcribed *in vitro* from the cDNA of the μ1 sodium channel. Oocytes were assayed 3-5 days later, by the two-electrode voltage clamp technique, and 1-3μA sodium currents were typically recorded. These currents showed two phases in their inactivation, a "fast" component and a "slow" component, as expected. The fast component could be selectively eliminated by lowering the holding potential to -120mV, while the slow component could be eliminated by raising the holding potential to -60mV. The time constant of the slow inactivation depended on the amplitude of the depolarization step. The time constant decreased with depolarization up to about -30mV, and then unexpectedly increased. Both components were equally sensitive to block by TTX with a K_d of about 6nM. Oocytes injected with poly(A)$^+$ RNA from rat brain showed only fast inactivating currents, as found in normal neuronal tissue. It does not appear that the fast and slow inactivating components were due to two separate populations of channels, as the peak conductances were not reduced when, for example, the fast component was eliminated. This work was supported by NIH grants NS09660 and NS15879.

85.10

MOLECULAR CLONING OF A HUMAN BRAIN SODIUM CHANNEL GENE. C.M. Lu, T.A. Rado*[1], R.E. Powers* & G.B. Brown, Dept. of Psychiatry and Behavioral Neurobiology and [1]Division of Hematology/Oncology, University of Alabama at Birmingham, Birmingham, AL 35294.

A DNA fragment from a human brain sodium channel gene has been cloned by polymerase chain reaction (PCR). The primers for PCR were designed based on published rat and eel sodium channel amino acid and cDNA sequences. Analysis of these sequences has revealed extensive interspecies homology and sequence conservation. The assumption, therefore, is that amino acid sequences which are most highly conserved in other species would also be conserved in the human gene. Using the BIGPROBE computer program, which takes into account species preferences in codon usage, a pair of primers, 20 nucleotides each, has been designed based on sequences flanking those conserved regions in rat and eel. A 212bp PCR product was obtained when human genomic DNA was used as templates. This DNA fragment was cloned into M13 and subsequently sequenced, revealing 87% and 91% homology with rat brain sodium channel gene subtype I and II respectively and containing no intron. The presence of RNA transcripts of this gene in human brain tissue was demonstrated by the use of PCR on first strand cDNA (RT-PCR) synthesized from various sources. The RNA transcripts of this gene were found present in human brain tissue but not in T84 cells, a human colonic carcinoma line with well defined ion currents in which no voltage gated sodium mediated excitability has been observed. Two approaches including screening of human brain cDNA libraries and RACE (Rapid Amplification Of cDNA End) technique have been used to extend this clone. This partially cloned gene is the first information concerning the molecular structure of human sodium channels and will serve as a starting point for future studies on the molecular biology of human sodium channel.

85.11

ENHANCEMENT OF SODIUM CURRENTS IN THE XENOPUS OOCYTE EXPRESSION SYSTEM BY A COMPONENT OF FUNNEL-WEB SPIDER VENOM. J.C. Dreixler and J.P. Leonard. Dept. of Biol. Sciences, U. of Illinois at Chicago, Chicago, Il 60680.

We used Xenopus oocytes to express rat brain total RNA in order to study the effects of a pure synthetic analog of funnel-web spider toxin (synthetic FTX) on voltage-dependent sodium currents. Using a two electrode voltage clamp technique, currents obtained with 100 nM tetrodotoxin (TTX) application were subtracted from control currents in order to specifically measure TTX-sensitive sodium currents. A second family of currents was obtained, using the same paradigm, with or without application of synthetic FTX (100 nM). The peak inward TTX-sensitive sodium currents increased to 128 $\pm$ 9% (s.e.m.) of control with 100 nM of synthetic FTX versus 96 $\pm$ 10% of control without ($P < 0.05$; n= 6 oocytes). The enhancement develops over a 2-3 min period after synthetic FTX application and seems to be irreversible. This pure component had no obvious effects on calcium-activated chloride tails in the oocytes. Thus the synthetic FTX may mediate the excitatory effects seen by previous investigators using funnel-web spider venom while the calcium channel blocking action may be mediated by a separate component of the venom. We thank Pfizer Central Research and Natural Products Sciences Inc. for the synthetic analog of FTX. (Supported by NIH NS26432)

85.12

IDENTIFICATION AND *IN SITU* LOCALIZATION OF α SUBUNIT OF VOLTAGE-SENSITIVE SODIUM CHANNELS FROM *DROSOPHILA* USING POLYCLONAL ANTIBODIES. D. PAURON, M. AMICHOT* and J.B. BERGE*. I.N.R.A., Station de Recherches de Nématologie et de Génétique moléculaire des invertébrés, 123, bd Francis Meilland, B.P. 2078, 06606 Antibes Cedex, France.

Two genes homologous to vertebrate sodium channels have been cloned in *Drosophila melanogaster*. We have subcloned two cDNA fragments corresponding to the 3' end of one of these genes (the DSC1 one) in the expression vector pEX. The first fragment is 270 base pairs (bp) long and contain the sequence corresponding to a part of the region S2 and regions S3 and S4 of domain d. The other one comprises the S5 and S6 regions of domain d and is 680 bp long. Rabbits were immunized with the fusion products of these constructions. The polyclonal antibodies obtained were tested on purified membranes prepared from *Drosophila* heads in immunoblotting experiments. Both of them specifically recognize a protein with an apparent Mr of 250,000-260,000. This result is in good agreement with the reported size of the α subunit of Na channels present in nervous tissue as well as in other tissues in vertebrates and in invertebrates.

We have also used these antibodies as markers of Na channels in frozen sections of adult *Drosophila*. The antibodies clearly stain structures in the central nervous system but no specific labelling can be detected in the rest of the body. These data strongly suggest that DSC1 is a structural gene coding for a functional Na channel. It is expressed at least at the adult stage where it seems to be restricted to the nervous system.

ION CHANNELS: MODULATION AND REGULATION I

86.1

GAP JUNCTION ANTIBODY ATTENUATES ELECTRICAL COUPLING BETWEEN THE GOLDFISH MAUTHNER (M-) CELL AND ITS 8TH NERVE AFFERENTS. Daniel P.Yox, Donald S. Faber and Bruce J. Nicholson*. Depts. of Physiology and Biological Sciences, University at Buffalo, Buffalo, NY 14214.

A highly specific antibody to the 43kD gap junction protein (Cx43) of rat detects this protein in myocytes, glia and CNS neurons, and has been shown to block intercellular coupling in the former. Because the M-cell receives mixed electrotonic and chemical input from the saccular fibers of the 8th nerve, this presented an opportunity to analyze the modulatory role of the electrical component of this input. In five experiments, intracellular injections of affinity purified anti-Cx43 reduced the coupling potential produced by 8th nerve stimulation by 14-88%. This effect lasted the duration of the experiment (20min-2hr). Also, anti-Cx43 injections produced either no change or an increase (up to 97%) in the chemically mediated EPSP. Resting potential was unchanged, and antidromic spike height, a measure of M-cell input resistance, was either unchanged or reduced. Injections of pre-immune IgG at similar concentrations had no effect on resting potential and antidromic spike height (N=3), while electrotonic coupling was either unchanged or increased slightly.

This suggests that anti-Cx43 recognizes an epitope in M-cell gap junctions and that the resultant uncoupling enhances chemical transmission at these terminals. One explanation for the latter effect is that uncoupling increases evoked transmitter release by increasing presynaptic spike duration or amplitude. (Supported by DHHS: NS08819, NS15335, NS37109.)

86.2

VOLTAGE DEPENDENCE AND RECTIFICATION AT RAT CONNEXIN 26 AND 32 JUNCTIONS EXPRESSED IN XENOPUS OOCYTES. L.C. Barrio, T. Suchyna*, T. Bargiello, R. Roginski, R.S. Zukin, B. Nicholson* and M.V.L. Bennett. Albert Einstein College of Medicine, Bronx, NY 10461 and *SUNY Buffalo, Buffalo, NY 14260.

cRNAs encoding rat Cx26 or 32 were injected into defolliculated Xenopus oocytes. Antisense cDNA oligonucleotides to Xenopus Cx38 were coinjected to block endogenous coupling selectively. 24 hours later, vitelline membranes were removed and oocytes were paired. After 24-48 hours dual voltage clamp was used to measure junctional conductance (g_j). In Cx26 pairs "instantaneous" g_j (time resolution $\approx$10ms) increased by about 1.3/V for depolarization of either cell, and decreased similarly for hyperpolarization of either cell (range $\pm$100mV). This result indicates dependence on inside-outside voltage (V_{i-o}). For these pairs slow (τ>1s) decreases in g_j up to 80% were caused by polarization of either cell. Decreases were nearly equal for equal de- and hyperpolarization of either cell indicating dependence on transjunctional voltage (V_j) and not on V_{i-o}. V_j at which the change was half maximal (V_o) was c. $\pm$85mV. The g_j/V_j relation was well fit by a Boltzmann relation, with sensitivity parameter A of 0.17, indicating an equivalent movement of c. 4 electron charges through the applied field. Cx32 pairs formed junctions with no V_{i-o} dependence but with V_j dependence similar to that of Cx26; Boltzmann parameters were $V_o \approx 55$mV and A$\approx$0.09mV. Oocytes expressing Cx26 formed rectifying junctions with oocytes expressing Cx32. Instantaneous g_j depended linearly on V_j with a slope of $\approx$3.3/V over a range of $\pm$100mV with positive V_j defined as positive on the Cx26 side. Large positive V_j caused large (>90%) slow decreases in g_j with V_o and A more characteristic of Cx26 than of 32. In summary, 1) both Cx26 and 32 encode gap junctions that are voltage dependent, and 2) hemichannel properties are changed in the heterotypic 26/32 junctions, which exhibit fast g_j changes that may be similar to those of rectifying electrical synapses.

86.3

HETEROGENEITY BETWEEN BRAIN AND HEART CONNEXIN43 R.Kadle and B.J.Nicholson*. Dept of Biol. Sciences, SUNY at Buffalo, Buffalo, NY 14260.

Western blots of rat heart ventricle and brain homogenates reveal different electrophoretic mobilities of Connexin43 (Cx43). While heart ventricle contains a major 43 kD band and a minor 39-40 kD doublet, the brain has only the lower doublet, with the 39 kD band being the more prominent of the two. The 39-40 kD doublet is not a proteolytic product of the 43kD band since both are recognized by N- as well C-terminal antibodies. Alkaline phosphatase treatment of immunoprecipitated material from heart ventricle reduces the 43 kd band to 39 kD, but has no effect on the brain pattern. Thus the 39 kD band appears to be a non-phosphorylated form of Cx43. This is further supported by the observation that the in vitro translated Cx43 also migrates at 39kD and that phosphate incorporates only into the 43kD band in myocyte cultures. These observations suggest that different tissues maintain different overall levels of Cx43 gap junctional phosphorylation, with brain gap junctions being largely dephosphorylated. Supported by AHA 88-115F (RK) and NIH HL37109-05 (BJN).

86.4

HUMAN CONNEXIN 43: FUNCTIONAL PROPERTIES DETERMINED IN STABLY TRANSFECTED HEPATOMA CELLS. G. Fishman*, A.P. Moreno, L. Leinwand*, and D.C. Spray. Depts. Microbiology & Immunology and Neuroscience, A. Einstein College of Medicine, Bronx, N.Y. 10461

Connexin 43 (Cx43) is the major gap junction protein in the cardiovascular system and is also expressed elsewhere, including between astrocytes and between leptomeningeal cells in brain. To compare the properties of the human Cx43 channel to those of other gap junctions, a communication-deficient human hepatoma cell line (SKHep1) was transfected with a vector containing the full length coding sequence of human Cx43 (Fishman *et al.*, (1990) **J. Cell Biol.**, in press). After 3-4 weeks of selection, individual cells were injected with Lucifer Yellow and clones showing strong dye transfer were picked and subcultured. Pairs of these cells were voltage clamped using the dual whole cell method. Junctional conductance (g_j) in pairs of the transfected cells averaged >10 nS, compared to a very low level of coupling in the parental cell line. Voltage dependence of g_j was evaluated with transjunctional voltage (V_j) steps of $\pm$ 70 mV. For V_j < 50 mV, g_j was constant (to within 10%); at higher voltages, g_j declined, reaching minimal values (still >50%) at the highest potentials. Unitary conductances of the human Cx43 channels were measured after g_j was reversibly reduced by exposure to halothane (2 mM). Three channel sizes were recorded. The smallest, about 30 pS, were the size of the channels occasionally recorded in the parental cell line. The others, 60 and 90 pS, were similar to sizes recorded from gap junction channels in other tissues expressing Cx43. All channels were distinct from 120-150 pS channels expressed in SKHep1 cells transfected with Cx32 cDNA (Eghbali *et al.*, (1990) **PNAS** 87:1238). Connexin type thus appears to be a primary determinant of physiological properties of gap junction channels.

86.5

HUMAN CONNEXIN43 CHANNELS: UNITARY CONDUCTANCE IS ALTERED BY TREATMENTS THAT ACTIVATE PROTEIN KINASES AND IN MUTANTS LACKING PHOSPHORYLATION SITES. A.P. Moreno, G. Fishman*, L. Leinwand*, and D.C. Spray. Depts. Neuroscience and Microbiology & Immunology, A. Einstein Coll. Medicine, Bronx, N.Y.

Gap junctions formed between SKHep1 cells after stable transfection with human connexin43 (Cx43) cDNA exhibit three channel sizes: 30 pS, due to low level expression of an endogenous channel, and 60 and 90 pS corresponding to Cx43 (see Fishman *et al.* abstract). This raises the question of why there are multiple channel sizes associated with expression of a single connexin. The amino acid sequence of human Cx43 predicts numerous phosphorylation sites on its carboxyl terminus. To determine whether modification of these sites might influence unitary conductance, we compared event histograms from cell pairs before and after exposure to 8Br-cAMP (1 mM) and phorbol ester (TPA, 100 nM). Within minutes of treatment, the relative frequency of the largest channels decreased and that of the 60 pS events increased. Thus, treatments that activate kinases A and C may change unitary conductance of the gap junction channel. The potential phosphorylation sites in the human Cx43 sequence might also be necessary for posttranslational processing and entry into the surface membrane. To test this hypothesis, cells were stably transfected with vectors containing mutagenized human Cx43, in which a stop codon was inserted in the 3' portion so as to encode proteins of about 32 and 26 kDa. Transfectants expressing these mutations were coupled; thus phosphorylation of Cx43 is not required for insertion or assembly of functional channels. Single channel conductances were about 168 and 48 pS for the longer and shorter constructs. Together, these findings indicate that the carboxyl terminal region of Cx43 is a domain influencing the unitary conductance of the Cx43 channel.

86.6

LONG-TERM STIMULATION OF Na^+/Ca^{2+} EXCHANGE BY Mg·ATP IN GIANT EXCISED MEMBRANE PATCHES: PRIMARY PROTEIN OR LIPID EFFECT?. D.W. Hilgemann & A.Collins / UTSW / Dept. of Physiology / Dallas, Tx., 75235.

Outward Na^+/Ca^{2+} exchange current in giant excised cardiac sarcolemmal patches is strongly stimulated by 'cytoplasmic' Ca^{++} and Mg·ATP (Hilgemann, NATURE 334: 242, 1990). $[Ca^{++}]_i$ is effective in the absence of ATP, and Mg·ATP (0.5 to 8 mM) is effective in the absence of $[Ca^{++}]_i$ (10 mM EGTA). For the following reasons, involvement of a (serine) protein kinase in the ATP action appears unlikely: 1) H7 and peptide kinase inhibitors do not block, 2) ATP-gamma-S is ineffective, and 3) exogenous phosphatases do not accelerate decay of the ATP effect ($t_{1/2}$>15 min). The following findings support further testing of a possibility that the ATP action could involve establishment of lipid asymmetry by a phospholipid 'flipase': 1) Oxidizing agents, known to inhibit flipase activity, are highly effective blockers of $[Mg{\cdot}ATP]_i$ action from the external (pipette) side without inhibiting exchange (or sodium pump) current. 2) Reveral of ATP action is greatly accelerated by cytoplasmic diamide (1mM), which induces aminophospholipid flip. 3) Negatively charged detergents (<10μM) imitate ATP action while positively charged detergents (<1 μM) imitate reversal of ATP action.

86.7

ADENOSINE RECEPTORS MODULATE THE Na^+-Ca^{++} EXCHANGER IN CEREBRAL NERVE ENDINGS. L. Annunziato, M.Taglialatela, A.M.Rossi, L.M.T. Canzoniero, A. Fatatis and G.F. Di Renzo Inst. Pharmacology 2nd Sch. of Medicine, University of Naples and Chieti Via S. Pansini, 5 80131 Naples, Italy.

It is now well accepted that Adenosine (A), by a presynaptic mechanism, prevents depolarization-induced release of various neurotransmitters in several brain regions. Although it has been proposed that A may prevent Ca^{++}-dependent neurotransmitter release by blocking Ca^{++} entry through Voltage -operated Channels, the results are conflicting.

The aim of the present study was to investigate the role played by A on the activity of the Na^+-Ca^{++} exchanger when this antiporter operates as a Ca^{++}-influx pathway in Percoll-purified rat brain synaptosomes.

The methylxantine A receptor antagonist, theophylline(0.3-10 mM), dose dependently reinforced Na^+-dependent $^{45}Ca^{++}$ uptake elicited by extracellular Na^+ removal (145 mM choline). This effect could not be ascribed to the inhibition of phosphodiesterase since either 8 Br-cAMP or forskolin failed to enhance $^{45}Ca^{++}$ influx due to the activation of the Na^+-Ca^{++} exchanger.

30 min. exposure of synaptosomes to 2.5 and 10 U/ml of the enzyme adenosine deaminase (ADA), which catabolizes A to inosine, caused an enhancement of the Na^+ dependent $^{45}Ca^{++}$ uptake. The selective agonist of A1 receptor, L-PIA (100 nM), which is not metabolized by ADA, completely counteracted the ADA-induced reinforcement of Na^+-dependent $^{45}Ca^{++}$ uptake, whereas the rather selective A_2 receptor agonist, NECA (100 nM) was uneffective.

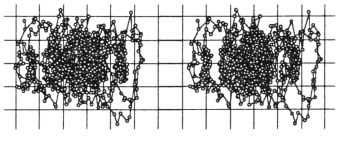
Collectively, these results are compatible with the hypothesis that A endogenously released from brain synaptosomes inhibits the activity of the Na^+-Ca^{++} antiporter when this exchanger works as a Ca^{++} influx pathway.

(Supported by C.N.R. and M.P.I. 60%-40% Grants to L.A., S.D.R.)

86.8

AN INHIBITOR OF ARACHIDONIC ACID METABOLISM INCREASES THE AMPLITUDE OF SPONTANEOUS VOLTAGE FLUCTUATIONS IN NEURONS K.S. Madden, G.D. Lange, A. Prasad, and J.L. Barker Laboratory of Neurophysiology and Instrumentation and Computer Section, NINDS, NIH Bethesda, MD 20892

We are using the acetylenic analogue of arachidonic acid, ETYA (5,8,11,14-eicosatetraynoic acid), to probe the function of essential fatty acid (EFA) metabolism in spinal cord cells from the embryonic (E7.5-9) chick. In these studies, the drug's effects were assessed using conventional whole cell patch methods for recording intracellular voltages. Under control conditions, the membrane potential spontaneously fluctuated regardless of whether acutely dissociated or cultured cells were used. Superfusion with ETYA (0.01-1 mM) increased the magnitude of the fluctuations. Less often, it also hyperpolarized cells (range: 3-10 mV). Power spectra of the voltage noise showed a ten-fold increase in the signal amplitude with no change in the shape of the power density function. The corner frequency closely approximated the measured membrane time constant. The data were then analyzed using an algorithm developed to identify chaotic behavior [Albano et al, *Phys. Rev. A* 38(6): 3017 (1988)]. The voltage time series consistently generated the diagnostic of a deterministic system, a geometric display describing a chaotic attractor as shown in the stereogram below. The knotted configuration of this display can differ between cells, but it appears to be a generally stable characteristic of individual cells. ETYA seems to "relax" the knots within the display. The relationship between this specific observation and the ability of ETYA to block EFA metabolism is not yet clear.



86.9

DIFFUSION AND DURATION OF PHYSIOLOGICAL INACTIVATION PRODUCED BY MICROINFUSIONS OF LIDOCAINE. D.C. Smith, R.L. Berry, M.L. Maring, M.A. Statnick*, C.L. Faingold and R.A. Browning. Dept. of Psychology and School of Medicine, Southern Illinois University-Carbondale, IL 62901.

Lidocaine hydrochloride is a local anesthetic which reversibly blocks sodium channels. As such, when microinfused into discrete brain loci, it reversibly blocks action potential generation. While we have demonstrated the effectiveness of such microinfusions in vivo (see Smith et al., Neurosci. Abst. 1983,84,85,87,88) in reversibly altering behavior, the diffusion characteristics and duration of inactivation following lidocaine microinfusion into the brain remained to be quantified.

To quantify the diffusion, different volumes of ^{14}C-lidocaine were infused over a 2 min period into the dorsal (0.5, 0.75, or 1.0µl) or ventral (0.5 or 1.0µl) hippocampus (dH or vH), or into the inferior colliculus (IC, 0.5µl). The brains were removed and frozen within 6-10min post-infusion. Frozen 300µm sections through the area of infusion were obtained and a template was used to obtain 9-35 frozen tissue punches/section. Radioactive counts were obtained for each punch and plotted on drawings of the sections as a function of the percent of radioactivity present at the infusion site. For all areas infused, and for all volumes, diffusion remained confined to the injected structure. Within the IC, diffusion of 0.5µl lidocaine was largely restricted to +/-900µm A-P, +/-1mm M-L and +/-1mm D-V. Peculiarities in diffusion patterns were noted in the dH and vH, where diffusion appeared to follow laminar contours, and these will be illustrated.

To determine the duration and extent of inactivation following lidocaine infusion, current source density (CSD) analysis was applied to field potential profiles obtained in the dH by electrical stimulation of the perforant path pre- and post-lidocaine. The CSD analysis revealed a complete absence of both sinks and sources 3-8 min post-lidocaine which gradually recovered over the next 30-120 min to baseline levels. The extent and pattern of tissue inactivated was closely associated with that seen in the diffusion experiments.

86.10

COMPUTER SIMULATION OF I_{AHP} KINETICS. P.Pennefather[1], F. Sala[2]* and A. Hernandez-Cruz[3]*. Howard Hughes Med. Inst., Stony Brook, NY;1, Fac. Pharmacy, Univ. of Toronto, ON;2, Dept. Neuroquimica, Univ. Alicante, Spain;3, Roche Inst. Mol. Biol, Nutley, N.J.

The relation between the time course of the Ca-activated K-current, I_{AHP}, and calcium loads induced by action potentials (APs) in bullfrog ganglion neurons has been characterized empirically by Goh and Pennefather[1]. We have now used that data to place constraints on simulations of the fate of calcium after it enters the neuron during trains of AP's. We used the calcium diffusion model of Sala and Hernandez-Cruz[2] which considers the effects of buffers of defined capacity, mobility and kinetics on the diffusion of calcium. Two principal types of buffers are considered: fast buffers (B_f) and slow buffers (B_S). We have simulated I_{AHP} by considering a third type of buffer, B_{AHP}, restricted to the outermost shell. We estimate that each AP produces 6 nA.ms of I_{Ca}. We first set out to generate an I_{AHP} that had an amplitude close to maximal and decayed roughly exponentially. The assumption that activation of B_{AHP} was cooperative proved essential. We assumed that activation resulted from occupancy of three equivalent binding sites for Ca with K_D=0.1 µM and k_{on}=10^8/M/s. Acceptable results were obtained with a relatively immobile B_f with [B_f]=40 µM, K_D=5 µM, k_{on}=10^8/M/s. Higher values of [B_f] reduced the amplitude of I_{AHP} and gave rise to a slow tail. With [B_f] at least 20 µM and setting other parameters of the model similar to those used previously[2], we reproduced the relation between I_{AHP} decay half-time and AP train length (log-log slope = .55) observed by Goh and Pennefather[1]. As also was observed[1], the model predicted that a 3-fold reduction of the calcium influx per AP causes a 3-fold shift to the right in that relation.
1, Neurosci. Abst. *14*, 1089, 1988;2, Biophys. J., *57*, 313, 1990

86.11

ARACHIDONIC AND OTHER UNSATURATED FATTY ACIDS BLOCK ACh-ACTIVATED Cl CURRENT AND OTHER 'FAST' TRANSMITTER RESPONSES IN *APLYSIA* NEURONS. V. Březina. Howard Hughes Med. Inst., Columbia University, 722 W. 168 St., New York, NY 10032.

Aplysia neurons exhibit three main types of response to transmitters: 'fast' Na and Cl currents, and a 'slow' K current. K-current responses have been suggested, because they are mimicked by arachidonic acid (AA) metabolites and blocked by inhibitors of their production, to be mediated by AA metabolites acting as second messengers. Unexpectedly, I have found that certain 'fast' responses, whose speed and activation in cell-free patches make second-messenger mediation unlikely, are equally or more sensitive to the inhibitors and fatty acids. Thus, the Cl-current response to ACh was blocked by the phospholipase-A_2 blocker BPAB (50% at <5 µM), by the lipoxygenase blocker NDGA (2 µM), by the cycloxygenase blocker indomethacin (10 µM), and by the lipo- and cycloxygenase blocker BW755C (<50 µM). Superfusion of 10-60 µM AA (20:4) or any of the unsaturated fatty acids 22:6, 22:4, 20:3, 20:2, 18:3 and 18:2 blocked >50% (often >90%) of the current. At least NDGA and indomethacin also blocked the 'fast' GABA-activated Cl and histamine-activated Na currents (but not the 'fast' Na-current response to FMRFamide). Leaving aside the possibility of action at sites unrelated to AA metabolism, these results are most consistent with the hypothesis that the inhibitors block steady fatty acid release and metabolism, leading to accumulation of free fatty acids that then directly block certain 'fast' transmitter-activated Cl and Na channels.

86.12

MULTIPLE PATHWAYS MODULATE CAN CURRENT IN SNAIL NEURONS. Don Partridge, Dieter Swandulla*, and Thomas Müller* Dept. of Physiol., Univ. of N.M., Albuquerque, N.M. 87131; Neurophysiology Dept., Max-Planck Institute, Planegg-Martinsried, F.R.G.

Calcium activated non-specific (CAN) current provides an important depolarizing drive in many bursting and secretory cells. In the bursting neurons of snail ganglia, CAN channels open in response to intracellular calcium and the resultant current provides the maintained depolarization for bursts.

We have studied the CAN current in the fast burster neuron of Helix aspersa and Helix pomatia following fast quantitative pressure injections of Ca^{++} into the cell. We have previously shown that CAN current amplitude is depressed by a cAMP-dependent phosphorylation process (*J. Physiol.* in press). Bath application of 5-HT causes a reversible reduction in CAN current that can be blocked by pre-injection of the cells with protein kinase A inhibitor. Injection of GTPγS leads to an increase in CAN current. Cholera toxin application, however, causes a reduction in CAN current similar to that observed with 5-HT. This latter effect implicates an involvement of an α_s subunit of a G protein.

The most parsimonious explanation of these results is that the 5-HT receptor is linked via an α_s to adenylate cyclase but that a second G protein-dependent avenue for modulation of CAN current exists. This might be an effect through an α_i on the adenylate cyclase or an effect at another point in the phosphorylation process.

86.13

ISOLATION AND CHARACTERIZATION OF ENDOGENOUS PROTEIN PHOSPHATASE INHIBITORS FROM *APLYSIA*. S. Endo, S. Shenolikar[1]*, A. Eskin[2], and J.H. Byrne. Depts. of Neurobiology and Anatomy, and Pharmacology[1], Univ. of Texas Medical School, Houston, TX 77225, Dept. of Biochem. and Biophys. Sci.[2], Univ. of Houston, Houston, TX 77204.

The phosphorylation state of a given protein reflects the activities of both protein kinases and protein phosphatases (PrPs). Although the role of cAMP-dependent protein kinase is well-recognized in the induction and the maintenance of sensitization, the role of PrPs in these processes remains unknown. Recent biochemical studies indicate that PrPs in *Aplysia* are similar to those of mammals, including their substrate specificity and sensitivity to the mammalian inhibitor I-2 and okadaic acid (OA), a specific inhibitor of PrP-1 and PrP-2A. Moreover, OA alters cAMP-dependent membrane currents in voltage clamped pleural sensory neurons (Endo et al., 1989; Ichinose et al., this volume). To further examine the regulation of PrPs in *Aplysia*, we have isolated and characterized endogenous inhibitor(s) of PrP in *Aplysia* tissues.

An inhibitor fraction was obtained from a trichroloacetic acid precipitate of a 100,000xg supernatant of whole central nervous system or buccal muscle. Inhibitory activity was analyzed using PrP-1 and PrP-2A purified from rabbit skeletal muscle and ^{32}P-phosphorylase *a* as a substrate. The heat-stable inhibitor fraction strongly inhibited PrP-1 (50 % inhibition was obtained in approximately 0.1 mg/ml) but did not inhibit PrP-2A. The activity of this trypsin-labile inhibitor(s) was reduced by incubation with alkaline phosphatase. These data suggest the presence of an *Aplysia* PrP inhibitor(s) which is subject to regulation by reversible protein phosphorylation, in a manner similar to mammalian inhibitor-1. Regulation of the inhibitor by phosphorylation could have profound effects on the second messenger cascade that contributes to behavioral sensitization in *Aplysia*.

86.14

ROLE OF PROTEIN PHOSPHATASES IN FMRFamide-, SEROTONIN-, AND cAMP-DEPENDENT MODULATION OF MEMBRANE CURRENTS IN SENSORY NEURONS OF *APLYSIA*. M. Ichinose, S. Endo and J.H. Byrne. Dept. of Neurobiology & Anatomy, Univ. of Texas Medical School, Houston, TX 77225.

Recent biochemical studies indicate that protein phosphatases (PrPs) in *Aplysia* are similar to those of vertebrates including their inhibition by okadaic acid (OA) (Endo et al., 1989; this volume). To examine the contribution of PrPs to the modulation of membrane currents (I_m), we measured the responses to brief applications of 5-HT, 8-bromo-cAMP and FMRFa in pleural sensory neurons (SNs) voltage clamped at approx. -30 mV. Intracellularly applied OA by itself mimicked the effects of 5-HT and cAMP and produced an inward shift in I_m associated with a decrease in input conductance (G_{in}). OA apparently occluded the responses to either 5-HT or cAMP. For example, as the OA-induced shift in holding I_m occurred, the amplitudes of the responses became smaller. Moreover, the durations of the responses were greatly increased (from initial values of 1 min to new values of up to 20 min). The FMRFa-induced outward I_m was reduced in the presence of OA. This suggests that FMRFa acts, at least in part, via activation of phosphatases. Injections of PrP-1 and PrP-2A, isolated from rabbit skeletal muscle, into SNs mimicked the effects of FMRFa, producing an outward I_m associated with an increase in G_{in}. Moreover, in the presence of the PrPs 5-HT-induced inward I_m were potentiated. The effects were greater in the presence of PrP-2A than PrP-1. These data suggest that: 1) there are relatively high basal activities of PrPs in the SNs; 2) PrPs contribute significantly to the recovery time course of 5-HT and cAMP responses; 3) the PrPs responsible for the dephosphorylation are OA-sensitive PrP-1 and/or PrP-2A; and 4) the response to FMRFa depends at least in part on the activation of PrPs.

86.15

MICROCYSTIN-LR IS A POTENT PROTEIN PHOSPHATASE INHIBITOR AND PROLONGS THE SEROTONIN- AND cAMP-INDUCED CURRENT IN SENSORY NEURONS OF *APLYSIA*. J.H. Byrne, M. Ichinose, S. Endo, S.D. Critz and S. Shenolikar[1]*. Depts. of Neurobiology and Anatomy, and Pharmacology[1], Univ. of Texas Medical School, Houston, TX 77225.

Recent biochemical studies indicate that protein phosphatases (PrPs) in *Aplysia* are similar to those of mammals including their substrate specificity and sensitivity to I-2 and okadaic acid (OA), a specific inhibitor of PrP-1 and -2A (Endo et al., 1989, 1990). In addition, OA prolonged the duration of both cAMP- and serotonin(5-HT)-induced membrane currents in voltage clamped pleural sensory neurons (SNs) (Ichinose et al., 1990).

Microcystin-LR (MCYST-LR) is a hepatotoxic cyclic heptapeptide purified from *Microcystis aeruginosa*. We examined whether it possessed PrP inhibitory activity against vertebrate PrPs using ^{32}P-phosphorylase *a* as a substrate. MCYST-LR strongly inhibited both PrP-1 and PrP-2A purified from rabbit skeletal muscle (IC_{50} of both PrP-1 and -2A was approximately 10^{-10} M). Moreover, both crude extracts and salt-extracted PrP from particulate fractions of abdominal ganglia of *Aplysia* were completely inhibited by MCYST-LR at concentrations greater than 10^{-8} M.

If MCYST-LR acts as an inhibitor of PrPs in *Aplysia*, it would be expected to enhance cAMP-dependent processes. Consequently, we examined its effect on responses to application of either 5-HT or cAMP in SNs voltage clamped at ~ –30 mV. By itself, intracellular application of MCYST-LR induced an inward shift of holding current and decreased input conductance, effects similar to those of 5-HT and cAMP. In addition, in the presence of MCYST-LR, the recovery of both 5-HT- and cAMP-induced inward currents was markedly slowed (from the control of about 1 min to more than 15 min). This prolongation of recovery from the response peak is similar to the effect of intracellularly applied OA, and may be induced by the persistence of cAMP-dependent protein phosphorylation. These data suggest that MCYST-LR is a potent inhibitor of PrPs and that MCYST-LR may be a useful probe to investigate the actions of PrPs in neuronal function.

86.16

PHORBOL ESTERS MODULATE $I_{K,Ca}$ IN SENSORY NEURONS OF *APLYSIA*. S.D. Critz and J.H. Byrne. Dept. of Neurobiology & Anatomy, University of Texas Medical School, Houston, TX 77225.

Although much is known about the modulation of membrane currents by cAMP-dependent kinase in sensory neurons of *Aplysia*, less is known about their modulation by protein kinase C (PKC). A two-electrode voltage-clamp was used to measure current responses to 200 ms pulses from -50 mV to membrane potentials of -20, -10, 0, +10, and +20 mV in isolated clusters of pleural sensory neurons. Responses were first measured in ASW (+150 μM TTX) and again after application of phorbol 12,13-dibutyrate (PDBu) or phorbol 12-myristate, 13-acetate (PMA) (max. DMSO=.05%). Phorbol esters had little effect on membrane current at pulses from -20 mV to 0 mV. Above 0 mV there was complex modulation of membrane current, the most prominent effect of which was an increase in outward current at the end of the pulse. PDBu was more potent than PMA and its effects were dose-dependent (threshold≈50 nM, saturation≈1.5 μM). Neither inactive 4α-phorbol ester nor DMSO alone had any effect on membrane current. In 2.0 mM TEA, which blocks a calcium-activated K^+ ($I_{K,Ca}$) current in these neurons (Baxter & Byrne, 1989), the ability of PDBu to modulate membrane current was reduced suggesting that PKC increased $I_{K,Ca}$.

To examine whether PKC acted directly on $I_{K,Ca}$ or indirectly via modulation of Ca^{2+} currents (Braha et al., 1988), sensory neurons were voltage-clamped at a fixed potential (-35 mV) and Ca^{2+} (500 mM) was injected directly into the cells (10 ms pulses of 100-900 nA at 50 Hz for 1-2 s) about every 60 s from a separate electrode. After three stable outward currents were elicited, PDBu (500 nM) was applied. PDBu led to a 5-fold increase in response to Ca^{2+} injection, consistent with an enhancement of $I_{K,Ca}$. This effect of PDBu persisted when experiments were repeated in ASW that contained reduced Ca^{2+} (0.5 mM) and 10 mM $CoCl_2$ to block inward calcium current. These data indicate that PKC may affect $I_{K,Ca}$ directly, and suggest multiple roles for the activation of PKC in sensory neurons.

86.17

EXTRACELLULAR TUMOR NECROSIS FACTOR INDUCES A DECREASED Na^+ CONDUCTANCE IN IDENTIFIED NEURONS OF APLYSIA KURODAI. M. Sawada, N. Hara* and T. Maeno*. Dept. Physiol. and Cent. Res. Lab., Shimane Medical Univ., Izumo 693, JAPAN.

The ionic mechanism of the extracellularly ejected recombinant human tumor necrosis factor-alpha (rhTNF) on the membrane of identified neurons R9 and R10 of Aplysia kurodai was investigated with conventional voltage-clamp, micropressure ejection, and ion substitution techniques. Micropressure- ejected rhTNF caused a marked hyperpolarization in the unclamped neuron. Clamping the same neuron at its resting potential (-60 mV) and reejecting rhTNF with the same dose resulted in the development of a slow outward current (I_o(TNF)) associated with a decrease in input membrane conductance. I_o(TNF) was decreased by depolarization and increased by hyperpolarization. The extrapolated reversal potential of I_o(TNF) was approximately +10 mV. I_o(TNF) was sensitive to changes in $[Na^+]_o$ but not to changes in $[Ca^{2+}]_o$, $[K^+]_o$ and $[Cl^-]_o$ and was resistant to ouabain (10^{-4} M), tetraethylammonium (5 mM) and 4-aminopyridine (5 mM). Ion substitution and pharmacological experiments suggest that I_o(TNF) in identified neurons R9 and R10 of Aplysia is due to a decreased Na^+ conductance but not due to an activation of the Na^+-K^+ pump and that immunomodulator such as TNF is capable of influencing directly the nervous system function as well as the immune system.

86.18

DOWN REGULATION OF VOLTAGE DEPENDENT SODIUM CHANNELS INITIATED BY SODIUM INFLUX IN DEVELOPING NEURONS. B.Dargent, P. Cau and F. Couraud. Biochimie, CNRS UA 1179 Fac. de Médecine Nord, Bd P. Dramard, 13326 Marseille Cedex 15-France

In order to address the issue of whether regulatory feedback exists between the electrical activity of a neuron and ion channel density, we investigated the effect of Na^+ channel activators (α-scorpion toxin, batrachotoxin and veratridine) on the density of Na^+ channels in fetal rat brain neurons *in vitro*. A partial but rapid ($t_{1/2}$ = 15mn) disappearance of surface Na^+ channels was observed as measured by a decrease in the specific binding of ^{3}H-saxitoxin and ^{125}I-β scorpion toxin and a decrease in specific $^{22}Na^+$ uptake. Moreover, the increase in the number of Na^+ channels which normally occurs during neuronal maturation *in vitro*, was inhibited by chronic channel activator treatment. The induced disappearance of Na^+ channels was abolished by tetrodotoxin, was found to be dependent on the external Na^+ concentration and was prevented when either choline, a non permeant ion, or Li^+, a permeant ion was substituted for Na^+. Amphotericin B, a Na^+ ionophore and monensin were able to mimick the effect of Na^+ channel activators while a KCl depolarisation failed to do this.This feedback regulation seems to be a neuronal property since Na^+ channel density in cultured astrocytes was not affected by channel activator treatment nor by amphotericin B. The present evidence suggests that an increase in intracellular Na^+ concentration, whether elicited by Na^+ channel activators or mediated by a Na^+ ionophore, can induce a decrease in surface Na^+ channels and therefore is involved in down regulation of Na^+ channel density in fetal rat brain neurons *in vitro*.

PEPTIDES: PHYSIOLOGICAL EFFECTS I

87.1

VASODILATOR PROSTAGLANDINS MAY SERVE A PROTECTIVE ROLE DURING THE DEVELOPMENT OF HYPERTENSION IN THE SHR. **D.R. Luthin , W.H. Cline. Dept. of Pharmacology, Southern Illinois Univ. Sch. of Med., Springfield, IL 62794-9230.**

Prostaglandins (PGs), specifically PGI_2 and PGE_2, have been shown to decrease norepinephrine(NE) release from presynaptic nerve terminals and to act as vasodilators postjunctionally. It has been proposed that the modulation of noradrenergic neurotransmission by endogenous PGs is diminished in the aging adult spontaneously hypertensive rat(SHR). To determine the role of PGs in modulating responses to either periarterial nerve stimulation (PNS) or angiotensin II (ANG II) perfusion during the development of hypertension in the SHR, the cyclooxygenase inhibitor, indomethacin (INDO) at a concentration of 3 μM was perfused continuously throughout the experiments in the presence or absence of ANG II (10 nM) in the isolated perfused mesenteric vasculature of 4-6 weeks old SHR and age-matched Wistar-Kyoto (WKY) rats. INDO alone enhanced perfusion pressure responses (PPR) to PNS at 8-14 Hz in the SHR and at 12-14 Hz in WKY preparations. PPR to exogenous NE (1.5-44 nmol) were not altered in either SHR or WKY. PNS-induced NE release was enhanced only at 14 Hz in the SHR and not in WKY preparations. ANG II alone enhanced PPR to PNS at 10-14 Hz in the WKY, but had no effect on SHR preparations. PPR to exogenous NE were enhanced at 6-44 nmol in SHR, but were not altered in WKY preparations. NE release was enhanced only at 4 Hz in the SHR, and was unaltered in WKY preparations. Simultaneous perfusion of INDO and ANG II significantly potentiated PPR to PNS at 6-14 Hz in SHR, and at 8-14 Hz in WKY preparations. PPR to exogenous NE were also significantly enhanced in both WKY and SHR preparations. NE release was not altered in SHR, and was significantly decreased at 10-14 Hz in WKY preparations. These data indicate that vasodilator PGs are released in response to PNS and ANG II perfusion, and act primarily postjunctionally, with the effect being greater in SHR preparations. These data also suggest that these vasodilator PGs may serve a protective role during the development of hypertension in the SHR. **Supported by the American Heart Association, Illinois Affiliate.**

87.2

EFFECTS OF THYROID DENERVATION ON THYROID BLOOD FLOW AND VIP CONTENT. M. Michalkiewicz*, L.J. Huffman* and G.A. Hedge. Physiology, West Virginia University, Morgantown, WV 26506.

The presence of VIP in thyroid nerve fibers and its stimulatory effect on thyroid blood flow (TBF) have prompted us to study the effects of denervation on TBF and thyroid VIP content. Male Sprague-Dawley rats (220-250 g) were anesthetized prior to either sham surgery or one of the following bilateral denervations: superior cervical ganglionectomy (SCGX); superior laryngeal neurectomy (SLNX); inferior laryngeal neurectomy (ILNX). Two weeks after surgery, the following measurements were made: thyroid VIP concentrations (by RIA); TBF (using ^{141}Ce-labeled microspheres); plasma levels of TSH, T_3, and T_4 (by RIA); and thyroid weight (TW). SLNX, but not SCGX or ILNX, caused a significant 62% decrease in thyroid VIP concentrations (when compared with shams). TBF, TW, and plasma levels of TSH, T_3, and T_4 were not affected by any of the denervations. Conclusion: The majority of thyroidal VIP is derived from the SLN (parasympathetic nerve supply). The lack of change in TBF or hormone secretion two weeks after thyroid denervation may be due to a functional reorganization of the autonomic pathways to the thyroid. (NIH DK35037; NSF DCB-8904470).

87.3

RELEASE OF SUBSTANCE P, SUBSTANCE K AND CALCITONIN GENE-RELATED PEPTIDE FROM THE RAT TRACHEA

X.-Y. HUA & T.L. YAKSH Dept. of Aneasthesiology, Univ. of California, San Diego, CA92093

Substance P(SP), Substance K(SK) and Calcitonin gene-related peptide (CGRP) exist in primary sensory afferents which innervate the airway, including the trachea. The present study is to investigate if activation of the peripheral primary sensory terminals in the trachea could evoke the release of these neuropeptides. The trachea (below larynx to bifucation) was dissected and perfused intraluminally with Krebs solution(37°C: 0.2ml/min). After lyophilization, the perfusates were subjected to RIA for determining the peptides. K^+(70mM-120mM) and capsaicin(10^{-8}M-10^{-4}M) caused a concentration-dependent increase in the level of CGRP,SP and SK in the perfusates. The effect was attenuated in the absence of calcium. Two exposure to K^+ did not diminish peptides release in the second exposure, but capsaicin desensitized the tissue to subsequent capsaicin exposure. The result suggests that the peripheral terminals of primary afferents in the trachea possess releasable stores of these 3 peptides. Such release may create the changes in airway reactivity which are observed in the presence of irritant stimuli.

Peptides	Basal	K+120mM	Caps10-5M
	(fmol/fraction)		
SK	0.7±0.7	1.1±1.1	26.4±4.3
SP	1.8±0.6	3.9±2.0	16.2±5.2
CGRP	13.4±1.4	96±25	148±20

87.4

THE RESPONSE OF NEURAL ELEMENTS OF RAT DURA MATER IN AN EXPERIMENTAL MODEL OF SAH. B.G. MULLEN*, M.J. FRITTS* AND J.T. KELLER. Depts. of Neurosurg. U. of Cincinnati, The Christ Hosp., J.N.G. Inst. of Med. Res. Cinti, OH 45219

While the effect of subarachnoid hemorrhage (SAH) upon neurotransmitter/neuropeptide content has been examined in perivascular nerves, its effect on dural nerves and mast cells is unknown. The density of calcitonin gene related peptide (CGRP) and neuropeptide Y (NPY) in perivascular nerve fibers and serotonin(5-HT) in mast cells was examined immunocytochemically following the production of SAH. Cisternal subarachnoid injections of either 0.3ml autologous blood (experimental) or 0.3ml buffered lactated ringer's solution (sham-operated controls) were performed in Sprague-Dawley rats which were subsequently sacrificed/examined at 6,24, or 48 hours or 6 days. The pattern and intensity of labeling of CGRP fibers was similar in all animals and in each time period following SAH. The intensity of NPY labeling of perivascular nerves and 5-HT labeling of the mast cell population was diminished in both 6 and 24 hour animals (experimental and sham-operated controls) and returned to normal (non-operated controls) at 48 hours and 6 days. The changes in 5-HT and NPY content in dural mast cells and nerve fibers, respectively, may reflect a general response to the increase in intracranial pressure following SAH. These substances are also of interest with regard to their potential role in vascular headache and neurogenic inflammation.

87.5

PLASMA EXUDATION INDUCED BY HUMAN ALPHA-CGRP AND ITS SEQUENCES IN RAT SKIN. K. Tuominen, A. Koskinen+[1] and H. Uusitalo. Dept. of Anatomy, Eye Res. Lab. Univ. of Helsinki, Finland, [1]Dept. of Chemistry, Univ. of Surrey, Guilford, UK.

CGRP is a potent vasodilator and may have a role in neurogenic inflammation. The activity of alpha-hCGRP and its fragments to induce plasma extravasation in the rat skin was studied by using intravenous Evans Blue and ^{125}I human serum albumin. CGRP and CGRP fragments in concentrations of $2x10^{-11}$ - $2x10^{-8}$ mol/20 µl were intradermally injected in dorsal skin. Plasma exudation 30 min after the injections was expressed as blood plasma leakage in a skin sample. A significant increase in Evans blue area and plasma exudation were induced by at doses $2x10^{-10}$ - $2x10^{-9}$ mol. $CGRP_{8-37}$ induced both plasma exudation and extravasation equipotently to whole CGRP. The effects of $CGRP_{15-24}$ and $CGRP_{15-37}$ were smaller and $CGRP_{28-37}$ has only a minor effect. Based on the present findings it is evi-dent that CGRP can induce changes in rat skin in concen-tration of $2x10^{-10}$ mol resembling those induced by neuro-genic inflammation. It is further suggested that sequence $_{15-24}$ is important in this respect. Based on the previous data it seems evident that CGRP has two active epitopes: one in the amino-terminal end (cysteine loop) and the other in $CGRP_{15-24}$.

This work was supported by TEKES, Helsinki.

87.6

NEUROPEPTIDE Y AND OPIOIDS MODULATE RESPONSES TO NERVE ACTIVATION OF THE PERFUSED RAT MESENTERY. Y.J. Li* and S.P. Duckles. Dept of Pharmacol, Coll of Med, Univ of Calif, Irvine, CA 92717.

In perfused rat mesentery the contractile response to transmural nerve stimulation (TNS) was the net result of both constrictor and dilator nerve activation. In control tissues TNS produced a frequency-dependent increase in perfusion pressure, which was potentiated by pretreatment with capsaicin ($3x10^{-7}$ M, 20 min) to deplete sensory transmitters. When adrenergic nerves were blocked with guanethidine ($5x10^{-6}$ M) and methoxamine ($3x10^{-6}$ M) was added to increase smooth muscle tone, TNS caused a dilator response which was abolished by capsaicin, but not by propranolol or atropine. Treatment with indomethacin (10^{-6} M) resulted in a significant increase in the vasodilator response to TNS, but no change in dilation to exogenous calcitonin gene related peptide. NPY (10^{-8} M) significantly potentiated vasoconstrictor responses to TNS, but suppressed the capsaicin sensitive vasodilation. Mu, delta or kappa opioid receptor agonists, DAMGO, ([D-Ala^2,NMe-Phe^4,Gly-ol]-enkephalin; $5x10^{-8}$ M), DPDPE ([D-Pen^{2,5}]-enkephalin; 10^{-7} M) and EKC (ethylketocyclazocine; $5x10^{-8}$ M), had no effect on the vasoconstrictor response to TNS. In contrast, DAMGO and DPDPE significantly inhibited vasodilator responses to TNS, but EKC was without effect. After treatment with indomethacin, DAMGO still inhibited the vasodilator response, but DPDPE was no longer effective. Our results show that both adrenergic vasoconstrictor and capsaicin-sensitive sensory vasodilator nerves can contribute to the control of vascular tone in the rat mesentery. Prejunctional control of transmitter release by NPY or opioid receptors is dependent on the specific nerve type as well as, in some cases, the participation of endogenous prostaglandins. *NIH #DK36289.*

87.7

CALCITONIN GENE-RELATED PEPTIDE (CGRP) MEDIATES NERVE STIMULATION-INDUCED VASODILATION IN RAT MESENTERY S.P. Han, X. Chen, L. Naes and T.C. Westfall Dept. of Pharmacol., St. Louis Univ. School of Medicine, St. Louis, MO 63104

The mediator of nonadrenergic-noncholinergic (NANC) vasodilation was studied in the isolated perfused rat mesenteric vascular bed precontracted with methoxamine. Norepinephrine release was abolished by guanethidine. Periarterial nerve stimulation elicited a frequency-dependent vasodilation which was resistant to atropine and propranolol but sensitive to tetrodotoxin and capsaicin indicating its neurogenic nature. The NANC vasodilation was significantly attenuated by CGRP receptor desensitization, infusion of CGRP antiserum or hCGRP (8-37), a CGRP receptor antagonist. Vasodilation induced by exogenous CGRP was similarly attenuated by these procedures. Infusion of CGRP antiserum or hCGRP (8-37) also elevated the basal perfusion pressure in the mesentery suggesting that the vascular tone is tonically regulated by endogenous CGRP. The NANC vasodilation was smaller in amplitude and shorter lasting in the mesentery from SHR than that from normotensive rat. These data suggest that primary sensory nerve-derived CGRP 1) mediates NANC vasodilation, 2) tonically regulates vascular resistance and 3) its deficiency may play a role in pathogenesis of hypertension (Supported by HL 26319 and HL 35202).

87.8

NEUROPEPTIDE Y AND GALANIN: OPPOSING CENTRAL AND PERIPHERAL ACTIONS ON GASTRIC ACID SECRETION. J.G. Geoghegan,* D.C. Lawson*, S.S. Schiffman, T.N. Pappas*. Duke University Medical Center, Durham, NC 27710.

Several brain-gut peptides have opposite central and peripheral effects on gastric function. This study compares the central and peripheral actions of Neuropeptide Y (NPY) and Galanin (GAL) on meal-stimulated gastric acid secretion. Six gastric fistula dogs with cerebroventricular guides were given peptide (NPY or GAL) or carrier (control) by bolus intraventricular injection or IV infusion.

Results: Central NPY increased gastric acid (control: 13.6±2.3 mmol/hr; NPY 500 pmol/kg: 30.6±6 mmol/hr*), and peripheral infusion inhibited gastric acid (Control: 22.9±2.7 mmol/hr; NPY 500 pmol/kg/hr: 15.5±2.6 mmol/hr*). Central GAL augmented gastric acid (Control: 11.5±2.1 mmol/hr; GAL 62.5 pmol/kg: 21.8±1.9 mmol/hr*). This is the opposite of its reported peripheral inhibitory effect on stimulated gastric acid. (*$p<0.05$)

Conclusions: NPY and GAL both have opposing central and peripheral effects on gastric acid secretion.

87.9

SINGLE FIBER VAGAL AFFERENT RESPONSES TO INTRAGASTRIC SALINE INFUSIONS AND CELIAC ARTERY CHOLECYSTOKININ INFUSIONS IN RATS. G.J. Schwartz, P.R. McHugh & T.H. Moran, Johns Hopkins Univ. Sch. Med., Dept. Psychiatry, Balto., MD. 21205.

A role for the vagus has been proposed in the inhibition of food intake elicited by gastric distension and cholecystokinin (CCK). We examined the electrophysiological responses of single vagal afferent nerve fibers to intragastric (i.g.) saline infusions and celiac artery (c.a.) CCK infusions. Two response patterns were identified. One pattern (N=12) demonstrated increased vagal firing both in response to 2 ml i.g. saline infusions and to 0.1 ml c.a. infusions of CCK, while the other (N=4) showed increased firing during i.g. infusions but not following CCK infusions. Fibers demonstrating the first pattern were further investigated to determine the relationships between 1) i.g. infusion parameters and firing rate and 2) c.a. CCK dose and firing rate. When the i.g. infusion volume was held constant at 2 ml, firing rate did not change with increasing i.g. infusion rate over a 0.1-2.5 ml/min range. However, if the i.g. infusion rate was held constant at 0.1 ml/min, the firing rate increased with increasing i.g. infusion volume over a 0.5-2.0 ml range. In response to CCK infusions, both firing rate and response duration increased over a 10-1000 pmol dose range, while response latency decreased. Finally, CCK pretreatment enhanced the response to a subsequent 2 cc i.g. saline load. The results demonstrate that some, but not all, vagal fibers responding to intragastric infusions respond to CCK, and suggest that the effects of these stimuli interact at a peripheral level. (Supported by NIH grant DK19302).

87.10

SPINAL AND PERIPHERAL NEUROPEPTIDE Y (NPY) DIFFERENTIALLY ALTER DUODENAL AND COLONIC INTRALUMINAL PRESSURE. S.Wager-Srdar, B.Ghazali* & J. Davison*, Dept. of Medical Physiology, Univ.of Calgary, Calgary, Alberta, Canada

NPY is widely distributed in the central nervous system, spinal cord and gastrointestinal (GI) tract of several species, including rats. Hellstrom, et al. reported that intravenous (IV) administration of NPY to rats inhibited the propagation of the migrating myoelectric complex (MMC) and the GI transit of ^{51}Cr. Nitecki, et al. demonstrated that NPY had opposing effects on the fasting MMC following central or peripheral injections in dogs. We compared the effects of NPY administered intrathecally (INTH) at the level of T4 (n=11) and (IV) via the femoral vein (n=6) on the intraluminal pressure in the duodenum and colon of urethane anesthesized rats. The duodenum and colon were perfused with saline via indwelling catheters and pressures were recorded simultaneously during a baseline (BL) period, saline (5ul), or following NPY (200 pmole in 5 ul of saline). In contrast to BL (4.17 ± 0.4 cm H_20), NPY (IV) decreased colonic pressure (1.37 ± 0.33 cm H_20), $p<0.003$. Colonic pressure was unchanged following NPY (INTH) compared to BL. The responsiveness of the colon to NPY following INTH or IV injection was found to be statistically different, $F(1,15)=8.07$, $p<0.01$. Following saline (INTH), BL duodenal pressure was (2.55 ± 0.32 cm H_20). However, following NPY (INTH), duodenal pressure exhibited a biphasic response with inhibition (1.63 ± 0.35cm H_20), $p<0.05$, followed shortly by excitation (3.97 ± 0.39 cm H_20), $p<0.001$, compared to BL. In contrast to BL (1.53 ± 0.21 cm H_20), duodenal pressure appeared to be increased by NPY (IV) (3.97 ± 1.05 cm H_20), $p=0.05$; however, this observation was variable. These observations indicate that NPY may be having an effect on GI muscle tone which altered intraluminal pressure. However, the effect of NPY on intraluminal pressure in the colon appeared to be mediated through independent neural pathways or receptor populations following spinal or peripheral administration. This work was supported by Medical Research Council of Canada. S.W-S. is a National Institutes of Health post-doctoral fellow and B.G. is a Saudia Arabia government post-doctoral fellow.

87.11

CCK FACILITATES GANGLIONIC TRANSMISSION IN THE GUINEA PIG GALLBLADDER VIA A PRESYNAPTIC CCK-A RECEPTOR. G.M. Mawe, Department of Anatomy and Neurobiology, The University of Vermont, Burlington, VT 05405

Cholecystokinin (CCK) is named for its ability to cause contractions of smooth muscle fibers, resulting in an emptying of the gallbladder. There has been some dispute as to whether, in addition to its direct effect on smooth muscle, CCK acts through the ganglionated plexus of nerves in the wall of the gallbladder. In this study, intracellular recording techniques were used to test for direct and presynaptic effects of CCK on gallbladder neurons.

Direct application of CCK had no direct effect on the membrane potential or input resistance of gallbladder neurons. However, CCK had a presynaptic facilatory effect on nicotinic transmission in gallbladder ganglia. CCK (0.1 μM) increased the amplitude of fast EPSPs in gallbladder ganglia, but it did not affect the amplitude or duration of responses to exogenously applied acetylcholine. CCK caused a 3-fold increase in the quantal content, measured from fast EPSPs recorded in low Ca^{2+}/high Mg^{2+} solution. The selective CCK-A receptor antagonist L-364,718 (1 nM) blocked the presynaptic facilatory effect of CCK, but the selective CCK-B antagonist L-365,260 (10 nM) did not.

Results from this study indicate that CCK does not affect gallbladder ganglion cells directly. However, CCK does have a facilatory effect on nicotinic transmission that is mediated by CCK-A receptors in gallbladder ganglia. Supported by NS26995.

87.12

EFFECTS OF GALANIN ON THE FUNCTION OF PANCREATIC ISLET SECRETION AND ELECTRICAL ACTIVITY OF B CELL. X. W. Fu*, and X. Y. Shen. Dept. of Physiology, Shanghai Med. Univ., Shanghai, 200032, China.

The present study was designed to investigate the effects of galanin on the insulin and glucagon secretions of isolated rat islets and electrical activity of mice B cell by incubation, radioimmunoassay and intracellular microelectrode techniques. Galanin 0.3 μmol/L had a more pronounced inhibitory effect on insulin secretion in culture medium with a glucose concentration of 5.5 mmol/L than in medium with 2.7 or 20 mmol/L of glucose. Galanin did not affect glucagon release. Addition of galanin (0.15 and 0.3 μmol/L) to the perfusion medium inhibited spontaneous electrical activity with decreases in frequency and amplitude of the spike of B cell. Verapamil (0.03 - 0.09 mmol/L) blocked dose-dependently the depolarization induced by high K+ (50 mmol/L). Galanin also attenuated the depolarization induced by high K+, which showed the same results as the effect of verapamil. The results suggest that galanin inhibits insulin release and electrical activity of B cell by blocking of voltage-dependent Ca2+ channels.

EXCITOTOXICITY I

88.1

ELEVATION AND OSCILLATION OF $[Ca^{2+}]_i$ AND INTERCELLULAR COMMUNICATION IN GLIAL CELLS IN RESPONSE TO TRAUMA AND GLUTAMATE. A. Charles, J. Merrill*, E. Dirksen* and M. Sanderson*. Depts. of Neurology and Anatomy and Cell Biol., UCLA School of Med., Los Angeles, CA 90024.

Mechanical trauma to a single cell in a mixed glial cell culture induced an increase in intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) in the traumatized cell. This Ca^{2+} response was communicated cell-by-cell to surrounding cells. $[Ca^{2+}]_i$, measured by digital fluorescence imaging and fura-2, increased from about 75 nM to 500-700 nM. As $[Ca^{2+}]_i$ recovered to resting levels, cells displayed asynchronous $[Ca^{2+}]_i$ oscillations with a periodicity of 5-20 sec. In the absence of extracellular calcium, trauma induced a similar response except that $[Ca^{2+}]_i$ decreased in the traumatized cell and the $[Ca^{2+}]_i$ oscillations in the surrounding cells were decreased in amplitude and duration. Glutamate (100 μM) induced a simultaneous $[Ca^{2+}]_i$ increase in most cells, followed by asynchronous oscillations of $[Ca^{2+}]_i$ having greater periodicity than the oscillations induced by trauma. Based on similarities of the Ca^{2+} response of glial cells to trauma and excitotoxic concentrations of glutamate, we propose that communicated changes in $[Ca^{2+}]_i$ initiate the response of glial cells to injury. Supported by Smokeless Tobacco Res. Council, Inc. and the CF Foundation.

88.2

CALCIUM LOCALIZATION IN RETINA IN GLUTAMATE TOXICITY AND ISCHEMIA IN VITRO. M.S. Burns and C.M. Panattoni*. Ophthalmology Research Laboratories, UCDavis, Davis, CA 95616.

Increased intracellular calcium (Ca_i^{2+}) is thought to be a mechanism of toxic cell death in both neural and non-neural cells. Increases in Ca_i^{2+} can arise from redistribution of intracellular Ca and/or influx from extracellular calcium. To test if extracellular Ca influx occurs in retinal cell toxicity, and to localize exogenous Ca influx, we incubated retinas in the stable isotope, ^{42}Ca, and imaged total Ca content in retinal cell layers using Secondary Ion Mass Spectrometry (SIMS).

Rat retinas were incubated in modified Ames' medium for up to 6 hours under normal conditions or 5 mM glutamate or ischemia (100% N_2) and then prepared for SIMS analysis by low temperature freeze drying. Electrolyte localizations were compared to non-incubated control retinas.

Approximately 35% of the total retinal Ca was exchanged for ^{42}Ca in 6 hours of normal incubation conditions. The tracer was equally distributed in all retinal layers. With glutamate incubation, 50% of the endogenous total Ca content was lost from the inner plexiform layer (IPL), but little loss was seen in other retinal layers. Exogenous ^{42}Ca uptake occurred in all layers, but was greater in the inner nuclear (INL) and IPL. In ischemia, total retinal Ca loss was seen in all areas, but uptake occurred predominantly in the INL and IPL. Cell death in the INL was greatest in the glutamate treated retinas. Cell death under ischemic conditions was similar to normal incubation.

These results indicate that there is influx of exogenous Ca into retinas made toxic by either glutamate or ischemia, and there is preferential localization in the layer comprised by amacrine and bipolar cell synaptic processes and glial (Müller) cell processes.

Sponsored by the Office of Naval Research.

88.3

ENERGY METABOLISM, CALCIUM HOMEOSTASIS AND EXCITOTOXICITY IN VITRO. A. Schurr, P. Lipton[1], C.A. West* and B.M. Rigor. Dept. of Anesthesiology, Univ. of Louisville Sch. of Med., Louisville, KY 40292, and [1]Dept. of Physiology, Univ. of Wisconsin Med. Sch., Madison, WI 53706.

The excitotoxic hypothesis postulates glutamate (Glu) and aspartate (Asp) to have a major role in the neuronal damage inflicted by various brain disorders. Disturbed energy metabolism and ion homeostasis have been implicated as contributing factors in excitotoxicity. The rat hippocampal slice preparation and electrophysiology were used to study the relationships between oxygen, glucose, Ca^{2+}, Mg^{2+}, Glu, Asp, N-methyl-D-aspartate (NMDA) and neuronal damage. Under standard conditions (95% O_2, 10 mM glucose) Glu and Asp were innocuous. However, the rate of recovery of neuronal function (evoked population spike) after hypoxia and reoxygenation decreased with elevated [Glu], [Asp] or [NMDA] in the artificial cerebrospinal fluid (ACSF). Omitting Ca^{2+} from, or elevating Mg^{2+} in, the ACSF during hypoxia, abolished excitotoxicity. NMDA enhanced hypoglycemic neuronal damage, effect that was intensified by low-Mg^{2+} ACSF and antagonized by Ca^{2+}-free ACSF. Lactate protected against, while the glycolytic inhibitor 2-deoxyglucose sensitized slices to, NMDA-enhanced, hypoglycemic neuronal damage. These results indicate that the excitotoxins have only a secondary role in hypoxic and hypoglycemic neuronal damage and are innocuous when energy metabolism is adequate and calcium homeostasis is maintained.

88.4

EXCITATORY AMINO ACID EVOKED CALCIUM INFLUX AND CALCIUM-DEPENDENT NEUROTOXICITY IN RAT CORTICAL CULTURES. W. C. Zinkand,* C. Thompson,* A. I. Salama, and J. Patel. ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

Several groups have shown that removal of extracellular calcium both during and after an EAA insult will provide neuroprotection. In primary cultures of rat cerebral cortex, we have observed that EAA-induced neurotoxicity is dependent on extracellular calcium both during and after exposure to EAA's. Incubation of cells with glutamate (Glu) or N-Methyl-D-Aspartate (NMDA) for 5 min causes an influx of calcium as monitored by $^{45}Ca^{++}$ and Fluo-3. Eighty to ninety-nine percent of the cells treated as such are destined to die within 24 hours as measured by release of lactate dehydrogenase to the cell culture media. Neuroprotection, as well as abolition of the primary Ca^{++} influx, can be obtained by co-incubating EAA's with the NMDA-associated channel blocker MK-801 or the strychnine-insensitive glycine antagonist 7-chlorokynurenic acid (7-CK). In addition, a secondary influx of calcium following washout of the EAA was observed. This secondary influx is not blocked by the presence of MK-801, 7-CK, the NMDA receptor antagonist CPP, or the inorganic calcium channel blocker lanthanum. Cytotoxicity, however, can be blocked by removing extracellular calcium concomitantly with the secondary calcium influx suggesting a role in EAA neurotoxicity.

88.5

CALCIUM CHANNEL ANTAGONISTS AT NANOMOLAR CONCENTRATIONS ATTENUATE NMDA RECEPTOR-MEDIATED NEUROTOXICITY OF RETINAL GANGLION CELLS IN CULTURE. Jeffrey T. Offermann, Nikolaus J. Sucher, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital & Progr. in Neurosci., Harvard Med. Sch., Boston, MA.

Dihydropyridine calcium channel antagonists block a prolonged or 'L-type' component of voltage-gated Ca^{2+} current in patch-clamp recordings of postnatal rat retinal ganglion cells (Karschin and Lipton, *J. Physiol.* 1989;418:379). In the present study on these neurons, 500 -1000 nM nifedipine or nimodipine completely blocked the toxic effects of either 200 μM *N*-methyl-D-aspartate (NMDA) or an endogenous glutamate-related compound present in the retinal cultures. Previous data have shown that the neurotoxicity engendered by these agents can also be completely prevented by selective NMDA antagonists (200 μM APV or 20 μM MK-801; Hahn et al., *PNAS* 1988;85:6556). Under our culture conditions, ≥96% of each dihydropyridine is bound to rat serum, yielding an effective free concentration of approximately 20 nM. It is interesting to compare these results with those of murine cortical cultures in which 30-100 μM nifedipine is necessary to attenuate toxicity due to even weak NMDA agonists (Weiss et al.,*Science* 1990;247:1474). The present findings suggest, at least in our preparation, that NMDA receptor-mediated toxicity is dependent on both receptor-activated and voltage-gated Ca^{2+} channels.

88.6

DELAYED CALCIUM INCREASES IN RAT HIPPOCAMPAL NEURONS FOLLOWING GLUTAMATE EXPOSURE. R.D. Randall and S.A. Thayer Dept. of Pharmacology, Univ. of Minnesota, Minneapolis, MN 55455.

The intracellular free calcium concentration, $[Ca^{2+}]_i$, was measured in single rat hippocampal neurons grown in primary culture using indo-1 based microfluorimetry. Neurons exposed to glutamate (100 μM) for 5 min, in the presence of Mg^{2+}, responded with large increases in $[Ca^{2+}]_i$ (4.5±0.8 μM; n=17). Each cell was observed for a total of 3 hours. Following removal of glutamate, $[Ca^{2+}]_i$ returned to basal levels (16 of 17) indicating that at this time the ability of the cell to buffer the Ca^{2+} load had not been compromised. The cells subsequently displayed a period of $[Ca^{2+}]_i$ spike activity of variable duration (108±15 min). $[Ca^{2+}]_i$ spikes were also observed in control cells and could be blocked with 1 μM tetrodotoxin. Finally the $[Ca^{2+}]_i$ gradually increased to μM levels for the remainder of the recording period (14 of 17). Control cells (6 of 14) also displayed a slight increase in $[Ca^{2+}]_i$ toward the end of the recording period. Glutamate treated cells displayed profound morphological changes including, axonal swelling, distortion of the soma, and in some cases death (n=5). These data suggest that three Ca^{2+} mediated events may contribute to excitatory amino acid-induced neurotoxicity including an initial exposure or triggering event, a period of synaptically driven Ca^{2+} spiking, and a large sustained elevation in $[Ca^{2+}]_i$.

88.7

CALCIUM-INDEPENDENT ARACHIDONIC ACID-INDUCED CELL DEATH IN HIPPOCAMPAL PYRAMIDAL CELLS IS A RESULT OF FREE RADICAL GENERATION. B.E. Alger and D.O. Keyser. Dept. of Physiology, Sch. of Med., Univ. of MD, Baltimore, MD 21201.

Arachidonic acid (AA) release is associated with cytotoxicity but the mechanism of action has not been studied in single neurons. We have used whole-cell voltage-clamp to investigate neuronal death observed following application of AA (50 μM) to acutely isolated hippocampal neurons (n=55). Five to seven min following application of AA an increase in background current noise at the holding potential of -50 mV occurred that was followed by a gradual inward shift in holding current and, after 15 min, cell death. This sequence was not altered by inhibitors of AA metabolism or by buffering of extracellular and intracellular calcium to ~10 nM. The free radical generating system, xanthine (XA) and xanthine oxidase (XO) applied extracellularly resulted in cell death. Superoxide dismutase (SOD; 90 U/ml) or a cocktail of free radical scavengers, blocked AA-induced and XA+XO-induced cell death for up to 30 min suggesting the effect was due to superoxide radical formation. To determine if the change in holding current was due to movements of particular ions, we altered the concentrations of Ca^{2+}, Ba^{2+}, Na^+, K^+ and Cl^- intra- and extracellularly. Neither these changes nor substitution of Tris CH_3SO_3 for permeant ions altered the onset of AA-induced cell death. Our results indicate that AA can cause cell death via generation of oxygen free radicals and this effect is independent of Ca^{2+} and other physiological ions tested.

88.8

THE EXCITOTOXIN β-N-OXALYL-L-α,β-DIAMINOPROPIONATE (β-L-ODAP) IS A POTENT ASTROGLIAL TOXIN. C.G. Hatalski, H.S. Roberts* and R.J. Bridges, Departments of Psychobiology and Neurology, University of California-Irvine, Irvine, CA 92717.

Astrocytes play a key role in excitatory amino acid (EAA) neurotransmission by transporting and metabolizing L-glutamate. Damage to these cells would be expected to reduce the efficiency of these processes and, thus, increase the probability of excitotoxic neuronal damage. We found that the EAA agonist that is the causative agent in human neurolathyrism, β-L-ODAP, is a potent gliotoxin. Primary cultures of cortical astrocytes were prepared and purified from Sprauge-Dawley rat pups (McCarthy and deVellis, *J. Cell Bio.* 85, 890, 1980). β-L-ODAP was dissolved in media and added to confluent secondary cultures of type I astrocytes. The cells were observed for 48 hours and cell survival was determined by quantifying lactate dehydrogenase activity in both the culture medium and the remaining cells (Koh and Choi, *J. Neurosci. Meth.* 20, 1987). At low concentrations of β-L-ODAP (500μM), treated astrocytes show dramatic morphological changes (e.g., increased cell swelling and vacuoles). Increasing the concentration of β-L-ODAP, resulted in lysis of the cells. The potency of this effect (LD_{50} approximately 2mM) is similar to that observed with the well known gliotoxin, L-α-aminoadipate. In contrast to β-L-ODAP, the α-L- and β-D- isomers, as well as L- and D-γ-N-oxalyl-α,γ-diaminobutyric acid, were not toxic. The results of these studies suggest that the CNS damage induced by β-L-ODAP may include a pathological effect on astrocytes, as well as neurons.

88.9

BMAA ACTIVATES AN IONIC CURRENT WHICH IS BLOCKED BY EXTRACELLULAR CALCIUM. C.N. ALLEN, D.O. Carpenter[1] and P.S. Spencer, Cntr. Res. Occ. & Envir. Tox., Or. Hlth Sci. Univ Portland, Oregon 97201 and [1]Wadsworth Cntr for Labs & Res., Albany, New York 12201.

BMAA is a neurotoxic amino acid present in seeds of the false sago palm, Cycas circinalis, a plant which is etiologically linked to Western Pacific Amyotrophic Lateral Sclerosis Parkinsonism-Dementia (ALS/P-D). Ionic currents were recorded from cultured hippocampal neurons using the whole-cell voltage clamp technique. BMAA (0.5 mM) in the absence of bicarbonate activated inward currents at membrane holding potentials more negative than 0 mV. Increasing the BMAA concentration to 2 mM caused an apparent current desensitization. The BMAA currents were not blocked by 1 mM Mg, but increasing the extracellular Ca concentration from 0.1 mM to 3 mM reduced the current amplitude 90%. The IC_{50} of the Ca block was estimated to be 0.65 mM. Sodium bicarbonate (20 mM) potentiated BMAA currents in solutions containing 3 mM Ca while currents recorded in 0.1 mM Ca were not potentiated. Addition of sodium bicarbonate to solutions containing calcium results in the formation of calcium-bicarbonate complexes which reduce the free calcium concentration. These data suggest that bicarbonate may potentiate BMAA-activated currents in part by reducing extracellular calcium. (Supported by NIH-NINCDS grant NS-23807).

88.10

BASIC FIBROBLAST GROWTH FACTOR ATTENUATES NMDA RECEPTOR MEDIATED NEUROTOXICITY IN STRIATAL NEURONS

A. Freese, S. Finklestein, and M. DiFiglia, Department of Neurology, Mass. General Hospital & Harvard Medical School, Boston, MA 02114.

We have recently characterized the neurotoxicity of glutamate (glu) in primary striatal cultures (Freese, et. al., *Brain Res.*, 1990, in press). As shown by Mattson, et. al. (*J. Neurosci.* 9:3728, 1989), Fibroblast Growth Factor (FGF) reduces the neurotoxicity of glu in hippocampal cultures. In the current study, the neuroprotective effect of basic (b)FGF was further evaluated in striatal cultures.

In separate experiments, striatal cultures were incubated with or without bFGF (6 pM) from the day of plating for 12-18 days in culture. These cultures were then exposed to 3 mM glu, 1 mM Quinolinate (QA), or 1 mM Kainate (KA) for 3 h., and survival of neurons was quantitated. Neurons exposed to bFGF and glu had a survival of 86+/-2% (n=21 dishes, 7 experiments), whereas those exposed to glu only had a survival of 54+/-3% (n=19, 7 exps). Striatal neurons exposed to QA (an agonist of the NMDA receptor) had a survival of 96+/-2% (n=12, 4 exps) with bFGF and 74 +/-4% survival (n=12, 4 exps) without bFGF. Surprisingly, when exposed to KA, survival of neurons was not affected by preincubation with bFGF (survival of 64+/-6% with and 64+/-10% without bFGF; n=5, 2 exps). Quisqualate is not toxic in these cultures. Control cultures, not exposed to glu, QA, or KA, had a survival of 99+/-1% with and 98+/-1% without bFGF. The protective effect of bFGF on glu neurotoxicity was observed in cultures treated as little as 2 h. before glu exposure.

These results suggest that bFGF markedly protects striatal neurons from glu excitotoxicity by attenuating NMDA receptor function. Whether such an effect is mediated by the receptor or post-receptor events remains to be determined. *Supported by NIH grant NS 16367 to MD.*

88.11

A ROLE FOR GLUTAMATE UPTAKE IN MODULATING GLUTAMATE NEUROTOXICITY IN CULTURES OF RAT CEREBRAL CORTEX. K.M. Harris, D. Rulf, and P. A. Rosenberg. Dept. of Neurology, Children's Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

Previous results [Neuroscience Lett., 103, 162, 1989] have shown that cortical neurons in astrocyte-poor cultures are killed by glutamate via an NMDA receptor dependent mechanism, but are one hundred times more sensitive to the toxicity of glutamate than neurons in astrocyte-rich cultures. This suggests that the vulnerability of neurons to glutamate toxicity may be dependent upon normal astrocyte function.

In the present study, the neurotoxicity of glutamate was compared with that of NMDA, a poorly transported glutamate agonist. Cortical cultures were exposed to selected concentrations of NMDA for 20-24 hours. Unlike glutamate, NMDA had similar potencies as a neurotoxin in astrocyte-rich and in astrocyte-poor cultures. One explanation for the different pharmacology of glutamate and NMDA is the presence of an active uptake system for glutamate in astrocyte-rich cultures. We found that by substituting choline for sodium, the potency of glutamate as a neurotoxin in astrocyte-rich cultures was greatly increased. These data are consistent with glutamate uptake playing an important role in determining the vulnerability of cortical neurons to glutamate toxicity.

88.12

SYNTHESIS OF GLUTAMATE FROM GLUTAMINE CONTRIBUTES TO EXTRACELLULAR GLUTAMATE ACCUMULATION ACCOMPANYING GLUTAMATE NEUROTOXICITY IN CORTICAL CULTURES. P. A. Rosenberg. Dept. of Neurology, Children's Hospital and Program in Neuroscience, Harvard Medical School, Boston, MA 02115.

The neuronal mechanism(s) contributing to extracellular glutamate accumulation accompanying neuronal injury and death have not been well characterized. In order to isolate the neuronal contribution to extracellular glutamate accumulation, cultures of embryonic cerebral cortex were used in which astrocyte proliferation was stringently suppressed by early exposure to the mitotic inhibitor cytosine arabinoside (at 4 days in vitro). Killing of neurons in these cultures by exposure to 10 μM glutamate in the absence of glutamine resulted in no detectable increase in the extracellular glutamate concentration following a 20-24 hour incubation, using HPLC with ninhydrin detection. In contrast, when neurons were killed by exposure to glutamate plus glutamine (2 mM), substantial amounts of glutamate could be demonstrated in the extracellular medium. Using ^{14}C-glutamine, it was possible to show that the extracellular glutamate derived from glutamine. These results suggest that extracellular glutamate accumulation accompanying glutamate mediated neurotoxicity at least in part depends upon the synthesis of glutamate from glutamine, and is not simply due to release of pre-existing stores.

88.13

ELECTROPHYSIOLOGY OF GLUTAMATE EXCITOTOXICITY IN CULTURED HIPPOCAMPAL NEURONS: MEMBRANE POTENTIAL AND NMDA CHANNEL ACTIVITY. S. Sombati, W.W. Anderson, R.B. Lee* and R.J. DeLorenzo. Dept. of Neurology, Med. Col. of Virginia, Richmond, VA 23298.

Electrophysiological changes due to short and long-term glutamate (GLU) application were studied in cultured rat hippocampal pyramidal neurons.

Membrane potential (MP) recordings were performed using whole-cell current patch-clamp. Long (10 min) application of 500 uM GLU caused rapid, sustained depolarization from -60 mV resting potential (RP) to 0 mV. After GLU application, 60% of the neurons remained depolarized (-35 to 0 mV) throughout the recording session (20-90 min) (n=15). The remaining neuron MPs recovered, although the membrane conductance at RP was twice that of pre-GLU. After brief (10-30 s) application of 500 uM GLU, all cells returned to RP within 5 s.

Because NMDA receptors are crucial for GLU excitotoxicity, we also studied NMDA channel activity during GLU application using cell-attached patch-clamp. When the cell was bathed in saline + TTX, and the pipette contained 10-500 uM NMDA (plus saline; 0-Mg, TTX, 10 uM glycine), 45-50 pS channels occurred with a reversal potential about 60 mV above RP. With no NMDA in the pipette, channels of this type were not observed. At >= 50 uM NMDA, the channels underwent slow desensitization. With 200-500 uM NMDA in the pipette, application of 500 uM GLU to the bath caused NMDA channel amplitude to decrease to 0, indicating membrane depolarization. The patch membrane was then hyperpolarized by 50-60 mV to a potential where the amplitude of NMDA channels was about -3 pA. During GLU application the channel activity first decreased and then increased above pre-GLU controls. Burst lengths were 1-9 s vs 100-500 ms pre-GLU. Channel activity then disappeared 30-60 min after GLU application.

The MP and non-invasive NMDA channel recordings show that 10 min GLU application produces long-lasting membrane depolarization. This post-GLU depolarization could enhance excitotoxic effects by removing the Mg block of the NMDA channel. The transient increase in NMDA channel activity could contribute to, or be the result of, excitotoxic damage.

88.14

WITHDRAWN

88.15

NEUROPROTECTIVE EFFECTS OF IFENPRODIL AGAINST GLUTAMATE TOXICITY IN HIPPOCAMPAL CELL CULTURES. I.A. Shalaby, M. Prochniak*, and "B. Chenard*. Pfizer Central Research, Groton, CT 06340

We have examined the neuroprotective efficacy and potency of the novel NMDA antagonists ifenprodil and SL-82,0715 against glutamate induced neurotoxicity in hippocampal cell cultures. 2 to 3 week hippocampal cultures from fetal rats were exposed to glutamate or NMDA with or without antagonist for 15 minutes. The drugs were washed out and cells allowed to remain in the media for 24 hours following the glutamate exposure. Glutamate and NMDA induced dose - dependent increases in LDH reaching 3 fold above control levels. Morphological examination confirmed the neuronal specificity of the degeneration. The putative antiischemic compounds ifenprodil and SL-82,0715 blocked the glutamate (1 mM) toxicity with IC_{50}s of 200 nm and 1 μM, respectively. The protective effects of ifenprodil were not due to its potent alpha-1 adrenergic receptor antagonism as prazosin had no neuroprotective activity up to 10 μM. Similarly, the neuroprotective effects of ifenprodil were not due to its sigma receptor activity (Karbon et. al., Eur. J. Pharmacol. 176, 247-, 1990), as sigma ligands such as DTG, haloperidol, and 3-PPP were not neuroprotective up to 1 μM. Ifenprodil does not act through the modulatory strychnine insensitive glycine binding site, as exogenous glycine failed to reverse its neuroprotective effect. We conclude that the antiischemic agents ifenprodil and SL-82,0715 are potent neuroprotective compounds in vitro, and attenuate glutamate - induced neurotoxicity by mechanisms related to their NMDA antagonist properties.

88.16

IS THERE A LINK BETWEEN N-METHYL-D-ASPARTATE RECEPTORS AND SIGMA RECEPTORS IN METHAMPHETAMINE-INDUCED NEUROTOXICITY IN MICE? P.K. Sonsalla, D.E. Vitagliano and I.A. Terleckyj. Dept. Neurology, UMDNJ-Robt. Wood Johnson Med. Sch., Piscataway, NJ 08854.

The N-methyl-D-aspartate (NMDA) receptor is comprised of the NMDA recognition site, the co-agonist glycine site, and several modulatory sites (including phencyclidine, Mg^{++}, Zn^{++} and possibly polyamine sites). Excitotoxicity mediated by NMDA receptors can be altered by compounds which act at these various sites. We have found that competitive and noncompetitive NMDA antagonists which interact with several of the sites mentioned above protect against the neostriatal dopaminergic damage induced by methamphetamine (METH); these findings indicate that the excitatory amino acids play an important role in this neurotoxicity. Recently, it has been suggested that there may be a link between the putative haloperidol-sensitive sigma site and NMDA receptors. If so, we hypothesized that compounds with high affinity for this site should modify the neurotoxic effects of METH. Of the compounds tested, only BMY 14802 provided protection against the METH-induced decrements in neostriatal TH activity and DA content. None of the other compounds at the doses tested (rimcazole, DTG, (+)3-PPP, dextromethorphan, 1-butaclamol, or clorgyline) altered these METH-induced changes. These findings indicate 1) that the activation or inhibition of these putative sigma receptors is unlikely to be an important aspect of METH-induced neurotoxicity, 2) that the sigma receptor does not appear to be linked to NMDA receptors and 3) that the neuroprotection afforded by BMY 14802 against damage induced by METH may be due to some property other than its ability to bind to the sigma site.

88.17

DIFFERENTIAL EXPRESSION OF C-FOS mRNA FOLLOWING KAINIC ACID ADMINISTRATION. G. Tocco, S. S. Schreiber, P. C. May, R. F. Thompson, M. Baudry. Program in Neuroscience, University of Southern California, Los Angeles, CA 90089-2520.

Systemic or intracerebral injection of the neurotoxin, kainic acid (KA), in rats induces limbic seizures and extensive neuronal damage throughout the limbic system. Since the proto-oncogene c-fos increases in rodent brains after seizure activity, we were interested in determining the regional expression of c-fos at different times following KA administration.

Rats received KA (8-10 mg / kg i. p.) and were killed at various time intervals following seizure induction. The levels of expression of the c-fos mRNA were quantified by in situ hybridization. One hour after the induction of seizures , coronal sections at the level of dorsal hippocampus exhibited a generalized increase in the expression of c-fos message. However, 12 hours and up to three days after the onset of seizures, the increase in c-fos expression was mostly confined to CA3 pyramidal cells. These results suggest that prolonged c-fos expression may be a charatcteristic of cells that are selectively vulnerable to KA.

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EXCITOTOXICITY II

89.1

PHYSIOLOGIC and ANTICONVULSANT EFFECTS OF AN INHIBITOR OF GABA-T: *IN VIVO* CEREBRAL MICRODIALYSIS STUDY. R.S.K. Young, M.J. During, W.J. Aquila*; Yale Medical School; New Haven, CT.

To investigate the potential anticonvulsant effects of increased extracellular fluid levels of GABA, Aminooxyacetic acid (AOAA), an inhibitor of GABA-aminotransferase (GABA-T), was administered parenterally to rabbits. AOAA (400 mg/kg) produced high voltage slowing of the EEG, followed by marked attenuation. *In vivo* microdialysis of cerebral cortex showed a bimodal response to AOAA with either significant increase in glutamate, aspartate, and GABA, or, little change in these amino acids (Table). Administration of either flurothyl or bicuculline (2 mg/kg) produced seizure in the low GABA, but not the high GABA group. Animals in whom levels of extracellular fluid GABA are raised by AOAA have marked protection from seizure.

	GLUT.	ASPART.	GABA
HIGH GABA GROUP	12.5±7.9*	2.1±1.6*	1.2±0.8*
LOW GABA GROUP	2.4±1.3	0.2±0.2	.02±.004

(μM/L dialysate; Mean±SE; N = 6; *, $p < 0.01$ vs. control value by Analysis of variance)

89.2

EFFECTS OF INTRAOCULAR ADMINISTRATION OF N-methyl-D-aspartate (NMDA) ON THE RETINA OF THE ADULT RAT. R. Siliprandi, R. Canella*, R. Zanoni*, G. Carmignoto and N. Schiavo*. Fidia Research Laboratories, Abano Terme (PD), Italy.

We investigated the effect of a single intravitreal injection of NMDA (from 2 nmoles to 200 nmoles) on the adult rat retina. One week after injection, morphological analysis of Nissl-stained, transverse sections of retinae showed a dose-dependent loss of cells in the ganglion cell (GC) layer. Quantitative analysis of Nissl-stained, whole-mounted retinae showed that the administration of 20 nmoles NMDA resulted in a 60% loss of cells with somal diameter exceding 8 μm. Choline acetyltransferase (ChAT) activity also decreased in NMDA treated retinae in a dose-dependent manner (ED_{50} ~ 10nmoles). The results indicate that GCs and cholinergic retinal neurons (presumably cholinergic amacrine cells) of the adult rat retina are highly vulnerable to NMDA and represent a useful and reproducible model system for in vivo studies of pharmacological agents able to limit neuronal damage produced by the excitotoxic action of NMDA.

89.3

MECHANISMS UNDERLYING EXCITOTOXICITY ASSOCIATED WITH METABOLIC INHIBITION. W.J. Nicklas and G.D. Zeevalk. Dept. of Neurology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway NJ 08854.

Numerous studies have suggested that CNS ischemic damage is mediated by excitatory amino acid (EAA) associated excitotoxicity. We have made use of an ex vivo preparation of E13 chick neural retina to evaluate effects of chemically-induced hypoglycemia (iodoacetate, IOA) or hypoxia (KCN) and their relationship to acute excitotoxicity. Histology and amino acid release were used as indices of acute excitotoxicity. The mild excitotoxic lesion produced by either drug alone was completely inhibited by NMDA, but not non-NMDA, antagonists. A more robust lesion produced by combination of IOA and KCN was only partially prevented by NMDA antagonists; non-NMDA antagonists showed little protection but a combination of both types of EAA antagonists afforded considerable protection. Aspartate or glutamate efflux was not increased in mild lesions but medium levels of these EAAs were elevated with more severe metabolic inhibition. Increasing $[K^+]_o$ also produced excitotoxic-like lesions; the histological damage at 55 mM $[K^+]_o$ was almost completely prevented by MK-801. Tetrodotoxin (TTX) only minimally protected against elevated K^+, NMDA, IOA or IOA-KCN mediated toxicity. The data are consistent with energy depletion initially causing NMDA receptor activation via membrane depolarization, i.e., mild lesions are prevented by NMDA antagonists. As the damage becomes more robust, non-NMDA mediated toxicity is apparent. This may have relevance to the disparate literature findings concerning the efficacy of NMDA antagonists as anti-ischemic agents.

89.4

SUBSTITUTED GUANIDINES: CORRELATION BETWEEN PCP RECEPTOR BINDING AND IN VITRO NEUROPROTECTION. C.J.KIRK*, N.L. REDDY*, J.B.FISCHER, R.N.MCBURNEY*, A.C. SERVER, E.WEBER[1]*, J.F.W. KEANA[2]*. Cambridge NeuroScience Research, Inc. Cambridge, MA 02139, [1]Dept. Pharmacol., U.C. Irvine, Irvine, CA 92717, [2]Dept. Chemistry, U. Oregon, Eugene, OR 97403

Much interest has been generated concerning the potential neuroprotective qualities of compounds such as MK-801 which have a high affinity for the PCP receptor. We report here on the correlation between PCP binding affinities and in vitro neuroprotection exhibited by a series of substituted guanidine compounds. Displacement of ^{3}H-MK-801 from rat brain membranes was used to determine PCP binding affinities and selected drug candidates were subsequently tested in an in vitro excitotoxicity assay. The toxicity assay was based on the measurement of lactate dehydrogenase (LDH) released into the medium by dead and dying neurons following a brief exposure to glutamate in the presence or absence of a putative neuroprotective compound. Drugs with nanomolar affinities gave neuroprotection in the micromolar range. The binding affinity (IC_{50}) was plotted against the ED_{50} for neuroprotection for each drug tested. The resultant graph indicated a very strong correlation between PCP affinity and neuroprotection, and suggests that binding studies may be reliable predictors of neuroprotective activity.

89.5

DIFFERENTIAL LOSS OF [^{3}H]MK801, [^{3}H]SCH23390 BINDING SITES AND CAT ACTIVITY FOLLOWING QUINOLINIC ACID-INDUCED LESIONS OF RAT STRIATUM. A.B. Norman, M. Kolmonpunporn, L.M. Ford and P.R. Sanberg. Div. of Neuroscience, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

The excitotoxin quinolinic acid (QA) is thought to exert its neurotoxic actions by activation of the NMDA glutamate receptor. The measurement of excitotoxin-induced damage in rat striatum generally employs the neurochemical assay of choline acetyltransferase (CAT) or glutamate decarboxylase (GAD). Theoretically, neurons which express NMDA glutamate receptors should be primarily lost following QA lesions. Furthermore, D_1 dopamine receptors should also provide a good index of neurotoxicity as these receptors are located entirely on intrinsic striatal neurons. Male Sprague-Dawley rats received bilateral intrastriatal injections of QA (75,100,150 nmol) or vehicle (n=6 per group). After 21 days striata were dissected and stored at -70°C.

	Control	75 nmol	100 nmol	150 nmol
CAT (umoles/	14.3±0.7	12.7±0.7	10.9±0.7	9.1±1.2
(hr/g tissue)	(100)	(88.8)	(76.2)	(63.6)
[^{3}H]MK801	28.2±1.4	22.2±2.6	15.3±0.8	14.9±1.0
(pmoles/g tissue)	(100)	(78.7)	(54.3)	(52.8)
[^{3}H]SCH23390	65.0±1.7	38.0±6.3	22.9±2.6	18.8±4.7
(pmoles/g tissue)	(100)	(58.5)	(35.2)	(28.9)

Values in parentheses represent % control.

CAT containing neurons in rat striatum are relatively resistant to QA-induced toxicity and therefore may not represent an appropriate neurochemical assay. D_1 dopamine receptors are very sensitive to QA-induced toxicity and may represent a very good neurochemical assay. Interestingly, the loss of only 47% of [^{3}H]MK801 binding sites indicates that a population of [^{3}H]MK801 binding sites may not be associated with QA-sensitive NMDA receptors. Supported by NINDS.

89.6

A PARALYTIC DOSE OF DYNORPHIN 1-13 IN THE RAT SPINAL CORD FAILS TO ENHANCE THE RELEASE OF EXCITATORY AMINO ACIDS BUT DECREASES THE EXTRACELLULAR CONCENTRATION OF GLUTAMINE. D. H. Smullin, S. R. Skilling* and A. A. Larson. Department of Veterinary Biology, University of Minnesota, St. Paul, MN 55108.

Excitatory amino acid (EAA) antagonists have been shown to protect against dynorphin (DYN)-induced neurotoxicity in the spinal cord (Brain Res 507 1-5 1990). We tested the hypothesis that the neurological damage produced by intrathecal DYN, therefore, involves facilitated release of neurotoxic concentrations of the EAA neurotransmitters aspartate (Asp) and glutamate (Glu) into the spinal cord ECF. Using microdialysis, Asp and Glu release in the lumbar spinal cord of conscious rats was monitored before and after intrathecal injection of 20 nmoles of DYN 1-13 . While 100% of the animals became paralyzed within 10 min of the DYN injection, we did not observe a significant increase in the average release of either Asp or Glu during the 60 min collection period following injection. A portion of the animals did show release of Asp and Glu, however, there was no correlation between release and the degree of neuronal damage visualized histologically. These results argue against EAA release as a mechanism for DYN-induced paralysis or neurotoxicity. We did observe, however, a significant decrease (40%) in the ECF concentration of glutamine (Gln) following DYN in all of the animals tested. Previous research has shown that, in the absence of Gln, Glu is not neurotoxic (J. Neurochem 52 1694-1699) and that EAA antagonists can inhibit Gln-induced changes in Glu release (Abstr. Soc Neurosci. 15 480, 1989). The present results, therefore, support the hypothesis that DYN-induced neurotoxicity involves an enhanced uptake of Gln and its subsequent conversion into ammonia and glutamate within the neuron. Alternatively, the decrease in extracellular Gln could result in a decrease in its conversion to GABA. Loss of this inhibitory transmitter could also play a role in the neurotoxic effects of DYN. (Supported by USPHS Grants DA04090, DA00124, DA04190, DA07234 and CA01342)

89.7

SUBSTANCE P[5-11] INHIBITS NMDA-INDUCED NEUROTOXICITY BOTH *IN VITRO* AND *IN VIVO*. J.B. O'Neill, H. Jaffe*, J.M. Hill, G.M. Barbour*, L.D. Kwart*, D.I. Lambie*, W.D. Bowen[1], M.R. Ruff*, and C.B. Pert*. Peptide Design L.P., Germantown, MD 20874, [1]Brown University, Providence, RI 02912.

Recently we isolated a peptide from rat brain with phencyclidene-like properties and identified it as substance P (1). We now report that a heptapeptide fragment of substance P, SP[5-11], which we have termed alpha-neuroprotectin (αNP), potently inhibits binding of the NMDA receptor antagonists, [^{3}H]-TCP and [^{3}H]-CGS 19755, to rat brain membranes, without affecting binding of [^{3}H]-DTG to the sigma receptor (guinea pig membranes). NMDA-induced toxicity of mouse hippocampal neurons was potently and completely prevented by αNP (EC_{50}, 1pM) and substance P. αNP was not itself neurotoxic at concentrations up to 10^{-5} M under these conditions.

Additionally, *in vivo* administration of αNP protected against NMDA lethality in newborn Sprague Dawley rat pups in a dose-dependent fashion. Further studies showed that an NMDA-induced behavioral deficit in young rats, as measured by observing the air righting reflex, was also prevented by αNP. Structure-activity and receptor binding studies indicate that the neuroprotection isn't mediated through the classical substance P receptor. Therefore, αNP antagonizes the neurotoxic effects of NMDA *in vitro* and *in vivo* by a mechanism probably related to the interaction of this peptide with the NMDA receptor complex.

1)Jaffe, H., *et al., Int. J. Biochem.*, **22**: 239 (1990).

89.8

NEUROPROTECTIVE PHARMACOLOGY OF AMPA AND QUISQUALATE INDUCED BRAIN INJURY. M.V. Johnston, J.W. McDonald and W.H. Trescher, The Johns Hopkins University and Kennedy Institute, Baltimore, MD 21205.

The neuroprotective characteristics of antagonists of quisqualate (QUIS) and NMDA receptors, and anticonvulsant drugs were evaluated against AMPA and quisqualate induced brain injury in perinatal rats. PND 7 rats received unilateral intrastriatal stereotaxic injections of either 10 nmol AMPA or 100 nmol QUIS and either a potential neuroprotective compound or an equivalent volume of saline (n = 5-10 per group). MK-801 and anticonvulsant drugs were administered i.p. 15 minutes after intrastriatal excitotoxin injection and CNQX was co-injected with excitotoxins intrastriatally. The severity of brain injury was quantitated 5 days later by comparison of the weights of injected (I) and contralateral (C) cerebral hemispheres; %damage=100(C-I)/C. Data are presented as %protection (mean±SEM), a term that reflects the degree of damage in neuroprotective groups vs saline treated groups. Coinjection of CNQX, a competitive ionotropic quisqualate receptor antagonist, markedly reduced both AMPA and QUIS neurotoxicity (AMPA, 10 nmol CNQX = 37±2% protection, $p<0.05$, 20 nmol CNQX = 68±7% protection, $p<0.001$; QUIS, 10 nmol CNQX = 43±15% protection, $p<0.05$, 20 nmol CNQX = 67±7% protection, $p<0.01$). Treatment with the non-competitive NMDA receptor antagonist MK-801 (1 mg/kg, i.p.) significantly reduced both AMPA (32±8% protection, $p<0.01$) and QUIS (21±4% protection, $p<0.05$) toxicity. Combined treatment with both CNQX (10 nmol) and MK-801 (1 mg/kg) produced near complete neuroprotection (AMPA, 92±5% protection, $p<0.001$; QUIS, 79±13% protection, $p<0.001$). The anticonvulsant drugs diazepam (1-10 mg/kg) and phenytoin (40-80 mg/kg) effectively limited QUIS toxicity but not AMPA induced brain injury. The maximal protection against QUIS toxicity was 69±17% for diazepam (10 mg/kg, $p<0.01$) and 46±22% for phenytoin (80 mg/kg). The data provide an initial characterization of the in vivo pharmacology of non-NMDA receptor mediated excitotoxic brain injury.

89.9

MK801 TRANSIENTLY DECREASES CEREBRAL TEMPERATURE IN PERINATAL RATS. C.-K.Chen, J.W.McDonald, W.H.Trescher and M.V. Johnston, Kennedy Research Institute and The Johns Hopkins University School of Medicine. Baltimore, MD 21205

MK801, a noncompetitive NMDA receptor antagonist, has neuroprotective activities on focal ischemic-hypoxic and NMDA-induced brain injury in perinatal rats (McDonald et al, 1987). Recently it has been suggested that hypothermia induced by MK801 may account for its neuroprotection. In this study, we investigated the effect of temperature on NMDA-induced brain injury and the effect of MK801 on temperature in perinatal rats.

In 7-day-old rats, under ether anesthesia, NMDA (25 nmol) was injected into the right striatum and the rats were then put in an incubator at various temperatures(40°, 36°, 33°, 31°, 28° and 25°C) for 2 hr. The degree of unilateral brain injury was assessed by disparities in cerebral hemisphere weights 5 days later. Lower ambient temperature decreased the degree of brain injury and there was a linear correlation between the ambient temperature and injury(R^2=0.991). To study the relationship between the body and ambient temperatures, a filament-thin temperature probe was inserted into the right striatum of an additional group of 7-day-old rats. Rat pups were then put in an incubator at 36°, 33° and 30°C. There was a linear correlation between cerebral, skin and ambient temperatures (R^2≥0.88). To assess the effect of MK801 on body temperature, MK801(1mg/kg) was injected ip into rat pups. The cerebral temperature decreased slightly (0.98±0.08°C, n=4) immediately after the injection and then increased gradually and coincided with the controls 40 min later. From the correlation between temperature and brain injury(2.48% change damage/°cerebral temp.), we estimated that the temperature difference induced by MK801 could decrease NMDA-induced brain injury by 2.4%.

In this model, there is an excellent correlation between ambient, skin and cerebral temperatures. Although MK801 transiently decreased cerebral temperature, our data indicate that this change accounted for less than 3% of the drug's neuroprotective effect.

89.10

Effects of L-N^G-monomethylarginine and Sodium Nitroprusside on Kainate Mediated Toxicity in Rat Cerebellar Granule Cell Cultures. W.E. Lyons, P.S. Puttfarcken, and J.T. Coyle, Dept. of Psychiatry. Johns Hopkins Sch. of Med., Baltimore, MD 21205.

The characterization of kainate (KA) induced toxicity in rat cerebellar granule cell cultures, has demonstrated a lack of correlation between osmotic imbalance, receptor expression, and KA mediated cell death (Kato, K., et al. *submitted*). This discrepancy suggests that other events may be critical for the induction of a KA mediated toxic response. To elucidate a mechanism associated with KA mediated toxicity, the involvement of receptor linked transmembrane processes was examined in cerebellar granule cell cultures. Previous studies have demonstrated that KA receptor activation ultimately leads to the synthesis of nitric oxide (NO) from arginine in slices of rat cerebellum (Garthwaite et al., 1989). In the current study, the role NO may play in the toxic response elicited by KA was evaluated. Dose response curves performed in the presence of varying concentrations (100 nM to 1 mM) of L-N^G-monomethylarginine (MA), a competitive inhibitor of the NO generating enzyme, indicated that MA produced a dose-related depression of maximally stimulated KA (500 μM) toxicity. At a concentration of 300 μM, MA produced a maximum inhibition of approximately 50-60%. Furthermore, the addition of a subtoxic dose (300 μM) sodium nitroprusside (NP), a compound which spontaneously generates NO, greatly potentiated KA induced toxicity. KA was more potent in eliciting a toxic response in presence of 300 μM NP, as indicated by the leftward shift in the KA dose response curve. These data suggest that NO may be involved in mediating the toxic effects of KA in cerebellar granule cell cultures.

89.11

THE DEVELOPMENTAL TIME COURSE AND IONIC DEPENDENCE OF KAINATE MEDIATED TOXICITY IN CEREBELLAR GRANULE CELLS. P.S. Puttfarcken, K. Kato, W.E. Lyons, and J.T. Coyle, Dept. of Psychiatry. Johns Hopkins Sch. of Med., Baltimore, MD 21205.

In the presence of physiological concentrations of Mg^{2+} and in the absence of glycine, the mechanisms associated with kainate (KA) induced toxicity were examined in cerebellar granule cell cultures. Pharmacological characterization indicated toxicity was mediated exclusively through the KA receptor in these cells. Two separate components of toxicity were observed and differentiated according to time of onset, morphological change, and ionic dependence. Acute toxicity, as determined by the release of lactate dehydrogenase (LDH) after 30 min of 1 mM KA exposure, was apparent betweeen 8-11 days in culture (DIC) and was dependent on both Na^- and Cl^-. Removal of Ca^{2+} intensified acute toxicity. Vulnerability to acute toxicity did not correlate with KA receptor expression (5 DIC) nor receptor mediated Cl^- influx (8 DIC). Delayed neurotoxicity, as determined by LDH release 24 hrs after KA exposure, was apparent after 8 DIC and dependent on Cl^-. Removal of Ca^{2+}, during the 30 min drug exposure, did not alter delayed toxicity. Under conditions in which acute toxicity was not detected (-Na^-), KA mediated cell death was still apparent. These observations, together with the difference in onset and ionic dependence, suggest that the mechanisms involved for each component are different.

89.12

PROLINE: A POTENTIAL EXCITOTOXIN IN THE MAMMALIAN CENTRAL NERVOUS SYSTEM. S.W. Helm*, D.B. Reichling and A.B. MacDermott. Dept. of Physiology and Center for Neurobiology and Behavior, Columbia University, New York City, NY 10032.

The amino acid proline (Pro) has been implicated as an endogenous excitatory agonist, with partial activity at the NMDA receptor (Nadler, et al, 1989). We have attempted to further define the receptor subtypes involved in Pro's excitatory activity. Multiple concentrations of Pro were screened for excitatory activity using whole cell recording. Solutions were applied to voltage-clamped embryonic rat spinal cord neurons in culture, and the responses compared to those obtained with glutamate (Glu). Application of 3.0 mM Pro or 1.0 uM Glu resulted in currents of nearly equal amplitude. APV (50 uM) suppressed the response to Pro by 25-50% in all cells tested, and glycine (5.0 uM) potentiated the response to Pro, consistent with an effect on the NMDA receptor. An additional 25-50% of the Pro response was inhibited by CNQX (30 uM). In about 50% of the cells screened, part of the current activated by Pro was resistant to APV and CNQX. Thus Pro activates both NMDA and non-NMDA subtypes of Glu receptor and, in addition, can induce an APV/CNQX-insensitive response. When applied to cells loaded with the calcium indicator indo-1, 3.0 mM Pro resulted in a significant increase in $[Ca^{2+}]_i$. This effect was virtually eliminated by the addition of 10 uM Mg^{2+}, suggesting the NMDA receptor as the primary route of calcium entry following Pro activation. The significance of Pro's excitatory action is two-fold. First, the APV/CNQX-insensitive component of the Pro response suggests a possible unique role for Pro in excitatory transmission. Second, the Pro-induced elevation in $[Ca^{2+}]_i$ implicates Pro as a potential neurotoxin in the mammalian CNS.

89.13

EXTRACELLULAR GLYCINE, BUT NOT GLUTAMATE, REMAINS ELEVATED AFTER TRANSIENT GLOBAL CEREBRAL ISCHEMIA IN THE RABBIT. A. J. Baker,* M. H. Zornow, M. S. Scheller,* T. L. Yaksh, S. R. Skilling,* D. H. Smullin, A. A. Larson and R. Kuczenski Neuroanesthesia Research Lab., Univ. of California, San Diego, La Jolla, CA 92093 & Univ. of Minnesota, St. Paul, MN 55108

The neuronal injury that results from transient global cerebral ischemia continues to evolve in the post-ischemic period. Significant elevations in extracellular concentrations of putative mediators of ischemic neuronal injury (*eg* glutamate or dopamine) however, return to baseline immediately upon reperfusion. This raises questions about the mechanisms of post-ischemic injury. Using a model of transient global cerebral ischemia in the rabbit, extracellular hippocampal glutamate, aspartate and glycine, and striatal dopamine and serotonin were measured using microdialysis with high temporal resolution during and after ischemia. The concentrations of glutamate and aspartate increased to peak levels that were dependent on ischemic duration but returned briskly to baseline levels with reperfusion. There was a massive elevation of extracellular dopamine, which also returned to baseline after reperfusion. Glycine concentrations increased during ischemia but in contrast to the previous neurotransmitters, remained elevated throughout the 80 min reperfusion period. Glycine has recently been shown to facilitate the activity of glutamate at the NMDA receptor. The sustained elevation of glycine concentrations after ischemia therefore, may explain the apparent ongoing toxicity of glutamate in the reperfusion phase despite prompt return of glutamate to baseline concentrations. These results may also have implications for new avenues of therapeutic intervention.

89.14

CHANGES IN BINDING TO THE N-METHYL-D-ASPARTATE (NMDA) RECEPTOR COMPLEX IN RAT HIPPOCAMPAL SUBREGIONS AT VARIOUS TIMES FOLLOWING RECOVERY FROM INSULIN-INDUCED HYPOGLYCEMIA. L.P.Miller, D. Panchison*, and R.N.Auer, Lab Neurosci Vet Adm Med Ctr, Dept Pharm, Georgetown Univ Sch Med, Wash D.C.20007 & Dept Path, Hlth Sci Ctr, Univ Calgary Alberta, Canada T2N 4N1

Insulin-induced hypoglycemia with attending isoelectric(ISO)EEG leads to neuronal damage in the neostriatum, hippocampal dentate gyrus (DG), subiculum, CA1 and outer cortical regions (Auer et al 1984). Cumulative evidence now supports excitotoxic mechanisms (Wieloch 1985) as an underlying cause of striatal cell damage.The present investigation explored the etiology of cell death in the hippocampus by examining the effect of 30 min hypoglycemia-induced ISO on binding to NMDA receptor complex within this region. We examined 4 sets of experimental animals: controls, 30 min ISO + no recovery,30 min ISO + 1 day recovery and 28 day recovery. Using 12 micron coronal sections components of the NMDA receptor complex were characterized in various subregions of the dorsal hippocampus as: A) 3H-glycine binding to the modulator site, B) 3H-TCP binding to the ion channnel portion of the complex and C) 3H-glutamate binding to the recognition site. Quantitative autoradiography was performed using a LOATS RAS 1000 image analysis. Our results showed differential changes :

PERCENT OF CONTROL (*p<0.050)

	CA1			DG-external blade			DG-crest		
Treatment	Gly	Glut	TCP	Gly	Glut	TCP	Gly	Glut	TCP
ISO 30 min	77*	108	102	86	112	102	82	106	103
" + 1D rec	81*	94	93	88	95	94	66*	96	93
" + 28D rec	87	79*	71	86	89*	80	69*	75*	80*

89.15

RAT STRAIN DIFFERENCES IN KAINIC ACID (KA) NEUROTOXICITY III: INFLUENCE OF AGE. G.T. Golden, G.G. Smith*, J.H. Kulp*, P.F. Reyes and T.N. Ferraro. VA Med. Ctr., Coatesville, PA 19320 and Thomas Jefferson Univ., Philadelphia, PA 19107.

Adult Wistar-Furth (WF) and F344 rats are exceptionally sensitive to the neurotoxic effect of KA, reliably demonstrating EEG and behavioral status epilepticus in response to a 10 mg/kg, sc dose. Responses of SD and LEH rats are much more variable. We now report dose-response data for juvenile (35-40 days old) and adult (70-90 days old) WF, F344, SD and LEH rats. Juvenile male WF (n=51), F344 (n=55), SD (n=60), LEH (n=50) and adult male WF (n=48), F344 (n=52), SD (n=52), LEH (n=53) rats were given KA 6, 8, 10, 12 or 14 mg/kg, sc. In adults, WF and F344 strains demonstrated the greatest sensitivity and most reliable convulsant responses to KA; SD and LEH strains were less sensitive and and showed more variable convulsant responses. Regardless of strain, all juvenile rats exhibited greater sensitivity and less variable convulsant responses to KA compared to adults. This was most evident in juvenile SD and LEH rats, however the rank order of sensitivity and seizure proneness observed among juveniles was the same as adults (WF = F344 > SD > LEH). Mortality rates were equivalent for adults and juveniles when collapsed across all doses. Compared to adults, however, juvenile mortality rates were higher at the lower KA doses (6, 8 and 10 mg/kg) and lower at the higher doses (12 and 14 mg/kg). Results suggest that while seizure sensitivity to KA decreases with age, genetic factors may regulate the expression of this resistance. (Supported in part by VA funds.)

89.16

OUABAIN INCREASES EXTRACELLULAR GLUTAMATE AND ASPARTATE IN HIPPOCAMPAL SLICES. J. E. Madl. Dept. of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523.

Ouabain and other cardiac glycosides can produce signs of CNS toxicity when given in high doses. Ouabain is an inhibitor of the Na/K ATPase and has been reported to release several types of neurotransmitters, including GABA. Extracellular accumulations of glutamate (Glu) and aspartate (Asp) have been implicated in many types of CNS disease and dysfunction. The possibility that ouabain might cause extracellular accumulations of Glu and Asp was therefore examined in hippocampal slices.

Transverse, 400µm sections of hippocampus were obtained from adult rats and washed 3 times, 15 min per wash, in Hank's balanced salt solution with an additional 10mM glucose and 10 mM HEPES (HBSS, pH 7.3) to remove amino acids released by cutting the slice. Slices were incubated for various periods of time in 4 ml of the same HBSS in a humidified incubator under 5% CO_2. The concentrations of Glu and Asp in the supernatants of slices incubated with 100 µM ouabain were increased more than 10-fold over the supernatant of control slices. In contrast, supernatant concentrations of the asparagine increased only 2-3 fold.

These results suggest that ouabain may cause a selective extracellular rise of Glu and Asp. This extracellular rise may be due in part to decreased Na-dependent uptake of Glu and Asp by glia from decreases in the Na-gradient of the plasma membrane. The extracellular rise of Glu and Asp may contribute to the CNS signs of toxicity seen with cardiac glycosides.

89.17

SPINAL CORD ISCHEMIA-INDUCED ELEVATION OF EXTRACELLULAR AMINO ACIDS AS MEASURED BY MICRODIALYSIS. R.K.Simpson, C.S.Robertson, and J.C.Goodman, Dept.Neurosurg.,Baylor Col.of Med.,Houston,TX,77081

A simple yet reliable model of spinal cord ischemia has been developed by inserting a catheter into the abdominal aorta of rabbits and inflating the balloon just inferior to the renal arteries. Recent investigations have shown that paraplegia is consistently reproduced if the balloon is inflated for 20 minutes after loss of the N_3 component of the somatosensory evoked potential (SEP). Because of its high reliability, this model has been successfully used to study several biochemical events that occur as a consequence of ischemia-induced spinal cord damage. Our study focused on the role of excitatory amino acids in acute, severe spinal cord injury by measuring changes in the extracellular concentrations of amino acid neurotransmitters using microdialysis techniques. Dialysis tubing, 0.2 mm in diameter, with a molecular weight cut-off of 6000 was inserted through the spinal cord via a laminotomy and perfused with artificial cerebrospinal fluid at 5 µl/min. Samples were collected prior to, and during the ischemic period immediately following loss of the N3 SEP componentproduced by sciatic nerve stimulation. Samples were also collected immediately upon reperfusion, and 20 min, 40 min, and 60 min after reperfusion. Glutamate, aspartate, glutamine, asparagine, glycine, taurine, valine, and leucine were measured in the microdialysis perfusate by high pressure liquid chromatography. The concentrations of glutamate, glycine, and taurine were significantly higher during ischemia and reperfusion than controls. Delayed elevations in the concentrations of asparagine and valine were also detected. The elevation of glutamate is consistent with the hypothesis that excitotoxins may mediate neuronal damage in the ischemic spinal cord. Increased extracellular concentrations of asparagine and valine may reflect preferential use of amino acids for energy metabolism under ischemic conditions. The significance of increased levels of inhibitory amino acid neurotransmitters is unclear and is the subject of a future study.

89.18

SELECTIVE NEURON DAMAGE IN THE RAT SPINAL CORD INDUCED BY ACROMELIC ACID. S. Kwak[1], H. Aizawa*[1], M. Ishida[2] and H. Shinozaki[2]. [1]National Institute of Neuroscience, Tokyo 187, [2]The Tokyo Metropolitan Institute of Medical Science, Tokyo 113, Japan.

A single systemic injection of acromelic acid, a novel kainate analogue, caused long-lasting spastic paraparesis in the rat. Two rats developed paraparesis were neuropathologically examined one week and three months after the injection, respectively. Numerous chromatolytic neurons with marked reactive gliosis were scattered in the gray matter of the spinal cord of the rat paralytic for one week. Degenerated neurons were most abundant at lumbar and sacral segments but scanty in laminae I, II and IX of Rexed. The cytometry on the 1st sacral segment disclosed that the number of small size neurons was significantly decreased in both rats. The rest of the central nervous system was intact except for the slight increase of gliosis in CA4 of the hippocampus. Above findings indicate that acromelate selectively destroyed interneurons in the caudal spinal cord presumably through excitotoxic mechanism, which induced spastic paraparesis in the rat. These behavioral and neuropathological changes in the rat induced by acromelic acid are quite different from those by kainic acid and suggest that acromelic acid may act on a different glutamate receptor subtype from the kainate receptor in the rat central nervous system when injected systemically.

89.19

VISUALIZATION OF GUANYLATE CYCLASE IN CULTURED CNS NEURONS. Jonathan Chen, Bing Chang*, Michael Heller*, and Ferid Murad*. Abbott Laboratories, Pharmaceutical Product Division, Abbott Park, IL 60064

The relevance between glutamate neurotoxicity and cGMP accumulation is fundamental to the understanding of many neurological disorders. Glutamate induced neuron death may be attributed to cGMP increases intracellularly. Therefore, guanylate cyclase (GC), the enzyme that catalyzes cGMP formation through EDRF/NO may be essential for the process of neuronal degeneration.

In order to test this hypothesis, rat embryonic CNS neurons were cultured. Fluorescent immunocytochemical studies showed that soluble GC specific monoclonal antibodies react quantitatively with cell bodies and dendritic - axonal processes. Results also showed sodium nitroprusside stimulated cGMP accumulation, indicating that the cultured CNS neurons express functional soluble GC activity *in vitro*.

In summary, we have found cultured CNS neurons a plausible model for cyclic GMP and guanylate cyclase studies. Whether EDRF/NO affects the process of neuronal degeneration is the central interest for our future studies.

90.1

AN IN VIVO MODEL OF NMDA EXCITOTOXICITY IN RATS: SUPRASPINAL VERSUS SPINAL SITES OF ACTION. L. Robles, E. Echevarria, J. E. Moreton, J. B. Long and F. C. Tortella, Neuropharmacology Br., Div. of Neuropsychiatry, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

We recently described an in vivo EEG and behavioral model of NMDA excitotoxicity in rats (Robles et al., FASEB J. 4: 1990) where i.v. NMDA (100 mg/kg) produced a continuum of responses marked by 1) initial non-convulsive (NC) spike-wave EEG complexes followed by 2) episodes of vigorous behavioral "running fits" (RFs) immediately preceding 3) full clonic "popcorn" (PC) convulsions and 4) culminating in tonic forelimb extension and death. However, it was observed that the EEG associated with the PC convulsions was not always reflective of ictal activity prompting speculation that supraspinal and spinal sites of action may be involved. This issue was studied by treating rats, under identical experimental conditions, with i.c.v. (12.5 nmol; n=5) or i.t. (25 nmol; n=9) administered NMDA. The immediate response to i.c.v. NMDA was PC convulsions associated with high-amplitude EEG slow-waves and intermittent spikes followed by multiple episodes of NC EEG seizures. At higher doses rostrally-directed scratching and brief RFs emerged several minutes postinjection. In contrast, the excitotoxic profile for i.t. NMDA was temporally reversed: i.e. immediate (albiet moderate) RF activity with ataxia followed by jacknife posturing of the body, caudally directed preening, and vocalization. Although EEG spike-wave NC seizures occurred several minutes postinjection, PC convulsions were not observed after i.t. NMDA.

90.2

AGE- AND DOSE-RELATED EFFECTS OF INTRAHIPPOCAMPAL IBOTENIC ACID ON NEURAL DEGENERATION AND MEMORY-BASED LEARNING IN THE INFANT RAT. N.J. Lobaugh, D.S. Norton*, & A. Amsel. Dept. of Psychology and Inst. for Neuroscience, University of Texas at Austin, 78712.

Ibotenic acid (IBO) is an effective neurotoxin in the dorsal hippocampus in 5 and 7-day-old (DO) rat pups (Cook & Crutcher, Neurosci. 18:79, 1986; Steiner, et al., Brain Research, 307:117, 1984). In the present study, four unilateral injections of IBO were made through the extent of the hippocampus in either 11 or 15 DO rat pups, using 1 or 5 µg/µl (in 1 or .5 µl) with 3 or 7 days survival. The hippocampus was more susceptible to IBO at 11 than at 15 days: 1µg resulted in an absence of pyramidal- and granule-cell layers, while 5µg produced complete destruction of the hippocampus at the injection sites. At 15 days, 1µg produced minimal damage that was restricted to injection sites, and 5µg produced damage similar to that seen in the 11-DO hippocampus after 1µg. Fink-Heimer degeneration was not seen in 11-DO pups, or in 15-DO pups that received 1µg injections. In 15-DO pups that received 5µg/µl injections, 62% showed degeneration in the medial corticohabenular tract, with no apparent cell loss in the habenula or in septum.

We assessed the behavioral effects of this lesion following bilateral injections of IBO using a discrimination task that requires intact short-term memory of the previous trial, and found only small effects of the lesion at the 1µg dose: Pups injected at 15 days and allowed to survive 7 days showed a reduction in the magnitude of the discrimination compared to saline controls. This grant was supported by NSF Grant BNS 8609877.

90.3

KAINATE NEUROTOXICITY IN AGED RATS: A MODEL FOR STUDYING DOMOATE DEMENTIA IN AGED HUMANS. D.F. Wozniak, G.R. Stewart, B. Fry*, L. Kettinger* and J.W. Olney, Washington University, St. Louis, MO 63110.

Domoate (Dom) is a kainic acid (KA) analog recently implicated in a human seafood poisoning incident in Canada which resulted in convulsions and death or longterm neuropsychological deficits (profound memory loss) in some victims, with elderly individuals being the most severely affected (J. Teitlebaum, in I. Hynie & E. Todd, Eds, *Proc. Symp. on Domoate toxicity*, in press, 1990). Dom expresses neurotoxic (excitotoxic) activity *in vitro* by an action at the KA subtype of glutamate receptor and, when administered to adult rats, Dom mimics KA in causing status epilepticus and a severe seizure-related brain damage syndrome (See Stewart et al., Soc. Neurosci, Abst., 1990). Because Dom is prohibitively expensive, we explored the feasibility of using KA to study the age dependency of Dom neurotoxicity. Young (6 mos), middle age (12 mos) and old (24 mos) rats (n = 30, 23, 24 respectively) were compared for sensitivity to the KA neurotoxic syndrome. Doses of KA (mg/kg sc) that induced the syndrome in 50% of young, middle age and old rats (ED_{50} and 95% confidence levels) were 9.04 (7.61-10.99), 3.87 (2.36-5.56), 2.06 (1.02-3.31) respectively. Thus, sensitivity increased markedly in the 6 to 12 mos interval and continued to increase, but at a slower pace, between 12 and 24 mos. Whether the age-related sensitivity to KA neurotoxicity is mediated by a CNS or peripheral mechanism warrants further study. Resolving this question in KA-treated rats may help clarify the extreme sensitivity of aged humans to Dom neurotoxicity. Supported by MH14677 (DFW), AG05681 & MH38894 (JWO).

90.4

EFFECT OF COMPETITIVE AND NON-COMPETITIVE EAA ANTAGONISTS ON DOMOIC ACID TOXICITY IN MICE. S.M. Strain* and R.A.R. Tasker. Dept. of Anatomy & Physiology, Atlantic Veterinary College, Univ. of P.E.I., Charlottetown, P.E.I., Canada, C1A 4P3

In December of 1987, a number of individuals suffered seizures and memory loss following the consumption of contaminated mussels. The toxin responsible was identified as domoic acid (DOM), an excitatory amino acid (EAA) similar to kainic acid. The present study was designed to investigate the effects of both competitive and non-competitive EAA antagonists on i.p. DOM toxicity. Female CD1 mice (20-25g) were injected (i.p.) with an ED50 (100 ug) of DOM and were scored for toxicity behaviors for 60 minutes. Antagonists (or saline) were injected 15 minutes before DOM. Antagonists tested were DNQX (50 & 100 mg/kg), MK801 (0.1 & 0.3 mg/kg), kynurenic acid (200 & 400 mg/kg), GDEE (50 & 75 mg/kg), and dextrorphan (20 & 40 mg/kg). DNQX, MK801, kynurenic acid and GDEE produced no significant change in behavioral toxicity scores when compared to either vehicle control or antagonist control values. Dextrorphan (20 mg/kg), however, produced a 50% reduction in the time spent in seizure activity and significantly decreased the overall toxicity score ($p<0.01$). We are currently investigating the pharmacological and histopathological effects of dextrorphan on DOM toxicity in greater detail.

90.5

SYNERGISM BETWEEN EXCITATORY AMINOACIDS IN CANADIAN TOXIC MUSSELS. A. Novelli, J. Kispert*, S. Gascón*, M. Baraldi and V. Zitko*. Dept. Biochem., Sch. of Med. 33006 Oviedo, Spain; Lab. of Bioph., I.S.A.S., 34014 Trieste, Italy; Lab. Mol. Biol., NINDS, Bethesda, MD 20892, USA; Lab. of Pharmac., Sch. of Pharm., 41100 Modena, Italy; Marine Chem. Div., Biol. Station, St. Andrews, N.B. E0G2X0,Canada.

During the period November-December 1987, more than 100 Canadians suffered from acute intoxication after eating cultured mussels. For many of them, symptoms included disorientation, confusion and memory loss, long-lasting at times. The mussels were found to contain high amounts of domoic acid (DA), a rare excitatory amino acid, agonist at the NON-NMDA type of excitatory aminoacid receptors. We used rat cerebellar neurons in primary culture (10-30 days in vitro) as an experimental system where to characterize the neurotoxicological properties of a "toxic mussels" extract (TE). Neurotoxic effects of TE, DA on cerebellar neurons in primary culture were compared following 24 hrs exposure in the growth medium. Purified DA was less neurotoxic (Tox_{50}~7,4µM) than comparable concentrations of DA within the TE (Tox_{50}~1µM DA). Quisqualic acid (QA=100µM) selectively antagonized DA neurotoxicity. However, full protection from TE neurotoxicity was achieved only by simultaneous addition of QA + APV(1mM) or QA + Mk-801(1µM), indicating the presence of NMDA receptor agonists. A "normal mussels" extract (NE) was than tested for neurotoxic effects. NE induced a dilution-dependent neurotoxicity which was fully antagonized by APV or MK-801. Similarly, glutamic acid (GA) neurotoxicity (Tox_{50}~13µM) was antagonized by APV or by MK-801. Both TE and NE resulted to contain similar amounts (in µmol/gr fw) of aspartic acid (AA) and GA . Neurotoxicity by NE occurred at higher concentrations (Tox_{50}~20µM AA+GA) than neurotoxicity by TE (Tox_{50}~8µM AA+GA). On the other hand, 50% neurotoxicity was produced by TE with an internal concentration of DA~1µM, lower than the concentration required by purified DA (~7.4 µM). A synergism factor (SF) between excitatory aminoacids equal to ~ 3.3 was calculated. We suggest that SF may have been responsible for the neurological problems reported in humans who ingested "toxic mussels".

90.6

BEHAVIORAL INDEX OF A "WINDOW OF VULNERABILITY" TO PERINATAL MONOSODIUM GLUTAMATE. K. Fisher*, R. Turner*, J. Kleim*, G. Pineault*, and M. J. Saari, Neuroscience Research Unit, Nipissing University College, North Bay, Ontario. P1B 8L7.

Perinatal monosodium glutamate (MSG) administration to rats causes significant organ system dysfunction, developmental anomalies and behavioral deficits. Injections administered after day 10 post partum do not appear to lead to similar damage. In this experiment we demonstrate that rat pups are maximally influenced by MSG when the amino acid is administered before 6 days of age. Male and female Wistar rat pups were cross fostered and injected with MSG (4g/kg; 20% aqueous solution; s.c.) or saline vehicle on days 2, 3, 5, 7 and 9 post partum. The surface righting reflex was tested twice daily from day 3 to day 9. The results support the interpretation that rat pups are particularly sensitive to MSG during the first 5 days post partum and that this sensitivity is reduced by day 7 to control levels. It is likely that the long lasting neurotoxic effects of MSG administration in rats is due to a specific vulnerability present in the early post natal period.

90.7

EXTREME SENSITIVITY OF INFANT ANIMALS TO GLUTAMATE TOXICITY: ROLE OF NMDA RECEPTORS. G.J. Wang, J. Labruyere*, M.T. Price, J.W. Olney. Washington University, St. Louis, MO 63110.

Infant animals are much more sensitive than adults to hypothalamic damage following oral or subcutaneous administration of glutamate (Glu). That this can be attributed to "weakness" of blood brain barriers during infancy is a common misconception; the arcuate hypothalamic (AH) nucleus, like other circumventricular organs, has no blood brain barrier protection either in adulthood or infancy. In theory, Glu excitotoxicity can be expressed through either N-methyl-D-aspartate (NMDA), kainic acid (KA) or quisqualic acid (Quis) receptors. However, in view of evidence that NMDA receptors are hypersensitive and non-NMDA receptors hyposensitive to excitotoxic stimulation during development, Glu neurotoxicity in the immature CNS may be expressed predominantly through NMDA receptors. Supporting this view is evidence that, in the infant rat, NMDA antagonists provide excellent protection against hypoxic/ischemic brain damage (thought to be mediated by endogenous Glu). Here we show that NMDA receptor antagonists, when co-administered systemically with Glu to immature rats, are highly effective in preventing Glu from destroying AH neurons. This suggests that Glu neurotoxicity in the immature AH is mediated primarily by NMDA receptors; thus, the tendency of immature NMDA receptors to be hypersensitive to excitotoxic stimulation may help explain the extreme sensitivity of immature AH neurons to Glu-induced damage. Supported by HD24237, DA05072 & RSA MH38894 (JWO).

90.8

ONTOGENETIC DEPLETION OF SOMATOSTATIN AND NEUROPEPTIDE Y IN THE RAT STRIATUM. M.A. Musgrave*, R.J. Boegman, J.V. Milligan. Dept. of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Somatostatin (SS) and neuropeptide Y (NPY) are two neuropeptides implicated in several neurodegenerative disorders including Alzheimer's and Huntington's diseases. The actual role played by these peptides in disease states is as yet undetermined. The consequences of neuropeptide depletion in terms of neuromodulatory and behavioral effects are not well investigated. With this in mind, the focus of the present study was to excitotoxically deplete SS and NPY in a discrete brain area to develop a possible working model for further investigations of neuropeptide influence. The area chosen for investigation was the striatum due to the colocalization of both peptides with the enzyme NADPH-diaphorase. Rat pups were lesioned with N-methyl-D aspartate (NMDA) at either of two points in post natal development (PND 23 or PND 30). Lesions were accomplished using volumetric doses (0, 0.5, 1, 2, or 3 μl) of NMDA at one of two concentrations (50 nm/μl or 25 nm/μl). At both PND ages, a double histochemical staining technique for NADPH-diaphorase and Acetylcholinesterase (AChE)-positive neurons revealed an approximate 75% depletion of NADPH-diaphorase positive staining, compared to the contralateral side, with either 2 or 3 μl of NMDA (25 nm/μl). At both volumes AChE-positive staining was found to be unchanged compared to the contralateral side. The neuromodulatory and behavioral consequences of such depletions are currently being investigated. (Supported by the Medical Research Council of Canada).

90.9

FOCAL INJECTIONS OF EXCITOTOXINS ELEVATE MET-ENKEPHALIN LEVELS IN THE RAT STRIATUM AND GLOBUS PALLIDUS. B.B. Ruzicka and K. Jhamandas. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

To develop a model of the striatopallidal enkephalin deficit associated with Huntington's disease, we examined the sensitivity of the enkephalinergic neurons to four excitatory amino acids (EAA's) - N-methyl-D-aspartate (NMDA, 50-150 nmol), quisqualate (QUIS, 26-106 nmol), kainate (KA, 0.5-7 nmol) and quinolinate (QUIN, 18-288 nmol) - following single injections into the right striatum of the rat. Levels of met-enkephalin-like immunoreactivity (ME-i.r.) in the striatum and globus pallidus were estimated by RIA seven days post injection. Each of the four EAA's produced dose-related and bilateral elevations in ME-i.r. in both the striatum and globus pallidus. The rank order of apparent efficacy of the EAA's, based on the magnitude of the maximal effect, was QUIN = KA > NMDA = QUIS. The rank order of apparent potency, based on the doses producing a maximal effect, was KA > QUIS > QUIN > NMDA. QUIN (72 nmol)-induced increases in pallidal ME-i.r. were attenuated by the EAA receptor antagonists, kynurenate (KYN, 36 nmol) and CPP (1.8 nmol). KYN (52 nmol), but not CNQX (1.4-5.3 nmol), also reduced the QUIS (53 nmol)-induced elevations in pallidal ME-i.r. Injections of CPP (1.8-3.6 nmol) into the striatum contralateral to the QUIN (72 nmol) infusion did not block the QUIN-induced response in the contralateral hemisphere, but it did decrease this response in the ipsilateral pallidum, suggesting that contralateral changes in ME-i.r. did not involve the mobilization of an endogenous EAA. These results show that activation of EAA receptors increases ME-i.r. in the striatum and globus pallidus at seven days following exposure to EAA's, a finding possibly unique to enkephalinergic neurons.
(Supported by the Medical Research Council of Canada)

90.10

QUINOLINIC ACID-INDUCED BRAIN DAMAGE PREVENTED BY N-METHYL-D-ASPARTATE ANTAGONISTS. C.M. WRAY* AND R.J. BOEGMAN. DEPT. PHARMACOLOGY AND TOXICOLOGY, QUEEN'S UNIVERSITY, KINGSTON, ONTARIO, CANADA, K7L 3N6.

The ability of two selective N-methyl-D-aspartate (NMDA) receptor antagonists to protect against the neurotoxicity induced by the endogenous tryptophan metabolite quinolinic acid (Quin) was evaluated in the rat striatum. MK-801 {(+)-5-methyl-10.11-dihydro-5H-dibenzo(a,d)cycloheptene-5,10,-imine maleate} partially antagonized the toxic effects of QUIN on striatal acetylcholinesterase (AChE) and NADPH-diaphorase neurons, increasing cell survival to 66% and 47% respectively when administered 1 hour after an infusion of Quin. In contrast, an intravenous dose of CPP {4-(3-phosphonopropyl)-2-piperazine-carboxylic acid} partially protected only AChE-containing neurons against a lower dose of QUIN. However, when co-injected with Quin, CPP fully protected NADPH-d neurons. Our results indicate that striatal neurons containing NADPH-d or AChE show a differential response to Quin-induced neurotoxicity and that some can be rescued from neurotoxin-induced cell death even after pre-exposure to the neurotoxin alone for up to four hours. Supported by the Medical Research Council of Canada and the Huntington's Society of Canada.

90.11

NMDA EVOKES THE RELEASE OF SOMATOSTATIN FROM STRIATAL NEURONS IN PRIMARY CULTURE. J.S. Williams, I. Berbekar* and S. Weiss. Neuroscience Research Group, University of Calgary, Calgary, AB, Canada T2N 4N1.

It has been suggested that the resistance of specific neuronal phenotypes, e.g. striatal somatostatinergic neurons, to NMDA-induced neurotoxicity may be due to a paucity of this subtype of excitatory amino acid (EAA) receptor on these neurons. A highly sensitive radioimmunoassay was used to examine the actions of EAAs on the release of endogenous somatostatin-like immunoreactivity (SLI) from mouse striatal neurons in primary culture. Indirect immunocytochemistry of these neuronal cultures suggested that 2-4% of the neurons contained SLI. During a 15 min incubation period, 47±10 fmoles of SLI were released from 14 days in vitro striatal neurons, cultured in 35mm dishes. In the presence of 100μM NMDA, an additional 112±21 fmoles of SLI were released (+238%); the NMDA-evoked release was dose-dependent (EC_{50}, 20μM), attenuated in the absence of added Ca^{2+}, potentiated in the absence of added Mg^{2+} and unaffected by the presence of 1μM TTX. The selective antagonists APV (100μM) and MK-801 (1μM) blocked the NMDA-evoked release of SLI. Kainate was equieffective yet 5-fold less potent (EC_{50}, 100μM) than NMDA in evoking SLI release; quisqualate was marginally effective. The results of this study suggest that NMDA and KA receptors are present on striatal somatostatinergic neurons in primary culture.

Supported by the Medical Research Council of Canada.

90.12

HALOPERIDOL ATTENUATES QUINOLINATE TOXICTY IN THE STRIATUM M.F.Mazurek and S.Garside. McMaster University Medical Centre, Hamilton, ONT, L8N 3Z5 Canada.

Excitotoxicty mediated by the NMDA receptor has been implicated in neuronal damage associated with such disorders as stroke, epilepsy and Huntington's disease. We have investigated the ability of the dopamine D-2 receptor antagonist haloperidol to modify NMDA-mediated toxicity in the striatum. The specific NMDA agonist QUIN was injected into the right striatum of rats pretreated with one of: (1)saline (controls); (2)haloperidol 0.2mg/kg (LOW HAL); (3)haloperidol 2.0 mg/kg (HIGH HAL); (4)6-OH DA lesion of the nigrostriatal tract. Animals were sacrificed by decapitation 7 days later. Brains were sliced in the coronal plane and photographed. Lesion size was estimated using two measures: ranking of lesion size from photographs by blinded observers; and quantitation of lesion size using the Bioquant II programme. The size of the lesion was significantly attenuated by both measures in the HIGH HAL and the 6-OH DA groups. LOW HAL animals had lesions comparable to those of controls.

These results suggest that dopamine D-2 receptor activation is important for the expression of excitotoxicity in the striatum. Haloperidol might help prevent neuronal damage in stroke and Huntington's disease.

90.13

KAINIC ACID TOXICITY IN STRIATUM IS PARTIALLY BLOCKED BY ANTICONVULSANT MEDICATION. S.Garside and M.F. Mazurek, McMaster University Medical Centre, Hamilton, ONT, L8N 3Z5, Canada.

Intrastriatal injections of Kainic acid (KA) produce lesions not only of the striatum but also of many extrastriatal brain regions. It is believed that these 'distant' lesions represent epileptic-induced brain damage rather than direct KA-receptor-mediated toxicity. We investigated the possibility that a portion of the striatal damage from intrastriatal KA injections might also result from epileptic activity. KA 10 nmols was injected into the right striatum of rats pretreated with one of: (1) saline (controls); (2) dilantin 50 mg/kg (DIL) (3) phenobarbital 5 mg/kg (PB). Animal were sacrificed 7 days later. Brains were sliced in the coronal plane and the magnitude of the lesion was assessed by morphological and biochemical criteria. The size of the lesion (as assessed by the Bioquant II programme) was significantly attenuated by 35% in DIL animals and by 28% in the PB group. Somatostatin immunoreactivity was reduced by only 17% in DIL and 8% in PB striatum vs. 60% depletion in controls. Substance P immunoreactivity was reduced by 60% in DIL and 53% in PB vs. 83% in controls.

At least part of the striatal damage produced by intrastriatal injection of KA may be due to indirect or 'distant' effects similar to those operative at extra-striatal sites.

90.14

DISAPPEARANCE OF BEHAVIOURAL HYPERACTIVITY IN RATS WITH KAINIC ACID-INDUCED STRIATAL LESIONS. J.C.S. Furtado and M.F. Mazurek, McMaster University Medical Centre, Hamilton, ON, L8N 3Z5, CANADA.

Rats with kainic acid (KA) lesions of the striatum are known to demonstrate nocturnal hyperactivity. We have used Digiscan Animal Activity Monitors to study the time course of behavioural changes in rats with KA- or quinolinic acid-(QUIN) induced striatal lesions. Rats received 1) saline (CONT), 2) 10 nmol KA or 3)240 nmol QUIN injected stereotaxically into the right striatum. For a period of 10 weeks post-surgery, KA-lesioned animals demonstrated a profile of nocturnal hyperactivity, when compared to the CONT group, on most behavioural parameters, including total distance travelled, rest time, number of movements and movement time. By contrast, the QUIN-lesioned animals were not hyperactive in relation to controls. By 16 weeks post-surgery, and thereafter, neither the KA- nor the QUIN-lesioned animals were distinguishable from controls.

These results suggest that the hyperactivity induced by KA striatal lesions may disappear over time. The attenuation of hyperactivity may have important consequences for the interpretation of behavioural changes in animal models of Huntington's Disease.

ACETYLCHOLINE I

91.1

CHOLINERGIC PROPERTIES OF A MURINE SEPTAL CELL LINE, SN56.B5.G4. J.K. Blusztajn and A. Venturini*. Dept. of Pathology, Boston Univ. Sch. of Med., Boston, MA 02118.

We investigated the processes of acetylcholine (ACh) synthesis and release in the SN56.B5.G4 cell line derived from the fusion of neurons of the mouse postnatal day 21 septum and the murine neuroblastoma cells, N18TG2 (Hammond D.N., *et al., Science*, 234: 1237, 1986). The cells expressed choline acetyltransferase activity and, when incubated with [^{14}C]choline, accumulated [^{14}C]ACh. This [^{14}C]ACh was synthesized from choline taken up by a high-affinity transport (apparent K_m=4.6±0.7 μM) which required Na^+. (Substitution of Li^+ for Na^+ in the medium inhibited the [^{14}C]ACh accumulation by 70%.) The spontaneous and evoked release of [^{14}C]ACh from the cells (measured at 5 mM and 40 mM extracellular K^+, respectively) was small and variable. In order to investigate whether ACh release could be enhanced by cellular differentiation, the cells were incubated for two days with 1 mM dibutyryl cAMP. This treatment resulted in neurite extension but had no effect on [^{14}C]ACh accumulation. However, the dibutyryl cAMP-treated cells were capable of both spontaneous and evoked ACh release (7% and 17% of their content, respectively, over a 30 min period). These data demonstrate that the SN56.B5.G4 cells exhibit a number of features of the cholinergic phenotype and may be useful as models of septal cholinergic neurons in long term culture.

Supported by NSF BNS8808942 and Center for Alternatives to Animal Testing grants.

91.2

MOLECULAR ANALYSIS OF THE CHOLINE ACETYLTRANSFERASE STRUCTURAL GENE FROM THE NEMATODE C. ELEGANS. A. Alfonso-Pizarro*, K. Grundahl* and J. B. Rand. Program in Molecular and Cell Biology, Oklahoma Medical Research Foundation, Oklahoma City, OK 73104.

We have cloned and begun to analyze the cha-1 gene complex from the soil nematode Caenorhabditis elegans. cha-1 is the structural gene for choline acetyltransferase (ChAT), the enzyme which synthesizes acetylcholine, and we wish to investigate its spatial and temporal control during neural development. In addition, genetic evidence suggests that the ChAT protein in C. elegans may consist of multiple structural and functional domains, and we are interested in determining the organization of the gene. We used the transposable element Tc1 to "tag" and clone part of the cha-1 complex, and we then isolated genomic clones spanning most of the region. The genomic clones were used to isolate cDNAs and to analyze cha-1 mutants. Sequence data from cDNAs indicate regions with homology to pig and Drosophila ChAT amino acid sequences. Using the cDNAs as probes on Northern blots, we have identified a low-abundance mRNA of approximately 2 kb. In addition, we have identified and mapped insertions and deletions associated with 10 independent mutations in the cha-1 complex.

Supported by grants from NIGMS and NSF.

91.3

CIS-REGULATORY ELEMENTS OF THE *DROSOPHILA* CHOLINE ACETYLTRANSFERASE GENE. T. Kitamoto*, K. Ikeda and P.M. Salvaterra. Beckman Research Institute of the City of Hope, Duarte, CA 91010.

Biochemical and immunocytochemical studies have shown that the expression of *Drosophila* choline acetyltransferase (ChAT) is subject to exquisite temporal and spatial regulation. Although our previous studies have indicated the importance of translational regulation of ChAT mRNA, the main regulation of ChAT expression is expected to be accomplished at the transcriptional level. As a first step toward understanding the molecular mechanism of transcriptional control of ChAT expression, we have analyzed the *cis*-regulatory elements of the ChAT gene by P-element mediated germ line transformation.

Fusion of a 7.4kb 5'-flanking sequence to *E.coli lacZ* gene directed expression of ß-galactosidase in the optic lobe and the antenna of transformed flies with a pattern which corresponded closely to the distribution of endogenous ChAT protein. Furthermore, the fusion of this 7.4kb sequence to ChAT cDNA resulted in the rescue of the lethality of Cha^{ts-1}, a temperature sensitive ChAT allele. 5'-deletions of the 7.4kb fragment to 3.3kb or 1.2kb in the *lacZ* fusion genes led to a more restricted pattern of ß-galactosidase expression in specific regions of the optic lobe. These results indicate that different subsets of cholinergic neurons may show different transcriptional regulation of the ChAT gene. (supported by NIH-NINCDS)

91.4

CHARACTERIZATION OF RAT FIBROBLASTS GENETICALLY MODIFIED TO EXPRESS DROSOPHILA CHOLINE ACETYLTRANSFERASE (dChAT). M. SCHINSTINE[1], C. WARD[3], M.B. ROSENBERG[2], T. FRIEDMANN[2*], R.L. WHITING[3], AND F.H. GAGE[1]. DEPT. OF NEUROSCIENCES[1] AND PEDIATRICS[2], UNIV. OF CALIF.-SAN DIEGO, LA JOLLA, CA 92093 and SYNTEX RESEARCH[3], PALO ALTO, CA 94304.

Choline acetyltransferase catalyses the formation of acetylcholine (Ach) from choline and acetyl-CoA. In the present experiments, the genes encoding for Drosophila choline acetyltransferase (generously donated by Dr. P.M. Salvaterra) and neomycin resistance were introduced into fibroblasts using retroviral vectors derived from Moloney murine leukemia virus. This study reports on the characterization of dChAT expressed by G418-resistant rat fibroblasts. Most studies were conducted on the fibroblast cell line Rat1.

The success of dChAT infection was confirmed immunocytochemically, biochemically, and by Northern blot analysis. The level of dChAT enzyme expressed by infected Rat1 fibroblasts (Rat1/ChAT) was measured using a modification of the Fonnum assay. This procedure revealed an enzyme activity of ~300 nmoles Ach/hr/mg protein. No activity was detectable in uninfected cells. The presence of dChAT within Rat1/ChAT cells was accompanied by a constitutive secretion of Ach. The level of extracellular Ach, measured by HPLC with electrochemical detection, was ~30 uM/20 hr/25 cm^2 flask. Comparatively, intracellular levels of Ach were 0.99 uM. There were no detectable levels of Ach, either secreted or intracellularly, in cultures of uninfected fibroblasts. Secretion of Ach in Rat1/ChAT cells was modulated by extracellular choline in a dose-responsive manner. Levels of secreted Ach were ~128% greater, as compared to controls, when 0.5 mM choline (the highest concentration used) was added to the culture medium. Choline had no effect on uninfected Rat1 cells. Additional studies using other compounds to control Ach secretion are currently in progress. Moreover, we will report on the transplantation of primary fibroblasts, genetically modified to secrete Ach, into the brain.

Supported by a Univ. of Calif. President's Fellowship to M.S. and NIA-08514.

91.5

TWO SPECIES OF HUMAN CHOLINE ACETYLTRANSFERASE mRNA WITH DIFFERENT PROTEIN CODING DOMAINS. W.L. Strauss, R. Zhang and M.V. Lorenzi. Dept. of Pharmacology, Univ. of Miami Sch. of Med., Miami, FL 33101

The existence of multiple forms of the enzyme choline acetyltransferase (ChAT) has been reported by a number of laboratories. In order to determine whether ChAT protein isoforms might be translated from different mRNAs, restriction fragments isolated from a clone of the human ChAT gene were hybridized to Northern blots of RNA isolated from CHP134 human neuroblastoma cells and adult human nucleus basalis. One of these probes, pChAT1.2, contains an exon with 86% and 73% identity to the regions of porcine and rat ChAT mRNA which code for the amino termini of the corresponding proteins. pChAT1.2 detects two transcripts in RNA isolated from CHP134 cells and human nucleus basalis. Both the 6000 and 2300 nt mRNAs detected with pChAT1.2 are large enough to code for a protein with the reported size of human ChAT. A second restriction fragment, pChAT5, contains an exon with 96% identity to porcine ChAT mRNA and 94% identity to rat ChAT mRNA. The region of identity between these sequences corresponds to amino acids 203-263 of the predicted rat and porcine ChAT proteins. In contrast to pChAT1.2, a probe prepared from pChAT5 detects the 2300 nt form of ChAT mRNA, but not the 6000 nt form, in both CHP134 cells and human nucleus basalis. The ability to distinguish between the two species of human ChAT mRNA using a probe that contains a protein-coding exon suggests that each mRNA encodes an isoform of ChAT with a unique amino acid sequence. It is not clear how the corresponding translation products are related to previously identified ChAT isoforms.

91.6

ANALYSIS OF EPITOPES ON CHOLINE ACETYLTRANSFERASE (ChAT) USING MONOCLONAL ANTIBODIES (Mabs). C. Cozzari*, J. Howard*, and B. Hartman. Istituto Biologia Cellulare CNR, Roma (Italy), and Dept. Psychiatry, Univ. of Minnesota, Minneapolis (MN).

Twelve different monoclonal antibodies against rat brain Chat (affinity constants from 10^7 - 10^{11}) were prepared. The immunohistochemical localization in rat brain did not differ between MAbs, but the intensity and detail was enhanced with the high affinity MAbs. The antigenic domains of ChAT and their evolutionary modifications, as indicated by cross-reactivity, were studied. The relative location of the epitopes were determined by assessing how binding of MAbs against a given epitope affected binding of MAbs against all other epitopes. The 12 MAbs were separately conjugated to Sepharose and allowed to bind ChAT. The Sepharose linked MAb-ChAT complexes were then separately incubated with each 125 I-labeled MAb. Comparison of results obtained with all pairs showed the existence of at least three distinct immunogenic domains. The main domain contained the epitopes that produced the three highest affinity MAbs plus several others. With two exceptions these epitopes were present on all non-primate mammals (rat, mouse, pig,beef) but not primates (monkey and human) nor non-mammals (bird, turtle, fish). The first exception was an epitope highly conserved in all species. The second exception was an epitope on the"edge" of the main domain which was conserved in all mammals (including primates) but not non-mammals. This epitope overlapped with an epitope in the second domain with a similar cross-reactivity profile (conserved in all mammals but not in the other classes). The third domain (widely separated from the other two) contained an epitope which was conserved in all species with the interesting exception of the primates.

91.7

SPECIFIC IMMUNOPRECIPITATION OF CHOLINE O-ACETYLTRANSFERASE FROM RAT HIPPOCAMPAL NERVE TERMINALS REVEALS AN ENDOGENOUS FORM OF PHOSPHORYLATED ENZYME. B.M.Schmidt and R.J.Rylett. Dept. Physiology, Univ.Western Ontario, London. N6A 5C1

The enzyme choline O-acetyltransferase [ChAT; EC 2.3.1.6] catalyses the formation of acetylcholine in cholinergic nerve terminals. Little is known about the regulation of this enzyme although interest has recently increased due to its apparent association with neuropathological conditions such as Alzheimer's disease. Subcellular fractionation of synaptosomes prepared from hippocampus of rat brain was used to isolate cytosolic (cChAT) and membrane-associated (mChAT) pools of the enzyme. Selective activation of mChAT followed depolarization of the nerve terminals by both 40mM potassium (124+3% control) and 50uM veratridine (124+5% control), similar to findings by Carroll (1986). In order to test the hypothesis that this enhanced activity involves phosphorylation of the protein, a common regulatory process in the central nervous system, we have developed a method to isolate each enzyme pool from a crude synaptosomal preparation using immunoprecipitation. Rat hippocampal synaptosomes were incubated with [^{32}P]-inorganic phosphate to label ATP pools and achieve protein phosphorylation, then ChAT was isolated by immunoprecipitation. SDS-PAGE and autoradiography reveals an endogenous pool of ChAT which exists in the phosphorylated state. This is in agreement with the findings of Hersh et al. (1989) for whole rat brain. (Supported by a grant from MRC Canada).

91.8

STRUCTURE-FUNCTION RELATIONSHIPS OF HUMAN BUTYRYLCHOLINE-STERASE (CHE) BY THE IN OVO PRODUCTION OF RECOMBINANT CHE MUTEINS.

L.F. Neville*, A. Gnatt*, Y. Lowenstein*, R. Padan*, S. Seidman*, and H. Soreq. Dept. of Biological Chemistry, The Life Sciences Institute, Hebrew University of Jerusalem, Jerusalem 91904, Israel.

To characterize the effects of point mutations on CHE function, synthetic RNAs derived from abnormal human chromosome 3 (Gnatt et. al., Cancer Res. 50:1983, 1990) and other various libraries were injected into Xenopus oocytes and their CHE activities assessed. Asp70 to Gly substitution markedly reduced succinylcholine (SuCh) interaction as well as exhibiting decreased binding to CHE inhibitors. An additional Ser425 to Pro mutation rendered CHE "atypical" since SuCh and dibucaine binding was abolished. Additional CHE muteins containing single Pro425, His114, or Tyr569 mutations failed to modify CHE ligand binding.

These data demonstrate that Asp70 functions in anionic site binding of CHE inhibitors but only some choline substrates since CHE muteins bound other choline substrates normally. The synergistic effect of Pro425 with Gly70 suggests that the 2 conserved domains harboring ser425 and asp70 in all cholinesterases lie adjacent with each other.

91.9

CHARACTERISTICS OF SOLUBILIZED [^{3}H]HEMICHOLINIUM-3 BINDING SITES FROM RAT STRIATAL TISSUE. T.K. Chatterjee and R.K. Bhatnagar, Dept. of Pharmacology, University of Iowa, Iowa City, IA 52242

We have reported that [^{3}H]hemicholinium-3 ([^{3}H]HC-3) binds to choline uptake sites and that these binding sites show ATP-dependent affinity conversion (J. Neurochem. 54: 1500, 1990). Here we show that [^{3}H]HC-3 binding sites could be solubilized using 0.2% deoxycholate in 50 mM glycylglycine buffer pH 7.4 containing 200 mM NaCl and assayed in 50 mM glycylglycine buffer containing 200 mM NaCl, 0.2% BSA, 50 µM CaCl2, 1 mM $MgCl_2$ with final detergent concentration of 0.05%. The solubilized preparation bound [^{3}H]HC-3 with a K_D of 8.9 nM and a B_{max} of 574 fmol/mg protein. Similar to the membrane bound form, the solubilized sites showed ATP-dependent conversion to a low affinity state with a K_D of 23.4 nM and B_{max} of 587 fmol/mg protein. Unlike the membrane bound form, the solubilized [^{3}H]HC-3 binding sites showed sensitivity to certain trypsin inhibitors. Para-aminobenzamidine, benzamidine and aprotonin inhibited [^{3}H]HC-3 binding with IC-50s respectively of 200 µM, 410 µM and 0.51 trypsin inhibitor units/ml. Other trypsin inhibitors, PMSF (1 mM) and α_1 antitrypsin (1mg/ml) did not affect the binding. Taken together, these results suggest that choline transport process might involve ATP-dependent phosphorylation of choline transporter sites. The significance of sensitivity to certain trypsin inhibitors remains uncertain. Nevertheless, certain endogenous proteins having such an inhibitor domain (e.g., A4 amyloid) might well play a modulator role in choline binding and transport. Supported by DAMD contract #17-87-C-8113.

91.10

STEREO-SELECTIVITY OF THE SODIUM-DEPENDENT HIGH-AFFINITY CHOLINE UPTAKE SYSTEM. **S. Ferguson, M. Diksic, and B. Collier.** Dept. Pharmacology and Therapeutics, Mc Gill University, Montreal, Canada H3G 1Y6.

Choline and its analogues are limited in their use for **in vivo** visualization of the central cholinergic system as they act as substrate for choline phosphotransferase (CPK), the first step for phospholipid synthesis, and eventually accumulate in the phospholipids of all cells. To attempt to circumvent this difficulty we have studied the isomeric forms of α- and β-methylcholine (MeCh), as well as a non-hydroxyl choline analogue. In rat cortical synaptosomes choline uptake (1 μM) was selectively inhibited by the stereoisomeric analogues. (R)-α-MeCh and (S)-β-MeCh (IC_{50} = 10 μM and 55 μM) were better able to block uptake than their stereoisomers (IC_{50} 40 μM and 180 μM, respectively). ^{3}H-labelled (R)-α-MeCh and (S)-β-MeCh as well as the non-hydroxyl analogue could be transported by rat synaptosomes (K_T 9 μM, 6.8 μM, 50 μM, respectively), while their stereoisomers could not. In addition, the stereo-analogues served as poor substrates for CPK **(in vitro)**, only (S)-α-MeCh was well phosphorylated with respect to choline. The non-hydroxyl analogue was not phosphorylated. These results suggest that (R)-α-MeCh and the non-hydroxyl choline analogue may be useful for **in vivo** visualization of cholinergic neurons as they are unlikely to accumulate in the phospholipids of non-cholinergic cells. Supported by AHAF (Alzheimer's program)

91.11

STIMULATION OF [^{3}H]-HEMICHOLINIUM-3 BINDING IN CORTICAL OR HIPPOCAMPAL TISSUES AFTER TREATMENT WITH β-CARBOLINES OR MDL 26,479. J.A. Miller and P.A. Chmielewski*. Merrell Dow Research Institute, Cincinnati, OH 45215.

[^{3}H]-Hemicholinium-3 ([^{3}H]HC-3) binds to the high affinity choline uptake (HACU) site. Increased turnover of acetylcholine in cholinergic nerve terminals results in increased HACU and increased [^{3}H]HC-3 binding. Several cognition enhancing drugs have been shown to increase HACU in cortical or hippocampal tissue and may enhance memory, in part, by this mechanism. In this study, β-carboline inverse-agonists (.1-10 mg/kg) and the triazole, MDL 26,479 (1-5 mg/kg), were administered to rats i.p., the hippocampi and cerebral cortices were removed and homogenized. [^{3}H]HC-3 binding was measured in membranes prepared from these tissues.

Following treatment with the β-carbolines, methyl 6,7-dimethoxy-4-ethyl- β-carboline-3-carboxylate (DMCM), ethyl β-carboline- 3-carboxylate (β-CCE) and methyl β-carboline- 3-carboxylate (β-CCM), resulted in increased [^{3}H]HC-3 binding ex vivo in rat cortical and hippocampal membranes. Additionally, treatment with the triazole MDL 26,479 resulted in increased [^{3}H]HC-3 binding in cortical but not hippocampal membranes. Unlike the β-carbolines, MDL 26,479 is not convulsant even at high doses. This may indicate that MDL 26,479 may prove effective in enhancing memory as has been reported for β-carbolines, but without serious toxicity such as seizures.

91.12

HEMICHOLINIUM MUSTARD INHIBITION OF HIGH AFFINITY CHOLINE TRANSPORT: Na^+ DEPENDENCE AND EFFECTS ON [^{3}H]-HEMICHOLINIUM-3 BINDING. K.H. Gylys, H.M. Vargas and D.J. Jenden. Dept. of Pharmacology and BRI, UCLA School of Medicine, Los Angeles, CA 90024.

Hemicholinium mustard (HCM), a nitrogen mustard analog of hemicholinium-3 (HC3), has been shown to be a potent irreversible inhibitor of high affinity choline transport (HAChT; Smart, 1981; Gylys *et al.*, 1989). The present work expands our pharmacological characterization of this compound by examination of the sodium dependence of HCM effects. Rat brain synaptosomes were incubated with HCM for 10 min. in sodium-free medium. This preincubation was followed by 3 washes in fresh buffer; HAChT was then measured in a subsequent 4 min. incubation. In the absence of sodium, HCM inhibition of HAChT was reduced by 85%. This result demonstrates that HCM inhibition is sodium dependent, an important criterion for HAChT specificity. [^{3}H]-HC3 binding techniques were then used to examine in more detail the irreversible effects of HCM. Preincubation of rat brain membranes with 1μM HCM for 10 min., followed by washing, resulted in a 70% decrease in binding capacity (B_{max}) of [^{3}H]-HC3. These results indicate the suitability of HCM as an affinity ligand for future studies of HAChT. (Supported by MH17691).

ACETYLCHOLINE—RECEPTORS: MUSCARINIC I

92.1

THE IDENTIFICATION OF MUSCARINIC RECEPTOR SUBTYPES IN PC12 CELLS. A. Takashima* and J. G. Kenimer, Lab. Cell Physiology, CBER, FDA, Bethesda, MD 20892

We have previously presented pharmacological and biochemical evidence that muscarinic stimulated phosphatidyl inositol(PI) hydrolysis and norepinephrine (NE) release in PC12 cells are independent events controlled by separate muscarinic receptor subtypes (Takashima and Kenimer J. Biol. Chem. 264, 10654-10659, 1989). Moreover, these distinct muscarinic stimulated events are differentially expressed during the cell cycle and during cell growth. Muscarinic-stimulated NE release is maximal in the G1 cell cycle phase whereas muscarinic-stimulated PI hydrolysis is at a minimum. In order to identify these muscarinic receptors, we utilized a method based on the polymerase chain reaction (PCR). Poly (A^+) mRNA was isolated from PC12 cells synchronized at G1 phase and was reverse transcribed with an oligo(dT) primer. The synthesized cDNAs were amplified by PCR using a set of degenerate primers corresponding to sequences within the 3rd and 6th transmembrane region of five reported rat muscarinic receptor subtypes (m1-m5). After 35 amplification cycles, agarose gel electrophoresis revealed two distinct PCR products, one approximately 800 bp in size and the other approximately 900 bp. These cDNA fragments were ligated into the pGEM3zf(-) vector and subcloned. The partial sequence analysis of these cDNA fragments reveals high homology with the rat m1 muscarinic receptor subtype.

92.2

DIFFERENTIAL EFFECTS OF SODIUM FLUORIDE OR ORTHOVANADATE TREATMENTS ON MUSCARINIC RECEPTOR-MEDIATED INOSITOL PHOSPHATE METABOLISM IN TE671/RD, SH-SY5Y AND PC12 CELLS. M. Bencherif and R.J. Lukas. Division of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Cells of the TE671/RD human clonal line express a pharmacologically-identified M3 muscarinic acetylcholine receptor (mAChR) subtype coupled to inositol phosphate (InsP) metabolism through a G-protein sensitive to cholera toxin and insensitive to pertussis toxin. We also have confirmed the presence of similar mAChR subtypes, but with distinctive toxin sensitivities, on cells of the SH-SY5Y human neuroblastoma (Lei et al., 1988) or the PC12 rat pheochromocytoma (Horwitz, 1989). Studies on functional coupling of mAChR demonstrate that NaF treatment enhances mAChR-mediated Ins monophosphate accumulation in PC12 cells, but not in TE671/RD or SH-SY5Y cells. The NaF effect has complex kinetic and dose-dependent character, but is not due to AlF_4 contamination. By contrast, sodium vanadate treatment induces increases in the resting and stimulated levels of Ins mono-, bis- and tris-phosphate in all three cell lines. Other studies suggest that the vanadate effect most likely involves inhibition of Ins phosphatases, whereas the mechanism(s) and potential species-specificity of the NaF effect needs to be elucidated. The results indicate that functional M3-type mAChR activity is differentially sensitive to agents acting at G-proteins or other, down-stream sites.

92.3

THE MUSCARINIC AGONIST OXOTREMORINE-M ACTIVATES CATECHOLAMINE SECRETION AND $^{45}Ca^{++}$ UPTAKE IN BOVINE CHROMAFFIN CELLS: EVIDENCE FOR A NOVEL TYPE OF CHOLINERGIC RECEPTOR. E. Heldman , M. H. Shirvan and H.B. Pollard. Lab. Cell Biol. and Genetics, NIH, Bethesda, MD 20892

Bovine chromaffin cells secrete catecholamines (CA) in response to nicotinic but not muscarinic agonists. Chromaffin cells from most other species, however, secrete in response to muscarinic agonists, and it puzzled us that while bovine cells possessed functional muscarinic receptors they were nonetheless not coupled directly to secretion. To examine this in more detail we tested the effect of a series of muscarinic agonists on secretion and $^{45}Ca^{++}$ uptake in bovine chromaffin cells. We found that the full muscarinic agonist oxotremorine-M (Oxo-M) induced a robust CA secretion. Oxotremorine evoked only a moderate secretion at the highest concentration tested (1mM). By contrast, muscarine, pilocarpine, bethanechol, and McN-A-343 did not elicit any secretory response. Secretion by both Oxo-M and nicotine was inhibited by hexamethonium and high concentrations of atropine (100 μM). Secretion induced by nicotine and Oxo-M, but not by high K^+ was blocked by the selective ganglionic M-1 agonist, McN-A-343, indicating a direct action of McN-A-343 on the receptor rather than indirect effect on the secretory mechanism. Secretion induced by Oxo-M and nicotine was also relatively insensitive to both kappa bungarotoxin, an effective ganglionic nicotinic blocker, and to alpha bungarotoxin, a specific blocker of muscle nicotinic receptors. Pretreatment with either nicotine or Oxo-M led to desensitization to a repeated dose of either agonist. However, homologous desensitization was more profound. Oxo-M also induced $^{45}Ca^{++}$ uptake by chromaffin cells. These data suggest that the "nicotinic" receptor in bovine chromaffin cells may have some muscarinic character, and is distinct from those nicotinic receptors heretofore described in ganglia, brain or muscle. We have tentatively named this unusual receptor the "muscatinic receptor."

92.4

VOLTAGE-DEPENDENCE OF NICOTINIC BUT NOT MUSCARINIC RECEPTOR-MEDIATED SECRETION IN PORCINE ADRENAL CHROMAFFIN CELLS. Y. XU and E.J. Forsberg. Dept. of Physiology, Univ. of Wisconsin, Madison, WI 53706.

Catecholamines and ATP are co-localized in granules of adrenal medullary chromaffin cells, and are co-released upon stimulation by cholinergic agonists. In bovine chromaffin cells, nicotinic, but not muscarinic, acetylcholine receptors have been shown to mediate cholinergic agonist-evoked catecholamine and ATP release. In contrast, we have found that both nicotinic and muscarinic receptor selective agonists could evoke ATP release in porcine chromaffin cells. The release of ATP was monitored on-line using a photoluminescence, luciferin/luciferase, assay. Methacholine (a selective muscarinic receptor agonist) stimulated ATP release in a dose-dependent fashion with an EC_{50} of about 200 μM. The methacholine response was totally blocked by 1 μM atropine. Methacholine also caused an increase in cytosolic Ca^{2+} monitored by the Ca^{2+} sensitive dye fura-2. Predepolarization of the cells with high extracellular K^+ (40-100 mM) did not block methacholine-evoked ATP release and the increase in cytosolic Ca^{2+}, but did block responses mediated by nicotinic agonists. Extracellular application of EGTA (30 s before adding agonist) reduced muscarinic receptor-mediated ATP release by about 70% both in normal and predepolarized cells, but totally blocked nicotinic receptor-mediated secretion. These experiments suggest that in porcine chromaffin cells nicotinic responses are mediated by activation of voltage-dependent Ca^{2+} influx, while muscarinic responses are mediated by both voltage-independent Ca^{2+} influx and by intracellular Ca^{2+} mobilization.

92.5

EFFECT OF ANTAGONISTS TO SUBTYPES OF MUSCARINIC RECEPTORS ON MOTONEURONS OF THE LOBSTER CARDIAC GANGLION. J. Freschi. Neurology Dept., Emory Univ., Atlanta, GA 30322.

Muscarinic agonists evoke a voltage-dependent slow inward current in motoneurons of the lobster cardiac ganglion (Freschi & Livengood, J. Neurophysiol. 62:984, 1989). To determine the subtype of muscarinic receptor that mediates the cholinergic current, various antagonists, having specificity for subtypes of mammalian muscarinic receptors, were applied to neurons under voltage clamp. The muscarinic agonist methacholine was used in all of these experiments (K_D = 0.45 mM; nH = 1.08). The M1 agonist McN-A-343 had no effect. From the competition curves of antagonists vs. 1 mM methacholine, slope factors and antagonist K_i values were calculated. Slope factors were all close to 1. The results (-log K_i) in order of potency were: atropine (7.69) > pirenzepine (6.37) ≥ 4-DAMP (6.09) > methoctramine (5.37) ≥ HHSiD (5.08) = (R)-HHD > (S)-HHD (4.0). Neither AF-DX 116 nor gallamine had an effect at 10^{-4} M.

This profile of antagonist potencies is unlike that for any of the mammalian muscarinic receptor subtypes. Although the terms "M1-like" and "M2-like" have been used in studies of various invertebrate muscarinic receptors, invertebrate nervous systems appear to have their own unique profiles and will require their own nomenclature.

92.6

Stimulation of Smooth Muscle Contraction by Anatoxin-a by Its Interaction with Nicotinic Receptors. Peter K. Chiang, D. L. Butler, N. D. Brown, A. D. Wolfe, and Peter Kostka, Dept. of Applied Biochemistry, Walter Reed Army Institute of Research, Washington D. C. 20307-5100

Anatoxin-a is an exotoxin that has been isolated from the blue green algae Anabaena flos, and is known to cause death among waterfowl, livestock and fish that inhabit or frequent water areas with high algae bloom. Synthetic anatoxin-a was tested for its effect on guinea pig ileum contraction. It was found that anatoxin-a stimulated ileum contraction with a potency similar to acetylcholine (ACh). Even though the stimulation could be blocked by both atropine or hexamethonium, no specific inhibition of the binding of [^{3}H]N-methylscopolamine to ileum plasma membranes was observed in the presence of anatoxin-a. In comparison, the stimulation of ileum contraction by ACh was not blocked by hexamethonium or tubocurarine, thus indicating that the action of anatoxin-a might possibly be at the presynaptic nicotinic receptor sites. Furthermore, anatoxin-a failed to modulate the secretion of alpha amylase from pancreatic acinar cells, a process that is controlled by muscarinic receptors. Anatoxin-a also failed to inhibit acetylcholinesterase or butyrylcholinesterase. It may be inferred that anatoxin-a binds to presynaptic nicotinic receptors, thus releasing endogenous ACh, which in turn causes ileum contraction by interacting with the postsynaptic muscarinic receptors.

92.7

ANTICHOLINESTERASES REVEAL A MUSCARINIC INHIBITION OF NICOTINIC TRANSMISSION IN THE CAT SUPERIOR CERVICAL GANGLION. M. Bachoo* and C. Polosa. Physiology Dept., McGill Univ., Montreal, Canada H3G 1Y6.

In anesthetized cats, under partial nicotinic block (hexamethonium infusion) and pretreated with pirenzepine (50 µg/kg), methacholine (25-100 µg) injected into the arterial supply of the superior cervical ganglion depressed dose-dependently the postganglionic compound action potential (CAP) evoked by preganglionic stimulation. The depression was blocked by the M2-muscarinic antagonists AFDX (125-300 µg/kg) or pancuronium (200-400 µg/kg). These antagonists did not enhance the amplitude of the CAP evoked by repetitive preganglionic stimulation. Eserine (0.1-0.5 mg/kg) depressed the amplitude of the CAP in a dose-dependent manner at all frequencies above 0.5 Hz. This depressant effect was blocked by AFDX or pancuronium at doses which block the methacholine-evoked depression. A similar depression, also sensitive to AFDX and pancuronium, was produced by the anticholinesterases neostigmine and edrophonium. Thus, nicotinic ganglionic transmission is depressed by an M2-mediated mechanism. This mechanism is activated by exogenous agonists but not by ACh released by the nerve terminals, unless an anticholinesterase is present. The data suggests that the M2 receptor of the sympathetic ganglion synapses has properties (e.g. location, number, affinity for the transmitter) resulting in resistance to synaptic activation. Funded by MRC and QHF.

92.8

ACTIVITY OF MUSCARINIC AGONISTS AND ANTAGONISTS AT CLONED M1 MUSCARINIC RECEPTORS AND RAT SUPERIOR CERVICAL GANGLION. H.W.G.M. Boddeke*, G. Gmelin Sandoz Pharma, Sandoz Preclinical Research, CH 4002 Basle, Switzerland.

In this study the effects of muscarinic agonists and antagonists in A9-L cells transfected with m1 muscarinic receptors were compared with the responses of the same compounds measured in rat superior cervical ganglia, an in vitro model for m1 receptors.

A9-L cells were cultured in Dulbeccos Modified Eagle medium supplemented with 10% fetal calf serum. Increases in intracellular calcium were measured using the calcium indicator fura-2. In rat superior cervical ganglia muscarinic agonist-induced slow depolarizations were recorded extracellularly.

In contrast to muscarinic antagonists a tenfold lower potency of agonists at cloned receptors compared to m1 receptors in rat superior cervical ganglion was observed. Good correlations for both agonist pD2 values (r=0.80) and antagonsit pA2 values (r=0.94) at cloned m1 receptors and rat ganglion were found.

These results indicate that the effects of muscarinic agonists and antagonists at cloned m1 receptors can be used to predict effects of muscarinic compounds in functional in vitro models.

92.9

EVIDENCE FOR INHIBITORY NICOTINIC AND FACILITATORY MUSCARINIC RECEPTORS ON CHOLINERGIC NERVE TERMINALS OF THE RAT URINARY BLADDER. G.T. Somogyi and W.C. de Groat, Departments of Pharmacology and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

Cholinergic modulation of ^{3}H-acetylcholine (ACh) release was studied in the body of the urinary bladder (UB) of the rat which was prelabelled with ^{3}H-choline. ^{3}H-choline uptake was very prominent in the UB body where there is a dense cholinergic innervation. Electrical field stimulation in the presence of 1 µM hemicholinium-3 markedly increased ^{3}H-ACh outflow from the superfused tissue. Eserine (ES) in 1, 5, 25 µM increased the evoked release of ACh in a dose dependent manner by 64%, 220% and 306%, respectively. Atropine (1 µM) alone increased the release of ACh by 44% but atropine decreased by 48% the amount of ACh release in the presence of 5 µM ES. McN-A 343 (50 µM) an M1 muscarinic receptor agonist increased the release of ACh by 750%. Pirenzepine (1 µM) inhibited the ES-induced increased release by 63%. The nicotinic antagonist D-tubocurarine (50 µM) (DTC) significantly increased the ES-induced release by 471%, whereas, DTC alone did not change the release of ACh. We conclude that in ES treated UB, M1 receptors are activated leading to an increased ACh release. This increased release was depressed by the specific M1 blocker pirenzepine. However, ES also unmasked an inhibitory nicotinic mechanism. Block of this mechanism with DTC further increased ACh release in the presence of ES. Supported by USPHS Grant MH 30915, NSF BN-82-08348, NIH AM 316 888.

92.10

CEREBROVASCULAR MUSCARINIC CONSTRICTION IS MEDIATED BY M_1 RECEPTORS COUPLED TO PHOSPHOINOSITIDE (PI) TURNOVER. F. Dauphin* and E. Hamel. Cerebrovasc. Lab., Montreal Neurological Institute, Montréal, Québec, Canada.

The muscarinic receptor subtype involved in the acetylcholine (ACh)-mediated constriction of the cat middle cerebral artery was pharmacologically characterized in endothelium-denuded vascular segments. Cholinergic agonists (ACh, carbachol, methacholine and oxotremorine) elicited contractile responses which corresponded to 50-70% of the maximal constriction induced by 127 mM K^+ with similar affinities (pD_2 values ranging from 4.65 to 4.85) except for oxotremorine (pD_2 of 5.85). Non-selective (atropine) or selective M_1 (pirenzepine, UH-AH 371), M_2 (AF-DX 116, methoctramine and AQ-RA 741) and M_3 (4-DAMP and HHSiD) muscarinic antagonists potently inhibited the ACh-induced constriction. Their order of potency (pA_2 values from 10.48 to 6.23) was: UH-AH 371 > atropine ≥ 4-DAMP > HHSiD ≥ pirenzepine ≥ AQ-RA 741 ≥ AF-DX 116 > methoctramine. The carbachol-induced stimulation of PI turnover was investigated in [^{3}H]-myo-inositol prelabeled cat cerebral arteries and characterized using selective muscarinic antagonists. Carbachol induced a dose-dependent increase in PI-hydrolysis which was blocked by 4-DAMP, pirenzepine and UH-AH 371 with high potency (-logIC_{50} values of 8.92, 7.87 and 7.84, respectively) while AF-DX 116 was less potent (-logIC_{50} of 7.07). These results indicate that a M_1 muscarinic receptor coupled to PI turnover mediates this ACh-induced constriction. Supported by the MRC of Canada and Dr. Karl Thomae GmbH.

92.11

m2 AND m4 MUSCARINIC RECEPTOR SUBTYPES INHIBIT CALCIUM CURRENTS IN DNA-TRANSFECTED NG108-15 NEUROBLASTOMA HYBRID CELLS. H. Higashida[1], *M.Hashii[1], *K.Fukuda[2], *S. Numa[2] & D.A.Brown[3]. [1]Dept.Biophysics, Univ. Kanazawa Med.Sch., Kanazawa 920, Japan, [2]Dept.Med.Chem. & Mol.Genet., Kyoto Univ.Fac.Med., Kyoto 606, Japan and [3]Dept.Pharmacol., Univ. Coll., London WC1E6BT, U.K.

Stimulation of muscarinic acetylcholine receptors (mAChR) inhibits Ca currents (I_{Ca}) in nerve cells. To determine which molecular subtype of mAChR is responsible, inhibition of I_{Ca} in clones of NG108-15 cells transfected with DNA for mAChRI-IV (K.Fukuda et al., 1988:Nature,335, 355) was measured. I_{Ca} was recorded at 33-35°C with "whole-cell" patch clamp electrodes using 50 mM Ba as charge carrier and 1 s commands to +20 mV from -80 mV. Maximum inhibition at 1 mM ACh in non-transfected cells was 12.8±1.8% (mean ± s.e.m., n=36). All clones of mAChRII- and mAChRIV-transformed cells showed a significant increase in inhibition to between 18.1 and 23.1%. In contrast no increase in inhibition was detected in vector-transfected cells, or in pooled clones of cells transfected with DNA for mAChRI or mAChRIII. Inhibition did not correlate with total mAChR number measured by ^{3}H-QNB binding. Hence, I_{Ca} in these neuronal cells is preferentially inhibited by molecularly defined mAChRII and mAChRIV.

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92.12

SIGNALING THROUGH THE MUSCARINIC RECEPTOR-ADENYLATE CYCLASE SYSTEM OF THE HEART IS BUFFERED AGAINST GTP OVER A RANGE OF CONCENTRATIONS. E. J. Ehlert and B. E. Rathbun*. Dept. of Pharmacology, Col. of Med., Univ. of Calif., Irvine, California 92717

The influence of GTP on muscarinic receptor occupancy and inhibition of adenylate cyclase activity was investigated in well washed homogenates of the rat myocardium. In these homogenates, the highly efficacious muscarinic agonist oxotremorine-M was without effect on adenylate cyclase activity in the absence of exogenous GTP, but caused a maximal 38% inhibition of the enzyme in the presence of 0.1 μM GTP. Increasing the concentration of GTP to 0.1 mM caused small to moderate increases in the maximal inhibition of adenylate cyclase elicited by oxotremorine-M and in the concentration of this agonist required for half-maximal inhibition of the enzyme. In contrast, the same change in the concentration of GTP (0.1 μM - 0.1 mM) caused a relatively large increase (46-fold) in the concentration of oxotremorine-M necessary for half-maximal receptor occupancy. Similar observations were made for the highly efficacious muscarinic agonist carbachol. Our results show that GTP increases receptor coupling efficiency and decreases agonist affinity and that these two effects oppose one another so that the level of muscarinic agonist-mediated inhibition of adenylate cyclase activity remains relatively constant over a range of concentrations of GTP. We have also used a model to predict the influence of GTP on receptor binding properties and agonist-mediated inhibition of adenylate cyclase activity and have calculated theoretical results generally consistent with the experimental observations. *Supported by N.I.H. Grant NS 26511.*

92.13

PHYSIOLOGICAL ACTIONS OF AN M1 MUSCARINIC AGONIST, AF102B. A. Fisher, D. Gurwitz*, R. Haring*, Z. Pittel*, E. Heldman* and M. Segal*#, Israel Inst. Biolog. Res., Ness-Ziona and #The Weizmann Inst., Rehovot, Israel.

The effects of acetylcholine (ACh) and of the M1 muscarinic agonist AF102B were compared in CA1 neurons of rat hippocampal slices. ACh produces a slow depolarization associated with decreased persistent K^+ current (I_K), a blockade of a Ca^{2+}-dependent I_K underlying the slow AHP, a blockade of the m-current and a reduction in EPSP's evoked in these cells by stimulating stratum radiatum. These effects are known to be mediated by M1, M2, M3 muscarinic receptors. AF102B (microperfusion: 10mM, 1-2nl microdrop) blocked AHP and caused an associated increase in the number of action potentials evoked in response to a long depolarizing current pulse. This effect of the drug was blocked by low concentration of the M1 antagonist, pirenzepine (100 uM, 10nl microdrop). AF102B (10-50mM, 10nl) caused a slow depolarization and an associated increase in input resistance, but did not affect EPSP's at any concentrations used. In line with these discriminative effects, AF102B did not potentiate phosphoinositides (PI) turnover but rather attenuated carbachol (CCh)-induced PI turnover in rat cortex (m1, m2, m3, m4) and SK-N-SH cells (mostly m3) and an associated CCh-induced mitogenesis in these cells. Cu^{2+}-pretreatment of cortical homogenates uncovered the agonistic binding parameters of AF102B. In summary, AF102B as a selective M1 agonist displays a unique profile in central neurons. Supported by Snow Brand, Japan.

92.14

OLIGONUCLEOTIDE PROBE FOR THE RAT MUSCARINIC ACETYLCHOLINE RECEPTOR M3 MRNA HYBRIDIZES WITH A MURINE MRNA UNDER HIGH STRINGENCY CONDITIONS IN THE CNS OF TWO STRAINS OF MICE. L. Tsiokas, S.C. Zhang*, J.J. McArdle and M. Watson. U. of Med. & Dent. of N.J.- N.J. Med. Sch., Newark, N.J. 07103.

In situ hybridization (ISHH) studies were done in male Sprague-Dawley rats and two strains of mice with synthetic oligonucleotide probe complementary to 4-48 or 4-51 base sequence of m1-m5 (NEN, Boston) muscarinic acetylcholine receptors (mAChR). Quantitative ligand autoradiography using mAChR selective ligands was also done on adjacent CNS slices. Probe was 3'-end labeled by terminal deoxynucleotidyl transferase and ^{35}S-dATP, (>1,000 Ci/mmol, NEN), 3'-end tails had 10 bases (specific activity>2.4x10^9 dpm/ug). Probe was purified by NENSORB 20 cartridges and recoveries were ~65%. Sections (10um) of 84d old long and short-sleep (LS/SS) mouse brain were incubated in hybridization buffer with 1x10^{6}dpm probe (25°C; 24h) in a humid chamber. Washes were done in moderately high stringency conditions (T_m=55°C; 2xSSC). After post-hybridization procedures slices were air-dried and apposed to Hyperfilm-ßmax (Amersham). ^{35}S brain paste standard was used to quantitate autoradiograms (4wk; 0-4°C) via a DUMAS image analysis system. Except for dentate gyrus which was densely labeled in LS mice, rat m3 probe showed a distribution consistent with rat m3 mAChR density in hippocampus, striatum and cortex, but with significantly less expression in SS mice. These data suggest the rat m3 probe can be used to clone the murine m3 mAChR. Support from MH-43024.

92.15

CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST AN m1 MUSCARINIC RECEPTOR PEPTIDE.

C.P. Bolden, D.J. McCormick*, C. Krco* and E. Richelson, Mayo Foundation, Jacksonville, FL 32224 and Rochester, MN 55905

Three monoclonal antibodies (mab's) were raised against a synthetic peptide corresponding to a unique portion of the amino terminal extracellular domain of the human m1 (Hm1) and rat m1 (Rm1) muscarinic receptor (MR). Comparison of the amino acid sequences of the different subtypes of the MR indicates that there is little conservation in the amino terminal domain. This observation permits the production of mab's to MR subtypes that will likely be subtype selective/specific. The three mab's produced to date (oligopeptide VSPNITVLAPGKGPW) recognize a single protein (~80 kD) by Western blot analysis in membranes prepared from rat cortex. The m1 oligopeptide inhibits mab binding in this preparation in a dose-dependent manner. ELISA indicates the mab's demonstrate little or no cross reactivity with synthetic peptides corresponding to a portion of the amino terminal domain of the Rm3, Rm4, porcine m2 (Pm2) or Hm4 receptors. In addition, these antibodies have no apparent effect on specific muscarinic antagonist binding or muscarinic receptor-mediated responsiveness. (Supported by MH27692 from the USPHS)

92.16

PROBING THE STRUCTURE-FUNCTION RELATIONSHIP OF THE M_1 AND M_2 MUSCARINIC CHOLINERGIC RECEPTORS. J. Lai, J.W. Bloom*, W.R. Roeske and H.I. Yamamura. Depts. Pharmacology and Internal Medicine, Univesity of Arizona Health Sciences Center, Tucson, AZ 85724.

The structurally defined rat m_1 and m_2 muscarinic receptor gene products expressed in vitro (murine fibroblast B82) are distinguished by both their ligand binding characteristics and their functional coupling via distinct G-proteins. The two gene products resemble pharmacologically the brain M_1 and cardiac M_2 type muscarinic receptors, respectively. Pirenzepine is selective for the m_1 receptor (K_i= 13 nM) and himbacine, methoctramine and AF-DX 116 are selective for the m_2 receptor (K_i= 10.2 nM, 44 nM and 92 nM, respectively). The m_1 receptor is coupled to the hydrolysis of inositol lipids (EC_{50}= 15.8 μM) whereas the m_2 receptor is inversely coupled to adenylate cyclase (EC_{50}= 1.6 μM), the latter mediated by a G-protein 1000x more sensitive to pertussis toxin (1 ng/ml) than that mediating the former. These well defined characteristics of the m_1 and m_2 receptors are essential to the analysis of a series of m_1/m_2 chimeric receptors to delineate structural components pertaining to the distinct nature of the two receptors. Supported in part by AHA, USPHS and ADCRC.

92.17

AMPLIFICATION OF GENES ENCODING THE RAT MUSCARINIC RECEPTOR SUBTYPES BY POLYMERASE CHAIN REACTION (PCR). E.V.Varga,K.Kashihara,J.Bloom, J.Lai,W.R. Roeske and H.I.Yamamura. Dept. of Pharmacol. and Internal Med., Univ. of Arizona, Tucson, AZ 85724.

PCR is an efficient method for amplification of genes from the genomic DNA for the purpose of in vitro expression. This technique has previously been employed to amplify and express the rat m_2 muscarinic receptor gene. The error rate of the PCR was 1 nucleotide substitution in 841 base pairs. The rat m_3 gene was amplified by PCR in a similar manner and showed 1 nucleotide difference compared with the published sequence. The deduced amino acid sequence demonstrated one mismatch at residue 349 (Val>Ala). Because we have found the same sequence in three of four different PCR products, we do not attribute the difference to the PCR. The coding region of the m_5 gene was also amplified, but for this amplification we found 3 nucleotide substitutions in 1536 base pairs. We conclude that DNA sequence analysis is a requisite for the use of PCR products.

92.18

EXPRESSION OF 5 HUMAN MUSCARINIC RECEPTOR SUBTYPES IN HeLa CELLS. S. Pepitoni*, J. Borkowski*, R. Mallon* and R.D. McQuade, Schering-Plough Research, Bloomfield, New Jersey, 07003.

Researchers have previously cloned at least 5 subtypes of human muscarinic receptors (Bonner et al., Science, 1987). We have obtained these clones from Dr. Bonner and have successfully inserted them into a eukaryotic expression vector under the control of the SRα-promoter. Subsequently, the muscarinic receptor subtypes were transiently expressed in HeLa cells. The receptor gene products from these cells were then analyzed for their binding profiles and for their ablilities to stimulate phospholipase C.

Receptor binding experiments were performed with the non-selective radioligand, ^{3}H-quinuclidinyl benzilate (QNB). These studies demonstrated that all 5 of the receptor subtypes were expressed. Subsequent studies were undertaken to examine the abilities of these receptors to bind to the m-1 selective radioligand, ^{3}H-pirenzepine. The m-1 receptor exhibited the highest degree of binding to ^{3}H-pirenzepine, however the m-3 and m-5 subtypes were also labeled to a lesser degree. The m-2 receptor did not demonstrate any labeling with the ^{3}H-pirenzepine.

Studies were then initiated to examine the abilities of these expressed receptor subtypes to mediate stimulation of phospholipase C. Phospholipase C is the enzyme responsible for the metabolism of phosphotidyl inositol (PI)) and for the generation of the second messenger IP3. Stimulation of the m-1 receptor by 1 mM oxotremorine-M resulted in an approximately 7-fold increase in PI turnover and this stimulation was virtually eliminated by preincubation with 10 μM atropine. The m-3 and m-5 receptors also stimulated PI turnover, albeit to a lesser degree, while stimulation of m-2 sites did not. These data suggest that the m-1 receptor is linked to stimulation of phospholipase C and that m-2 receptors must be associated with some other second messenger system.

92.19

EXPRESSION AND PHARMACOLOGICAL STUDIES OF A HUMAN M1 MUSCARINIC RECEPTOR CARRYING A MYC EPITOPE AT N- OR C-TERMINAL. T.V. Dam, P. Bouchard*, P. Payette*, M. Dennis* and F. Gossard*. National Research Council of Canada, Biotechnology Research Institute, Montréal, Québec, CANADA.

In an attempt to study the human muscarinic receptor 1 subtypes (Hm1) with immunochemical methods, a synthetic oligo cDNA coding for 10 amino acids of the C-myc protein was inserted by mutagenesis in the Hm1 gene. The synthetic sequence was inserted in frame at the N- or C-terminal ends of the protein. The mutated gene was then inserted in the expression vector pSVP4 under the SV40 early promoter and introduced in Neuro-2A neuroblastoma cells (N2A) (ATCC # CCL131). To determine the binding properties of the myc-tagged receptors, membranes prepared from N2A cells, stably transfected with the constructs, were labeled with [^{3}H]-N-methyl-scopolamine (NMS) in a Tris-HCl buffer, pH 7.4 at 4°C containing 2 mM $MgCl_2$. The results showed that the myc-tag at the C-terminal did not affect the expression of Hm1 receptor both in the affinity (Kd) and the maximum number of sites (Bmax). Experiments are in progress to determine if the Hm1 muscarinic receptors tagged with a myc epitope retain fully their biological activity. (Supported by FRSQ, NRC, MRC).

ACETYLCHOLINE—RECEPTORS: NICOTINIC I

93.1

EFFECT OF PHOSPHORYLATION ON LIGAND BINDING TO THE NICOTINIC ACETYLCHOLINE RECEPTOR. J.M. Herz, W. Xian*, P. Berryhill* and B. Schmid *. Inst. for Neuroscience and Cell Research Inst., Univ. of Texas, Austin, TX. 78713

The nicotinic acetylcholine receptor (nAChR) can be phosphorylated by a protein tyrosine kinase both in vivo and in vitro. Since it has previously been demonstrated that the nAChR isolated from Torpedo californica electroplax is phosphorylated on tyrosine residues, it is of interest to determine the functional significance of this finding. To examine whether ligand binding properties are modified by phosphorylation, receptors in Torpedo membranes were phosphorylated by activation of an endogenous tyrosine kinase using [γ-^{32}P]ATP in the presence of PKI and in the absence of detergent. Samples were analyzed by SDS-PAGE and autoradiography. Incorporation of ^{32}P into nAChR subunits was quantitatively determined by gel excision and liquid scintillation counting. High stoichiometric incorporation was achieved after incubation for 60 min with 200μM ATP. Monoclonal anti-phosphotyrosine antibodies recognized receptor subunits in Western blots. Phosphorylated membranes were washed and resuspended in an appropriate buffer for ligand binding. Fluorescent ligands specific for the agonist/antagonist sites and the noncompetitive inhibitor site have been used to determine binding affinities and maximal number of sites. This has allowed us to correlate changes in binding parameters with phosphorylation. Supported by AHA Texas Affiliate 89G-401.

93.2

THE EFFECTS OF CHRONIC CORTICOSTERONE ADMINISTRATION ON SENSITIVITY TO NICOTINE AND NICOTINIC CHOLINERGIC RECEPTOR BINDING ARE REVERSIBLE. J.R. Pauly, E.U. Grun and A.C. Collins. Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

Studies from our lab have demonstrated corticosterone (CCS) modulation of sensitivity to nicotine in mice. Chronic CCS administration reduces sensitivity to nicotine and also the number of CNS receptors labeled by alpha-bungarotoxin (BTX) by 40-70%. This study examined the time course for normalization of nicotine sensitivity and BTX binding following CCS pellet removal. C3H animals were adrenalectomized (ADX) and implanted with hormone pellets (60% CCS) which were removed after 1 week. Animals were tested for nicotine sensitivity, plasma CCS and BTX binding 0, 1, 3, 5, 7 and 9 days following pellet removal. Plasma CCS levels returned to ADX levels 9 days after pellet removal. Nicotine sensitivity was normalized 7 or 9 days after pellet removal, depending on test measurement. CCS-induced reductions in BTX binding were also reversed following pellet removal; the time course depended on brain region. In cerebellum, binding returned to ADX control levels by day 5. Binding in midbrain, cortex and the colliculi increased to control values by day 9. In other regions (striatum, hippocampus and hypothalamus), binding remained 40-50% below control binding at the 9 day time point. Supported by DA-05131 and a grant from R.J. Reynolds Tobacco Co.

93.3

ACUTE EXPOSURE TO NICOTINIC AGONISTS DOWN REGULATES CELL SURFACE NICOTINIC ACETYLCHOLINE RECEPTOR ON THE TE671/RD CLONAL CELL LINE. A.M. Joy and R.J. Lukas. Division of Neurobiol., Barrow Neurol. Institute, Phoenix, AZ 85013.

Cells of the TE671/RD line express a muscle-type nicotinic acetylcholine receptor (nAChR). The regulation of cell surface nAChR was studied using a radiolabeled alpha-bungarotoxin (I-Bgt) binding assay peformed on adherent cells in culture. Exposure to 1 mM nicotine resulted in an apparently maximal 50% down regulation of cell surface I-Bgt binding sites within 10 minutes of drug treatment. These sites were not recovered up to four hours after removal of nicotine. Scatchard analysis revealed no change in K_D, but a decrease in B_{max} for remaining specific I-Bgt binding sites at saturation. Down regulation of nAChR also occurred during acute treatment with nicotinic antagonists pancuronium (1 mM, less than 50% down regulation) or d-tubocurarine (0.1 mM, more than 50% down regulation), while simultaneous treatment of cells with nicotine and antagonist produced a level of down regulation seen with antagonist treatment alone. Treatment of cells with agents presumed to block clathrin coated pit-mediated endocytosis, such as 2-10 mg/ml bacitracin, or in hypotonic medium (0.2 M sucrose) failed to block nicotinic ligand-induced nAChR down regulation. This acute phase of nAChR down regulation precedes a more slowly evolving up regulation of surface nAChR induced by nicotinic agonist treatment.

93.4

STAUROSPORINE DECREASES THE EXTENT OF RECOVERY FROM DESENSITIZATION AT SNAKE TWITCH FIBER NICOTINIC RECEPTOR-CHANNEL COMPLEXES. Jean C. Hardwick and Rodney L. Parsons. Department of Anatomy & Neurobiology, University of Vermont, Burlington, VT 05405.

Desensitization of nicotinic receptor-channel complexes occurs during prolonged exposure to cholinergic agonists such as carbachol. The recovery from desensitization, following removal of agonist, is a biphasic process with an initial fast phase followed by a slower component. Further, as recovery proceeds, the rate of desensitization produced by subsequent exposure to agonist is increased. The present study was done to test whether protein phosphorylation was involved in either the recovery from desensitization or the acceleration of desensitization with subsequent agonist exposure. Snake twitch fiber endplates, maintained in an isotonic potassium proprionate solution and voltage-clamped to +30 mV, were perfused with 540 μM carbachol to initiate the activation-desensitization sequence. The endplate region was then washed and re-perfused with agonist at different times to determine the time course and extent of recovery. Pretreatment with the protein kinase inhibitor staurosporine (10 nM to 0.5 μM) for 15 min produced a concentration-dependent decrease in the final extent of recovery of both carbachol-induced currents and MEPC amplitudes without any significant effect on the initial fast phase of recovery. Staurosporine had no effect on the initial peak carbachol-induced current, initial rate of desensitization, or the acceleration of desensitization occurring with subsequent applications. Also, staurosporine had no direct effect on MEPC amplitude or timecourse in fibers voltage-clamped to either -100 mV or +30 mV, indicating that activation kinetics were not altered. We conclude that recovery from desensitization has at least two components; a rapid staurosporine-insensitive component and a slower component which requires phosphorylation and is staurosporine sensistive. Supported by NIH grants NS08580 (JH) and NS25937 (RP) and a MDA grant (RP).

93.5

EFFECTS OF OXIDIZING AGONISTS ON NICOTINIC RECEPTORS. R.H. Loring, Y. Xie and G.S. Jones, Jr.* Dept. of Pharmaceutical Sciences, Northeastern University, Boston, MA 02115

One feature of all known nicotinic receptor subtypes is that reduction of an invariant disulfide by dithiothreitol (DTT) substantially decreases the responsiveness of the receptor to agonists. Therefore, we have synthesized nicotinic agonists with the ability to reoxidize disulfides for use as potential probes at the nicotinic agonist binding site. Dithio-bisacetylcholine (DT-ACh) and dithio-bis(1,1 dimethyl, 4-acetylpiperazinium) (DT-DMAP) were tested as nicotinic agonists in the chick retina preparation (Loring, Br. J. Pharm. 99:207, 1990). The EC_{50} for nicotinic stimulation by DT-ACh was 10^{-4}M while that of DT-DMAP was > 1 mM. DTT (2 mM) substantially blocks (> 85% in 20 min) retinal responses to the nicotinic agonist dimethylphenylpiperazinium (DMPP) while the response of NMDA receptors is enhanced 2-8 fold (Aizenman et al, Neuron 2:1257, 1989). Single applications (2 sec) of DT-ACh (EC_{50} 20 μM) or DT-DMAP (EC_{50} 30 μM) restored the DMPP responses of DTT-treated retinas while leaving the NMDA responses undiminished. Both DT-ACH and DT-DMAP displaced the binding of the neuronal nicotinic antagonist, ^{125}I-neuronal $_5$bungarotoxin (^{125}I-NBT), to homogenates of chick retina with IC_{50}'s of ≈10^{-5}M. DT-ACh was tested for the ability to reoxidize DTT-reduced receptors from chick retina or Torpedo by preventing affinity alkylation by bromoacetylcholine (BAC)$_4$. Alkylation by BAC was indirectly measured as the permanent inhibition of ^{125}I-NBT binding in washed retina homogenates. DT-ACh (10^{-6} M) protected against subsequent BAC alkylation in DTT-treated retinas. Similarly, oxidation by DT-ACh (EC_{50} 10^{-7}M) protected subsequent binding of ^{125}I-α-bungarotoxin to reduced and BAC-treated Torpedo receptors. These results suggest that DT-ACh and DT-DMAP are potent and selective oxidizing agonists of nicotinic receptors. Supported by NS22472.

93.6

EFFECTS OF REDUCING AGONISTS ON NICOTINIC RECEPTORS. Y. Xie, R.H. Loring and G.S. Jones, Jr.* Dept. of Pharmaceutical Sciences, Northeastern University, Boston, MA 02115

The vicinal disulfide bond found in the nicotinic agonist binding site is believed to be strained and therefore, is easily susceptible to reduction by dithiothreitol (DTT) and other agents (Kao and Karlin, J. Biol. Chem. 261:8805, 1986). However, nicotinic agonists, but not antagonists, protect against DTT reduction of the disulfide by inducing a conformational change in the receptor (Damle and Karlin, Biochemistry 19:3924, 1980). We asked whether a reducing group present on an agonist itself would have access to the susceptible disulfide bond. Reduction of dithio-bisacetylcholine (DT-ACh) by DTT or ditho-bis(1,1 dimethyl-4-acetylpiperazinium) (DT-DMAP) by $NaBH_4$ yielded products with strikingly different characteristics than the parent compounds, when tested in the chick retina preparation (Loring, Br. J. Pharm. 99:207, 1990). Unlike DT-ACh, the presumed reduction product, mercaptoacetylcholine (ACh-SH) was susceptible to the action of acetylcholinesterase, but in the presence of 10^{-6}M neostigmine, ACh-SH was more efficacious than DT-ACh. The presumed reduction product of DT-DMAP, 1,1-dimethyl-4-mercaptoacetylpiperazinium (DMAP-SH), was at least 10 fold more potent than DT-DMAP (EC_{50} DMAP-SH = 500 μM). DMAP-SH and ACh-SH were equipotent (IC_{50}'s ≈10^{-5} M) with the parent oxidized compounds in displacing the binding of the neuronal antagonist, ^{125}I-neuronal bungarotoxin, to chick retinal homogenates. Both DMAP-SH and ACh-SH activated nicotinic responses in chick retina without giving evidence of reducing nicotinic receptors. Experiments are underway to see if desensitization by ACh-SH or DMAP-SH gives evidence of reducing nicotinic receptors at the agonist binding site. Supported by NS22472.

93.7

PHARMACOLOGY OF THE NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR α4β2 EXPRESSED IN XENOPUS OOCYTES. Pilar, G., P. Charnet*, C. Labarca*, B. Cohen*, N. Davidson, and H.A. Lester. Div. Biology, Caltech, Pasadena, CA 91125.

Two to 7 days after co-injection with 12-25 ng apiece of mRNA from α4 and β2 cDNA clones (S. Heinemann), macroscopic conductances were induced by superfusion of ACh (0.5-5 μM) at 13° C. Responses desensitized by about 1/3 with a time constant of 30 sec and displayed a Hill coefficient near unity. ACh-induced conductance decreased at less negative potentials and there was little outward current at positive potentials. Voltage-jump relaxations (+50 mV → -10 to -150 mV) were described by a single exponential; the rate constant decreased slightly at less negative voltages. Hexamethonium (Hx) decreased ACh-induced currents by 50% at 4-8 μM. Hx also increased the rate constant of voltage-jump relaxations in a dose-dependent fashion, consistent with open-channel blockade with a forward binding rate of 2×10^7 $M^{-1}s^{-1}$. This resembles channel blocking rates, e.g. for QX-222, at muscle receptors. However, QX-222 (20-40 μM) had little or no blocking effect on the receptors. In repetitive jumps to more negative voltages, the Hx blockade was increased, suggesting that the channel can close on the bound Hx molecule; this Hx use dependence was not observed with expressed muscle receptors. In single-channel data (100 mM KCl), predominant conductances were 21.7, 12.5, and (less frequently) 34 pS. Average channel open time was quite variable (0.5-10 ms) with little voltage dependence. The α4β2 pharmacology shares some of the characteristics of the muscle of ganglionic receptors; but the differences suggest a distinct functional receptor class that may be present on CNS neurons. Support: NS-11756, NS-10338, and MDA.

93.8

TIME-DEPENDENT MATURATION OF ACETYLCHOLINE RECEPTOR CHANNEL ACTIVITY IN THE PLASMA MEMBRANE. L. Li* and M. G. McNamee. Dept. of Biochem. & Biophys., Univ. of California, Davis, CA 95616.

Time-dependent maturation of acetylcholine receptor (AChR) channel activity in the plasma membrane was studied using *Xenopus laevis* oocytes injected with *in vitro* synthesized *Torpedo californica* AChR RNA transcripts. The surface ^{125}I-α-bungarotoxin (α-BGT) binding assay showed that the surface-expressed AChR level was similar on Day 2 and Day 3 following microinjection of RNAs into the oocytes. In contrast to their toxin binding ability, the expressed AChRs were slower to acquire their ion channel activity. Voltage-clamp experiments at holding potentials of -80 mV showed that for the same individual oocytes the whole cell current response to 1 μM ACh increased by 10 to 33 fold from Day 1 to Day 2, and by another 2 to 4 fold from Day 2 to Day 3 . The *normalized* channel activity of surface expressed AChRs, measured as the ACh-induced conductance per fmol of surface α-BGT binding sites, increased by 3 to 5 fold from Day 2 to Day 3 . These results suggest that maturation of AChR ion channel function may require processes that occur after the receptor is inserted in the plasma membrane. (Supported by USPHS Grant NS 22941).

93.9

MOLECULAR DYNAMICS OF ION CHANNEL FORMING TRANSMEMBRANE SEGMENTS OF THE NICOTINIC ACETYLCHOLINE RECEPTOR

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High-temperature (800K) molecular dynamics was performed on the transmembrane segments of the Torpedo nicotinic acetylcholine receptor (nAChR) in order to define a structural template model of this region within ligand-gated ion-channels (LGICs). A 20 picosecond trajectory was computed for the alpha subunit M1 to M3 region, using a starting structure with residue positions 209-236, 244-265 and 275-299 in a helical conformation (phi -60, psi -40), and the intervening linker regions in an extended conformation (phi -120, psi 120). Within the first 10 picoseconds of the dynamics trajectory the three helical segments folded into an antiparallel bundle. Several features of the final structural model are consistent with experimental data that indicate a partly hydrophilic surface of the M2 segment contributes to the wall of the ion-channel. The packing of annular phospholipids around the pentameric transmembrane region of the receptor has also been modelled. The number of phospholipid molecules predicted from the modelling studies is in accordance with previous measurements made on Torpedo receptor-enriched membranes (Barrantes, Crit. Revs. Biochem. Mol. Biol. 24: 437, 1989).

93.10

RECTIFICATION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR CURRENTS.

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We studied nicotinic acetylcholine receptor (nAChR) currents in embryonic Xenopus spinal neurons and rat pheochromocytoma (PC12) cells using whole cell and single channel patch clamp techniques. In cell attached patches, single channel conductances were 21 pS for Xenopus neuron nAchR (amphibian solutions, 86 mM Na), and 48 pS for PC12 cell nAchR (mammalian solutions, 160 mM Na). Whole cell inward currents were carried by mono- and divalent cations, and were blocked by curare. Unlike nAChR currents in muscle, those in neurons and PC12 cells show inward rectification. We observed in whole cell recordings that peak current I-V relations during brief applications of 50-100 uM ACh or nicotine were almost linear at voltages more negative than -10 mV, but showed a sharp bend at positive voltages such that outward current was virtually absent.

We investigated the possible role of Mg-dependent block of nAChR channels in generating this rectification by recording whole cell nAChR currents during dialysis with Mg-free solutions containing 10 mM EDTA and 5 mM Na-ATP. I-V relations were determined at the peak of nAChR current using voltage ramps from -50 to +60 mV, or voltage steps from -70 mV to +60 mV. We observed partial relief of rectification during dialysis, as indicated by gradual development of a small amount of outward current at positive voltages. However, even after extensive dialysis with Mg-chelating solutions (up to 40 min), substantial rectification remained. We did not observe rectification of single channel nAChR currents in outside-out patches using the same Mg-free internal solutions. These observations suggest that an additional mechanism(s) is responsible for the rectification seen in whole cell current. We are presently characterizing channel opening probability as a function of voltage in the absence of Mg.

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93.11

REGULATION OF FOUR DISTINCT NICOTINIC ION CHANNEL TYPES IN SYMPATHETIC NEURONS FOLLOWING INNERVATION IN VITRO.

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We have studied neuronal nicotinic acetylcholine receptors (nAChRs) of chick sympathetic ganglion neurons following innervation *in vitro*. Before their *in vivo* innervation these neurons respond to ACh with three different classes of channel openings, referred to as S(mall), M(edium) and L(arge) openings (Moss et al., Neuron 3 (1989), p. 597). Following innervation in the animal, there is (a) an increase in frequency of all channel openings, (b) a shift in the conductances of S and M, (c) a shift in the major contributing classes from S & M in ED 10 to M & L in ED 17 and (d) a new class of higher conductance ACh activated channel openings (called extra-large; XL). To determine whether these changes were due to innervation *per se* we removed neurons prior to innervation *in vivo* (ED 10/11) and innervated them by preganglionic neurons *in vitro*. Under these conditions, 'ED 17-like' single channel characteristics are detected in innervated neurons within 48 hours. To directly examine the properties of newly inserted AChRs we have treated neurons with an irreversible ligand, bromoacetylcholine-bromide (BAC), to selectively block nicotinic AChRs. Based on macroscopic current analysis, we found that the nAChR turnover rate is ~ 2 % per hour (Gardette et al., in prep.). Monitoring the recovery of single channel openings at various times after BAC treatment of non-innervated ED 10/11 neurons, reveals that the proportional contribution of the different channel classes was altered such that the neurons had less S and M, but more openings of the L- type and in addition some XL openings. Thus, even before innervation *in vivo*, sympathetic neurons can express nicotinic receptors characteristic of later stages of development (eg. ED17-like) so that innervation may increase the rate of expression of "mature" AChR types rather than trigger these events *de novo*. *Supported by NIH and the McKnight foundation to L.W.R . and a NATO grant (#81-415) to A.B.B.*

93.12

NICOTINIC RECEPTOR SUBUNIT EXPRESSION IN CHICK CNS CORRELATES WITH ACH GATED SINGLE CHANNEL ACTIVITY,

J.P.Doyle*, S.E.Huck, A.B. Brussaard, M.D.Listerud, L.W.Role, Dept. of Anat. & Cell Biol, Ctr for Neurobiol. & Behav., Columbia Univ. P. & S., 630 W. 168th St., NY, NY 10032.

Both molecular (Wada et al., 1988) and recent biophysical (Mulle et al., 1990) data indicate that the habenular nucleus (HAB) of the rat is a rich source of neuronal nicotinic acetylcholine receptors (AChR). Our studies on peripheral neurons reveal important developmental changes in AChRs (Listerud et al., Brussaard & Role, this vol.), leading us to examine similar regulation of AChR channel expression in the chick CNS. We identify the chick HAB and lateral spiriform nucleus (LSN) by combined anti-choline acetyltransferase (gift of M. Epstein) and anti-AChR (gift of J. Lindstrom) staining. Northern blot analysis with an α3 probe detects hybridization to RNA from the LSN and the HAB, but not from cerebellum or optic tectum of ED16-19 chicks. We assayed subunit expression in adult LSN and HAB with the polymerase chain reaction using oligonucleotide primers which amplify α2, α3, α4, α5, and β2 and β4 cDNA sequences. α3 and α5 probes amplify cDNA sequences from both the LSN and HAB. Probes to β subunits amplify a product from HAB cDNA whose size and restriction map correspond to the β2 product. These probes also amplify a smaller product from both regions corresponding to neither β2 nor β4.

This diversity of subunits suggests the possibility of different channel classes. After papain dissociation, cells from the HAB and LSN survive for several days in culture. We have monitored ACh gated single channel activity in cell attached patches from isolated HAB neurons of ED11 and ED17 chicks. A single conductance class of about 34 pS was seen at both stages of development with approximately a ten fold increase in frequency observed in patches from ED17 neurons. A larger conductance class in patches from ED17 neurons was also observed in one set of recordings. Thus, neurons of the chick habenula express a variety of AChR subunits which may contribute to multiple classes of ACh activated channels and could provide a model for the developmental regulation of AChRs in the central nervous system. *Supported by NIH and the McKnight foundation to L.W.R.*

93.13

CALCITONIN GENE-RELATED PEPTIDE (CGRP) MODULATES NICOTINIC ACETYLCHOLINE RECEPTORS IN CHICK SYMPATHETIC NEURONS.

D.C. Valenta & L.W. Role, Dept. of Anat. & Cell Biol. and Ctr. for Neurobiol. & Behav., Columbia Univ. P & S, 630 W. 168th St., NY, NY 10032.

Chick sympathetic ganglion neurons receive input from both ACh- and CGRP-containing nerve fibers (New & Mudge, 1986; Tessier-Lavigne, unpub.). We have examined whether CGRP can regulate neuronal nicotinic acetylcholine receptor (nAChR) channel function by assaying effects of CGRP on whole-cell nicotinic sensitivity and agonist-induced desensitization in voltage-clamped chick sympathetic neurons *in vitro*. Incubating sympathetic neurons with CGRP (100-500 nM for > 3-5 mins.) increases the mean peak amplitude of nAChR-mediated currents by 1.5-2-fold vs. control (n =13). CGRP, when coapplied with submaximal concentrations of nicotinic agonist, also induces an ~30% increase in the mean rate of agonist-induced current decay (n = 16). These data suggest that CGRP enhances nicotinic sensitivity and the rate of nAChR desensitization. Since CGRP activates adenylate cyclase in other systems (cf Laufer & Changeux, 1987), we have assayed effects of elevating cyclic AMP levels on nAChR function in sympathetic neurons in parallel experiments. We find that treating sympathetic neurons with cholera toxin (CTX, 10 μg/ml, 60 min. preincubation) and cyclic AMP analogues (chlorophenylthio-cAMP, CPT-cAMP, 1 mM, 90 min. preincubation + coincubation; adenosine 3',5' monophosphorothioate S_p-isomer, S_p-cAMPS, 1 mM, 120 min preincubation) elicits a 1.6-2.1-fold increase in peak nAChR-mediated currents (n=25), results similar to those obtained in chick parasympathetic neurons (cf Margiotta et al, 1987). CTX can induce a 45% increase in the rate of current decay (n=5), however, both CTX and S_p-cAMPS significantly decreased the rate of current decay in some experiments (by 15-39%, n=10). Since neuronal nAChRs mediate synaptic transmission in sympathetic ganglia, the modulation of nAChR channels by CGRP and cyclic AMP may regulate synaptic function *in vivo*. *[Supported by NIH (NS22061), McKnight Fndn., Columbia Univ. (BRSG) to LWR & an NIH Predoctoral Fellowship to DCV.]*

93.14

NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR GENE EXPRESSION AND FUNCTIONAL BLOCK IN CHICKEN SYMPATHETIC GANGLIA NEURONS.

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Our lab is interested in the acetylcholine gated ion channels expressed by chick sympathetic neurons and the subunits that comprise these channels. Using sequence information (provided by M. Ballivet, U. Geneva) we have assayed AChR gene expression in RNA from sympathetic ganglia by the polymerase chain reaction (PCR) technique. We observe cDNA dependent PCR products in reactions containing synthetic oligonucleotide primers specific for the α3, α5, and β4 AChR subunit genes and have confirmed their identity by detailed restriction enzyme maps. Radioactive probes generated from these fragments and a β2 clone (M. Ballivet) hybridize under stringent conditions to four RNA species respectively (α3: 3kb, α5: 4kb, β2: 4.6kb, and β4: 2.7kb) in Northern blots of total RNA taken from embryonic sympathetic ganglia (ED11 and ED17).

To examine function, two tandem antisense oligos were designed against the 5' end of the unspliced β4 message. Isolated sympathetic neurons maintained in culture were first treated in a manner that covalently blocks over 90% of the surface AChRs (Brown & Kwiatkowski, Br. J. Pharmac. 1976, 56:128) and were then incubated with the oligos (20μm). After 48hrs, cells exposed to the antisense β4 oligos (n=15) had a dramatic reduction in ACh induced channel activity as compared to both a sense α3 oligo (n=10) and control media (n=14) co-cultures. These data suggest the molecular correlates for the several ACh channels we observe in these neurons (Moss et al. Neuron 1989 3:597). *Supported by NIH and McKnight Foundation (LWR), NSF Predoctoral Fellowship (MDL), NATO grant #81-415 (ABB).*

93.15

INTRACELLULAR CALCIUM ION TRANSIENTS ASSAY CHICK CILIARY GANGLION ACH SENSITIVITY. Jeremy B. Tuttle Dept of Neuroscience, Univ of Va Hlth Sci Cntr, Charlottesville, VA 22908

Ciliary ganglion neurons regulate ACh receptors via retrograde intercellular interactions (Jacob & Berg, J. Cell Bio, 105:1847 (1987), McEachern et al, J.Neurosci, 9:3899(1989)), but study of the mechanisms and signals responsible has been hampered by lack of a non-electrophysiological assay for ACh receptor function, necessitated by the large proportion of "silent" receptors in the neuronal membrane. ACh receptor regulation in vivo is mimicked by neurons in solitary culture, where ACh responses are retained if a solubilized muscle membrane fraction is included (J.B. Tuttle, Soc Neurosci Abs, 15: Pt 2, 1260, 1989). An assay for ACh responses was developed using receptor-mediated transient increases of intracellular free Ca ion imaged via the fluorescent Ca indicator INDO. Freshly isolated neurons or neurons on collagen in solitary culture for <24 hrs respond to ACh with dose-dependent Ca transients (1-100 uM ACh) which were blocked by curare and by removal of extracellular Ca, suggesting receptor activation is required to detect the Ca transients. After 4-5 da in vitro without active factors, responses were not obtained, paralleling electrophysiological measures of lost sensitivity. However, Ca responses to depolarization in high K saline were intact, demonstrating competent Ca channels and detection in the older cultures. Appropriate optics and culture density allow groups of neurons to be imaged simultaneously. While the Ca ion flux assay is less sensitive and less precise than measurements of single cell ACh-activated currents or voltage transients, it allows simultaneous data collection from many neurons and thus screening of extract fractions for activity. Also, using high spatial and temporal resolution, Ca transients and receptor localization by non-blocking fluorescent immunoprobes might be combined in live cells. Supported by the Muscular Dystrophy Association.

93.16

EXTRACELLULAR ATP MODULATES THE FUNCTION OF THE PERIPHERAL NICOTINIC RECEPTOR. R.A.M. Reis, R. Rozental and E.X. Albuquerque. Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, RJ 21941, Brazil and Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. of Med., Baltimore, MD 21201.

Previous work suggested that some substances secreted from motor nerve terminals could modulate the peripheral nicotinic receptor–ion channel complex (nAChR) function (Albuquerque *et al.*, 1979, *J. Physiol.* 297:423). ATP (adenosine–5'–triphosphate) is co–released at cholinergic synaptic junctions at concentrations up to 100 μM (Silinsky, 1975; *J. Physiol.* 247:145) with acetylcholine (ACh). To evaluate how ATP modulates the nAChR, single channel currents were recorded from junctional and perijunctional regions of the single fibers of the frog *Leptodactylus occelatus* interosseal muscle (patch–clamp technique, cell–attached configuration). ATP (0.01–0.1 μM) modified the kinetic properties of ACh–induced currents (0.4 μM); the major alteration observed was a marked increase in the mean number of events per burst. These effects were time– and voltage–dependent, i.e, they were enhanced by membrane hyperpolarization. Also, a slight decrease in the mean open time was noticed. It is worth noting that in contrast to previous report (Lu and Smith, 1989; *Neurosci. Abs.* 15:1300, 1989) ATP (0.01–0.5 μM) alone neither induced channel openings nor potentiated the agonistic effect of ACh. However, at higher concentrations (1–50 μM), a weak agonistic activity could be recorded. Our data suggest that ATP at nanomolar concentration range modifies junctional ACh activity which may be important in the modulation of the neuromuscular transmission. Support: FINEP, CNPq, UFRJ/UMAB Mol. Pharmacol. Training Program.

93.17

EFFECT OF METAPHIT ON THE PERIPHERAL NICOTINIC ACETYLCHOLINE (ACh) RECEPTOR. T. Tano[1,2], K. Rice[3], R.S. Aronstam[4], A.C. Oliveira[2], Y. Aracava[1,2] & E.X. Albuquerque, E.X.[1,2] [1]Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, RJ 21941, Brazil, [2]Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. Med., Baltimore, MD 21201, [3]Lab. Chem., NIH, NIDDK, Bethesda, MD 29892 & [4]Dept. Pharmacol. Toxicol., Med. Coll. Georgia, Augusta, GA 30912.

The actions of metaphit (1–[1–(3–isothiocyanatophenyl)cyclohexyl]–piperidine), a phencyclidine (PCP) analog, on peripheral nicotinic ACh receptors were studied using electrophysiological and biochemical techniques. Metaphit (50–100 μM) depressed the peak amplitude more markedly than it shortened the decay time constant of endplate currents recorded from the sartorius muscles of *Rana pipiens*. The current–voltage relationship was nonlinear from −60 to −200 mV. In cell–attached single channel recordings obtained from the perijunctional regions of interosseal muscle fibers of frog, a decrease of the frequency of channel openings was observed in the presence of metaphit (0.5–3.0 μM). Mean open time, burst time and single channel conductance remained unaltered. The sensitivity of denervated mammalian skeletal muscle to iontophoretic application of ACh was reduced by metaphit (2–16 μM). This effect was dependent on the concentration of metaphit and on the frequency of ACh pulses. Binding experiments using *Torpedo* electroplax membranes showed that metaphit inhibited the binding of [^{3}H]perhydrohistrionicotoxin, and this effect was further enhanced by the presence of the agonist carbachol. These data suggest that, in contrast to PCP which has multiple effects at peripheral nicotinic receptors, metaphit primarily enhances agonist–induced desensitization. Supported by FINEP, CNPq & NIH #P50–MH44211.

93.18

ANTAGONISM OF NEURONAL NICOTINIC RECEPTORS BY METHYLLYCACONITINE. S Wonnacott[1], A Drasdo[*1] & M Caulfield[*2] [1]Dept. Biochem., Univ. Bath, Bath BA2 7AY, UK and [2]Dept. Pharmacol., Univ. College London WC1E 6BT, UK.

Methyllycaconitine (MLA) possesses insecticidal and neuromuscular blocking activities. MLA competes for binding to muscle nicotinic receptors (nAChR) and brain [^{3}H]nicotine sites at μM concentrations, but inhibits [^{125}I]αbungarotoxin binding to rat brain at nM concentrations. We have examined its effects on functional neuronal nAChR in two preparations: (i) rat superior cervical ganglion, in which pre- and postsynaptic nicotinic responses were measured by extracellular recording (Brown & Marsh, Brain Res. 156: 187-191, 1978); (ii) rat striatal nerve terminals, in which the presynaptic nicotinic stimulation of [^{3}H]-dopamine release was measured by superfusion (Rapier et al., J.Neurochem. 54: 937-945, 1990). MLA at 25μM reduced pre- and postganglionic responses to DMPP (30μM) by 80% and 62% respectively. In the perfused synaptosome preparation, 10μM MLA inhibited nicotine-evoked [^{3}H]dopamine release by 80%. Thus ganglionic and brain nAChR are similarly sensitive to low micromolar concentrations of MLA, consistent with its K_i for brain [^{3}H]nicotine binding sites. MLA may be a useful novel antagonist for studying neuronal nAChR; its ability to discriminate between nAChR and brain αbungarotoxin binding proteins make it a valuable probe for exploring the roles of these related proteins in nervous tissue.

93.19

NICOTINE DIFFERENTIALLY AFFECTS THE SPONTANEOUS FIRING OF HIPPOCAMPAL PYRAMIDAL CELLS AND INTERNEURONS. D.A. Engstrom and G.M. Rose. Department of Pharmacology, UCHSC & Medical Research, VAMC, Denver CO 80262

Recent evidence suggests that changes in hippocampal nicotinic-cholinergic receptors occur with Alzheimer's disease. We are interested in understanding the functional consequences of such changes. A first step is to determine the physiological effects of nicotinic receptor activation upon different subclasses of hippocampal neurons.

Extracellular recordings of hippocampal CA1 pyramidal cells and interneurons were made using saline and drug-filled multi-barrel glass electrodes in male Sprague-Dawley and Fischer 344 rats. Animals were anesthetized with sodium pentobarbital (50-60 mg/kg, i.p.), placed in a stereotaxic apparatus and surgically prepared for acute recording. Pyramidal cells and interneurons within the hippocampus were isolated and identified using physiological criteria. Cell firing rates were sampled using a window discriminator and displayed on a stripchart recorder. Nicotine (barrel concentration: 1 mM) was applied using the technique of pressure microinjection.

The spontaneous firing rates of all pyramidal cells tested to date (36) were increased, and all interneurons (23) decreased, by nicotine. Preliminary results indicate that the effects of locally applied nicotine upon both pyramidal cells and interneurons can be blocked by curare, a neuromuscular-junction type nicotinic-cholinergic receptor antagonist. (Supported by NSF BNS8811486, the VAMRS and NIA AG04418).

94.1

CHARACTERIZATION OF A MOUSE DOPAMINE D2A RECEPTOR GENE AND DEVELOPMENTAL EXPRESSION OF THE RODENT mRNA D2A TRANSCRIPTS. K.J. Mack, R.D. Todd, and K.L. O'Malley. University of Wisconsin, Madison, WI 53792 and Washington University, St. Louis, MO 63110

Molecular techniques have identified at least three types of Dopamine D2 receptors. The rat D2A receptor gene is a large gene with multiple introns and an alternatively spliced exon, leading to the transcription of two alternatively spliced mRNA forms (O'Malley et al. Biochem. 29:1367, 1990). The D2B receptor gene product has the pharmacological profile of a D2 receptor, yet shares little sequence homology with the D2A receptor gene (Todd et al. PNAS 86:10134, 1989).

Using restriction endonuclease mapping, Southern blotting, and DNA sequencing, we have determined the organization of the mouse D2A receptor gene. Atypical for a G-protein coupled receptor, the mouse D2A gene spans at least 30kb of genomic sequence and contains 7 introns. There is at least 18kb of sequence between the first (noncoding) exon and the second (first coding) exon. This gene shares 99% amino acid homology with the rat gene and 95% homology with the human gene. The mouse gene can also be alternately spliced to form mRNAs for the $D2A_{415}$ and $D2A_{444}$ receptor proteins.

In order to understand the developmental regulation of the D2A gene, a PCR amplification technique was used to detect the multiple forms. An oligonucleotide primer set made from sequences in exon 7 and 8 was used to detect the total amount of D2A mRNA. A different primer set, which flanks the alternative exon (6), was used to detect the two forms of the D2 mRNA. Both D2A transcripts were detected as early as embryonic day 15 in the rat, at levels less than 15% of adult values. The D2A mRNAs then gradually increase to adult levels, as measured as a percentage of total mRNA.

94.2

DOPAMINE D-2 RECEPTORS AND ALTERNATIVELY SPLICED FORMS OF D-2 RECEPTOR mRNA IN RAT, BOVINE, AND HUMAN TISSUES. K.A. Neve, J.N. Joyce, V. Simonneaux, M. Ebadi, A. Spanoyannis, and R.L. Neve. VAMC, Portland, OR; Univ. of Pennsylvania Sch. Med., Philadelphia, PA; Univ. of Nebraska Coll. Med., Omaha, NE; and Univ. of Calif., Irvine, CA.

Two molecular forms of D-2 receptors can be distinguished on the basis of the presence ($D\text{-}2_{444}$) or absence ($D\text{-}2_{415}$) of a 29-amino acid exon. Both mRNAs were widely distributed in rat, bovine, and human tissues, but the ratio of the alternatively spliced forms varied. For example, $D\text{-}2_{444}$ was expressed more abundantly than $D\text{-}2_{415}$ in rat pituitary and neostriatum, whereas a number of other rat tissues expressed both mRNAs at similar levels. Distribution of the two mRNAs in bovine and human tissues differed in several respects from distribution in rat. Using the ligand [^{125}I]epidepride, we have characterized dopamine D-2 receptors in many tissues, including some with a low abundance of D-2 receptor mRNA. For some drugs, there were small but reliable differences in binding affinity among the tissues. For example, flupenthixol had an affinity of 2-3 nM in human cortex (frontal and temporal lobes) but an affinity of 0.5-1.0 nM in human putamen and accumbens. Also, the K_D of [^{125}I]epidepride for D-2 receptors varied from 18-80 pM among tissues, with the lowest values observed in human cortex, intermediate values in human and rat caudate-putamen, and highest values in bovine cortex, hippocampus, and caudate. The possibility that differences in drug binding affinity are related to differences in the ratio of $D\text{-}2_{415}$ to $D\text{-}2_{444}$ is being investigated. (MH 45372, MH 43852, HD 18658).

94.3

ANTI-PEPTIDE ANTIBODIES LOCALIZE ^{125}I-NAPS BINDING TO THE C-TERMINAL PORTION OF THE D_2 DOPAMINE RECEPTOR. C. David* and S. Fuchs. Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

N-(4-azido-3-^{125}I-iodophenethyl)-spiperone (^{125}I-NAPS) is a potent derivative of the D_2 dopamine receptor antagonist, spiperone. Photolabelling of bovine striatal or pituitary membranes, results in the specific labelling of a major 90 Kd protein band and 40 Kd, 20 Kd, and 14 Kd fragments, as well. In an attempt to develop specific reagents for the D_2 receptor, we raised antibodies against synthetic peptides of the rat D_2 receptor. Antibodies directed against peptides which correspond to cytoplasmic regions of the receptor, including the 14 amino acid C-terminal peptide, immunoprecipitate the 90 Kd band. Differential reactivity of the anti-peptide antibodies with various proteolytic fragments of the D_2 receptor indicates that ^{125}I-NAPS binds covalently to a carboxy terminal 140 amino acid fragment of the D_2 receptor. This implies that the photoaffinity ligand binds to a residue(s) located at the short extracellular stretch between the 6th and 7th transmembrane segment of the receptor or within these segments themselves. Further studies are being carried out to determine whether the receptor ligand binding site is located in this region or in another portion of the molecule which is conformationally in close proximity to this area.

94.4

CAN VOLTAGE-CLAMPED *XENOPUS* OOCYTES DETECT RECEPTOR-DRIVEN ALTERATION OF CYCLIC AMP LEVELS? Angela K. Birnbaum, Ping Y. Law, Sandra Roerig and George L. Wilcox. Dept. of Pharmacology, University of Minnesota, Minneapolis, MN 55455, U.S.A.

Xenopus oocytes form gap junctions with the follicular cells that surround them. The conductance of these gap junctions can be modulated by second messengers, such as cyclic AMP. The purpose of the present study is to exploit this phenomenon as an indicator of activation of receptors coupled to adenylate cyclase. The first part of the study examined follicle-oocyte junctions in oocytes injected with mRNA for human α_2 adrenergic receptors; the second part of the study examined oocyte-oocyte junctions in oocyte pairs injected with mRNA for β_2 and α_2 adrenergic receptors.

Stage V-VI folliculated oocytes were removed from mature female *X. Laevis*. Single oocytes were impaled with two 3M KCl-filled micropipettes (0.5 - 5 MΩ) and voltage-clamped with a two-electrode voltage clamp (0 to -100mV stepped or -10 to -25 mV constant). Currents were measured during and after application of ZK-62711, a phosphodiesterase inhibitor, isoproterenol, a β agonist, and tramazoline, an α_2 agonist. Isoproterenol elicited an outward current which reversed near -100mV. This effect was likely mediated by endogenous β receptors in the follicular cells. The current was presumably carried by K^+ ions through K^+ channels in the follicle cells. This effect of isoproterenol was enhanced by ZK-62711 pretreatment. Tramazoline did not appear to alter this current by itself, but co-administration of tramazoline with isoproterenol sometimes decreased the isoproterenol effect slightly.

Stage V-VI oocytes were defolliculated by 2h collagenase digestion, injected with mRNA for β_2 and α_2 adrenergic receptors and mechanically devitillinized before juxtaposition. After three days, some coupling between oocytes was observed when each oocyte was independently voltage clamped. Coupling was variably affected by isoproterenol and tramazoline. Xenopus oocytes may be useful in expression of receptors using cyclic AMP as a second messenger. (Supported by NIDA R01 grants 01933 and 04274).

94.5

REGULATION OF DOPAMINE D2 RECEPTOR GENE EXPRESSION O. Civelli, L. Vallar, J. Meldolesi, H. Van Tol, L. Thambi, J. Bunzow and D. Grandy. Vollum Institute, OHSU, Portland, OR 97201 and University of Milan, Milano, Italy

We have characterized two isoforms of the D2 dopamine receptor which are generated by alternative splicing. Several aspects of expression and stimulation of these two isoforms have been studied. At the level of the gene it has been found that the 5'untranslated region of the mRNA is interrupted by a long intron. This intron may play a regulatory role in D2 receptor gene expression. The long isoform of the D2 receptor has also been expressed via recombinant vaccinia virus infection. This expression system may be of value in efforts to raise antibodies specific to the D2 receptor. At the cellular level dopamine stimulation of the receptor has been found to affect different second messenger systems depending on the environment of the receptor. In fibroblasts, the short isoform is able to inhibit adenylyl cyclase activity and phosphoinositol turnover. In lactotrophs, stimulation of the same isoform inhibits adenylyl cyclase, has no effect on inositol phosphate levels and permits G protein-mediated coupling of the receptor to K+ channels. These second messenger effects were analyzed following stimulation of the long isoform and will be discussed in regard to the G proteins present in these cell lines.

94.6

D2 DOPAMINE RECEPTOR mRNA IS EXPRESSED IN DORSAL ROOT AND PETROSAL GANGLIA OF RAT. D.A. Bayliss, M. F. Czyzyk-Krzeska, K.B. Seroogy, E.R. Perl and D.E. Millhorn, *Department of Physiology, University of North Carolina, Chapel Hill NC 27599*

A number of primary sensory neurons of rat petrosal ganglia (PG) express tyrosine hydroxylase (TH) and are thought to be dopaminergic. In contrast, only a few dorsal root ganglia (DRG) cells of rat express TH. *In situ* hybridization was used to determine if neurons of PG and DRG might also express the mRNA for the presumptive dopamine autoreceptor (*i.e.* D2 receptor). Two synthetic oligonucleotides, complementary to different regions of D2 receptor mRNA, were labeled with [^{35}S] and applied as a cocktail to sections (10 μm) of fresh frozen ganglia. A number of PG neurons were found to contain D2 receptor mRNA; the topographic location of these cells within PG suggest that D2 receptor may be expressed in putative dopamine cells. In DRG, a substantial subpopulation of neurons also expressed D2 receptor mRNA. The number of D2 receptor mRNA-containing cells in DRG was much greater than the number of TH-expressing cells. These latter results indicate that D2 receptor mRNA may be found in primary sensory cells which are not dopaminergic. (NIH HL33831)

94.7

QUANTITATIVE MEASUREMENT OF RAT DOPAMINE D2 RECEPTOR mRNA SPLICE VARIANTS BY SOLUTION HYBRIDIZATION. L.A. Snyder,*Y. Shifman, J.L. Roberts, S.C. Sealfon. Fishberg Center of Neurobiology, Mt. Sinai Medical School, New York, NY 10029.

Two D2 receptor mRNAs which are splice variants of a single gene exist in the rat brain. In order to investigate the regional distribution and regulation of the two mRNA forms, we developed a sensitive and reproducible solution hybridization/nuclease protection assay. This assay was used to examine the subtype specific distribution of D2 mRNA in the brain and pituitary of adult male rats. The mRNA encoding the longer form of the D2 receptor is found in highest levels in the pituitary neurointermediate lobe, followed by levels in the anterior lobe, and in the striatum. Much lower levels are seen in other brain areas. In contrast, the mRNA encoding the shorter form of the D2R is found in highest levels in the striatum, followed by intermediate levels in the pituitary and low levels in other brain areas. Thus the ratios of the two D2 splice variant mRNAs varies widely across tissues, with the mRNA encoding the shorter form of the D2 receptor comprising 10-50% of the total D2R mRNA. The highest ratios of long to short D2R subtype mRNA is found in the pituitary (~9:1) while lower ratios (~2:1 and 1:1) are found in the striatum and the midbrain respectively. Using a probe that does not discriminate splice forms, we have demonstrated that haloperidol treatment increases the D2R mRNA level in the rat NIL, but not the anterior pituitary (Mol Cell Endo. 1989. 67:101-105) and are determining if this regulation is specific to either splice form.

94.8

ANTIBODIES TO SYNTHETIC PEPTIDES CORRESPONDING TO SEGMENTS OF THE DOPAMINE (DA) D_2 RECEPTOR. J.Y. Lew, K.Y. Lee, M. Goldstein and A.Y. Deutch. Neurochem. Res. Lab., New York Univ. Med. Ctr, N.Y., N.Y. 10016 and Dept. Psych. and Pharmacol., Yale Univ. Med. Ctr., West Haven, CT 06516.

To characterize the DA D_2 receptor by immunochemical and immunohistochemical procedures, several peptides corresponding to discrete AA sequences of the DA D_2 receptor were synthesized and used as antigens for generation of polyclonal antibodies in rabbits. One peptide, D_2-1, containing the AA 10-20 from the N-terminal segment, and four peptides, D_2-3, D_2-4, D_2-4a and D_2-5, containing the AA 221-235, 261-270, 292-315, and 332-343 from the third cytoplasmic loop of the DA D_2 receptor, respectively, were synthesized.The synthetic peptides were coupled to bovine thyroglobulin or to a purified protein derivative of tuberculin and rabbits were immunized with conjugates corresponding to 50-200 μg of peptide. The antisera were tested by solid phase ELISA procedure and by Western immunoblots. Anti D_2-3, D_2-4, D_2-4a and D_2-5 interact with several solubilized striatal proteins (M.W. 34-120 KDa) and with one cerebellar protein (34 KDa). Preimmune serum does not recognize the immunoreactive proteins. Histochemical studies indicate that anti-D_2-4 and D_2-4a recognize neurons in the substantia nigra, pars compacta and medium size striatal neurons. The staining specificity for the DA D_2 receptor is now under investigation. These studies were supported in part by Grants NIMH 02717 and NINCDS 06801.

94.9

DOPAMINE TYPE D_2 RECEPTOR-SPECIFIC ANTIBODIES. J.W. Brock, S. Farooqui, and C. Prasad. Neuroscience Lab, Pennington Biomed.Res.C., Baton Rouge; Dept. of Med., LSUMC, New Orleans, LA 70808.

Antibodies have been raised in preparation for mapping the location of dopamine type D_2 receptors in brain. Rabbits were innoculated with keyhole limpet haemocyanin-conjugated oligopeptide identical to amino acid sequence 77-86 of the D_2 receptor. In the D_2 receptor, this sequence is located between transmembrane domains 3 and 4. Monthly blood samples were taken and booster injections given. Antibody titer was determined by ELISA. The antibody was reactive only with amino acid sequence 77-86 (titer, 1:15,000 on ELISA). The antibody did not react with 2 analogues of the peptide or a peptide representing amino acid sequence 1-15. These data suggest that the antibody is selective against dopamine D_2 receptor protein. (Supported by Dept. of Army, grant #1788G8023)

94.10

ORGANIZATION AND EXPRESSION OF THE HUMAN $D2_A$ GENE. K.Y. Gandelman, S. Harmon*, K.J. Mack, R.D. Todd, and K.L. O'Malley. Department of Anatomy & Neurobiology and Psychiatry, Washington University School of Medicine, St. Louis, MO 63110.

The dopamine D2 receptor has been implicated in the pathophysiology of a number of disorders. Because detailed description of its gene locus will aid in understanding putative mutant alleles and polymorphisms in human, we have isolated the complete human $D2_A$ gene. Like the rat $D2_A$ gene (O'Malley et al. (1990) Biochemistry 29: 1367-1371), the human gene contains at least eight exons and spans at least 45 Kb. Exons 3-8 are clustered within 9 Kb of genome. Exon 2 is separated from exon 1 by at least 30 Kb. We and others have shown that alternative utilization of exon 6 gives rise to alternative $D2_A$ transcripts. Despite the extreme size of intron 1, no alternative transcripts can be detected in basal ganglia and pituitary using polymerase chain reaction analysis. The relative abundance and tissue distribution of the alternative $D2_A$ transcripts were examined in twenty different human brain regions. The relative expression of the two transcripts varied by at least 200-fold across the brain region surveyed. As expected, high levels of transcripts were detected in caudate, putamen and pituitary. Moderate levels were detected in the locus coeruleus and amygdala. In contrast to the rat brain, very low levels of transcripts were detected in cortical regions.

94.11

A68930: A POTENT AGONIST SPECIFIC FOR THE D-1 DOPAMINE RECEPTOR. M.P. DeNinno, R. Schoenleber, R. MacKenzie, D.R. Britton, K.E. Asin, C. Briggs, J.M. Trugman and J.W. Kebabian. Neuroscience Research Division, Abbott Laboratories, Abbott Park, IL 60064 and Department of Neurology, University of Virginia, Charlottesville, VA 22908 (JMT).

A68930 [1R, 3S] 1-aminomethyl-5,6-dihydroxy-3-phenylisochroman HCl, is a potent (EC_{50} = 2.5 nM), partial (intrinsic activity = 66 percent of dopamine) agonist in the dopamine-sensitive adenylate cyclase model of the D-1 dopamine receptor. In contrast, A68930 is a much weaker (EC_{50} = 3,920 nM) full agonist in a biochemical model of the D-2 dopamine receptor. The orientation of the 3-phenyl substituent in the molecule is critical for the affinity and selectivity of the molecule towards the D-1 receptor. A68930 also displays weak alpha-2 agonist activity but the molecule is virtually inactive at the alpha-1- and beta-adrenoceptors. When tested in rats bearing a unilateral 60HDA lesion of the nigro-neostriatal neurons, A68930 elicits contralateral turning that is antagonized by D-1 receptor selective doses of SCH 23390 but not by D-2 receptor selective doses of haloperidol. When administered to these 60HDA lesioned rats, the molecule increases the accumulation of 2 deoxyglucose in the substantia nigra on the lesioned side of the brain. When tested in normal rats, A68930 elicits hyperactivity and, at higher doses, produces a forelimb clonus.

94.12

EXPRESSION AND CHARACTERIZATION OF D_2 DOPAMINE RECEPTOR ISOFORMS IN TRANSFECTED MAMMALIAN CELL LINES. Mario S. Rinaudo, Frederick J. Monsma, Jr., Lauren E. Black, Lawrence C. Mahan*, & David R. Sibley. ETB, NINDS, and LCB, NIMH, NIH, Bethesda, MD 20892.

We recently described the cloning of two variants of the D_2 dopamine (DA) receptor which are derived from alternative RNA splicing (*Nature* 342: 926-929, 1989). To investigate the functional properties of the short (D_{2S}) and long (D_{2L}) isoforms of the D_2 receptor, we have stably expressed both cDNAs in CHO cells. Following transfection, several clonal cell lines were isolated which exhibited D_2 receptor binding with B_{max} values ranging from 0.5 to 5 pmol/mg protein as determined by ^{3}H-methylspiperone (^{3}H-MSP) binding. Cell lines expressing ~1-2 pmol/mg protein of each isoform were chosen for direct comparisons. Both receptor isoforms revealed a K_D for ^{3}H-MSP of ~60 pM. Competition assays for ^{3}H-MSP binding by DA antagonists to both D_{2L} and D_{2S} exhibited identical affinities and rank orders of potency. Inhibition of ^{3}H-MSP binding by DA revealed the presence of high and low affinity agonist binding states with both receptor forms exhibiting similar values for the affinities (K_H and K_L) and proportions of the agonist binding states. In addition, both receptor isoforms show a complete loss of high affinity agonist binding with the addition of guanine nucleotides. Both of the D_2 receptor isoforms expressed in CHO cells exhibit DA-induced inhibition of forskolin-stimulated cAMP accumulation by ~60% with EC_{50} values of ~100 nM. In each case, the DA inhibition of cAMP accumulation is prevented by pretreatment of the cells with pertussis toxin. Finally, for both receptor isoforms, pretreatment of the cells with 100 μM DA for 4 to 24 hrs results in desensitization of the ability of DA to inhibit cAMP accumulation. These data suggest that the D_{2L} and D_{2S} receptors are functionally similar in their ligand binding properties and linkage to adenylyl cyclase inhibition.

94.13

MOLECULAR CLONING OF A D_1 DOPAMINE RECEPTOR LINKED TO ADENYLYL CYCLASE ACTIVATION. Frederick J. Monsma, Jr. Lawrence C. Mahan*, Loris D. McVittie, Charles R. Gerfen, and David R. Sibley. ETB, NINDS and LCB, NIMH, NIH, Bethesda, MD 20892.

We have used NS20Y cells, which express D_1 dopamine receptors linked to adenylyl cyclase activation, to investigate the molecular biology of this receptor subtype. In order to clone this D_1 receptor, the polymerase chain reaction (PCR) method was used to selectively amplify a D_1 receptor cDNA sequence from NS20Y cell mRNA. Poly $(A)^+$ RNA was used to synthesize cDNA by reverse transcription followed by PCR amplification with sets of highly degenerate primers derived from the transmembrane sequences of the previously cloned adrenergic, D_2 dopaminergic, and serotonin receptors. This resulted in the amplification of a novel cDNA sequence which exhibits considerable homology to various members of the G protein-coupled receptor family. This cDNA is most homologous with β-adrenergic receptors followed by α-adrenergic and D_2 dopaminergic receptors. It also predicts a 3rd cytoplasmic loop consisting of 54 amino acids and one phosphorylation site for the cAMP-dependent protein kinase. Most importantly, the predicted 5th transmembrane spanning domain contains conserved serine residues believed to be involved in catecholamine binding. Northern blot analysis reveals a transcript size of ~4 kb which is predominantly located in striatal tissue with lesser amounts in the cortex and retina. In contrast, no mRNA is observed in the cerebellum, hippocampus, kidney or pituitary. *In situ* hybridization analysis also indicates a high abundance of mRNA in the striatum as well as in the olfactory tubercle. To establish the identity of this receptor, a full-length cDNA was isolated from a rat striatal library and transiently expressed in COS-7 cells. ^{3}H-SCH23390, a D_1 selective radioligand, exhibited specific binding (K_D = 0.3 nM, B_{max} = 400 fmol/mg) only in transfected cells, thus confirming the D_1 identity of this clone.

OPIOIDS: BEHAVIOR

95.1

EVIDENCE FOR AN ENKEPHALINERGIC MECHANISM IN AMYGDALOID SUPPRESSION OF DEFENSIVE RAGE IN THE CAT. **C.L. Lu, M.B. Shaikh and A. Siegel. Dept. of Neurosciences, UMDNJ - New Jersey Medical School, Newark, New Jersey 07103.**

Recently, we have demonstrated enkephalinergic (ENK) inhibition of defensive rage behavior (DR) elicited from the periaqueductal gray (PAG) of the cat. The present study provides evidence that a major source of this inhibitory ENK input to the PAG arises from the amygdaloid complex. Cannula-electrodes were implanted into the PAG for the elicitation of DR as well as for infusion of the opioid antagonist, naloxone. Monopolar stimulating electrodes were also implanted into central amygdaloid (CE) sites from which suppression of DR could be obtained. Initially, concurrent stimulation of the CE at very low, subseizure current levels (0.1mA, 60hz) and PAG resulted in an immediate suppression of this response after 2-4 trials. Total suppression of PAG elicited DR ranged from 30-60 min following CE stimulation. Secondly, naloxone (1.0, 7.0 and 10.0 ug/0.25 ul) was infused intracerebrally into PAG sites from which DR was elicited prior to CE stimulation. Naloxone treatment blocked the suppressive effects of CE stimulation in a dose and time dependent manner. These data strongly suggest that potent amygdaloid suppression of defensive rage utilizes ENK as its transmitter at the level of the PAG. [Supported by NIH grant NS 07941-20, the Fogarty International Center FO5TW04110, and the Foundation of UMDNJ].

95.2

ACUTE AND LONG-TERM BEHAVIORAL, GLUCOCORTICOID AND OPIOID ADAPTATION TO SOCIAL STRESS K.A. Miczek, I. Fier*, J.M. McNamara*, G.F. Koob, C. Rivier. Dept. Psychology, Tufts University, Medford, MA 02155, Neuroscience, Res. Inst. Scripps Clinic; Peptide Biology Lab, Salk Institute, La Jolla, CA 92037.

Exposure to the stress of being attacked and threatened by a dominant opponent prompts rats to engage in a range of affective, behavioral and hormonal responses. The magnitude, sequence and time course of these responses, as monitored by audio- and videorecords, cardiovascular telemetry and blood samples, reveals the integration of glucocorticoid steroids, opioid peptides and catecholamines in brain and periphery. Immediately at the start of the social confrontation, ACTH and corticosterone rise, and this elevation as well as tachycardia, hyperthermia, ultrasonic distress calls, submissive postures and behavioral immobility persist for 1-2 h. Glucocorticoid and cardiovascular responses to repeated exposures to social stress remain unattenuated in the submissive rat. In dominant animals that engage in aggressive behavior, the glucocorticoid stress response is limited to the first 30 minutes of the encounter. Diazepam (0.3, 1 mg/kg) prevents the ACTH and corticosterone response in submissive rats, but they keep emitting ultrasounds. When challenged during the acute social stress with opiates, a potentiation of analgesia is seen. Within 24 h after exposure to the social stress, tolerance to the analgesic effects of *mu* opioid agonists becomes detectable that lasts for several months. However, within the same animals the morphine "cue" remains unaltered as assessed by the drug discrimination method. In contrast to the large release of glucocorticoids and opioids during the acute social stress response, central opioid receptor-mediated changes in pain perception persist for months.

95.3

COLD ACCLIMATION POTENTIATES HYPERTHERMIA AND OPERANT HEAT ESCAPE OF RATS CENTRALLY INJECTED WITH DAGO. J.R. Wilson and B.A. Howard*. Dept. of Psychology, Univ.Manitoba, Winnipeg, Canada, R3T 2N2.

Centrally injected opioids evoke hyperthermia, a response potentiated following chronic cold exposure (Wong & Tse, Int.J.Peptide Protein Res., 24:74, 1984). To determine if these responses are mu-receptor mediated, two studies examined the effects of intracerebroventricular (icv) DAGO, in cold- and non-cold-acclimated (CA,NCA) sialoadenectomized rats on (a) core temperature at normal ambient temperature, and (b) operant escape from a mild heat challenge. In Experiment I, rats were implanted with an icv cannula and an abdominal Mini-Mitter temperature transmitter. The CA and NCA rats received ip saline (n=14) or naltrexone (NLTRX), a mu-antagonist, (2mg/Kg) (n=14) followed 30 min by 1 of 3 icv doses of DAGO (0.0, 0.1, 1.0 µg/5µl). Pre- and post-DAGO core temperature and activity were monitored in a 21°C open field. Experiment II used the same drug protocol, however CA and NCA rats (n=28) were shaped to escape convective heat challenge by lever pressing and then tested during a mild heat (37°C) challenge. In Experiment I, DAGO induced a dose-dependent hyperthermia in NCA rats that was NLTRX reversible, independent of activity, and potentiated by CA. In Experiment II, CA enhanced the duration of heat escape for both pre- and post-DAGO injections. The greatest heat escape, obtained in CA rats at the 1.0 µg dose of DAGO, was NLTRX reversible. Thus, central opioid-induced hyperthermia appears to be mu-receptor mediated and CA potentiates the mu thermal reactivity. These findings supplement reports (Uehara, A., et al., Am.J.Physiol., 257:E336, 1989; Travis, K.A. & Boulant, J.A., Am.J.Physiol., 256:R560, 1989) that CA increase hypothalamic-adrenocortical responsiveness, and cross tolerances emerge between thermal and nonthermal homeostatic processes. (Supported by NSERC Grant A6404 to JRW).

95.4

REPRODUCTIVE EXPERIENCE ALTERS OPIOID-MEDIATED MATERNAL BEHAVIOR AND PROLACTIN SECRETION IN LACTATING RATS. P.E. Mann, C.E. Lupini*, P.M. Ronsheim*, & R.S. Bridges, Dept. Anat. & Cell. Biol., Harvard Med. Sch., Boston, MA.

Sensitivity to opiate disruption of maternal behavior (MB) declines as a function of reproductive experience. The aims of this study were to determine whether central sensitivity to beta-endorphin (BE)-induced disruption of MB as well as naloxone (NAL) inhibition of suckling-induced prolactin (PRL) release changes with reproductive experience. Multigravid and age-matched primigravid rats were implanted with bilateral MPOA cannula on days 13-15 of gestation. On postpartum days 5 (saline) and 6 (BE: 0.5 ug/0.4 ul/side), dams were tested for MB 30 min after infusions. Animals were catheterized on day 7. On days 8-9, pups were removed and 4 hr later blood samples were taken before saline (day 8) and NAL (day 9; 5 mg/kg, i.v.) injections. Pups were returned and blood was sampled 10-30 min postinjection. Pups were removed again at 30 min and samples were taken for 60 min. Multiparous (MULTIP) animals were significantly less sensitive to BE than primiparous (PRIMIP) rats. Suckling-induced PRL levels were significantly higher in PRIMIP than MULTIP rats. Moreover, in PRIMIP rats, NAL reduced suckling-induced PRL release, while failing to affect PRL release in MULTIP rats. These results indicate that alterations (tolerance) develop in the endogenous opioid system as a consequence of previous pregnancies and/or lactations. Supported by NIDA (DA04291) & NIH (HD19789).

95.5

EJACULATION INCREASES POSTICTAL BEHAVIORAL DEPRESSION IN MEDIAL PREOPTIC AREA KINDLED RATS: EVIDENCE FOR OPIOID INVOLVEMENT. R. Paredes*, C. Manero*, A.E. Haller*, R. Alvarado* and A. Agmo. Escuela de Psicología, Universidad Anahuac y Laboratorio de Formación Reticular INNN. México D.F.

It has been postulated that ejaculation is achieved when a reward threshold is reached through the release of opioid peptides during sexual activity. Others have postulated that the activation of GABA produces the inhibition of sexual behavior normally ocurring after ejaculation. The present experiments tested these hypothesis measuring the opioid dependent postictal behavioral depression (PBD) that ocurrs after electrical kindling.

Sexually experienced male rats were kindled in the medial preoptic area (MPOA) or the amygdala (AMG). Once kindling had been established the PBD was measured after a standard kindling stimulus applied 2 min after ejaculation. The PBD was significantly increased after ejaculation. This effect was seen in MPOA but not in AMG kindled rats. The effects of ejaculation on PBD were blocked by naloxone. No effect was observed on seizure intensity and afterdischarge duration after ejaculation. It appears that opioid release during sexual behavior enhances the effects of those release after a kindled seizure, increasing the duration of the PBD. These results confirm that opioid peptides, rather than GABA, are release after ejaculation.

95.6

INTERFERENCE BETWEEN OPIOID AND NONOPIOID MECHANISMS OF CALMING IN 10-DAY-OLD RATS E.M. Blass & L. Brunson*. Depts. of Psych. & Nutrition, Cornell University, Ithaca, NY 14853

To better understand mechanisms underlying adjustments to maternal separation, 10-day-old rats were separated from their mother for six consecutive 3-min. epochs at 34°C. Between each epoch rats contacted their anesthetized dam for either 1 min. (N=10) or 5 min (N=10). Rate of ultrasonic vocalization was the dependent measure. Ultrasonic vocalization peaked during the third isolation period and then declined for both contact groups. Rats that had only 1-min. contact between separation epochs vocalized more than 5-min. contact rats ($p < .001$).

To determine whether the vocalization decrease was opioid-mediated, rats in each contact group were injected (i.p.) with either naltrexone (0.5 mg/KG BW) or isotonic saline vehicle (experimenters were uninformed as to syringe contents) and tested as above. Rather than increase vocalization, naltrexone markedly decreased vocalization for both 1-min. and 5-min. contact groups ($p < .001$) suggesting that quieting influences during the separation epochs were cast by the mother through nonopioid pathways and that antagonizing endogenous opioid pathways enhanced the maternal effect.

It followed that a low dose of morphine should enhance rate of vocalization. Rats injected with .0625 mg/Kg BW emitted significantly more vocalizations than did control rats ($p < .001$) for both 1- and 5-min. groups.

Under circumstances of considerable duress, opioid and nonopioid mediated coping systems act antagonistically in Day 10 rats.

95.7

DIFFERENTIAL EFFECTS OF SPECIFIC ENDOGENOUS OPIOID SYSTEMS ON AFFECTIVE BEHAVIORS IN NEONATAL RATS. P. KEHOE, C. BOYLAN*, AND W. SHOEMAKER. Trinity College and Univ. of Conn. Health Center, Hartford, Ct. 06106.

Previous results show that endogenous opioid systems mediate affective responses in neonatal rats. Opioids modulate isolation-induced vocalizing and analgesia in infants. Morphine decreases calls and produces analgesia as well as promotes positive associations with novel stimuli in rat pups. The opioid systems consist of different receptor types (mu, delta and kappa) and morphine may exerts its effects primarily as a mu receptor agonist. Functional effects of these subsystems may vary producing differential behavioral outcomes. To assess the influence of the kappa receptor system on these neonatal behaviors, the kappa agonist, U-50,488H, was tested in both the positive conditioning paradigm and response to pain and isolation stress. In contrast to morphine's effects, the kappa agonist produced no positive associative conditioning. Moreover, U-50,455H significantly increased vocalizations but did produce an analgesic response that was naltrexone-reversible. Mu receptor functioning seems to have a positive valence in the above behaviors as opposed to a somewhat negative one for the kappa system. These differential effects may be in part due to the interaction of the dopaminergic and opioidergic systems.

95.8

EFFECT OF ß-CYCLODEXTRIN ON ANALGESIA PRODUCED BY INTRATHECALLY ADMINISTERED OPIOIDS. J. JANG, H. HILL* & T.L. YAKSH. Dept. of Anesth., Univ. of Calif., San Diego. CA 92093 & Fred Hutchinson Cancer Center,* Seattle, WA 98104

Hydroxypropyl-ß-cyclodextrin (CDEX), a crown ether, forms inclusion complexes in its hydrophobic cavity with the lipophilic portion of drug molecule by a non-covalent bond. This may increase water solubility of lipid soluble drugs and thereby reduce their vascular redistribution after intrathecal (IT) administration. CDEX may thus prolong the analgesic action and reduce the supraspinal actions of IT opioids. In this study, opioids (morphine, lofentanil, alfentanil and sufentanil) with and without CDEX (20%, 2%, 0.2%, 0.02% and 0.002% in sterile water) were administered in rats with chronic IT catheters. CDEX prolonged the duration of analgesia (52.5 hot plate: HP) produced by opioids and reduced the catalepsy otherwise produced by a supermaximal dose of IT morphine. The magnitude of the facilitatory effect was dependent upon CDEX concentration and varied with drug lipid partition coefficient. Drug levels in plasma and spinal cord at time of peak effect confirmed delayed redistribution. No toxicity was observed at highest CDEX (40%) concentration. Data indicate CDEX may be a useful IT vehicle for poorly soluble drugs .

IT Drug	HP Time -Effect Area:(MPE*min)/1000					
CDEX conc.	0%	0.002%	0.02%	0.2%	2%	20%
Mor (3ug/10ul)	4.6/1.3	5.4/1.1	11.0/0.4	3.0/0.9	2.3/0.9	3.2/0.5
Lof(0.01ug/10ul)	0.8/0.2	2.2/0.5	4.6/1.5	5.0/1.4	6.7/1.8	2.2/0.4

(mean /SE; N= 5-6 rats /dose) (Supported by DA02110)

95.9

BEHAVIORAL INTERACTIONS BETWEEN MORPHINE AND MK-801: ANALGESIA, TOLERANCE, DEPENDENCE AND LETHALITY. K. A. Trujillo and H. Akil, Mental Health Research Institute, University of Michigan, Ann Arbor, MI, 48109-0720.

The non-competitive NMDA receptor antagonist MK-801 has been found to have a unique and interesting profile of behavioral actions, suggesting that this drug might be of use in the treatment of a number of clinical disorders. In the present studies we examined behavioral interactions between MK-801 and the prototypical opiate agonist morphine, following acute or chronic treatment. Adult, male Sprague Dawley rats were administered saline or MK-801 (0.03, 0.1, or 0.3 mg/kg i.p.) followed 30 minutes later by saline or morphine sulfate (1.0, 3.0, or 10.0 mg/kg s.c.). Analgesia was assessed by the tail flick assay 60 minutes following the second injection. In chronic studies animals were treated twice daily for 9 days, as above, with MK-801 followed 30 minutes later by morphine (10 mg/kg s.c.), and analgesia determined on days 1, 3, 5, 7 and 9. On day 10, withdrawal was precipitated by naloxone (2.0 mg/kg s.c.) 60 minutes following morphine treatment (10 mg/kg s.c.).

Acutely, MK-801 had no significant effects on analgesia, nor did it significantly affect the analgesic actions of morphine. At 0.3 mg/kg, MK-801 dramatically potentiated the lethal effects of morphine, shifting the apparent LD50 approximately 10-fold, from 100 mg/kg to 10 mg/kg. In chronic studies MK-801 dose-dependently attenuated the development of tolerance to morphine. Although 0.03 mg/kg had no effect on the development of tolerance, animals pretreated with 0.1 mg/kg showed significant morphine analgesia on days 7 and 9, when saline pretreated animals were approaching complete tolerance. Additionally, MK-801 dose-dependently attenuated the number of escape jumps observed on day 10 during naloxone-precipitated withdrawal. These results suggest that excitatory amino acid systems may be important in several opiate-mediated behaviors. This work was supported by NIDA NRSA DA05336 (K.A.T.), and NIDA DA02265 and NIMH MH422251 (H.A.).

95.10

ARE CHANGES IN AMYGDALOID OPIATE BINDING CONTROLLED BY GONADAL STEROIDS, PHOTOPERIOD, OR A COMBINATION OF THESE VARIABLES? M.L. Tubbiola and E.L. Bittman, Dept. of Zoology, University of Massachusetts, Amherst, MA 01003

Short days (SD) reduce binding of ^{3}H-naloxone (NAL), an opiate antagonist, in the medial amygdala (M) of male golden hamsters (Mesocricetus auratus). This reduction may be due to SD-induced decreases in circulating testosterone (T), a steroid independent effect of photoperiod, or an interaction between T and daylength. To answer this question specific NAL binding was measured by quantitative autoradiography in 9 groups of male golden hamsters after 12 weeks exposure to long (LD) or SD. Hamsters were left intact (Int), castrated (Cas), castrated and immediately given T implants to maintain T (Cas+T), castrated and given T implants after 5 weeks of LD to reinstate T (Cas...T), or pinealectomized (Pinx). Short days reduced NAL binding in M, but not other amygdaloid nuclei or in the bed nucleus of the stria terminalis, of intact hamsters ($p < 0.05$). Pinealectomy reversed this effect ($p < 0.05$). Castration elevated NAL binding in M and other amygdaloid nuclei ($p < 0.05$). T reversed the rise in NAL binding only in Cas+T hamsters housed in LD. Although short days increased NAL binding in M of Cas+T hamsters ($p < 0.05$), photoperiod had no influence on NAL binding in Cas or Cas...T groups.

These findings indicate that T regulates NAL binding in several amygdaloid nuclei. Daylength affects opiate receptor concentrations in M only if T is maintained; however the photoperiod effect is reversed in Cas+T as compared to intact hamsters. Changes in M opiate binding may explain deficits in copulatory behavior of SD hamsters with T maintenance, and diminished responsiveness to exogenous androgens in chronic castrates. (Supported by NSF BNS86-16935 and NIMH RO1-44132.)

95.11

NALTREXONE DOES NOT DISRUPT ACQUISITION OR EXPRESSION OF PAVLOVIAN CONDITIONED INHIBITION OF FEAR J. Landeira-Fernandez, M. S. Fanselow, J. P. DeCola, & J. J. Kim. Dept. of Psychology, UCLA, Los Angeles, CA 90024

Naltrexone (NTX) enhances acquisition of excitatory fear conditioning but its effect on inhibitory conditioning is untested. A neutral stimulus becomes a conditioned inhibitor (CI) when it is presented without reinforcement in conjunction with another stimulus that is usually reinforced. On alternate days of a 16 day procedure, rats were exposed to either a tone paired with shock or a simultaneous tone/light compound that was not paired with shock. Half the rats were injected with NTX prior to the training sessions on days where they received tone/light compounds (CI Training Days). No drug was given on tone shock days. To assess CI, a fear baseline was established by exposing rats to a few shocks in the novel context. CI was indicated by a reduction in freezing during presentation of the the light. For this test, half of the subjects in each training drug condition received NTX; the others received saline. A separate control group was used to assess acquisition of CI. There was reliable CI but NTX did not reduce acquisition or expression of CI. These results indicate that endogenous opioids do not play a role in conditioned inhibition of fear.

95.12

MU OPIOID RECEPTOR ANTAGONISTS ENHANCE PAVLOVIAN FEAR CONDITIONING. M. S. Fanselow[1], J. J. Kim[1], S. Young*[1], J. P. DeCola*[1], J. Landeira-Fernandez[1], D. J. Calcagnetti[2], & F. J. Helmstetter[2]. [1]Dept. of Psychology, UCLA, Los Angeles, CA 90024 & [2]Dept. of Psychology, Dartmouth College, Hanover, NH 03755.

Opioid antagonists have two major affects on Pavlovian fear conditioning. First, they attenuate expression of fear-induced analgesia if given prior to testing. Second, they enhance acquisition of fear conditioning if they are given prior to training. Previous research using selective opioid antagonists, administered intracerebroventricularly, has implicated mu and delta but not kappa opioid receptors in the analgesia. The present studies used intracerebroventricular application of these selective antagonist treatments to determine which opioid receptor types result in the enhancement of conditioning. Two selective mu receptor antagonists, $Cys^2Tyr^3Orn^5Pen^7$-amide and naloxonazine dramatically enhanced fear conditioning as measured by the freezing response. Neither the delta opioid antagonists, 16-Methyl Cyprenorphine and naltrindole, nor the kappa opioid antagonist, nor-Binaltorphimine, enhanced conditioning. These data are consistent with our earlier suggestion that the μ_1 receptors mediating fear-induced analgesia also modulate acquisition of conditional fear.

95.13

CONDITIONED WITHDRAWAL BEHAVIOR AFTER FOCAL BRAIN STIMULATION. B.E. Thorn, T. Nicholson, G.E.Jones and C. Runge. Univ. AL, Tuscaloosa, AL 35487.

Previous reports indicate that "chronic" (2 hr.) focal brain stimulation (FBS) of the periaqueductal gray (PAG) followed by naloxone results in with opiate-like withdrawal (Williams & Thorn, 1984). In that study, animals served as their own controls, 1st given sham FBS & 4 days thereafter, given real FBS for 2 hrs followed by naloxone. This study examined whether the order of presentation of the sham vs. real FBS trial would influence the behaviors obtained. 16 rats received electrode implants electrodes into the ventral PAG. Half the animals were 1st given sham FBS, while the other half were 1st given 2 hrs of real FBS at the current intensity previously shown to produce analgesia. Following the trial, animals were injected with 1mg/kg naloxone and observed for opiate withdrawal signs. Animals receiving sham stimulation 1st showed a significant difference between the sham trial and the real trial in the number of opiate withdrawal signs, whereas animals receiving the sham trial 2nd did not. These results may be explained as a classical conditioning effect of brain stimulation.

95.14

THE EFFECTS OF LOW CURRENT TRANSCRANIAL ELECTROSTIMULATION & MORPHINE ON SOMATOSENSORY EVOKED POTENTIALS IN THE SOMATOSENSORY CORTEX & THALAMUS, C. Hymel* & M. Skolnick, Neurophysiology Res Center, Univ Texas Hlth Sci Cntr, Houston, 1343 Moursund, Houston, TX 77030

Application of low current transcranial electrostimulation (TE) produces naloxone-reversible analgesic effects on Sprague-Dawley rats. Alternate forms of TE have also been shown to potentiate the analgesic effects of morphine. The aim of this research is to determine the combined effects of TE and morphine on somatosensory evoked potentials (SEP's) in specific nuclear regions of the thalamus and somethetic cortex using single recording electrodes to test the ability of TE to potentiate the analgesic effects of morphine.

Sites for SEP recordings were taken from pilot data in which specific nuclear regions were found in the somatosensory cortex and thalamus in which SEP responses to electric shock applied to the rat tail were significantly suppressed after TE treatment. In-brain SEP potential recording techniques were exploited to record the aggregate electrical activity of the neurons in close proximity to the probe in response to tail shock stimulation.

The results show both TE and morphine, acting alone, suppress SEP's in target nuclei. The combination of TE and morphine at various doses increases SEP suppression above that of TE or morphine alone.

95.15

MORPHINE HYPERALGESIC EFFECTS AGAINST CHEMO-INFLAMMATORY PAIN IN LEGHORN COCKERELS. R. A. Hughes and K. J. Sufka. Dept. of Psychology, Iowa State University, Ames, IA 50011-3180.

We recently identified a biological model in which responses to a noxious thermal stimulus were enhanced by morphine. This atypical hyperalgesic effect is naloxone reversible (Hughes, R. A., Pharmac. Biochem. Behav., 35: 567, 1990) and has dose and temporal characteristics of morphine analgesia (Sufka, K. J. & Hughes, R. A., Physiol. Behav., 47:385, 1990. The present study extends this unusual opioid hyperalgesic effect to include chemo-inflammatory nociception. Formalin (1% sc) in the plantar region of the foot reliably elicited discrete footlifts in young leghorn cockerels. This effect was enhanced by morphine sulfate (2.5 mg/kg, im.) and reversed by naloxone (5.0 mg/kg, im). Other opioid mediated responses were not atypical. Body temperature and respiration were reduced by morphine; naloxone reversed these effects.

95.16

OPIOID-RECEPTOR MEDIATION OF MORPHINE-INDUCED HYPERALGESIA IN DOMESTIC FOWL. K. J. Sufka, R. A. Hughes, and J. Giordano. Dept. of Psychology, Iowa State Univ., Ames, IA 50011-3180.

Recent research in this lab demonstrated that morphine produced a strain-dependent, naloxone-reversible hyperalgesic response to a noxious thermal stimulus in domestic fowl. The present study attempted to identify opioid-receptor mediation of this atypical opiate effect. Patterns of morphine (1.25 - 5.0 mg/kg/ml, im) hyperalgesia were examined against pretreatment with the selective mu antagonist beta-funaltrexamine, delta antagonist naltrindole, or kappa antagonist nor-binaltorphamine (10 ug/5ul, icv) on a hot-plate test (60°C) in 14-day old domestic fowl. Morphine produced a dose-dependent hyperalgesia. Beta-FNA attenuated this hyperalgesic effect, while nor-BNI only partially reduced hyperalgesia at the highest morphine dose tested. Pretreatment with NTI failed to block morphine effects. These results suggest that atypical morphine hyperalgesia is mediated, in part, by mu and kappa receptors in domestic fowl.

95.17

ENHANCEMENT BY AMNIOTIC FLUID OF DIFFERENT LEVELS OF VS-INDUCED ANALGESIA. A.C. Thompson, P. Abbott, J.C. Doerr, E.F. Ferguson* and M.B. Kristal. Dept. of Psychology, University at Buffalo, Buffalo, NY 14260.

A substance (POEF) in amniotic fluid (AF) and placenta has been shown to enhance analgesia produced by morphine, late-pregnancy, footshock, and vaginal/cervical stimulation (VS). Several of our studies suggest that the degree of enhancement is a function of the amount of analgesia being generated. We have examined this effect further in non-pregnant rats by testing the effect of AF ingestion on several levels of VS-induced analgesia.

Rats received AF (1/4 ml) or saline by orogastric intubation. Ten min later, pain threshold (measured as tail-flick latency) was determined before and during application of VS pressures of either 75g, 125g, 175g, or 225g.

The statistical analysis of the pain threshold data found AF to enhance VS-induced analgesia at the 125-g level (average increase in pain threshold from baseline was 36.6% [± 13.8] among control rats and 75.1% [± 17.67] among AF-treated rats). No significant effects were produced by AF ingestion on stimulation of 75g, 175g, or 225g. As expected, the effect of 1/4 ml AF on VS-induced analgesia depended on the dose of VS. An unexpected effect of AF on the induction of pseudopregnancy (PSP) among rats receiving 225g of VS was found such that fewer AF-treated rats became PSP (10%) than control rats (44%).

Results are considered in light of the role of amniotic fluid ingestion and vaginal/cervical stimulation in periparturitional analgesia.

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95.18

AMNIOTIC-FLUID INGESTION ENHANCES ANALGESIA PRODUCED BY THE CENTRAL ACTION OF MORPHINE. J.M. Di Pirro, A.C. Thompson and M.B. Kristal. Dept. of Psychology, University at Buffalo, Buffalo NY 14260.

Ingestion of amniotic fluid (AF) and placenta has been shown to enhance the analgesia produced by morphine, late-pregnancy, footshock, and vaginal/cervical stimulation in the rat. The present studies examined whether the active substance in amniotic fluid (Placental Opioid-Enhancing Factor, *POEF*) affects the central or peripheral actions of morphine.

First, quaternary naltrexone (QN), an opiate antagonist that does not readily cross the blood-brain barrier, was used (8mg/kg, sc, or saline) to block peripheral opiate receptors. Rats were given QN 20 min prior to measuring baseline pain threshold (using a tail-flick latency [TFL] test). Immediately after, MS (3mg/kg, ip) or saline was given. AF (0.25ml) or beef bouillon was intubated orogastrically 15 min after MS injection. Pain threshold was then measured 15 min after intubation. The results indicate that QN attenuated MS-induced analgesia (by eliminating the peripheral effect), but did not prevent enhancement by AF.

Second, the effect of AF on analgesia induced by centrally-administered MS was assessed, both with and without systemic QN. Rats were given icv injections of MS (2.5μg/2.0μl) or saline. AF or control intubation occurred 10 min later. TFL was measured 20 and 40 min after intubation. The results show that AF significantly enhanced the analgesia produced by icv MS (about 92% above baseline at 20 min, about 98% above baseline at 40 min); our dose of MS, alone, induced only about a 10% elevation in pain threshold from baseline.

These results are consistent with the hypothesis that amniotic fluid ingestion primarily enhances the central action of morphine.

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BRAIN GLUTAMATE SYSTEMS

96.1

ENDOGENOUS INHIBITORS OF BRAIN GLUTAMATE DECARBOXYLASE. J.-Y. Wu, J. Bao*, M. Yarom, X.-W. Tang*, Y. H. Lee* and Y. Yan*. Department of Physiology and Cell Biology, University of Kansas, Lawrence, KS 66045.

The steady state level of GABA is believed to be determined by its synthetic enzyme, L-glutamate decarboxylase (GAD). Although GAD has been purified and studied extensively in terms of its cellular and subcellular distribution, little is known about its regulation. Hence, this study was designed to search for endogenous brain substances that can regulate GAD activity. Briefly, GAD was prepared from pig brain as previously described [J. Neurochem. 44, 957 (1985)]. The P_2 membranes thus obtained were suspended in 50 mM Tris-Citrate (pH 7.0) and subjected to freezing and thawing, followed by extensive extraction with 5 mM Tris-Cl, (pH 7.0). The extract was filtered through hollow fiber PM 10 (exclusive limit: 10,000 daltons) and the filtrate obtained was concentrated and used as the starting material for endogenous inhibitors (EIs). It was found that 50 μl of crude EI preparation inhibited 73% of soluble GAD activity. Similar results were obtained with membrane associated GAD. EIs were purified through Bio-Gel P-2 column and ion-exchange column. Two peaks of EI activity were obtained in Bio-Gel P2 column (exclusion limit: 2000-dalton), one was eluted after the void volume but slightly ahead of standard L-glutamate and GABA and the other one was eluted considerably behind the standard amino acids indicating that they are smaller than 2000-dalton. These inhibitors were excluded by cation exchanger but retained by anion exchanger suggesting that they are negatively charged. EIs were heat stable, were not chelated by EDTA and behaved as competitive inhibitors since they increased the K_m of L-glutamate for GAD without changing the V_{max}. The exact chemical structure of EIs remains to be determined. (Supported by NIH Grant NS20978 and BNS-8820581 from NSF).

96.2

THE APOENZYME OF GAD IS PRESENT PREDOMINANTLY AS THE 63-kDa FORM OF GAD IN SYNAPTOSOMES AND RAT BRAIN. D. L. Martin, S.B. Martin, S.J. Wu, and N. Espina. Wadsworth Center for Labs. and Res. NY State Dept. Health, Albany, NY 12201-0509, and Dept. Environ. Health and Toxicology, SUNY Albany.

Glutamate decarboxylase is regulated, at least in part, by the interconversion of inactive apoenzyme (GAD without bound cofactor, pyridoxal-P) and active holoGAD. Previous studies from several groups have shown the presence of 59-, 63- and 67-kDa forms of GAD in brain. We have investigated which forms are present as apoGAD in synaptosomes and in cortex, caudate, hippocampus and cerebellum of rat brain. Endogenous apoGAD was labeled by incubating synaptosomes or 2mm punches of each region for 15 min with ^{32}P-pyridoxal-P in Triton X-100-containing lysis buffer followed by reduction with $NaBH_4$ to covalently link the ^{32}P-pyridoxal-P to GAD. Proteins were separated by SDS-PAGE. Punches were obtained from 1mm slices of frozen brain from heads decapitated into liquid N_2. Synaptosomes contained a major labeled band at 63 kDa and remarkably few other strongly-labeled proteins. The 63-kDa band was identified as GAD by immunoaffinity chromatography with the GAD-1 antibody, and its labeling was blocked by 4-deoxypyridoxine-P, which inhibits labeling of purified GAD. Labeling at 59 and 67 kDa was barely discernable. More proteins were labeled in punches. Western blots of punch extracts were stained with 3 anti-GAD antibodies [1440 (Oertel et al., *Neurosci.* 6:2681, 1981), K2 (Houser et al., *Abst. Soc. Neurosci.*) and GAD-6 (Chang and Gottlieb, *J. Neurosci.* 8:2123, 1988] which recognize the 59-, 63-, and 67-kDa bands to varying degrees. The 63-kDa band was intensely labeled and corresponded to the major band stained with 1440 and GAD-6. A minor labelled band corresponded to the major K2-stained band. Labeling of each band was blocked by deoxypyridoxine-P and the labeled 63-kDa band was identified by immunoaffinity purification with GAD-1. Supported by Grant MH35664 from NIMH.

96.3

GABA TURNOVER AND GAD IN CULTURED GABAERGIC NEURONS FROM RAT CORTEX. K. Rimvall and D.L. Martin. Wadsworth Center for Labs. and Res., NY State Dept. Health, Albany, NY 12201.

Dissociated cells from prenatal (E20) rat cortex were cultured in serum-free, defined medium. Cells did not survive unless coverslips were inverted at 1 DIV, thus apparently providing them with a suitable microenvironment. Immunocytochemistry indicated that at least 80% of cells grown in serum-containing medium were GABAergic. Studies of the fraction of GABAergic neurons in serum-free medium are not yet complete. Biochemical studies were carried out with inverted, serum-free cultures. GABA turnover rates, as measured by inhibiting GABA-T with gabaculine, were approximately 20% of GAD activity, as measured by $^{14}CO_2$-trapping. Two anti-GAD antisera 1440 (Lab. Clin. Sci., NIMH) and K2 (Houser et al., *Abst. Soc. Neurosci.*, 15:488, 1989) and a monoclonal antibody GAD-6 (Chang and Gottlieb, *J. Neurosci.*, 8:2123, 1988) recognize the three forms of GAD described in the literature (59, 63 and 67 kDa) to varying degrees. Immunocytochemistry of inverted, serum-free cultures revealed that neuronal cell bodies and neurites are stained by 1440, K2 and GAD-6. Western blots of homogenized cultures with the same antibody/antisera confirm the presence of the 63- and the 67-kDa forms of GAD and possibly the 59-kDa form. The significance and *in vitro* development of the various GAD forms will be the subject of further investigations using this model system of GABAergic function. Supported by Grants MH35664 and NS853102 from USPHS-DHHS.

96.4

DISTRIBUTION OF IRON IN CHICK BRAIN: OVERLAP WITH GABA, TRANSFERRIN LOCALIZATIONS. M.M. Beck and J.M. Hill[1]. Dept. of Animal Sci., Univ. of Nebraska, Lincoln, NE 68583, and [1]Peptide Design, 12321 Middlebrook Rd., Germantown, MD 20874.

The role of brain iron is not well understood even though many facts about its distribution in mammals are known. It has been suggested that iron-rich areas of rat brain correlate with, among others, metabolic activity (Hill, J.M. and Switzer, R.C., III, Neurosci., 11, 1984) and GABA utilization (Hill, J.M., Brain Res., 342, 1985). To date, iron has not been demonstrated histochemically in appreciable amounts in avian brain. In the present study, brains of 10-day-old White Leghorn chicks were sectioned at 30 μm, thaw-mounted onto slides, air-dried, and stained with Perls'-DAB. Nuclei high in iron belonged, generally, to three functional groups: Auditory: An, La, MCC, FPL, PP; Vestibular: VeM, VeL, Ta, Cb; and Oculomotor: ROT, BCS, AL, QF, SAC, SGC. Extensive overlap was found between these areas and GABA localization in the chick (Lewis, P.A. and M.M. Beck, Neurosci. Abstr. 15, 1989). Both iron and GABA were primarily in glial cells, although several of the iron-containing nuclei also had many GABAergic terminals. Preliminary scans of ^{125}I-transferrin receptors in chick brain indicate that a similar distribution pattern exists. The results provide additional support for an interactive relationship between brain iron and GABA metabolism.

96.5

THE UPTAKE OF N-ACETYLASPARTATE INTO PRIMARY MURINE BRAIN CELLS IN CULTURE. O.H. Saab* and J.H. Neale. Department of Biology, Georgetown University, Washington, DC 20057.

N-Acetylaspartate (NAA), which is present in very high concentrations in the central nervous system, is produced by acetylation of aspartate and by the action of a dipeptidase on N-acetylaspartylglutamate (NAAG), and has been implicated in myelin biosynthesis. The objective of this research was to determine the fate of NAA in the extracellular/synaptic space following the neural release and hydrolysis of NAAG. Murine brain cells were maintained in dissociated cell culture for 14-17 days. The cells were then incubated with ^{3}H-NAA under different conditions in order to characterize the metabolism and transport. The results indicate a carrier mediated, sodium dependent transport mechanism for NAA with a K_M of about 1.5 uM. The neurons rather than the glial cells seemed most responsible for the uptake. The transport mechanism appears to be similar, but not identical, to other acidic amino acid transporters. Little evidence was obtained for the extracellular deacetylation of NAA.

96.6

CHARACTERIZATION OF SODIUM-DEPENDENT GLUTAMATE UPTAKE BY CLONAL CELL LINES. J.D. Sinor, J.S. Shumsky and M.B. Robinson. Children's Seashore House, Depts. Ped. and Pharm. Univ. of PA, Philadelphia PA,19104.

Previous studies have demonstrated that dihydrokainate (DHK) and α-aminoadipate (α-AAD) are region specific inhibitors of brain high affinity sodium-dependent glutamate uptake (Robinson, Soc Neurosci. Abs. 15 (1989) 602). Cerebellar uptake is insensitive to inhibition by DHK and is more sensitive to inhibition by α-AAD than brainstem and forebrain regions. The goal of these studies is to identify cell lines that express these pharmacologically distinct sodium-dependent uptake processes. The two cell lines characterized were a medulloblastoma, CHP 707, and C6 glioma. In both of these cell lines, greater than 80% of the accumulation of L-[3H]-glutamate was sodium-dependent. Eadie-Hofstee analysis of sodium-dependent glutamate uptake was consistent with a single site in C6 glioma with Km of 26 μM and a Vmax of 980 pmol/mg protein/min whereas the concentration dependence of glutamate uptake in CHP 707 supports the presence of high and low affinity sites. The sensitivity of the sodium-dependent glutamate uptake to DHK, α-AAD, and cysteine sulfinate (CS) was examined at a low concentration of glutamate (0.5 μM). In C6 glioma, the IC50's were as follows: DHK = 1300 μM, α-AAD = 720 μM, and 7.1 μM. These inhibition curves were consistent with a single site. In preliminary data, the sensitivity of sodium-dependent uptake by CHP 707 to these inhibitors was similar to that observed in C6 glioma. The ranked potencies of these inhibition data are more consistent with the uptake observed in forebrain or brainstem and are inconsistent with that observed in cerebellum.

96.7

TRANSFER AND EXPRESSION OF GLUTAMATE DECARBOXYLASE cDNA IN MAMMALIAN CELLS. C.H.J. Ruppert, N.J.K. Tillakaratne, M.G. Erlander, A. Torbati*, and A.J. Tobin, Dept. of Biology, UCLA, Los Angles, CA 90024.

Two cDNAs, coding for two different forms of the GABA-synthesizing enzyme glutamate decarboxylase (GAD_{65} and GAD_{67}) have been isolated in this laboratory. The availability of these cDNAs allowed us to design GAD-expressing cell lines. We cloned the coding regions of both cDNAs into the retroviral vector system of Miller and Rosman (Biotechniques 7, 980, 1989) and into plasmid vectors with inducible promoters. Stable clones of PC-12 and NIH3T3 cells show GAD activity, at about 10% the level present in brain homogenates. Retroviral vectors provide an efficient integration system with a wide host range that should enable us to program GABA production in many types of neuronal and non-neuronal cells, both in vivo and in vitro. This capability would allow us to approach questions concerning the specific functional properties of the two GADs in different cell types and the influence of GABA production on developmental processes in the nervous system. Supported by NS 20256 to AJT and a fellowship from the Deutsche Forschungsgemeinschaft to CHJR.

96.8

TONIC STIMULATORY EFFECT OF THE DOPAMINERGIC INNERVATION ON THE SYNTHESIS OF GABA IN SUBSTANTIA NIGRA PARS RETICULATA AND NEOSTRIATUM IN THE RAT. J.Aceves, B. Florán*, J.Benitez* and D.Martínez-Fong*. Dept. Physiology. CINVESTAV-IPN. 07000 México,D.F. México.

Dopaminergic innervation seems to stimulate the synthesis of GABA. Thus a decrease of GAD activity occurs in basal ganglia of Parkinsonian patients (McGeer and McGeer, J. Neurochem. 26:25, 1976), and chronic blockade of dopaminergic receptors with haloperidol reduces GAD activity in s. nigra reticulata and entopeduncular nucleus (Itoh, Psychopharmacology 79:169, 1983). Here we tested if the dopaminergic influence on the synthesis of GABA could be shown by unilaterally lesioning the dopaminergic nigrostriatal system with 6-hydroxydopamine and comparing the GAD activity in slices of s. nigra reticulata and neostriatum of the lesioned and intact sides. GAD activity was assessed by the accumulation of GABA after inhibiting GABA transaminase with aminooxyacetic acid (10 μM). After 2 weeks of the lesion, GABA accumulation in the lesioned side was markedly reduced in s. nigra reticulata (lesioned: 7.9 ± 0.3; intact: 18.9 ± 0.8 μg/mg prot., n=4, after 20 min of AOAA), and slightly but significantly ($P < 0.02$) reduced in neostriatum (lesioned 2.1 ± 0.1; intact: 2.9 ± 0.2; n=8). In s. nigra reticulata, GAD activity was reduced even 2 months after lesioning. These results support the idea of a tonic modulation of the synthesis of GABA by dopamine in the basal ganglia.

CARDIOVASCULAR REGULATION: BULBOSPINAL MECHANISMS

97.1

TONIC SYMPATHETIC CONTROL OF THE KIDNEY, SPLEEN AND HEART ORIGINATING FROM VENTROLATERAL PONTINE NEURONS IN RATS. K. Hayes, D.J. Beluli* and L.C. Weaver. The John. P. Robarts Research Institute and Department of Physiology, University of Western Ontario, London, Ontario, Canada.

Recent studies have demonstrated that regions outside the rostral ventrolateral medulla (RVLM) may contribute to tonic excitation of sympathetic nerves. We explored the ventrolateral pons to search for regions providing tonic control of arterial pressure (AP), heart rate (HR) and activity of renal and splenic sympathetic nerves. In rats anesthetized with urethane or Saffan, discharge of neurons in this region was inhibited by unilateral microinjections of glycine (50nl). Seven injections in urethane rats decreased AP by 17±7 mmHg, HR by 9±3 bpm and discharge of renal (17±8%) and splenic (19±9%) nerves. Eight injections in Saffan rats decreased AP by 19±7 mmHg, HR by 25±9 bpm and activity of renal (47±17%) and splenic (59±26%) nerves. These responses were evoked from sites 0.5-2.0 mm dorsal to the ventral surface and 1-3 mm rostral to the RVLM. In contrast, five injections of glycine (either anesthesia) into sites close to the A5 cell group caused increases in renal (22±2%) and splenic (14±1%) nerve activity with little or no change in AP or HR. These experiments demonstrate that tonic excitatory influences on sympathetic outflow affecting AP, HR and abdominal sympathetic nerves can be generated by neurons significantly rostral to the RVLM. These influences are particularly evident in Saffan-anesthetized rats. Moreover, tonic inhibition of renal and splenic sympathetic discharge may originate from neurons in the ventrolateral pons. (Support: Ontario Heart & Stroke Foundation).

97.2

DIFFERENTIAL CONTROL OF RENAL AND SPLENIC NERVES IS NOT DUE TO TOPOGRAPHICAL REPRESENTATION IN VENTROLATERAL MEDULLA. Beluli, D.J.* and L.C. Weaver. Robarts Research Inst. and the Dept. of Physiol. Univ. of Western Ontario, London, CAN

The capacity of the sympathetic nervous system to discharge differentially may be due to topographical organization within medullary vasomotor neurons. To search for topography in anesthetized rats, 15 nl (25 nMol) of the excitatory amino acid D,L-homocysteic acid (DLH) was microinjected into the ventrolateral medulla (VLM). The sympathetic discharge of either renal nerves (n=6), splenic nerves (n=7), or both nerves (n=6), was recorded. No topographical representation of the renal and splenic innervation could be found in the rostral or caudal VLM. Cardiovascular responses were elicited by DLH over a large area of the VLM, but the largest increases in renal and splenic activity occured after injection into the rostral VLM, while all the decreases in activity occured after injection into the caudal VLM. Although no topographical organization was found, a specific relationship in the magnitudes of responses between renal and splenic nerves was observed. Mean excitatory and inhibitory responses of splenic nerves were about half as large as those of renal nerves in all animals. When activity of both nerves was recorded simultaneously, splenic excitatory (rostral VLM) responses to DLH were only 44% of the renal responses and splenic inhibitory (caudal VLM) responses were 61% of the renal responses. In summary, mechanisms providing differential medullary control of renal and splenic nerves do not involve spatial organization of neuronal groups in the VLM. Support: MRC Canada & Rick Hansen Legacy Fund.

97.3

RESPONSES OF ADRENAL SYMPATHETIC PREGANGLIONIC NEURONS (SPNs) TO STIMULATION OF THE ROSTRAL VENTROLATERAL MEDULLA (RVL): EVIDENCE FOR FUNCTIONAL SPECIFICITY IN MEDULLOSPINAL SYMPATHETIC CONTROL. S.F. Morrison and D.J. Reis. Cornell University Medical College, Div. of Neurobiol., Dept. of Neurol. and Neurosci., New York, NY 10021.

Although reticulospinal sympathoexcitatory inputs from the RVL play a major role in regulating the discharge of sympathetic nerves innervating the heart and vasculature, the influence of these inputs on nerves innervating the adrenal gland is less well characterized. Thus, we examined the responses to single RVL stimuli (50-200 μA) of SPNs in spinal segments T7-T8 that innervate the adrenal gland in urethane/chloralose-anesthetized rats. Within the population of SPNs with axons in the greater splanchnic nerve, those antidromically activated from the adrenal nerve had significantly higher basal firing rates and slower axonal conduction velocities. Our previous results indicated that the RVL stimulus-evoked responses of splanchnic SPNs may be divided into four distinct patterns. The RVL-stimulus evoked responses of 80% of adrenal SPNs corresponded to a unique response pattern which was observed in only 10% of splanchnic SPNs. The response pattern of most adrenal SPNs was biphasic: an initial decrease in spontaneous firing lasting from 50-100 ms after the RVL stimulus was followed by an excitation which often consisted of several spikes over a 60-100 ms interval with a mean latency to peak activity of 136 ms. These results (a) support the hypothesis that differences in the RVL stimulus-evoked response profiles of SPNs correlate with differences in their function and (b) suggest the existence of unique inputs from the RVL to adrenal SPNs which may be consistent with the slow time course of glandular responses. Supported by NIH HL19874.

97.4

AORTIC DEPRESSOR NERVE STIMULUS-EVOKED INHIBITION OF SYMPATHOEXCITATORY NEURONS IN THE ROSTRAL VENTROLATERAL MEDULLA (RVL). L. Jeske, S.F. Morrison, S.L. Cravo*, and D.J. Reis. Cornell University Medical College, Div. of Neurobiol., Dept. of Neurol. and Neurosci., New York, NY 10021.

Baroreceptor stimulation inhibits the activity of reticulospinal sympathoexcitatory neurons in RVL through a polysynaptic pathway from the nucleus of the tractus solitarius (NTS) and involving unidentified neurons of the caudal ventrolateral medulla (CVL). To determine the temporal characteristics of this intramedullary pathway, we recorded the responses of antidromically-identified RVL-spinal neurons and postganglionic splanchnic sympathetic nerve activity (SNA) following paired-pulse stimulation of baroreceptor afferents in the aortic depressor nerve (ADN) of urethane-anesthetized, ventilated rats. RVL-spinal neurons exhibited arterial pulse-related activity and either slow (0.5-1.5 m/s; n=8) or rapid (3.4-8.3 m/s; n=14) antidromic conduction velocities. Stimulation of the ADN inhibited the spontaneous discharge of RVL neurons with an onset latency of 57 ms (range:40-76 ms) and a latency to complete silence of 67 ms. The duration of inhibition was directly related to stimulus intensity. ADN stimulation inhibited SNA with an onset latency of 96 ms and time to peak inhibition of 138 ms. Since (a) the RVL stimulus-evoked increase in SNA has an onset latency of 41 ms and peak latency of 68 ms and (b) the ADN to NTS delay is less than 10 ms, the intramedullary conduction time is approximately 55 ms. Given the distance between NTS and RVL, such a long delay is consistent with a multisynaptic intramedullary pathway mediating arterial baroreflex inhibition of RVL-spinal sympathoexcitatory neurons. Supported by NIH HL18974.

97.5

SEPARATE MECHANISMS BY WHICH SEROTONIN DEPRESSES INTRASPINAL AND GANGLIONIC SYMPATHETIC TRANSMISSION. D.N. Franz, S.C. Steffensen*, and L.C. Miner*, Dept. of Pharmacology, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

The marked increase in intraspinal transmission to sympathetic preganglionic neurons (SPGNs) produced by PDE inhibitors in spinal cats was nearly prevented by pretreatment with 5-HT precursors, 5-HTP or L-tryptophan, apparently by activating 5-HT receptors that are negatively coupled to adenylate cyclase. Microinjection of 5-HT (5 ug) into the SPGN neurophil also produced selective, marked depression with full recovery within 2 hr. Marked increases (100%) in intraspinal transmission to SPGNs followed microinjection of 1-2 ug of cyclic AMP analogs, forskolin, or RO 20-1724 into the SPGN neurophil. Transmission through isolated stellate ganglia in spinal cats was markedly depressed by serotonin (30-100 ug/kg, i.v.) for about 10 min. Prolonged depression (hours) was produced by i.v. 5-HTP (6-25 mg/kg) or by L-tryptophan (10-50 mg/kg, after 10 mg/kg of pargyline). 5-HTP did not affect postganglionic discharges from direct stimulation of ganglia blocked by mecamylamine, indicating that 5-HT must act presynaptically to depress release of acetylcholine from SPGN preganglionic terminals. In contrast to SPGNs, ganglionic transmission was not affected by i.v. RO 20-1724 or by microinjection of cyclic AMP analogs or forskolin into the ganglia. Although 5-HT appears to depress SPGN perikarya by inhibiting adenylate cyclase and reducing cyclic AMP levels, it depresses ganglionic transmission by reducing transmitter release from SPGN terminals through mechanisms that do not involve cyclic AMP.

97.6

PROJECTIONS FROM THE ROSTRAL VENTROLATERAL MEDULLA TO IDENTIFIED SPINAL SYMPATHETIC PREGANGLIONIC NEURONS AND SEROTONERGIC MEDULLARY RAPHE NEURONS IN RAT A.Zagon & A.D.Smith University Dept. of Pharmacology, Oxford OX1 3QT U.K.

The ventral part of the medulla plays an important role in the control of blood pressure. Both the rostral ventrolateral medulla (RVLM) and the caudal raphé nuclei in this area have been shown to project towards the intermediolateral cell column in the thoracic spinal cord. We have previously described a monosynaptic projection from the medullary raphé nuclei onto sympathetic preganglionic neurons (SPNs) (Bacon et al. Exp. Brain Res. 1990 79:587). In the present study we investigated if those parts of the RVLM which project to SPNs also project into the medullary raphé nuclei and if they have any connections with serotonergic neurons.

Axons arising from neurons of the medial part of the RVLM were labelled with *Phaseolus Vulgaris* Leuco-agglutinin (PHA-L). Cholera B-chain conjugated HRP (CB-HRP) was introduced into the left adrenal gland to retrogradely label SPNs. Sections of the spinal cord were processed to reveal CB-HRP and immunoreacted to reveal PHA-L. Brain stem sections were first processed for PHA-L immunohistochemistry and visualized using DAB to produce a brown reaction product. Then the sections were processed for 5-HT imunohistochemistry, visualized with benzidine giving a blue reaction product or with silver enhanced pre-embedding gold-staining.

Varicose PHA-L labelled axons of RVLM neurons were found in the intermediolateral cell column in close apposition to somata and proximal dendrites of retrogradely labelled SPNs. Occasionally labelled axons were found around more distal dendritic parts of SPNs in the intermediate and central gray regions of the thoracic spinal cord. Existence of direct monosynaptic connection between the labelled descending axons and the identified SPNs was established in the electron microscope. In all animals PHA-L labelled axons from the same injections were also seen in the caudal raphé nuclei. The greatest number of labelled axons was found in the raphé pallidus, where some of the axons entered from below the pyramidal tract. Varicose axons in close proximity to serotonin immunoreactive cell bodies and dendrites were observed in the raphé pallidus and ventral part of raphé obscurus.

Our results show a direct projection from the RVLM onto identified SPNs and raise the possibility that cardiovascular effects of the RVLM might be executed via serotonergic medullary raphé cells as well as directly via a monosynaptic link to SPNs.

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97.7

PATHWAYS MEDIATING AUTONOMIC RESPONSES ELICITED BY STIMULATION OF TONGUE AFFERENTS. S.J. Chen, M.M. Sawchuk*, G.V. Allen and D.F. Cechetto. Robarts Research Inst./Dept. of Physiology, Univ. of Western Ontario, London, Ontario N6A 5K8.

Nociceptive and gustatory stimuli evoke autonomic responses. The pathways mediating these responses are not completely known. Injection of horseradish peroxidase into the anterior left quadrant of the tongue was used to identify sites of afferent termination in the nucleus of the solitary tract (NTS) and the caudal spinal trigeminal nucleus (SNVc). In chloralose-anesthetized (150 mg/kg) rats electrical stimulation of the tip of the tongue increases arterial pressure, heart rate and sympathetic (renal) nerve discharge (SND). SND responses were evaluated using peristimulus-time histograms generated by single pulse stimulation. An initial increase in SND was observed at 71.2 (± SE 1.9) ms after the stimulation of the tongue. A synaptic blocking agent, cobalt (COB), was injected (10 mM, 300 nl) into areas of the NTS and SNVc identified by the anatomical results. Injections of COB were also made into the parabrachial nucleus (PB), the pontine A5 region and the ventral lateral medulla (VLM) which are likely sites of the second synapse mediating these responses. Only injections into the SNVc and A5 blocked the autonomic responses. It is concluded that autonomic responses are elicited by activation of pain receptors whose sequential pathway involves the SNVc, A5 and spinal cord preganglionic neurons.
(Supported by the Heart and Stroke Foundation of Ontario).

97.8

BASELINE AND REFLEX SYMPATHETIC ACTIVITY FOLLOWING COBALT INJECTIONS INTO THE VENTRAL LATERAL MEDULLA (VLM) AND SPINAL CORD. D.F. Cechetto and S.J. Chen. Robarts Research Inst./Dept. of Physiology, Univ. of Western Ontario, London, Ontario N6A 5K8.

Previous investigations suggested the VLM is primarily involved in maintainance of sympathetic tone and mediation of baroreceptor reflexes. In chloralose anesthetized (150 mg/kg) rats a presynaptic blocking agent, cobalt (COB), was injected into either the VLM or the spinal cord; baroreceptors were tested by either i.v. injection of phenylephrine or electrical stimulation of the aortic depressor nerve (ADN). Blood pressure, heart rate and sympathetic (renal) nerve discharge (SND) were continuously monitored. COB injection (10 mM, 300 nl) into the VLM (-1.0 mm caudal to 2.5 mm rostral to obex) did not change baseline SND at any site; however, injection into the rostral VLM (1.0 mm to 2.5 mm rostral to obex) attenuated (21% ± SE 5..9) baroreceptor reflexes, although complete reflex blockade was never obtained. Kynurenate (a postsynaptic excitatory amino acid blocker) injections (250 mM, 300 nl) evoked a large increase (87%) in baseline SND from rostral VLM sites and resulted in apparent block of baroreceptor reflexes. Intrathecal spinal cord injections (4 mM, 200 μl) of COB did not alter baseline SND but completely blocked baroreceptor reflexes. These results demonstrate that VLM afferents do not alter tonic SND and that intrinsic SND remains after removal of brain inputs to spinal preganglionic neurons. In addition, the rostral VLM is at least partially responsible for mediating baroreceptor reflexes.
(Supported by the Heart and Stroke Foundation of Ontario).

97.9

COCAINE INHIBITS GREATER SPLANCHNIC NERVE ACTIVITY IN ANESTHETIZED CATS. VFC Raczkowski#*, YM Hernandez*, KL Dretchen, RA Gillis* Department of Pharmacology, Georgetown University; & Children's Hospital#; Washington, DC 20007

We have shown that intravenous (IV) cocaine (COC) inhibits spontaneous cardiac sympathetic nerve activity (SNA) (FASEB JL 4:A1204,1990). These data contradict the commonly-held assumption that the sympathomimetic actions of COC result in part from increased central sympathetic outflow. They also appear to contradict the conclusion that COC increases centrally mediated SNA to the adrenal medulla (JPET 205:148-154,1978). Therefore, we recorded spontaneous SNA from the right greater splanchnic nerve, which innervates the adrenal medulla, during the administration of COC (4 mg/kg IV) in anesthetized (pentobarbital), paralyzed (vecuronium), mechanically ventilated cats with acutely denervated cardiovascular reflexes. Heart rate (HR) and arterial blood pressure (BP) were also recorded.

COC (n = 5) decreased SNA by 62 ± 6% ($p<0.01$). The maximal decrease of SNA occurred 69 ± 16 sec after drug administration. Initially, the mean BP decreased from 130 ± 12 mmHg to a minimum of 61 ± 14 mmHg ($p<0.01$). The maximal drop occurred at 91 ± 21 sec. A secondary increase in BP to a maximum of 153 ± 17 mmHg ($p<0.05$) occurred at 10.9 ± 3.7 min. HR was unaltered. In two other experiments, an equimolar dose of lidocaine (3.2 mg/kg IV) decreased SNA by an average of only 1.6%. The mean BP decreased from 113 mmHg to a minimum of 71 mmHg. The maximal drop occurred at 30 sec. The HR decreased from 164 to 149 bpm. These data indicate that, consistent with its ability to decrease cardiac preganglionic sympathetic outflow, COC also decreases sympathetic outflow to the adrenal medulla. These effects do not appear to be related to a local anesthetic action of the drug. Supported by NIH grant RO1 DA-05333.

97.10

NEUROCHEMICAL EVENTS IN THE SPINAL CORD DURING STIMULATION OF THE ROSTRAL VENTROLATERAL MEDULLA. G.A. Iwamoto, L.C. Abbott and T.G. Waldrop, Department of Veterinary Biosciences and Department of Physiology and Biophysics, University of Illinois, Urbana, IL 61801

Rostral ventrolateral medulla (RVLM) contains cells which project to the spinal cord (SC) and are responsible for maintaining blood pressure as well as participating in pressor responses. Several substances may mediate synaptic transmission of RVLM cells at the level of the SC. The purpose of this study was to determine if monoamines were involved.

Adult cats were anesthetized with alpha chloralose (60 mg/kg). The SC was exposed at T2-3. A concentric, flow-through microdialysis probe was placed just lateral to the dorsal root entry zone with the dialyzing portion of the probe (2 mm length x 0.25 mm dia.) localized to include the intermediolateral cell column. The medulla was exposed to allow placement of a stimulating electrode just ventral to the retrofacial nucleus. Control samples (15 min duration) were collected during stabilization of the probe for a period of 2 to 3 hr prior to stimulation. The RVLM was then stimulated for 15 min using repeated trains (40 Hz, 0.7 msec duration, 25 to 75 μA pulses) of 10 sec duration followed by 10 sec of no stimulation.

Increases in 5HT and/or 5HIAA accompanied the pressor response to RVLM stimulation in 6 of 9 animals. There were no changes observed in epinephrine or norepinephrine.

97.11

SPINAL NMDA RECEPTORS CONTRIBUTE TO CARDIOVASCULAR RESPONSES EVOKED BY HYPOTHALAMIC STIMULATION. J.P. Porter. Dept. of Physiol., Univ. of Louisville, L'ville, KY 40292

Spinal glutamate receptors participate in the pressor response evoked by activation of the ventrolateral medulla. In the present investigation, the effect of spinal NMDA receptor blockade with DL-2-amino-phosphono-valeric acid (AP5) on hemodynamic effects produced by hypothalamic stimulation was determined. In urethane anesthetized Sprague-Dawley rats, the changes in mean arterial pressure (MAP), heart rate (HR), and relative renal (RR) and hindquarter (HQR) vascular resistances produced by electrical stimulation of the paraventricular nucleus (PVN) or posterior hypothalamic area (PH) were determined, before and after intrathecal injection of AP5 (200 nmole in 10 μl). The AP5 significantly decreased baseline MAP and HR (n=6). Baseline RR tended to increase and HQR tended to decrease, but neither effect was significant. The increase in MAP and RR produced by hypothalamic stimulation of both sites was markedly reduced by the AP5. The decrease in HQR was reversed in 4 of 5 rats receiving PVN, and 2 of 4 rats receiving PH stimulation. These data suggest that spinal NMDA receptors participate in the cardiovascular responses evoked by hypothalamic stimulation. Whether the pathways mediating the effect involve activation of the ventrolateral medullary neurons that project to the spinal cord or direct hypothalamic-spinal projections is yet to be determined.

97.12

ROLE OF SPINAL N-METHYL-D-ASPARTIC ACID (NMDA) RECEPTORS IN SYMPATHOEXCITATION EVOKED BY GLUTAMATE STIMULATION OF THE ROSTRAL VENTROLATERAL MEDULLA (RVM) AND BY CEREBRAL ISCHEMIA. Michelle K. Bazil & Frank J. Gordon. Dept. of Pharmacology, Emory Univ., Sch. of Med., Atlanta, GA 30322.

Rats were anesthetized with urethane, paralyzed, vagotomized and artificially respirated. Sympathetic nerve activity (SNA) was recorded from the splanchnic nerve. The NMDA receptor antagonists D-AP7 (100 nmol) or CPP (12.5 nmol) were administered to the spinal cord via intrathecal (IT) catheters. Blockade of spinal NMDA receptors reduced mean arterial pressure (MAP) 29 ±4 mmHg and SNA 58 ±5%. Microinjections of L-glutamate (15 nmol/50 nl) into the RVM increased MAP 28 ±2 mmHg and SNA 97 ±29%. Pharmacologic blockade of spinal NMDA receptors reduced RVM pressor responses to 8 ±2 mmHg and evoked sympathoexcitation to 23 ±5% of control. In other experiments, cardiovascular and SNA responses were elicited by transient cerebral ischemia (CI). CI increased MAP 32 ±4 mmHg and SNA 75 ±19%. IT D-AP7 reduced CI pressor responses to 14 ±3 mmHg and SNA responses to 59 ±13% of control without affecting CI-induced increases in heart rate. IT administration of D-AP7 or CPP abolished the pressor and tachycardic effects of IT NMDA but not those of kainic acid or the quisqualate agonist AMPA. These results indicate that NMDA receptors in the spinal cord play an important role in the maintenance and bulbospinal regulation of sympathetic neural control of the circulation.

97.13

ROLE OF THORACIC INTERNEURONS IN CERVICALLY-EVOKED SYMPATHOINHIBITION IN THE RAT.

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We have previously described a cervical sympathoinhibitory system that inhibits both pre- and postganglionic sympathetic activity. We have been able to activate this system by electrical or chemical stimulation of the rostral cervical spinal cord only after C1 spinal transection or after inhibition of the rostral ventrolateral medulla with mucimol, leading us to hypothesize that this system is tonically active in the intact rat. Recordings from sympathetic preganglionic neurons (SPGN) suggested that the sympathoinhibition was due to the inhibition of presympathetic interneurons, not preganglionic neurons. In the present study, we tested the hypothesis that spinal interneurons which received somatic and visceral sensory information, and which might conceivably be excitatory antecedents to SPGN, were inhibited by cervical stimulation. Working in anesthetized, spinally-transected, and artificially-respired rats, we identified spinal interneurons which were excited by both somatic and visceral afferent stimuli. Most of these neurons also exhibited ongoing activity. Both the ongoing and evoked activities of the large majority of these neurons were inhibited by cervical electrical stimulation which also inhibited renal sympathetic activity. We conclude that the cervical "sympathoinhibitory system" may have a more general descending inhibitory function. Indeed, its sympathoinhibitory function may be mediated by inhibition of afferent activity which, in turn, excites SPGN. Supported by NIH Grant HL 16315.

97.14

Functional evaluation of cardiac, vasoconstrictor and renal sympathetic nerve activity (SNA) in the conscious spinal rat. K. Trostel* and J. Osborn, Dept. of Vet. Biol., U. of Minnesota, St. Paul, MN 55108

In anesthetized rats, cervical spinal transection (CST) increases renal SNA 2-3 fold resulting in decreased urinary sodium (Na) and water excretion (Osborn et.al., AJP 253:R619-R625, 1989). To determine whether there is functionally significant SNA in conscious spinal rats we conducted the following studies. First, mean arterial pressure (MAP), heart rate (HR) and plasma renin activity (PRA) were measured before and 24 hours after CST in 15 chronically instrumented Sprague-Dawley rats. Second, in the same rats, the response of functional indicators of SNA to combined alpha- and beta-adrenergic blockade (N=8) or saline vehicle (N=7) were evaluated 24 hours after CST. MAP, HR, urine flow (V), urinary Na excretion (UNaV), urinary potassium excretion (UKV) and PRA were determined before and after two hours of combined administration of phentolamine (2mg/kg + 1mg/kg/hr) and propranolol (2mg/kg + 1 mg/kg/hr). CST resulted in significant decreases of MAP (102.1±2.7 to 75.1±1.7 mm Hg), HR (338±12 to 292±7 bpm), and PRA (3.0±1.6 to 1.7±1.3 ng AI/ml/hr). Subsequent alpha- and beta-adrenergic blockade resulted in a small but significant increase in MAP (4mm Hg). There was no significant effect of adrenergic blockade on HR, V, UNaV, UKV or PRA. Based on these results, we conclude that sympathetic nerve discharge to the vasculature, heart, and kidney is either absent or ineffective in conscious spinal rats. NIH grant HL 39619.

97.15

SPINAL CONTROL OF CARDIAC FUNCTION IN THE RAT: ROLE OF DIFFERENT EXCITATORY AMINO ACID RECEPTOR SUBTYPES.

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In different groups of pentobarbital-anesthetized male Wistar rats (immobilized and artificially ventilated), L-glutamate (Glu; 0.9-1.77 nmole), N-methyl-D-aspartic acid (NMDA; 1-100 pmole), kainic acid (KA; 1-10 pmole), quisqualic acid (QA; 0.1-100 pmole) and (a-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA; 10 pmole) were microinjected into the right intermediolateral column (IML) at the T2 level. All these agonists produced tachycardia with little or no change in blood pressure. The effects of NMDA (100 pmole) were blocked by microinjections of D-AP7 (5 nmole). Kynurenic acid (2-4 nmole) blocked the effects of KA (3 pmole) and QA (1 pmole) but not Glu (1.77 nmole). The effects of Glu were blocked by glutamic acid diethylester (GDEE; 80 nmole); this dose of GDEE did not block the effect of carbachol (440 pmole). These results indicate that different subtypes of excitatory amino acid receptors, located in the IML at T2 level, may have a role to play in the spinal control of cardiac function.

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97.16

GLUTAMATE-CONTAINING AXON TERMINALS SYNAPSE DIRECTLY ON CHOLINE ACETYLTRANSFERASE-LABELED SYMPATHETIC PREGANGLIONIC NEURONS.

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We have proposed (Prog. Brain Res., 1989;81:159) that glutamate mediates the sympathoexcitatory effects of the vasomotor pathway from the rostral ventrolateral medulla (RVL) to the intermediolateral nucleus (IML): (A) glutamate-like immunoreactivity (GLU-LI) is in axon terminals making asymmetric synapses on IML neurons (B) neurons in the RVL are a source of this glutamatergic innervation, and (3) RVL stimulus-evoked excitation of sympathetic preganglionic neurons (SPNs) is blocked by glutamate receptor antagonists. To determine whether terminals with GLU-LI synapse upon cholinergic SPNs, coronal sections from the upper thoracic spinal cord were labeled immunocytochemically with antibodies to choline acetyltransferase (ChAT) (Crawford et al., Proc. Natl. Acad. Sci., 1982;79:7031) identified by the ABC reaction product and hemocyanin-conjugated glutamate (Hepler et al., J. Histochem. Cytochem., 1988;36:13) identified by silver-intensified immunogold labeling. Ultrastructurally, GLU-LI in the IML was in axon terminals containing a few mitochondria and numerous small, clear vesicles. Terminals with GLU-LI formed primarily asymmetric (excitatory) synapses on the shafts of medium and small dendrites as well as on dendritic spines of ChAT-labeled neurons. Frequently, more than one terminal with GLU-LI contacted the same target. These data indicate that SPNs are innervated by glutamate-containing terminals, suggesting a direct influence of glutamate on SPN membrane excitability. This finding is also consistent with a monosynaptic connection from vasomotor neurons of the RVL to thoracic SPNs. Supported by NIH HL18974.

97.17

PHARMACOLOGICAL STUDIES ON THE CARDIOVASCULAR RESPONSES TO INTRATHECAL ADMINISTRATION OF L- AND D-BACLOFEN IN THE RAT.

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We have previously demonstrated that the intrathecal administration of L-baclofen (7-700 nmol) to the thoracic spinal cord induces a decrease in arterial pressure and heart rate; D-baclofen (7-70 nmol), on the other hand, increases arterial pressure but has no effect on heart rate. Reports that D-baclofen antagonizes effects of L-baclofen and that phaclofen is an antagonist of baclofen prompted the present study. Anaesthetized (urethane; 2.5 g/kg, i.p.) male Sprague Dawley rats (300-350g) were used. In all cases, administration was intrathecal; control groups received vehicle. Effects of 70 nmol of L-baclofen were not modified by pretreatment 10 min beforehand with 700 nmol of D-baclofen (n=12). In contrast, 70 (n=10), but not 7 (n=7), nmol of L-baclofen blocked the cardiovascular effects of 700 nmol of D-baclofen. As a test for desensitization, D-baclofen was administered twice, 7 min apart, in one group of rats (n=5); the full-blown effect was observed with each of the two administrations. Phaclofen (2.5μmol) increased arterial pressure and heart rate [T9 (n=18) > T2 (n=14)]; these effects developed slowly over 5-10 min and persisted for 30 min, and produced total and partial block of the effects of 70 (n=9) and 350 (n=10) nmol of L-baclofen, respectively. (Supported by a Grant-in-Aid from the Québec Heart Foundation.)

97.18

ADRENAL MEDULLARY SECRETION WITH SPLANCHNIC STIMULATION IN SPINALLY TRANSECTED CATS.

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This project was undertaken to determine whether previously observed adrenal medullary hyperactivity that developed following high spinal cord transection in the cat (Stoddard, *J. Auton. Nerv. Syst.* 23:175-179, 1988) could be explained by increased sensitivity of the splanchnic nerve-chromaffin cell synapse. The splanchnic nerve was stimulated in acute (2-3 hr; N=7) or chronic (61-64 da; N=7), spinally transected (T3) cats that were decerebrate and unanesthetized. Stimulation (30V; 1 msec pulses) was applied at 3 and 30 Hz to deliver the same number of pulses in 3 min. Adrenal medullary secretion (ng/min) of catecholamines (CAs), [Met]enkephalin (ENK), and neuropeptide Y was measured. Both stimuli increased the secretion of CAs and neuropeptides in both acute and chronic animals. Low frequency stimulation increased the ratio of NE/EPI in acute animals; this ratio was unchanged by either stimulation in the chronic animals. Secretion of EPI and ENK at low frequency was greater in the chronic than in the acute animals. These data suggest there is some facilitation of the splanchnic nerve-chromaffin cell synapse that occurs over time following high spinal transection. However, it is likely that spinal mechanisms also contribute to adrenal medullary hyperactivity. [Supported by the Spinal Cord Research Foundation, PVA (SLS)].

97.19

EFFECTS OF 5,7-DIHYDROXYTRYPTAMINE ON CARDIOVASCULAR REGULATION IN THE AWAKE RAT.

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The effect of 5,7-dihydroxytryptamine (5,7-DHT) on cardiovascular regulation was studied in the male rat during normal activity and during applied cold stress. Blood pressure and heart rate were measured via tail artery cannulation one week following intrathecal administration of 5,7-DHT. The mean arterial pressure (MAP) was significantly less for the unstressed 5,7-DHT-lesioned rats than for the vehicle-injected controls. Preliminary results indicate that one month post-lesion this difference was no longer apparent. Cold stress caused a significant increase in MAP over the basal level in both the control and the experimental group.

Immunohistochemical analysis confirmed the loss of serotonin-immunoreactive fibers in the lesioned rats. Moreover an increase in calcitonin gene-related peptide (CGRP) immunoreactivity in fibers in the intermediolateral column of the spinal cord was observed a few months after the 5,7-DHT lesions. Studies are underway to determine the relationship between the apparent neuronal plasticity and change in cardiovascular function.

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98.1

REPETITIVE FIRING PROPERTIES OF NEURONS WITHIN THE MEDIAL NUCLEUS OF THE SOLITARY TRACT IN RATS. S.M. Johnson and R.B. Felder. Cardiovascular Center, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

The medial portion of the *nucleus tractus solitarius* (NTS) receives sensory information from baroreceptors and chemoreceptors located in the heart and great vessels. We examined the repetitive firing properties of medial NTS neurons by recording intracellularly from neurons using an *in vitro* brainstem slice preparation. The post-synaptic response resulting from electrical stimulation of the ipsilateral solitary tract was also studied. Our results (n=18 cells) showed that the resting membrane potential was -54 ± 8 mV (mean ± std. dev.) and the membrane resistance was 68 ± 38 MΩ (range = 23-164 MΩ). The action potential amplitude and undershoot were 70.0 ± 10.4 mV and 8.3 ± 4.6 mV, respectively. The action potential duration at half-height was 778 ± 166 ms. After-hyperpolarization was found in 8/15 cells, post-inhibitory rebound was found in 9/14 cells, and delayed excitation was found in 1/15 cells. In 9 of 10 cells tested, an action potential was evoked by stimulating the ipsilateral tractus solitarius. This is the first systematic examination of the repetitive firing properties of medial NTS neurons. These intrinsic neuronal properties, in addition to synaptic mechanisms, are important for afferent processing by NTS neurons.

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98.2

L-HOMOCYSTEIC ACID (LHCA), A POTENTIAL ENDOGENOUS TRANSMITTER IN THE BAROREFLEX. H. Ohta, S. Eversmann*, and W.T. Talman. Dept. of Neurol., VAMC and Univ. of Iowa, Iowa City, IA 52242.

We have hypothesized that glutamate is a transmitter at NMDA receptors in the baroreflex arc. NMDA antagonists block responses to NMDA and to baroreflex activation but not those to glutamate. Thus, an agent other than glutamate may be the endogenous agonist. We sought to evaluate the role of one such agent LHCA in cardiovascular regulation through the nucleus tractus solitarii (NTS) in 37 anesthetized rats, instrumented for recording arterial pressure (AP), heart rate (HR), and, in some, sympathetic nerve activity (SNA). Microinjections were made into NTS through micropipettes. Like NMDA, LHCA produced dose-related decreases in AP, HR, and SNA. The maximal response (decreased AP of 20.3±2.2 mmHg and HR of 26±2.8 bpm; $p<0.05$) occurred after a dose of 200 pmol; ED_{50}=20 pmol. The NMDA antagonists 2-APV and MK-801 significantly reduced responses to 20 pmol of LHCA and cardiovascular responses to baroreflex activation; MK-801, but not 2-APV, attenuated reflex responses of SNA. These data are consistent with a role for LHCA in baroreflex neurotransmission.

98.3

N-METHYL-D-ASPARTATE (NMDA) RECEPTOR BLOCKADE AND AFFERENT SYNAPTIC TRANSMISSION IN RAT MEDIAL NUCLEUS TRACTUS SOLITARIUS IN VITRO. M.C. Andresen and M. Yang. University of Texas Medical Branch, Galveston, TX 77550.

A variety of visceral afferents synapse on NTS neurons. Medial NTS receives substantial numbers of synaptic contacts from arterial baroreceptors. Reflex studies of the control of blood pressure and heart rate have implicated all three classes of excitatory amino acid receptors in these responses, but little is known about their cellular basis. Here, we studied the effects of blockade of NMDA receptors with the selective antagonist AP5 (2-amino-5-phosphonovalerate) on afferent synaptic transmission in mNTS. Neurons were recorded intracellularly from medial portions of NTS in a longitudinal slice of the rat medulla. The solitary tract (TS) was stimulated approx. 1-3 mm from NTS recording site at 0.5 Hz. Recording was limited to mNTS from the obex to 500 μ rostral to the obex bounded laterally by TS and the 4th ventricle. Some neurons were hyperpolarized to prevent spiking. TS stimulation evoked a short latency (2 msec) EPSP. AP5 was added to the slice surface near the recording electrode at 0, 1, 10, & 100 μM and measurements made at 2-3 min. We previously found that CNQX (non-NMDA blocker, 6-cyano-7-nitro-quinoxaline-2,3-dione) suppressed tract evoked EPSPs within 60 sec. AP5, however, had negligible effects on the EPSP even at 100 μM (n=8, $p>0.23$), but CNQX addition rapidly eliminated these EPSPs. Our results suggest that a non-NMDA receptor mediates these excitatory synaptic responses.

98.4

GLUTAMATE RELEASE IN THE NUCLEUS TRACTUS SOLITARIUS IS NOT AFFECTED BY BARORECEPTOR AFFERENTS. A.F. Sved and J.N Salter*. *Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

Previous studies have suggested that glutamate (Glu), or some other excitatory amino acid neurotransmitter, is the neurotransmitter of baroreceptor afferent nerves that project to the nucleus tractus solitarius (NTS). We have examined this hypothesis by determining the effect of afferent vagal stimulation or specific baroreceptor stimulation on release of endogenous Glu and aspartate (Asp) in the NTS. Microdialysis probes (200u diameter, 400u length of exposed dialysis membrane) were placed into the NTS of urethane anesthetized rats. Artificial CSF was perfused through the probe at 2 ul/min and 20 minute fractions were collected and analyzed for amino acids. By two hours after implantation of the probe, Glu and Asp levels in the dialysate fluid were stable (1.9±.4 pmol/20 ul and 3.5±.8 pmol/20 ul respectively). Neither bilateral electrical stimulation of vagal afferent nerves (2msec pulses, 500 uA, 10 Hz, for 20 minutes) nor increasing arterial pressure by 40 mmHg with an infusion of phenylephrine significantly altered the dialysate fluid levels of Glu, Asp, or any other measured amino acid (n=5 for each treatment). In contrast, increasing the K^+ concentration of the CSF perfused through the dialysis probe to 60 mM significantly increased the concentration of Glu (4.2±.7 fold) and Asp (3.2±.6 fold) in the dialysate fluid. Increasing the K^+ concentration increased the dialysate concentration of several other amino acids (i.e., GABA, taurine, glycine) and decreased the concentration of glutamine. Other amino acids were not affected. These data provide evidence that Glu is not the neurotransmitter of baroreceptor afferent nerves.

98.5

EVIDENCE THAT VAGAL AFFERENTS ARE NOT GLUTAMATERGIC. M.G. Backes and A.F. Sved. *Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

It has been suggested that baroreceptor afferent nerves release glutamate, or some other excitatory amino acid (EAA), at their terminals in the nucleus tractus solitarius (NTS). Glutamate analogs such as ^{3}H-D-aspartate (^{3}H-D-ASP), can be used to retrogradely label EAA-releasing neurons in the CNS. In the present study, this method was used to examine whether vagal afferents will transport ^{3}H-D-ASP retrogradely from the NTS to the nodose ganglion. ^{3}H-D-ASP (20-50 uCi in 25-100 nl) was injected into the NTS of rats at the level of the obex. In control rats ^{3}H-D-ASP was injected into the spinal trigeminal nucleus (Sp5). Following 14-48 hours, rats were anesthetized and perfused with 5% glutaraldehyde. Brains and nodose, petrosal, and trigeminal ganglia were removed. Cryostat sections (20 u) were direct-mounted onto gelled slides. Slides were defatted and dipped in Kodak NTB-2 emulsion. After various exposure times (1-6 months), slides were developed in D-19 and counterstained with cresyl violet. In 4 of 9 rats in which the injection was centered on the NTS, no retrogradely labeled cells were observed in the nodose or petrosal ganglia. In the other 5 rats, a small number (<10/ganglion) of retrogradely labled cells were found in the ipsilateral nodose ganglia. Many labeled neurons were always observed in the ipsilateral trigeminal ganglion following 3H-D-ASP injection into Sp5 (n=5). These data indicate that vagal and glossopharyngeal afferents do not specifically transport 3H-D-ASP retrogradely from the NTS, providing evidence that baroreceptor afferents are not glutamatergic.

98.6

AMINO-TERMINAL SUBSTANCE P FRAGMENTS IN CENTRAL CARDIOVASCULAR REGULATION: STRUCTURE SPECIFICITY AND BINDING SITES. M.E. Hall*, C.L. Miller*, N. Zahniser, J.M. Stewart. Dept. of Biochem., Biophys. & Genetics and of Pharmacology, Univ. of Colorado Medical School, Denver, CO 80262.

Substance P (SP) is a putative neurotransmitter (NT) of the baroreflex in the nucleus of the solitary tract (NTS). Recent work suggests that SP(1-7) produced by endopeptidase 3.4.24.11 is the NT, producing bradycardia and hypotension upon microinjection into the medial NTS of rats. Inhibition of 24.11 by phosphoramidon blocks depressor action of SP but not of SP(1-7). Classical tachykinin agonists such as the widely used metabolically stable C-terminal analog DiME-C7 and other C-terminal SP fragments are pressor in the NTS. Intra-NTS injections of SP N-terminal fragments and analogs show considerable structure specificity for hypotensive action, suggesting combination with a specific receptor. We report preliminary evidence for such a receptor. Incubation of membranes from rat brainstem (including the NTS) with 500 picomolar ^{125}I-Tyr-SP(1-7) showed specific binding of 5 fmoles/mg protein. This result provides initial evidence for a basis of action of SP(1-7) and related N-terminal SP peptides in the central nervous system.

98.7

EXCITATORY AMINO ACID (EAA) MODULATION OF CAROTID SINUS BARORECEPTOR ACTIVITY. G. Hajduczok, S.J. Lewis, M.J. Brody, and F.M. Abboud*. Depts. Internal Medicine, Pharmacology, and CV Center, Univ. of Iowa College of Medicine, Iowa City, IA 52242.

Recent studies from our laboratory have demonstrated that intravenous injection of the EAA analogue kainic acid (KA) produces a vasopressin mediated increase in arterial pressure in conscious ganglion-blocked animals. These responses to KA are abolished by chronic sinoaortic denervation. The purpose of the present study was to determine whether KA inhibits afferent baroreceptor discharge. In four chloralose anesthetized dogs, the left carotid sinus (CS) was vascularly isolated and multiunit baroreceptor activity (BA) was measured from the CS nerve during increases in carotid sinus pressure (CSP) before and during exposure of the CS to KA. Values below represent the percent of maximal BA during control.

CSP (mmHg)	50	75	100	125	150	175	200
Control	12±5	35±18	64±16	80±11	88±8	95±3	100
KA (10^{-8}M)	8±4	24±13	48±15	64±11	71±11	75±7	75±5

Exposure of the CS to KA resulted in a reversible inhibition of BA at all pressures. BA was also inhibited by KA at doses of 10^{-7} M and 10^{-6} M. This inhibitory effect appeared to be dose related. We conclude that the EAA analogue KA may suppress the baroreflex by inhibiting afferent baroreceptors.

98.8

COMPUTATIONAL MODELING SHOWS PRIMARY BARORECEPTOR AFFERENTS PRESENT PARALLEL, THRESHOLD-RECRUITED, TIME-DISTRIBUTED INPUTS TO THE NTS. *J.S. Schwaber, J.F.R. Paton, W.T. Rogers, K.M. Spyer. Neural Computation Group, E.I. duPont Co., Wilmington, DE 19880-0352 and Royal Free Hospital School of Medicine, London NW3 2PF.*

We are creating a computational model of the baroreceptor vagal reflex based on the literature and our own ongoing experiments. Modeling of baroreceptor A-fiber transduction and signal transmission properties has revealed a new and quite different perspective on the coding of cardiovascular information. The distribution of distinct pressure thresholds of these fibers yields a recruitement series across the pressure pulse that optimally encodes the shape, height and timing of the pulse as a parallel population response across the primary afferents. The rise time of the pulse pressure causes this recruitment series to be temporally dispersed. This time series is enhanced by an inverse correlation of conduction velocity with threshold. Thus, pulse pressure data are presented to the brain as a temporally dispersed population code. Interestingly, this computational result on the information coding of primary afferents matches well with (a) the dynamical, frequency-dependent response properties described for their second-order target cells in the NTS and (b) their apparently spatially distinct targets.

98.9

IS THERE A CENTRAL PATTERN GENERATOR IN THE MEDULLA FOR CONTROLLING CARDIOVASCULAR ACTIVITY? *J.F.R. Paton, J.S. Schwaber, W.T. Rogers. Neural Computation Group, E.I. duPont Co., Wilmington, DE 19880-0352*

In order to gain a better understanding of the baroreceptor reflex we are attempting to model the central neural mechanisms involved. Intracellular recordings of cells were made from rat brainstem slices within the dorsomedial and dorsal commissural nucleus tractus solitarius (NTS), the major regions receiving baroreceptor afferents and the NTS subgroups specifically concerned with cardiovascular control. Many cells in this area showed rhythmic ongoing discharge at a frequency of 5-10 Hz (mean 7.48, SEM +/-0.5 Hz) and were synaptically driven by electrical stimulation of both the ipsilateral tractus solitarius (ts; 2-5ms latency) and ventrolateral medulla (VLM; 3-7ms latency). In cases tested the neuronal discharge rate could be entrained by the frequency of ts and/or VLM stimulation within a preferred range. Inactivation of the VLM by cooling decreased the discharge frequency in some cells. Intracellular labeling of these active neurones demonstrated dendrites projecting medially and dorsomedially within the NTS and laterally into the ts. Their axons coursed out of the NTS ventrolaterally and in a rostral direction. The data provide some evidence for an oscillatory network between the NTS and VLM.

98.10

ELICITATION OF THE CAROTID SINUS BAROREFLEX AT LOW INTRASINUS PRESSURES. Qu, L. and Stuesse, S.L. Neurobiology Department, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.

In previous studies, we reported that baroreceptor fiber discharge can be elicited during low intrasinus pressure (ISP). In the present study, carotid sinus baroreceptor activity and its reflex effect were further investigated when ISP was varied in the range of 0-50 mmHg. In urethane-anesthetized rabbits with cut cervical sympathetic nerves, the carotid sinus region was isolated vascularly and perfused with Locke's solution equilibrated with 95% O_2 and 5% CO_2. Single unit discharges from baroreceptor or chemoreceptor fibers were tested for a response to pressure stimulation. Two groups of baroreceptors were identified: one had a mean pressure threshold of 47.8 mmHg and a maximal gain 0.238 impulses/sec/mmHg, and the other had a maximal gain of 0.13 impulses/sec/mmHg but no pressure threshold. Chemoreceptor discharges were also altered by changing ISP, but their activity was inhibited. Reflex changes in heart rate (HR), mean arterial blood pressure (MAP) and cardiac contractility were examined during bilateral carotid sinus perfusion in vagotomized rabbits with bilaterally cut aortic depressor nerves. Altering pulsatile ISP from 50 to 0 mmHg in 25 mmHg steps significantly increased MAP and decreased HR (Least squares liner regression, $P<0.05$). Increasing CO_2 in the perfusate by equilibrating the Locke's solution with 6% CO_2 or 12% CO_2 in 20% O_2 attenuated MAP responses but increased the cardiac responses, HR and cardiac contractility. Thus during carotid sinus hypotension, circulatory reflexes may be elicited which differ from those during normal blood pressure or hypertension. Supported by a grant from the American Heart Association.

98.11

EXCITATORY AMINO ACID RECEPTORS IN THE ROSTRAL DEPRESSOR AREA OF CAT MEDULLA DO NOT MEDIATE BAROREFLEX. C. W. Dempsey, D. E. Richardson, and C. J. Fontana. Lab. of Neurosurgery, Tulane University School of Medicine, New Orleans, LA 70112.

We have previously reported (Neurosci. Abst. 14:192, 1988) in anesthetized, atropinized cat a glutamate-responsive sympathoinhibitory area lying superior to the rostral ventral lateral medulla. When this region, designated as the rostral depressor area (RDA), is bilaterally inactivated by toxic microinjections of kainic acid, cardiovascular tone rises and PeSBR, the sympathetic component of phenylephrine-induced baroreflex, is 80% diminished. The present study was aimed at discovering if N-methyl-D-aspartic acid (NMDA) and alpha-amino-methylisoxazole-proprionic acid (AMPA) receptors mediate the tonal and baroreflexive functions of the RDA. Microinjection (40 nl) of either NMDA (20 pmol) or AMPA (3 pmol) into unilateral RDA produced acute hypotension and bradycardia lasting 5-10 min. Subsequent microinjection of the NMDA-complex glycine-site antagonist 7-chlorokynurenic acid (7CK, 50 pmol) blocked the NMDA-response and elevated heart rate for 30 min, but did not change PeSBR even when administered bilaterally. Microinjection of the AMPA-site antagonist dinitroquinoxalinedione (DNQX, 40 pmol) blocked the AMPA-response, but did not affect either tone or PeSBR. We conclude that NMDA- and AMPA-receptor mediation in the RDA is not significantly involved in these functions.

98.12

SINO-AORTIC DENERVATION ELIMINATES PRESSOR RESPONSES ELICITED BY MUSCIMOL INJECTIONS INTO THE NUCLEUS TRACTUS SOLITARIUS. A.M. McDonald and A.F. Sved. *Department of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

Local injection of muscimol (MUS), a $GABA_A$ agonist, into the nucleus tractus solitarius (NTS) of chloralose-anesthetized ventilated rats increases arterial pressure (AP) presumably by inhibiting tonically active neurons driven by baroreceptor afferents. The present study examined the effect of arterial baroreceptor denervation on this response to MUS. Male Sprague-Dawley rats were sinoaortically denervated (SAD) by bilaterally painting the carotid sinus with phenol and cutting the superior laryngeal nerve and the cervical sympathetic chain. Baroreceptor reflexes (phenylephrine-induced bradycardia and nitroprusside-induced tachycardia), tested in conscious rats one week following surgery, were totally absent in the SAD rats. Rats were then anesthetized with chloralose, artificially ventilated, and prepared for injections into the NTS (Catelli et al., Brain Res., 1987). In sham-denervated control rats, bilateral microinjection of MUS (100 pmol in 100 nl artificial CSF) into the NTS increased AP from 114±6 mmHg to 163±6 mmHg (n=7, $P<0.01$). In contrast, injection of MUS into the NTS of SAD rats did not influence AP (99±8 mmHg before, 99±8 mmHg after; n=6). Since MUS is known to inhibit neurons, the ability of MUS to increase AP in control rats indicates that it inhibits tonically active neurons in the NTS. The failure to elicit an increase in AP in the SAD rats suggests that, in the absence of arterial baroreceptor afferents, these neurons are not tonically active. These data imply that, in SAD rats, other inputs do not tonically excite NTS neurons that influence AP.

98.13

SELECTIVE INTERACTIONS OF ETHANOL WITH MEDULLARY NMDA AND GABA-A RECEPTORS INVOLVED IN BAROREFLEX REGULATION. K. Varga* and G. Kunos. LPPS, NIAAA, Bethesda, MD 20892

The effects of drugs and ethanol on blood pressure (BP), heart rate (HR) and baroreflex bradycardia were studied in urethane anesthetized rats. Ethanol, 1 g/kg i.v., or 0.2 µmol microinjected into the nucleus tractus solitarii (NTS) or the caudal ventrolateral medulla (CVLM), inhibited the reflex bradycardic response to i.v. phenylephrine. Intra-NTS injection of muscimol, 10-80 pmol/side, increased BP, HR, and inhibited baroreflex bradycardia. Intra-NTS ethanol markedly potentiated the pressor and tachycardic responses to intra-NTS muscimol. Glutamate, 0.5-2 nmol, microinjected into the NTS or the CVLM decreased BP and HR, and the effects in the CVLM but not in the NTS were inhibited by microinjection into the same site of 30 pmol of the NMDA-antagonist, MK-801. The effects of intra-NTS glutamate were inhibited by i.v. and intra-CVLM but not by intra-NTS ethanol. The effects of intra-CVLM glutamate were inhibited by i.v. and intra-CVLM ethanol. Thus, ethanol appears to inhibit NMDA-receptor function in the CVLM and to potentiate GABA-A receptor responses in the NTS, while non-NMDA glutamate receptors in the NTS are not directly affected. We propose that these selective and localized effects of ethanol on GABA-A and NMDA receptors account for the inhibition of the depressor baroreflex response, and could thus contribute to the well documented hypertensive effects of ethanol.

98.14

CONVERGENT EXCITATORY INPUTS FROM AREA POSTREMA AND AORTIC BARORECEPTORS ONTO NEURONS IN NUCLEUS TRACTUS SOLITARIUS (NTS). A.C. Bonham, Univ. California, Davis, Davis, CA 95616. E.M. Hasser, Univ. Missouri, Columbia, MO. C.M. Heesch, Univ. Kentucky, Lexington, KY.

Circulating arginine vasopressin (AVP) has been proposed to augment baroreflex-mediated sympathoinhibition by exciting area postrema neurons which project, ultimately, to baroreceptor-responsive neurons in the NTS (Bishop et. al., Circ. Res. 61:4 Suppl II:1-76-81,1987). If true, then some NTS neurons should receive convergent excitatory inputs from both area postrema and arterial baroreceptors. To test that hypothesis, we performed extracellular recordings of single unit activity in the NTS of anesthetized, artificially-ventilated rabbits. Area postrema neurons were activated by brief electrical stimuli (50-420µA, 0.03-0.07ms, 1.5Hz). Aortic baroreceptors were activated by single or twin electrical pulses (1-10v, 0.3-1ms, 1.5Hz) applied to the aortic depressor nerve (ADN). We recorded action potentials from 14 single postsynaptic cells excited by area postrema stimulation (onset latencies, 3-20ms). The cells were located - 0.2-0.7mm caudal to obex, 0.5-0.9mm ventral to the dorsal surface, and 0.1-0.6mm lateral to midline. Six of 10 cells tested were also excited by ADN stimulation (onset latencies, 60-120ms). In each of these cells, the waveform of the action potential evoked by stimulation of area postrema or ADN was identical. Furthermore, summation of inputs to individual NTS neurons was demonstrated during simultaneous threshold or subthreshold stimulation of area postrema and ADN.

In summary, brief electrical stimuli applied to area postrema evoked action potentials from NTS neurons, some of which also received excitatory input from aortic baroreceptors. These data suggest an excitatory interaction between baroreceptor and area postrema inputs to the NTS and provide a mechanism for AVP acting in area postrema to augment baroreflex-mediated sympathoinhibition.

98.15

THE CYTOARCHITECTURE OF THE CAUDAL NUCLEUS OF THE SOLITARY TRACT IN THE HUMAN MEDULLA. T.M. Hyde, S.K. Sigworth* and R.R. Miselis. CBDB, NIMH at St. Elizabeths Hospital, Wash., D.C., 20032.

In many species, the caudal nucleus of the solitary tract (NTS) has been divided into subnuclei on the basis of cytoarchitectural characteristics. In our study, sections from 8 normal human medullas were examined and computerized cell morphometry was performed on neurons within each subnucleus. Lying most caudally, the commissural subnucleus (Com) spans the midline, just behind obex, and contains small rounded neurons. The Com merges with the medial subnucleus (Med) anteriorly, and at this level other subnuclear divisions become apparent. The ventrolateral subnucleus (VL) lies in a triangular zone and contains large neurons. Rostrally, the VL is bounded medially by the interstitial subnucleus, which contains small cells intermingling with fibers of the tract. The intermediate subnucleus (Int) is largest at the midpoint of the rostrocaudal extent of the area postrema (AP), lies between the dorsal motor nucleus of the vagus (DMN) and the tract, and contains scattered large pigmented neurons. The dorsal subnucleus, containing densely packed large and medium-sized neurons, extends up and around the subnucleus gelatinosa (Sg). The Sg is a rounded cell-poor subnucleus lying ventrolateral to the AP, maximal in size at the rostral end of the AP. Composed of medium and small neurons, the Med is the largest subdivision, bounded by the Int, the dorsal subnucleus, the DMN, the AP, and the fourth ventricle. In conclusion, multiple subnuclei are identifiable in the caudal human NTS.

98.16

CAROTID BODY AFFERENT PROJECTIONS TO THE NUCLEUS TRACTUS SOLITARIUS (NTS) IN THE RAT: NEUROCHEMICAL HETEROGENEITY OF TARGET SUBNUCLEI. **J.C.W. Finley and D.M. Katz, Depts. of Medicine and Neuroscience, Case Western Reserve Univ. Sch. of Med., Cleveland, OH 44106**

Despite widespread interest in brainstem control of peripheral chemoreflexes, the anatomic and neurochemical organization of carotid body afferent pathways remains poorly defined. In the present study, anterograde transport of wheat germ agglutinin- horseradish peroxidase (HRP) was used to define projections from the carotid body to NTS; in addition, immunocytochemical staining was used to begin characterizing neurochemical properties expressed by neurons in the projection area. HRP (Sigma VI, 5-10% w/v) was injected unilaterally into the carotid body via glass micropipettes after vascular isolation of the tissue. 2-4 days later the carotid body, petrosal ganglion (PG) and brainstem were fixed, sectioned and processed for HRP histochemistry using modifications of Mesulam's (1976) TMB method. Labeled sensory neurons were segregated in the distal portion of the PG. The central processes of these neurons entered the medulla rostral to the obex, descended in the ipsilateral TS and ended in a highly restricted region of the caudal nTS. A dense network of fibers was present bilaterally in the nucleus commissuralis; at the level of the obex, fibers were also present in the medial and dorsal subnuclei of NTS. Immunocytochemical staining demonstrated that NTS regions receiving carotid body afferent projections contained both tyrosine hydroxylase- and beta-endorphin-positive neurons. Although postsynaptic targets of the sensory fibers remain to be defined, these findings indicate that carotid body inputs to the brainstem may interact with neurochemically diverse subpopulations of NTS neurons. **Supported by ALA Research Fellowship (JCWF) and HL39921-03.**

98.17

Baroreflex Function in Hypotensive Transgenic Mice Overexpressing Atrial Natriuretic Factor (ANF). Karen L. Cochrane and Loren J. Field*. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY 11724.

Conscious, chronically cannulated transgenic mice that have a 10 fold increase in ANF have mean arterial pressures (MAP) that are 30 mmHg lower than their nontransgenic littermates (Steinhelper, et al., 1990). Plasma renin activity and catecholamine concentration were not elevated. To determine if the baroreflex slope (BRS; interbeat interval (IBI) VS systolic arterial pressure (SAP)) is altered, arterial and venous cannulas were placed in 6 transgenic and nontransgenic mice and MAP and BRS (during phenylephrine infusion; 3 ug/kg, iv) assessed 1 day later. The MAP of transgenic mice was lower than the control mice (76±3 mmHg VS 102±3 mmHg). The transgenic mice BRS was half that of the nontransgenic mice (5±1 VS 11±1 msec/mmHg). The increase in SAP was comparable in both groups (93±2 to 151±3 and 125±4 to 190±5 mmHg) while the maximum IBI acheived was less in the transgenic mice (110±3 to 272±24 VS 109±4 to 341±28 msec). The blunted BRS was probably due to a lack of sympathetic withdrawal because BRS was abolished in transgenic mice (n=2 each) pretreated with atropine while those pretreated with atenolol had little alteration in BRS. (Supported by HL-38605).

98.18

CENTRAL VASOPRESSIN-INDUCED PRESSOR RESPONSES IN RATS WITH IMPAIRED BARORECEPTOR REFLEXES. B.Bagdan, Y.Takahashi*, Q.J.Pittman. Neuroscience Research Group, University of Calgary, Calgary, Alberta, CANADA

Arginine vasopressin (AVP), given intracerebroventricularly (ICV) elevates blood pressure (BP) and Heart Rate (HR) via neural mechanisms. We have asked if AVP activates the sympathetic system directly or if its pressor action results from an interruption of baroreceptor reflex control mechanisms. Rats anesthetized with Pentobarbital received electrolytic or sham lesions (verified histologically upon autopsy) in the nucleus tractus solitarius (NTS). After one week recovery rats were anesthetized with urethane and femoral artery and vein were cannulated. Methoxamine was given iv and 1/HR plotted against BP to provide an assessment of sensitivity of baroreceptors. Rats with low baroreceptor sensitivity (LBS; <0.6 msec/mmHg) had MAP of 72±4 mmHg, not significantly different (73±8mmHg) than sham lesioned rats with high sensitivity (HBS). LBS rats (n=9) given 100pmoles AVP ICV displayed pressor responses of 20±8mmHg and increases of HR of 44±9bpm. HBS rats (n=5) displayed pressor responses of 11±4 mmHg and increases in HR of 53±27bpm. Responses of the two groups were not significantly different. We conclude that the integrity of baroreceptor mechanisms is not required for AVP to exert its central pressor actions. Thus AVP may activate sympathetic mechanisms directly rather than produce increases in BP through creation of an anomalous baroreceptor signal.

98.19

AORTIC BARORECEPTOR FUNCTION IN LONG TERM STREPTOZOTOCIN DIABETIC RATS. P.J. Reynolds, M. Yang,* M.C. Andresen and S.K.S. Chang*. Oregon Health Sciences Univ., Portland, OR 97201 and Univ. Texas Medical Branch, Galveston, TX 77550.

Patients with diabetes mellitus have impaired autonomic control of heart rate. We have shown an impairment of the baroreflex control of heart rate in long-term streptozotocin (STZ) diabetic rats (J. Mol. Cell. Cardiol. 18:617-624, 1986). To determine if the defect is in the afferent limb of the baroreflex arc, activity of regularly discharging baroreceptors in relation to pressure and vessel diameter was studied in an *in vitro* aortic arch-aortic nerve preparation from 4 rats 52 weeks after the onset of STZ diabetes (D) and 5 age-matched control (C) rats. The baroreceptor threshold pressure (P_{th}) and suprathreshold pressure sensitivity (S_{th}) did not differ between groups. When related to vessel wall strain (e), however, the receptors of diabetic rats had a significantly lower threshold (e_{th}) and higher sensitivity to suprathreshold strain (S_e)(See table). Supported in part by grants from V.A. and ADA, Oregon Affiliate.

	P_{th} (mm Hg)‡	S_{th} (spike/sec/mmHg)	e_{th}	S_e (spike/sec/1%e)
C(78 units)	92.9 ± 1.5	1.27 ± 0.05	0.95 ± 0.03	1.01 ± 0.04
D(40 units)	97.8 ± 1.8	1.41 ± 0.07	0.79 ± 0.06*	1.47 ± 0.09*

‡ table values: mean ± S.E.M. *p<0.01

98.20

BARORECEPTOR-MEDIATED VASOPRESSIN RELEASE IN HUMAN AUTONOMIC FAILURE. H. Kaufmann*, E. Oribe*, M. Miller*, M. Wiltshire-Clement* and M. D. Yahr. Department of Neurology and Department of Geriatrics, Mount Sinai School of Medicine of the City University of New York, N.Y. 10029.

To investigate the role of vasopressin in the genesis of orthostatic hypotension of patients with primary autonomic failure we determined baroreceptor-mediated vasopressin release in 6 patients with this disorder. Three patients had pure autonomic failure (PAF) and 3 patients had multiple system atrophy with autonomic failure (MSA). Patients with MSA had a blunted vasopressin release in response to orthostatic hypotension whereas patients with PAF had a pronounced rise in the plasma concentration of vasopressin in response to orthostatic hypotension. The fall in blood pressure was more pronounced in the patients with MSA. Our preliminary results suggest that: 1) afferent baroreceptor pathways are spared in PAF, 2) blunted baroreceptor-mediated vasopressin release may be a marker for MSA, 3) the vasoconstrictor effect of vasopressin, released in response to baroreceptor stimulation, may moderate the fall in blood pressure in patients with PAF.

SUBCORTICAL SOMATOSENSORY PATHWAYS

99.1

ANATOMICAL INVESTIGATIONS OF AFFERENTS TO THE CERVICAL INTERNAL BASILAR NUCLEUS IN THE RAT: ANTEROGRADE, INTRA-AXONAL and IMMUNOCYTOCHEMICAL LABELLING STUDIES. D.P Crockett, S.L. Harris*, S. Maslany, J. Zhang* and M.D. Egger. Dept of Neuroscience & Cell Biology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854-5635.

The internal basilar nucleus (IBN) of Cajal, extending medially in the cervical dorsal horn from C4 to the cuneate nucleus (CN), receives a diverse input of afferent fibers. Intradermal injections of B-HRP into the palmar digits labelled primary afferents projecting to the IBN, as did intra-axonal injections of HRP into cutaneous and proprioceptive primary afferents. Spinal injections of *Phaseolus vulgaris* leucoagglutinin demonstrated dense projections of postsynaptic dorsal column fibers to the IBN. Antibodies to substance P (SP) labelled numerous fine terminals in the IBN in contrast to the CN, which contained fewer terminals with SP-like immunoreactivity. The SP-like immunoreactive fibers that were found in CN were most abundant caudally, near the pyramidal decussation. On the other hand, antibodies to CGRP (calcitonin gene-related peptide) labelled only a few terminals in the IBN and the caudal CN. Within the gracile nucleus, a few widely scattered fibers and terminals with SP-like or CGRP-like immunoreactivity were detected, particularly in the caudal pole of the nucleus. The IBN appears to be an important site of sensory integration and transmission to more rostral structures.

99.2

THE RACCOON LATERAL CERVICAL NUCLEUS: A SINGLE UNIT ANALYSIS. D. A. Simone and B. H. Pubols Jr. Good Samaritan Hospital & Medical Center, Portland, OR 97209.

Raccoon lateral cervical nucleus (LCN) neurons with receptive fields (RFs) located totally (N = 41) or partially (N = 19) on glabrous skin of the forepaw were examined for their response to light tactile stimuli.

88% of the neurons were in the ventral 1/2 of the nucleus, 12% in the dorsal half. 80% of those tested were antidromically activated from the contralateral thalamic ventrobasal complex or medial lemniscus.

Percentages of neurons classed as rapidly (77%) or slowly adapting (23%) did not differ significantly from those found among spinocervical tract (SCT) neurons. 85% were classed as light tactile and 15% as multireceptive units, also comparable to percentages found among SCT neurons. Exponents of power functions relating discharge rate during displacement ramp stimulation to ramp velocity (range for RA units = .710-.919; that for SA units = .448-.883) were similar to those found for SCT neurons. RF areas of neurons with RFs restricted to glabrous skin were significantly smaller for digital (N = 19; range = 10-129 mm^2) than for palmar units (N = 15; range = 38-257 mm^2), and, in both cases, were significantly larger than those for SCT neurons.

These results suggest that, except for increases in RF areas, properties of raccoon LCN neurons are similar to those of SCT neurons. [Support: NS-19486, USPHS.]

99.3

PROJECTIONS FROM LUMBOSACRAL DORSAL ROOT GANGLIA TO THE DORSAL COLUMN NUCLEI OF RATS. R. Giuffrida, P. Alario and A. Rustioni. Instituto di Fisiologia, Universitá di Catania, Italy, and Department of Cell Biology & Anatomy, University of North Carolina at Chapel Hill, NC 27599.

In a previous work we reported percentages and morphometric features of cervical dorsal root ganglion (DRG) neurons projecting to the dorsal column nuclei. We extended now the investigation to lumbosacral levels. Colloidal gold-labeled wheat germ agglutinin conjugated to inactive HRP (WGAapoHRP-Au) was pressure-injected (up to 0.8-1 μl) in cuneate and gracile nuclei of anesthetized rats. After two to three days the animals were perfused and ipsilateral DRGs were postfixed and embedded in paraffin. Retrogradely labeled neurons were identified after by silver enhancement of the gold particles. From counterstained 5 μm-thick sections, neurons displaying the nucleolus were considered for quantitative estimates. The percentage of labeled neurons varied in different ganglia and was highest, up to 15%, at L4. The greatest majority of retrogradely labeled neurons were of large size. The percentage reported for lumbosacral projections is considerably lower than that reported for cervical projections (up to 65%). Whether this results from a lower contribution to the gracile nucleus of all afferents or from the selective absence of one or more functional group of primary afferents in the gracile funiculus remains to be established.

99.4

OPTIMIZING IMMUNOGOLD E.M. DOUBLE-LABELING FOR AMINO ACIDS. K.D. Phend*, R.J. Weinberg and A. Rustioni, Dept. of Cell Biology and Anatomy, University of North Carolina, Chapel Hill, NC 27599.

Double-label immunocytochemistry using colloidal gold particles of different sizes has been used for electron microscopic visualization of two antigens in different or the same nervous elements. We have experimented with various protocols to use this technique for the demonstration of amino acid neurotransmitters, particularly glutamate, aspartate and GABA. The following preparatory steps seem to be required for optimal results: good perfusion (as judged by the EM tissue preservation) with 2.5% glutaraldehyde (EM grade), 0.5% paraformaldehyde (Sigma) and 0.1% picric acid, postfixation for four hours in same fixative, and overnight rinse in buffer. We routinely osmicate for one hour in 1% Os04, stain en bloc with 1% uranyl acetate, and embed in Epon-Spurr. Etching of sections is unnecessary with the use of 0.1% Triton in TBS, as suggested by DeZeeuw et al. (1988). Hot paraformaldehyde vapors have been used for double-labeling with primary antibodies raised in the same species, but this may impair staining for the second antigen, and treatment with heat alone (80°C) or with 10% formalin at room temperature is satisfactory. Reaction order of primary antibodies and choice of particle size may be crucial; this must be determined empirically.

99.5

PROJECTIONS OF PRIMARY AFFERENTS FROM THE DIGITS TO THE DORSAL HORN AND DORSAL COLUMN NUCLEI: WGA-HRP VS. B-HRP. S. Maslany, D.P. Crockett, J. Zhang* and M.D. Egger. Dept. of Neuroscience & Cell Biology, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854-5635.

Projections from cutaneous afferents to the dorsal horn (DH) and dorsal column nuclei (DCN) in the rat were examined using the following intracutaneously injected tracers: 25% free HRP, 2.5% WGA-HRP, a mixture of 25% free HRP and 2.5% WGA-HRP (WGA-HRP/HRP), or 0.1% B-HRP. After 3 days, the rats were perfused transcardially, transverse sections (60-μm thick) were cut and the HRP was reacted using the TMB-method. Data from 58 rats were analyzed. Injections of 25% free HRP produced no labelling. WGA-HRP and WGA-HRP/HRP produced similar projection patterns. In the DH, WGA-HRP and B-HRP labelled different subpopulations, with WGA-HRP labelling apparently biased toward smaller diameter fibers. For both forelimb and hindlimb digits, WGA-HRP labelled cutaneous afferents projected to Rexed's laminae I-III, with the densest label in lamina II. B-HRP labelled cutaneous afferents projected to laminae II-V, with the densest label in laminae III-IV. Comparisons of each digital projection map, using either WGA-HRP or B-HRP, indicated that, in the spinal cord, there was extensive overlapping among the labelled afferent fibers from these digits. Projections from forelimb digit 1 extended rostrocaudally from rostral C5 to rostral C6; from hindlimb digit 1 from caudal L3 to caudal L4. Projections from forelimb digit 5 extended from caudal C6 to rostral C8; for hindlimb digit 5 from caudal L4 to rostral L6.

In the DCN, WGA-HRP and the B-HRP labelled afferents projected more or less to the same locations for each digit, but B-HRP labelling was more intense. The DCN labelling was precisely restricted mediolaterally, with little or no overlap into adjacent areas. Labelling in the cuneate nucleus was less variable than that in the gracile nucleus.

99.6

TRIGEMINAL PRIMARY AFFERENT PROJECTIONS TO "NON-TRIGEMINAL " AREAS OF THE RAT CENTRAL NERVOUS SYSTEM (CNS). C. Marfurt and D. Rajchert. Northwest Center for Medical Education, Indiana University School of Medicine, Gary, IN 46408.

The central projections of rat trigeminal primary afferent neurons to various "non-trigeminal" areas of the CNS were examined by labeling the fibers with wheat germ agglutinin-horseradish peroxidase (WGA-HRP) transported anterogradely from the trigeminal ganglion. Terminal labeling was observed in the ipsilateral dorsal horn of the cervical spinal cord from C1-C7, and in contralateral pars caudalis and the dorsal horn of the spinal cord from C1-C5. Dense terminal labeling was observed in the ipsilateral paratrigeminal and solitary nuclei, whereas more moderate labeling was seen in the supratrigeminal nucleus and in the dorsal reticular formation. Small numbers of fibers were also observed in the cuneate, trigeminal motor, lateral and superior vestibular nuclei, and in the cerebellum. The latter fibers entered the cerebellum in the superior cerebellar peduncle and projected to the posterior and anterior lobes as well as to the interposed and lateral deep cerebellar nuclei. Most of the projections seen in this study originated from fibers in the dorsal part of the spinal tract of V, suggesting a largely mandibular origin. This study has demonstrated that somatosensory information from the head and face is transmitted directly to widespread areas of the CNS. These projections may function in trigeminospinal reflexes (dorsal horn from C1-C7), fusion of the sensory maps of the right and left sides of the head (contralateral projections), trigeminovisceral integration (paratrigeminal and solitary), control and integration of oral motor behavior (supratrigeminal and motor V), orofacial reflexes (reticular formation), and the coordination and stabilization of head posture and gaze (cuneate, vestibular and cerebellum).

99.7

AFFERENT PROJECTIONS FROM THE FACE TO THE BRAIN STEM OF MACAQUE MONKEYS. S.L. Florence and J.H. Kaas. Dept. Psych., Vanderbilt Univ., Nashville, TN 37240.

The topography of somatosensory inputs from receptors in the face to the trigeminal complex of the brain stem was examined by placing one or more injections of HRP conjugated to cholera toxin subunit B subcutaneously in different locations on the face of anesthetized macaque monkeys (Macaca fascicularis). Following brief transport periods, the animals were perfused, the brain stem was cut, and sections were processed for HRP, cytochrome oxidase (CO), or stained for cell bodies. Injections resulted in labeled afferent terminals in the pars caudalis, oralis and principalis. A somatotopy was evident in the pars caudalis so that projections from the eyelids were caudal while those from the lips were rostral. In the pars oralis, smaller projections than those seen in the pars caudalis appeared to reflect a second projection pattern, with the representation of the lips medial to the eyelid representation. The topographic representation of the face in the pars principalis may be oriented similarly. The CO preparations revealed dark and light regions in the complex that may relate to functional subdivisions and the locations of body part representations. Supported by NS16446 & NS08062.

99.8

EFFECT OF RETROGASSERIAN RHIZOTOMY ON THE LOCALIZATION OF CALCITONIN GENE-RELATED PEPTIDE AND GRIFFONIA SIMPLIFOLIA IN FELINE TRIGEMINAL NUCLEI. L.R. Johnson[1], M.A. Henry[2], C.D. Kullas*[2] and L.E. Westrum[2]. [1]Univ. of Colorado, Denver, CO 80262, [2]Univ. of Washington, Seattle, WA 98195

We are studying the normal organization and remodeling within trigeminal nuclei (TN) following trigeminal nerve lesions. Ten days following rhizotomy in adult felines, sections from TN were stained with the Fink-Heimer method, or reacted with the isolectin Griffonia simplifolia ($I\text{-}B_4$) that co-localizes with FRAP, or antibody to calcitonin gene-related peptide (CGRP). Terminal and axonal degeneration is seen throughout the ipsilateral TN with lesser amounts in contralateral caudal TN. $I\text{-}B_4$ and CGRP label was dramatically reduced throughout the ipsilateral sensory TN and remained essentially unchanged on the contralateral side. The ipsilateral trigeminal motor nucleus showed increased label for CGRP. $I\text{-}B_4$ and CGRP residual label have a similar distribution in the ipsilateral TN and are apparently in both degenerating fibers and a few normal axons. This study demonstrates that a significant portion of the CGRP and $I\text{-}B_4$ labeling is associated with a subset of trigeminal afferents. Ipsilateral residual labeling may be from cranial nerves IX and X or reactivity associated with degenerating trigeminal afferents. (Support: NIH DE04942, DE00219, UW Grad School Research Fund. LEW is CDMRC affiliate)

99.9

EFFECT OF RETROGASSERIAN RHIZOTOMY ON NERVE GROWTH FACTOR RECEPTOR IMMUNOREACTIVITY IN THE FELINE TRIGEMINAL NUCLEI. M.A. Henry[1], L.R. Johnson[2], C.D. Kullas*[1], L.E. Westrum[1], and M.A. Bothwell.[1] [1]Univ. of Washington, Seattle, WA 98195, [2]Univ. of Colorado, Denver, CO 80262.

We are studying the normal organization and response to injury within the trigeminal nuclei (TN) following lesions. Here, we present observations following unilateral trigeminal rhizotomy in adult felines on nerve growth factor receptor (NGFr) immunoreactivity (IR) within TN. Ten days following rhizotomy, Fink-Heimer stained sections revealed massive degeneration in the ipsilateral TN and modest degeneration in the caudal contralateral TN. Using a monoclonal antibody to the human NGFr, the contralateral TN showed a normal pattern of discontinuous pockets of IR. The pattern within the ipsilateral TN was drastically reduced, with the remaining IR most likely from the IX and X cranial nerves. Somata within the ipsilateral trigeminal motor nucleus (Vmo) showed an increase in NGFr-IR as compared to the contralateral side. This study indicates that NGFr-IR within the TN is associated with a specific subset of primary trigeminal afferents and Vmo shows increased NGFr-IR following rhizotomy. (Support: NIH DE00219, DE04942. UW Grad School Research Fund. LEW is CDMRC affiliate)

99.10

ORGANIZATION OF AXONS IN THE RAT'S TRIGEMINAL SPINAL TRACT. R.S. Crissman, D.A. Siciliano*, N.L. Chiaia, C.A. Bennett-Clarke, and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699.

Trigeminal (V) nucleus caudalis (SpC) receives input from a variety of different primary afferent fiber types that have distinct laminar distributions within this nucleus. We carried out several experiments to determine whether any such laminar organization was also present in the V spinal tract at the level of this nucleus. Immunocytochemistry for substance P and calcitonin gene-related peptide, and histochemistry for the lectin Bandierea simplicifolia-I (markers for small caliber and primarily unmyelinated fibers) all labelled numerous axons in the outer portion of the V spinal tract. Conversely, a monoclonal antibody directed against the 200 kD subunit of neuofilament protein (a marker for larger myelinated axons) labelled axons throughout the depth of the tract. The organization of the V spinal tract suggested by these experiments was confirmed by electron microscopy. In the outermost portion of the tract, the average ratio of unmyelinated to myelinated axons was 1.95:1; in the innermost portion of the tract, this ratio fell to 0.57:1. This decrease resulted almost completely from a reduction in the number of unmyelinated fibers in the inner part of the tract. Both our immunocytochemical, histochemical, and electron microscopic data thus indicate that the V spinal tract at the level of SpC has a clearly laminar organization. Supported by BNS 85 17537, DE 07734, RR 05700, and the State of Ohio Research Challenge.

99.11

ORIGINS AND TERMINATIONS OF TRIGEMINAL PROJECTIONS TO RAT SPINAL CORD. W. M. Falls, L. A. Smith*, M. S. Cook* and J. Stuk*. Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

Utilizing retrograde transport of WGA-HRP, neurons projecting to cervical and thoracic portions of rat spinal cord were labeled in trigeminal nuclei interpolaris (Vi) and oralis (Vo). Cell density was greatest following upper cervical injections and decreased significantly with each successive caudal injection. Cervical injections yielded bilateral cell labeling in ventrolateral magnocellular (vlVimc), border (brVi) and intermediate regions of rostral Vi and ventrolateral (VL), middle dorsomedial (MDM) and border (B) regions of caudal Vo. Thoracic injections produced bilateral cell labeling ventrally in rostral vlVimc and brVi and in caudal VL and B. Spinal projections of Vi and Vo neurons were studied using anterograde transport of PHA-L. Neurons in vlVimc projected contralaterally to cervical dorsal horns and thoracic ventral horns. MDM, VL and B cells bilaterally innervated cervical dorsal and ventral horns. DM neurons projected to these same areas at thoracic levels, but VL cells innervated only the dorsal horns. Vi and Vo efferents terminating at spinal levels were of three morphologically distinct types. These studies suggest complex interactions between neurons in specific portions of Vi and Vo and cells located in definitive regions of cervical and thoracic spinal cord. (Supported by N.I.H. Grant DE06725)

99.12

BRAINSTEM CONNECTIONS OF THE PARATRIGEMINAL ISLANDS IN THE RAT: A FLUORESCENT TRACER STUDY WITH TETRAMETHYLRHODAMINE DEXTRAN AND FLUORO-GOLD. D.W. Saxon* and D.A. Hopkins. Dept. of Anatomy, Dalhousie University, Halifax, NS, B3H 4H7

The paratrigeminal islands (PTI) of the dorsal trigeminal tract, which are involved in thermosensation and hibernation (Kilduff, 1988), receive afferent projections from the pharynx and oral cavity (Altschuler et al., 1989). In order to study the efferent projections of the PTI, injections of 5-10% tetramethylrhodamine dextran (TMR) (Schmued and Heimer, 1989) or a mixture of 10% TMR and 0.4% Fluoro-gold (FG) were made into the PTI. After injections of TMR into the PTI, anterograde labelling was present in the contralateral PTI, and in the nucleus of the tractus solitarius (NTS) intermediate, interstitial, ventrolateral and ventral subnuclei, primarily ipsilaterally. The PTI also project to the ipsilateral parabrachial complex, particularly the external medial and ventral lateral subnuclei. TMR was also present in the medial and external lateral subnuclei. After injections of TMR and FG in the PTI, anterograde and retrograde labelling were present in the contralateral PTI. Retrogradely labelled cells were present in the external lateral subnucleus of the PBN and Kolliker-Fuse nuclei; some of these cells were in fields of labelled axons. The results show that afferents to the PTI project directly and indirectly, via the NTS, to specific subnuclei of the parabrachial complex. Supported by MRC of Canada (Grant MT-7369).

99.13

2-DG LABELING PATTERNS IN THE TRIGEMINAL BRAINSTEM COMPLEX FOLLOWING SELECTIVE VIBRISSAL STIMULATION IN THE ADULT HAMSTER. P.A. Young, J.S. McCasland, T.A. Woolsey, R.W. Rhoades and M.F. Jacquin. Dept. of Anat. & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104; Div. of Exp. Neurol. & Neurosurg. & McDonnell Center for Studies of Higher Brain Function, Washington Univ. Sch. Med., St. Louis, MO 63110; Dept. of Anat., Med. Coll. of Ohio, Toledo, OH 43699.

Prior studies have shown that single vibrissa stimulation results in restricted foci of increased glucose utilization at all levels of the trigeminal (V) neuraxis. However, these patterns have been directly related to aggregates of cells and axon terminals corresponding to the vibrissae only at the cortical level. In the present study, we combined 2-DG labeling and cytochrome oxidase (CO) staining in individual sections to directly evaluate this relationship in the V brainstem complex. Ten hamsters had all of their large mystacial vibrissae trimmed, except for the left C3, and the right A1 and E4, vibrissae. After overnight fasting, 2-DG was injected and the animals behaved freely in the dark for 45 min. (McCasland & Woolsey, JCN, '88). Brainstems were processed for both CO staining and 2-DG autoradiography. CO patches were patterned in a vibrissal-like fashion in all of principalis' rostrocaudal extent and in caudal portions of subnuclei interpolaris and caudalis. 2-DG silver grains were densest above those CO patches corresponding to the left C3, and the right A1 and E4, vibrissae. There were, therefore, no consistent 2-DG foci in subnuclei oralis, or rostral interpolaris and caudalis. These data show clearly that patterns of increased glucose utilization following single whisker stimulation are well matched with CO patterns that reflect distributions of cells associated with that same vibrissa in the V brainstem complex. Support: DE07734, DE07662, NS17763 and EY04170.

99.14

TRIGEMINOCEREBELLAR PROJECTIONS IN THE ADULT RAT. J.J.A. Arends, T.J.H. Ruigrok*, T.A. Henderson and M.F. Jacquin. Dept. of Anatomy and Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104, and Dept. of Anatomy, Erasmus University, 3000 DR Rotterdam, The Netherlands (TJHR).

HRP, WGA-HRP and PHA-L tracing methods in conjunction with Zebrin-1 (mabQ113) immunohistochemistry, were used to study trigeminocerebellar projections in the rat. Massive HRP injections into the entire cerebellum retrogradely labeled cells in every trigeminal brainstem subnucleus. They were most numerous in subnucleus interpolaris (SpVi); subnucleus caudalis (laminae III-V) contained the smallest number.

Anterograde transport following tracer injections in SpVi labeled multiple patches of short parasagittal strips of mossy fiber rosettes in the cerebellar cortex. This discontinuous, patchy projection pattern may be the basis for prior electrophysiological demonstrations of 'fractured somatotopy' in the cerebellum (Welker, W.I., New Concepts in Cerebellar Neurobiology, Alan R. Liss, New York 1987, pp 239-280). Bilateral, but predominantly ipsilateral, terminations were observed in vermis (dorsal uvula; proximal lobules V-VIII) and in the hemispheres (Crura I and II, the simple lobule, the paramedian lobule, dorsal paraflocculus and lateral lobules II-V). Following injections at different dorsoventral levels of SpVi, differential projections were observed (as measured against the background of the Zebrin staining pattern), most notably in the uvula, the paramedian lobule and Crura I and II. Support: NIH DE07662, DE07734.

99.15

COMPARATIVE MAGNIFICATION OF THE VIBRISSA REPRESENTATION IN THE SUPERIOR COLLICULUS OF RODENTS AND CATS. C.-Q. Kao, J.G. McHaffie, M.A. Meredith, H.R. Clemo and B.E. Stein. Depts. Physiol. & Anat., Medical Coll. Va., Va Commonwealth Univ., Richmond, VA 23298

The vibrissa representation is an exquisite adaptation of the trigeminal system, reaching its zenith in the rodent, whose vibrissae are critical for survival. One might then expect that the essential role of vibrissae in facilitating the rodent's attentive and orientation behaviors via the superior colliculus (SC) would be reflected in an expanded representation of the cutaneous area encompassing them (the vibrissa pad) beyond that evident in species that make less use of vibrissae, such as cat. This has proved to be the case, with the proportions of the SC representing the vibrissa pad in mouse (44.4%) and rat (35.8%) being far larger than in cat (13.7%), and the proportion of the body devoted to the vibrissa pad being greater in mouse (1.12%) and rat (1.24%) than in cat (0.47%). When a relative index of magnification in the SC is calculated (% of body surface vs. % of SC devoted to it), the rat (28.9) and cat (29.2) appear to be the same, but the mouse exhibits a substantially greater expansion (42.2). Thus, the rodent vibrissa specialization can be reflected both in the nature of the stimuli that can activate SC neurons, and in an expanded representation of the perioral area containing them. Supported by grants NS 22543, EY05554 and BNS 8719234.

99.16

SOMATOTOPIC COMPONENT OF THE MULTISENSORY MAP IN CAT DEEP SUPERIOR COLLICULUS. M. A. Meredith, H. R. Clemo and B. E. Stein. Depts. Anatomy and Physiology, Med. Coll. Va., Va. Commonwealth Univ., Richmond, VA 23298.

While the somatosensory representation in the cat superior colliculus (SC) is well established, this region also contains maps of visual and auditory space and it is not known if, or to what extent, the somatosensory map participates with these others to form a comprehensive, multisensory representation. To examine this question, the receptive fields (RF) and patterns of modality convergence were examined in 198 somatosensory neurons in 21 chronically prepared, anesthetized (Ketamine/Acepromizine) cats. The somatosensory map was examined using procedures modified from McIlwain (J. Neurophysiol. 38:219, 1975), whereby dermal images were constructed by determining the histological distribution of neurons whose RFs encompassed a given body region. This process accommodated not only the relative largeness of some RFs encountered, but also their variations with depth in the SC. Although previously unreported representations of certain body regions were now revealed (e.g., tail, ventrum), the general organization of the somatosensory map is the same as described before (Stein et al., J. Neurophysiol. 39:401, 1976). When the pattern of convergence from other sensory modalities was evaluated, it was found that the overwhelming majority (125/198; 63%) of somatosensory neurons received visual and/or auditory inputs. These data strongly suggest that many features of this somatosensory map are similar to the visual representation also found here (Meredith and Stein, Soc. Neurosci. Abstr. 14:831, 1988), and this somatosensory representation is best considered a component of a multisensory functional unit that plays a critical role in effecting behavioral responses to a wide variety of stimuli. Supported by grants NS-22543 and BNS 8719234.

99.17

SOMATOSENSORY CORTICAL (SIV) INPUTS TO DEEP LAYER EFFERENT NEURONS IN CAT SUPERIOR COLLICULUS H.R. Clemo, M.A. Meredith and R.F. Spencer Depts. Physiology and Anatomy, Med. Coll. Va., Va. Commonwealth Univ., Richmond, VA, 23298.

While the afferents to as well as efferent targets of the superior colliculus (SC) are well established, the role of somatosensory cortical inputs in the control of specific SC output neurons is not known. Of the identified somatosensory cortices, SIV and para-SIV (which are located in the dorsal bank and fundus of the anterior ectosylvian sulcus) give rise to the heaviest corticotectal projection. As for output neurons of the SC, the involvement of tecto-reticulo-spinal neurons in the initiation of orientation behaviors is well documented. Therefore, by using double-labelling techniques, we sought to determine whether axons originating in SIV and para SIV contact tecto-reticulo-spinal neurons. Tecto-reticulo-spinal neurons were identified with the retrograde transport of HRP from injections into the dorsal tegmental decussation, while terminals of corticotectal axons were localized in the same animal (n=8) using PHA-L placed in SIV and para-SIV. Preliminary results show terminal labelling of cortical axons on the distal dendrites of HRP-filled neurons located in both the intermediate and deep grey laminae. These data demonstrate that somatosensory cortex has direct access to SC output neurons and thereby can modulate the activity of neurons that initiate orienting behaviors.

Supported by BNS 8719234, NS-22543, EY-02191.

99.18

MICRO-PUSH-PULL-PERFUSION USING CISTERNAL CEREBROSPINAL FLUID WITH MICROELECTRODE RECORDING IN THE CAT CUNEATE NUCLEUS. V.R. Roettger & M.D. Goldfinger, Department of Physiology & Biophysics, Wright State University, Dayton, OH 45401-0927.

Adult cats were anesthetized with Nembutal (40 mg/kg). The dura underlying the atlanto-occipital membrane was exposed. A 27-ga. needle was inserted into the cisterna magna; 5-6 ml of CSF was withdrawn at 200-500 ul/hr. During CSF collection, mean arterial blood pressure and heart rate were stable and averaged 120 mmHg and 4/sec respectively in 7 cats. For subsequent push-pull perfusion, the dura and arachnoid were cut and reflected to expose the brainstem pial surface. A small pial hole was made to admit the tips of the push-pull cannula (PPC).

The Gaddum PPC design (1) was miniaturized using 2 glass pipettes (ODs: 1,1.5 mm; IDs: 0.58, 1.12 mm). After each pipette was drawn to a fine tip, each tip was microforged to an outer diameter of 200 um(pull) or 100 um(push). The push pipette contained a glass filament. For electrical recording, a tungsten wire with an electrolytically-etched tip was fixed into the lumen of the push pipette such that the wire tip protruded 30-40 um from the pipette tip. The push pipette was inserted into the lumen of the pull pipette and positioned such that the push pipette tip protruded concentrically from the pull pipette tip; 22-ga. metal tubing provided access to the back of the pull pipette. Components were fixed in position with a heat-shrink collar and glue welds.

The PPC was positioned such that the push tip contacted cuneate nucleus tissue and the pull tip contacted both pushed CSF and air. CSF was pumped continuously through the inner/push tube at 20 ul/min. The outer/pull tube recovered CSF via coupling to a negative pressure trap. Perfusion efficiency was tested with respect to tube tip separation: a 200 um distance recovered 80% of pushed H_2O; a 50 um distance recovered 46%.

During perfusion with percutaneous electrical stimulation of the ipsilateral forepaw, evoked potentials and single-units were recorded from the PPC microelectrode. Averaged evoked potentials had similar waveform and FFT-derived spectral content as those recorded with conventional microelectrodes. These data suggest normal circuit function during push-pull perfusion with CSF.

(1) J.H.Gaddum, J. Physiol.(Lond.)155:1-2P, 1961.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS I

100.1

SOMATOSENSORY EVOKED RESPONSES IN VENTROBASAL THALAMIC NEURONS: AN INTRACELLULAR STUDY IN THE RAT. A. Núñez*, C. Barrenechea* and C. Avendaño. Dept. of Morphology, Medical Sch., Autónoma Univ., 28029 Madrid, Spain.

Intracellular recordings of thalamic relay neurons were obtained in order to study the spontaneous and stimulus-related activity in rat ventroposteromedial nucleus (VPm). Sprague-Dawley rats, weighing 200 g, were anesthetized with i.p. pentobarbital, paralyzed with i.p. vecuronium and artificially respired. Micropipettes filled with a 4% solution of HRP in 0.5 M KCl were lowered vertically into VPm. Sensory stimulation consisted of manually applied strokes or hair displacement on the receptive field. Impaled neurons showed stable membrane potentials below -50 mV and overshooting spikes. A few of them were succesfully labeled, drawn, and photographed. Peripheral stimuli elicited two kinds of response: 1, a depolarizing potential with a fast-rising slope (<5 ms) and low amplitude (<10 mV), with one or two spikes rising on it; 2, a slow depolarizing wave of larger amplitude (up to 20 mV) and longer duration (up to 100 ms), which triggered a burst of spikes. Both responses could occur spontaneously or locked to the stimuli, and their amplitude fluctuated. Depolarizing current pulses elicited neither of these activities, whereas hyperpolarizing pulses increased the amplitude of both, thus suggesting that both kinds of response have a synaptic origin.

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100.2

THE EFFECTS OF COOLING CORTICAL SOMATOSENSORY AREAS I (SI) OR II (SII) ON THE RESPONSIVENESS OF VENTROBASAL (VB) THALAMIC NEURONS TO FOREPAW MECHANICAL STIMULATION IN THE CAT. S. Ghosh, A.B. Turman*, G.M. Murray+, and M.J. Rowe*. School of Physiol. and Pharmacol., Univ. of NSW, and +Faculty of Dentistry, Univ. of Sydney, Sydney, Australia.

The functional significance of the descending projection from SI and SII to VB thalamus is poorly understood. This study's aim was to see if reversible inactivation by cooling of SI or SII affected the response properties of VB neurons to mechanical stimulation. In halothane anesthetized cats (n=15), the responsiveness of VB neurons to controlled mechanical stimuli (sinusoidal vibrations, step indentations) delivered to hairy or glabrous skin of the contralateral forepaw was studied before, during and after cooling of the forepaw representations of SI and/or SII. Response levels (imp./s) were unaffected in 75% of neurons (n=30); in 25% of neurons a reduction in response level was noted following inactivation of SI or SII. In all neurons responding to vibrotactile stimuli (n=29), the extent of phase locking to the vibration was unaffected by cortical cooling, whether or not response levels were changed. We conclude that the responses of VB neurons to low threshold mechanical stimuli are mainly unaffected by reversible inactivation of SI or SII; but in a proportion of neurons, a facilitatory corticothalamic influence was revealed.

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100.3

ANTIDROMIC IDENTIFICATION OF "COLD"-SPECIFIC LAMINA I CELL TERMINATIONS IN THE CAT THALAMUS. J.O. Dostrovsky and A.D. Craig. Dept. of Physiology, Univ. of Toronto, Toronto, Canada, M5S-1A8 and Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

Previous anterograde tracer studies have demonstrated several spinal and trigeminal lamina I projection sites in the thalamus, including a specific input to the n. submedius (Sm); previous physiological studies utilizing antidromic activation have suggested that lamina I cells that project to Sm include cells specifically responsive to innocuous cooling. The aim of the present study is to identify the terminal thalamic projections of "cold" spinothalamic and trigeminothalamic neurons by antidromic mapping with a mobile array of stimulation electrodes. In nine barbiturate-anesthetized cats an array was inserted into the region of Sm based on functional localization of the boundaries of ventrobasal thalamus. Single-unit extracellular recordings in lamina I were obtained from the contralateral medullary or cervical dorsal horn. Following antidromic activation of a "cold"-specific neuron, the array of stimulating electrodes was moved dorsoventrally at successive rostrocaudal locations in order to determine the focal sites from which the neuron could be antidromically activated. Complete histological reconstructions were done in each case. Twelve "cold" cells were activated from Sm. In all seven cells that were sufficiently well mapped, it was possible to demonstrate an additional separate projection to the putative thermoreceptive site on the dorsomedial aspect of VPM. Six cells also projected to the ventral aspect of VMb. These results demonstrate that specific thermoreceptive lamina I projections are distributed to several thalamic sites, including Sm.

(supported by NIH grants to J.O. Dostrovsky and A.D. Craig)

100.4

ORIGIN, COURSE, AND ULTRASTRUCTURAL TERMINATION OF DORSAL COLUMN NUCLEAR PROJECTIONS TO THE ROSTRAL PORTION OF THE POSTERIOR THALAMIC NUCLEUS IN THE RAT. R.J. Kosinski, P. Hlavac,* E.L. Bold and E.J. Neafsey. Depts. of Anatomy, The Chicago College of Osteopathic Med., 555 31st St. Downer's Grove, IL, 60515 and Loyola Univ. of Chicago, Sch. of Med.

The present study verified that the dorsal column nuclei (DCN) project to rostral portions of the posterior thalamic nucleus (Po) in rats by using the retro- and orthograde transport of WGA/HRP at light and electron microscopic levels. While anesthetized, black hooded rats were injected with a 2% solution of WGA/HRP (0.01-0.04ul) either within the DCN or rostral portions of Po, unilaterally. Animals survived for 24 - 48 hrs. Other animals received aspiration lesions of the DCN and were allowed to survive for 3 - 10 days. All animals were reanesthetized and perfused with a standard EM/HRP fixative. Brains were removed, sectioned, and processed for light and/or electron microscopy.

Results indicated that DCN projections to Po primarily originate from rostral and ventral portions of the contralateral DCN. These projections course with the medial lemniscus until the midbrain, where they diverge dorsally to distribute within Po, somatotopically. Degenerating or WGA/HRP-containing synaptic boutons were identified ultrastructurally to contain small, round vesicles and to form asymmetrical synapses with dindritic profiles and/or dendritic spines in a manner similar to that observed for other DCN projections.

100.5

WHISKER-EVOKED RESPONSES IN THE POSTERIOR NUCLEUS (PO) OF RAT. M. E. Diamond, M. A. Armstrong-James and F. F. Ebner, Center for Neural Science, Brown Univ., Providence, RI 02912

In rat, parallel 'lemniscal' and 'paralemniscal' pathways from the whisker follicles relay in VPM and PO, respectively, before terminating in separate zones of the somatic sensory cortex (barrels and septa). To learn more about the 'paralemniscal' pathway, we have compared the whisker-evoked responses of PO cells to those of VPM and cortical cells studied under similar conditions. The responses of 55 PO cells to controlled whisker deflection were measured in urethane-anesthetized rats. With a response criterion of ≥ 0.1 spikes/trial across 50 trials (after compensation for the high levels of spontaneous activity), 48 PO cells (87%) had an excitatory RF, 2 cells had an inhibitory RF, and 5 cells did not respond to whisker movement. The average excitatory RF was 4.3 whiskers (range 1-13). The mean response to the 'best' whisker was 0.45 spikes/trial; VPM cells gave a stronger response to the 'best' whisker: 1.12 spikes/trial (In Press).

The most striking finding was the long latency of PO cells even in response to the 'best' whisker (mean 22 ms; range 5-47 ms). Evoked activity in VPM (latency range 4-7 ms) thus initiates activity in cortex (latency range 7-25 ms) before the majority of PO cells discharge. This sequence of activation suggests that PO may influence the "late" communication among cortical barrel-columns. (Supported by NIH grant #NS 25907).

100.6

EFFECTS OF ATTENTION ON VP THALAMIC NEURONAL RESPONSES IN AWAKE MONKEY. T.J. Morrow and K.L. Casey, Depts. of Neurology and Physiology, Univ. of Michigan and V.A. Medical Ctr., Ann Arbor, MI 48105

A key question in neuroscience concerns the mechanism(s) by which the central nervous system filters incoming sensory signals and assigns importance or relevance to specific pieces of the sensory information being received. Previously we showed that the somatically evoked discharges of many primate ventral posterior (VP) thalamic neurons covary with the level of arousal (Brain Res. Bull, 21:433-438, 1988). Accordingly we chose to study the effects of attention on the responses of VP neurons in the awake monkey.

One African green monkey was trained to pull a bar to obtain a fruit reward under different experimental conditions: one involving attention to a visual cue, the other to a specific somatosensory stimulus. The responses of single neurons in the VP thalamus to somatic stimuli and to electrical stimulation of the spinal lemniscus (SL) were recorded during the behavioral task. Movements and EEG were monitored to identify periods of drowsiness, quiet waking and waking movement. Neural responses were separately analyzed by computer for changes related to elements in the behavioral paradigm and to shifts in arousal.

Five of the 8 neurons tested showed changes in somatic or SL evoked responses which correlated with behavioral task performance. During attention in the visual task, 3 units showed increased responsiveness and two were unaffected. Three units showed either an increased or decreased responsiveness during attention to a somatic cue applied concurrently to an area of the body outside of the unit's receptive field. One cell showed increased responsiveness during visual and decreased responsiveness during somatic trials. Modulation occurring during shifts in the state of arousal was mixed with respect to the direction of change in response to somatic stimuli. **Modulation of VP thalamic responsiveness can be attributed specifically to changes in attention and is distinct from those changes occurring during shifts in the general state of arousal.**

100.7

RESPONSE OF MONKEY VPM NEURONS TO AIR-PUFF STIMULATION DURING DISCRIMINATION AND ATTENTION TASKS. N. Tremblay*, N. Bastrash*, M.C. Bushnell, G.H. Duncan, Univ. Montréal, Canada H3C 3J7.

Many studies have reported slowly adapting (SA) and rapidly adapting (RA) neurons in ventrobasal thalamus, but no study has quantitatively investigated the discriminative properties of these neurons or their modulation by attention. Thus, the present study assesses the activity of single units in ventroposterior medial thalamus (VPM) of monkey during performance of air-puff discrimination and attention tasks.

One Rhesus monkey was trained to detect small changes in the intensity of air-puff and light stimuli. In the air-puff task, twenty-ms puffs of fixed intensity were delivered at 1Hz via a 2mm-diameter tube positioned 2mm from the monkey's face. After 4-7s, the puff intensity changed, cueing the monkey to release a lever for liquid reward. In the attention task, identical air-puff stimuli were presented on all trials, but for half the trials, the monkey earned reward only for attending to and detecting changes in the intensity of a light. Single units were recorded during the tasks and their receptive field and rate of adaptation to tactile stimulation were determined.

Within VPM, *both* RA and SA neurons were observed that responded to air-puff stimulation. Usually the response was excitatory, but clear examples of inhibition were observed time-locked to the air-puff stimuli. Both RA and SA neurons responded differentially to various air-puff intensities and responded to the smallest intensity detectable by the monkey. In 10 neurons tested for attentional modulation, there was no indication that activity was enhanced when the monkey attended to the air-puff ($p=0.44$), confirming results of previous multi-unit studies (Poranen and Hyvarinen, 1980).

Our results indicate that some SA and RA neurons in VPM are sufficiently sensitive to account for the fine tactile discrimination seen in primates. However, we have observed no evidence that the ventrobasal thalamus plays an important role in attentional modulation of tactile discrimination.

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100.8

ULTRASTRUCTURE OF SYNAPTIC CONNECTIONS IN THE THALAMUS FROM FIBERS ARISING FROM THE MEDIAL PARABRACHIAL NUCLEUS (MPB) OF THE RAT. A.M. Williamson, and H.J. Ralston, III, Dept. of Anatomy, UCSF, San Francisco, Ca. 94143.

Recent work by our laboratory (Ahlgren *et al.*, Soc. Neurosci. Abst. 15:157.2, 1989) and others (Yasui *et al.*, J. Comp. Neurol. 290:487, 1989) has shown that the MPB of the rat contains fibers which project rostrally into the thalamus and cortex. While the function of this projection is not known, the MPB contains cell bodies which are labelled by antibodies to calcitonin gene-related peptide (CGRP).

We examined this fiber projection to determine whether or not the varicosities in the thalamus, visible at the level of the light microscope, formed synaptic contacts and to define the nature of these contacts.

These experiments were conducted on male Sprague-Dawley rats which were injected with Phaseolus vulgaris leucoagglutinin (PHA-L) in the MPB. The animals were sacrificed after 7-10 days survival time, and the tissue was processed to demonstrate PHA-L using standard immunohistochemical techniques. The tissue was then processed for EM analysis. All procedures were in accordance with UCSF and NIH animal care guidelines.

Preliminary results suggest that PHA-L containing fibers in the gustatory nucleus and rostrally in the dorsal midline of the thalamus form both symmetric and asymmetric synaptic contacts onto small and large dendrites. No contacts onto other presynaptic elements or cell bodies have been seen. All labelled axonal elements were unmyelinated. Many appositions without synaptic specializations were also present.

This work was supported by NS-21445 from the NIH.

100.9

ELECTRON MICROSCOPY OF 5-HT AND TH IMMUNOREACTIVE AXON TERMINALS IN THE VP NUCLEUS OF CAT AND MONKEY THALAMUS. X.-B. Liu and E.G. Jones. Dept. of Anatomy and Neurobiology, Univ. of California, Irvine, CA 92717.

Physiological and morphological studies have shown that relay nuclei of the thalamus receive brainstem afferents and immunocytochemistry has revealed serotonin (5-HT) and noradrenaline fibers in the ventral posterior (VP) nuclei. However, the synaptic connections of these fibers in VP have nor been described. In the present study we have combined immunocytochemistry and EM analysis of serial thin sections to localize serotonin and tyrosine hydroxylase (TH) immunoreactivities in VP of cat and monkey.

Adult cats and monkeys were perfused with 4% paraformaldehyde in 0.1M phosphate buffer or in 2% paraformaldehyde and 0.2% glutaraldehyde in 0.1M phosphate buffer. Immunocytochemistry was carried out using polyclonal antisera and the ABC method. 5-HT immunoreactive fibers and terminals were distributed evenly in the lateral thalamus of cat and monkey. Within VPL, immunoreactive fibers were typically thin and bore many varicosities; in some instances the fibers or their fine branches closely surrounded neuronal somata. TH staining was not obtained in cat thalamus. In monkey, TH and 5-HT immunoreactive fibers had a similar staining pattern in VPL. Irregularly distributed varicosities could be seen along the immunoreactive fibers and their branches. Ultrastructural characteristics of 5-HT and TH immunoreactive elements are similar. About 100 immunoreactive terminals of both types were chosen for serial section EM study. Only 10% of 5-HT terminals and no TH terminals could be shown to form conventional synapses. These few formed asymmetrical synapses with proximal dendrites of relay cells and presynaptic dendrites of interneurons. The remaining immunoreactive terminals recognized by containing synaptic vesicles and mitochondria made contact with dendritic shafts and somata, but no synaptic contacts could be demonstrated. Supported by NIH grant no. NS22317.

100.10

INPUT-OUTPUT ORGANIZATION OF VPM RODS IN MONKEY THALAMUS. E. Rausell and E.G. Jones, Dept of Anatomy and Neurobiology, University of California, Irvine, Ca 92717.

The principal trigeminal nucleus of the monkey brainstem gives rise to fibers that terminate in elongated rod-like cell clusters within the ventral posterior medial (VPM) nucleus of the thalamus. The rods provide the physiological basis for the place and modality specific projection onto the first somatic sensory area, SI (Jones et al, *Exp. Brain Res.*, 62: 438, 1986). We have further characterized the organization of the afferent and efferent connections of the VPM rods, by means of the anterograde transport and immunocytochemical detection of iontophoretically injected *Phaseolus vulgaris* leucoagglutinin (Pha-L). Our findings demonstrate: a) The axonal terminations of electrophysiologically identified principal trigeminal neurons are restricted to the rods. b) In VPM, adjacent neurons that have the same stimulus-response characteristics lie in the same rod, and extend 800-950 μm in the anteroposterior dimension. c) Pha-L labeled fibers arising from the same rod enter SI perpendicularly from the white matter and each arborizes widely in a 4 to 6 mm long medio-lateral strip-like fashion. The terminations concentrate in the deeper part of layer III and in layer IV in the fundus of the central sulcus, extending 800-1500 μm anteroposteriorly. Within the strips, column-like foci of dense terminations alternate with foci of weak terminations in the same cytoarchitectonic field.

These data suggest that the thalamocortical input to SI from the VPM rods is provided by widely arborized axons that terminate in strips. Within the same cytoarchitectonic field, the strips contain areas of differential innervation density. This may provide the anatomical substrate for multiple representations within SI and the basis for activity dependent plasticity of the representation.

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100.11

THALAMOCORTICAL PROJECTIONS CORRELATE WITH STIMULUS-EVOKED METABOLIC ACTIVITY IN CAT SOMATOSENSORY CORTEX. W. Ma, D.E. Eslin, S.L. Juliano. Anatomy, USUHS, Bethesda, MD 20814.

We previously described the coincidence of evoked metabolic activity with cortico-cortical connections in the somatosensory cortex (SSC) and with retrogradely labeled neurons in the ventroposterior nucleus (VP) of the thalamus in primates. Those studies indicate the significance of connectivity in predicting functional activity. The current study seeks to determine the relationship between stimulus-evoked metabolic activity and thalamocortical projections in the SSC of the cat, although the precise functional and connectional relations may differ from those in primates. Double-barreled micropipettes were used to record thalamic units in VP responding to somatic stimuli, followed by iontophoretic injection of WGA-HRP into physiologically identified thalamic sites. Two days later, cats were injected with 2-deoxyglucose (2DG) and received the somatic stimulation found to best excite the thalamic neurons during recording. WGA-HRP injections led to column-like clusters of label in area 3b. Labeled fibers and terminals occurred primarily in layers IV and III and to a lesser extent in layer V; labeled cells were located largely in layer V. Following stimulation to the limbs, column-like metabolic patches coincided with the WGA-HRP label in somatotopically appropriate regions of area 3b. The metabolic patches generally overlapped with the WGA-HRP labeled fibers and terminals in layers IV and III. In addition, somatic stimulation to the limbs elicited metabolic activity in area 3a, where no WGA-HRP label was observed. The configuration of the evoked metabolic label was usually identical to the morphology of the WGA-HRP label. This finding suggests that evoked metabolic activity in SSC strongly correlates with cortical projections from VP neurons, although the functional roles played by corticocortical connections versus thalamocortical connections are yet to be elucidated.

100.12

THALAMOCORTICAL CONNECTIONS RELATED TO RESPIRATORY MUSCLE AFFERENTS IN THE CAT. J. L. Sallach, R. L. Reep and P. Davenport*. Dept. of Physiological Sci., Univ. Florida, Gainesville, FL 32610.

Previously we reported cortical responses to stimulation of respiratory muscle afferents in the cat. In conjunction with defining the pathways associated with these responses, the specific aim of this study was to identify thalamocortical connections within this system.

Respiratory muscle afferents were activated by stimulation of the left C5 portion of the phrenic nerve. Cortical activity was recorded in right SI using a silver ball electrode and/or tungsten microelectrode. After the region of primary activation was located, a microinjection of WGA-HRP or retrograde fluorescent tracer (DY or FB) was made, often with ^{3}H-leucine-proline. Injections were also made 2.0mm rostral and caudal, or 2.0mm medial and lateral to the primary area using different fluorescent tracers. The animals survived 2-7 days, were perfused, and their brains sectioned at 30-40μm. To facilitate localizing injection sites, a portion of the right hemisphere including SI was sectioned sagittally. The remaining brain was sectioned coronally.

The primary area is located in area 3a of SI, close to the 3a/3b border. Thalamic labeling associated with injections in the primary site is largely confined to the ventrobasal complex, particularly the ventromedial aspect of VPL (VPLm) mostly adjacent to the external medullary lamina, especially in the caudal aspect of VPL. The intralaminar and posterior nuclei also showed some labeling from injections in the primary site. Injections caudal or medial to the primary area produced labeling in the dorsolateral aspect of VPL (VPLl), also adjacent to the external medullary lamina. Rostral injections showed labeling in the ventrolateral and ventromedial nuclei. Anterograde labeling was comparable in location to that seen with the retrograde labeling from the primary area. Supported by NIH grant #HL37596.

100.13

THALAMIC CONNECTIONS OF THE FIRST (S-I), SECOND (S-II) AND PARIETAL VENTRAL (PV) SOMATOSENSORY AREAS IN NEW WORLD MARMOSETS (CALLITHRIX JACCHUS). Jon H. Kaas & Leah A. Krubitzer, Vanderbilt Univ., Nashville, TN 37240.

Small amounts of anatomical tracers were placed into three electrophysiologically defined representations (S-I, S-II, and PV) in seven marmoset monkeys. Using multiunit mapping methods, the receptive fields for much of the body surface were quickly defined, physiological boundaries were lesioned, and anatomical tracers including WGA-HRP and fluorescent dyes were injected into specific representations of body parts in these fields. Three types of injection strategies were used. First, numerous small injections of WGA-HRP were closely spaced within the entire mediolateral extent of S-I to form one long injection. In this way, the entire extents of thalamic nuclei projecting to S-I could be determined. Second, different anatomical tracers were placed into different body parts in the same representation so that the topographic organizations of thalamic nuclei could be determined. Finally, different anatomical tracers were placed in the representations of the same body parts of different cortical areas, allowing connectional similarities and differences to be readily assessed.

S-I receives topographic input predominantly from the ventroposterior nucleus (VP) of the thalamus and receives less dense input from the inferior (VPI) and superior (VPS) divisions of VP. In cases where strip injections filled almost the entire extent of S-I, VP was almost completely filled with labelled cell bodies and axon terminals. However, small unlabelled strips of cell sparse zones separating body part representations in VP were observed. Connections of S-II are most dense with VPI, VPS and the posterior nucleus (PO) of the thalamus, while connections of PV are most dense with PO, VPI and VPS and less dense with VP.

100.14

EVIDENCE FOR TRANSNEURONAL DEGENERATION OF INTERNEURONS IN THE VENTROPOSTERIOR NUCLEUS (VP) OF THE THALAMUS FOLLOWING CORTICAL ABLATIONS OF PRIMARY SOMATOSENSORY CORTEX IN MACAQUES (M. mulatta). J. Chmielowska, T.P. Pons and M. Mishkin. Laboratory of Neuropsychology, NIMH, Bethesda, MD.

An antiserum against the inhibitory transmitter gamma aminobutryic acid (GABA) was used to investigate the distribution of GABAergic cell bodies in VP of 2 normal and 3 operated hemispheres. In the operated hemispheres aspiration ablations of either all (areas 3a, 3b, 1 and 2) or portions (3a, 1 and 2 or 3b, 1 and 2) of the hand representations in primary somatosensory cortex were made at least 8 weeks prior to processing the tissue. In the normal hemispheres Nissl staining of cells and cytochrome oxidase (CO) activity was dense and uniform throughout the hand representation in VP and counts of GABA-positive neurons were on the order of 78.4/mm^{2}. In the operated hemispheres many of the cells in the hand representation in VP that project to cortex underwent degeneration and these regions stained only lightly for CO activity. Cell counts of GABA-positive neurons in these regions were 28.9/mm^{2}. Since it is unlikely that a large population of GABA-positive cells project directly to primary somatosensory cortex, their reduction after cortical ablation is most likely due to retrograde transneuronal degeneration of interneurons which have lost their local projection targets.

100.15

CO-LOCALIZATION OF RETROGRADELY-LABELED NEURONS AND LEU-ENKEPHALIN CYTOCHEMISTRY IN THE POSTERIOR NUCLEUS OF THE THALAMUS IN GREY SQUIRREL. H.J. Gould, III, R.H. Whitworth, Jr. and R.W. Rieck. Department of Anatomy, L.S.U. Medical Center, New Orleans, LA 70112-1393.

Thalamic nuclei connected with the parietal dysgranular cortex (PDC) (Gould et al., '89, J. Comp. Neurol., 287:38) were studied in the grey squirrel using a combination of retrograde transport of rhodamine microspheres and immunocytochemistry for localization of leu-enkephalin.

Neurons labeled with rhodamine were found in a narrow zone of thalamus along the dorsal border of the ventral posterior nucleus, including parts of both Pom and Pol described by Krubitzer and Kaas ('87, J. Comp. Neurol., 265:549). Within the field of retrogradely-labeled neurons, immunocytochemical techniques revealed a very sparse pattern of leu-enkephalin- immunoreactive fibers. Enkephalin labeling was also noted in the reticular nucleus of the thalamus (which is known to receive a dense projection from the PDC), within lateral portions of the ventral posterior nucleus related to the limbs, trunk and tail, and in the central lateral, central medial, and anterior group of thalamic nuclei.

These results are consistent with those of our earlier connectional studies. The co-localization of leu-enkephalin immunocytochemistry and neurons labeled with tracer injected into PDC, suggests that enkephalin may play a role in the modulation of interhemispheric circuits. (Supported by PHS-BNS 85-11090)

100.16

CLAUSTRAL PROJECTIONS TO FIRST SOMATOSENSORY CORTEX IN MACAQUE MONKEYS: A MULTIPLE RETROGRADE TRACING STUDY

A.Granato*, P.Barbaresi*, and D.Minciacchi. Inst. of Anatomy, and Lab. of Experimental Neurology, Catholic Univ., Rome, and Inst. of Physiology, Univ. of Ancona, Ancona, Italy.

The claustrum (Cl) is a subcortical structure reciprocally connected with the cerebral cortex. Previous anatomical and electrophysiological studies have demonstrated the existence of a somatotopic map in the cat's Cl. Claustral projections to primary somatosensory cortex (S1) have been also described in primates, but little is known about their topographic organization. In the present study we have investigated the somatotopic organization of Cl projections to S1 in macaque monkeys.

After electrophysiological identification of the face (S1fa) and hand (S1ha) representations in S1, fluorescent tracers were injected in these cortical regions. Tracer injections involved both areas 3b and 1. Two separate populations of retrogradely labeled neurons were observed in Cl: a first cell population surrounded dorsally the insular cortex, with S1fa neurons located laterally to S1ha neurons. More ventrally, a second cell population was observed in which S1fa cells were located rostrally to S1ha cells.

The ratio between the number of cells labeled in Cl and in the ventroposterior thalamic complex was significantly higher than that obtained in our previous studies in the cat (monkey: about 0.5; cat: about 0.3) (Granato et al., Eur. J. Neurosci. S. 2: 74, 1989). The monkey's Cl shows also a relative preference of projections to S1ha in respect to S1fa.

These data provide the first demonstration of ordered and possibly multiple somatosensory representations in the monkey's Cl. Furthermore, our comparison with projections arising from the thalamic relay indicate that the Cl represents, in monkeys, one of the major subcortical inputs to S1.

100.17

PARVALBUMIN AND CALBINDIN HISTOCHEMISTRY OF THE HUMAN THALAMUS. K.M. Harrington and N.W. Kowall. Neurology Service, Mass. General Hospital, Boston MA, 02114.

We examined the distribution of calbindin and parvalbumin immunoreactivity in normal adult human thalamus using monoclonal antibodies (Sigma). The anterior nucleus contained scattered parvalbumin and calbindin positive neurons and parvalbumin terminals. The medial dorsal nucleus contained many parvalbumin neurons and few calbindin neurons. The ventral anterior-lateral complex had parvalbumin terminals and a moderate number of weakly stained calbindin neurons. A few parvalbumin neurons were present in the reticular nucleus. Intralaminar and midline cell groups contained prominent calbindin neurons but few parvalbumin neurons. The lateral posterior nucleus had moderate numbers of parvalbumin and calbindin neurons. Scattered parvalbumin neurons and terminals and moderate numbers of lightly stained calbindin neurons were present in the lateral dorsal nucleus. Ventral posterior nucleus had a moderate population of parvalbumin neurons and a few calbindin neurons. Parvalbumin positive terminals and calbindin positive neurons were seen in the lateral geniculate. Parvalbumin and calbindin terminal fields defined the subthalamic nucleus and substantia nigra respectively. Our results show that parvalbumin and calbindin reactive neurons are generally segregated in the thalamus. Intralaminar and midline cell groups contain prominent calbindin neurons while parvalbumin neurons are concentrated in the medial dorsal and, to a lesser degree, the ventral posterior nucleus. The distribution of calbindin neurons in superficial cortical layers parallels the terminal fields of intralaminar thalamic nuclei which contain prominent calbindin positive cell groups. Similarly, parvalbumin local circuit neurons are concentrated in layer IV of cerebral cortex where projections from thalamic nuclei containing parvalbumin neurons terminate. Widely distributed but connectionally related nuclei may, therefore, be linked by shared biochemical features such as their content of specific calcium binding proteins.

100.18

CHEMOARCHITECTONIC DELINEATION OF VENTRAL TIER NUCLEI IN MACAQUE THALAMUS. L.J. Martin, J.C. Hedreen, D.L. Price and M.R. DeLong. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The cytoarchitecture of the macaque thalamus is well characterized, but chemoarchitectonic definitions of thalamic nuclei are less clear. To characterize the chemoarchitecture of ventral tier nuclei (i.e., ventral anterior pars magnocellularis and parvocellularis; ventral lateral pars oralis [VLo]; ventral lateral pars medialis; ventral lateral pars caudalis [VLc]; area X; ventral posterior lateral pars oralis [VPLo]); ventral posterior lateral pars caudalis [VPLc]; and ventral posterior medial [VPM]) in macaque thalamus, serial sections were stained for Nissl; acetylcholinesterase (AChE); cytochrome oxidase (CO); and glutamic acid decarboxylase (GAD). Ventral tier nuclei had distinct patterns of AChE staining. VLo had clusters of very dense, large, coarse boutons and fine varicose fibers surrounding AChE-positive neurons. VLo extended caudally and dorsally into the region usually labeled VLc. VPLo had a dense AChE innervation, with a pattern different from that of VLo, VLc, area X, and VPLc. CO and GAD staining delineated VLo and VPLo clearly from contiguous nuclei. Therefore, chemoarchitectonic patterns help to delineate thalamic ventral tier nuclei when cytoarchitectonic features are not distinct.

SOMATOSENSORY CORTEX AND THALAMOCORTICAL RELATIONSHIPS II

101.1

INTRINSIC SYNAPSES OF CALLOSAL PROJECTION NEURONS IN MOUSE SOMATOSENSORY CORTEX. E.L.WHITE AND D. CZEIGER, DEPT OF MORPHOLOGY, BEN-GURION UNIV. BEER SHEVA, ISRAEL.

Neurons in areas 1 and 40 of mouse SmI cortex were labeled by the retrograde transport of horseradish peroxidase (HRP) transported from severed callosal axons in the contralateral hemisphere. "Intrinsic" terminals of the local axon collaterals of these neurons were identified in areas 1 and 40, and their distribution and synaptic connectivity were examined. A post-lesion survival time of 3 days was chosen because by this time extrinsic callosal terminals were all degenerating, whereas the intrinsic terminals were labeled by HRP.

Intrinsic callosal axon terminals formed only asymmetrical synapses. Analyses of serial thin sections through layers II and III in areas 1 and 40 show 97 % of the intrinsic terminals (1215 total sample) synapse onto dendritic spines, likely those of pyramidal neurons; the remainder synapse onto dendritic shafts of both spiny and nonspiny neurons. The high proportion of axospinous synapses formed by intrinsic callosal terminals differs significantly ($p<<0.0001$) from the proportion of asymmetrical, axospinous synapses that occur in the surrounding neuropil where only about 80% of the asymmetrical synapses are onto spines. This result is in accord with previous quantitative studies of the synaptic connectivities of both extrinsic and intrinsic axonal pathways in the cortex (e.g. White and Keller, 1989): In all instances, axonal pathways are highly selective for the types of elements with which they synapse. NIH 20149 and BSF 86000-41

101.2

EFFECTS OF REPETITIVE AFFERENT DRIVE ON RESPONSIVITY OF SENSORIMOTOR CORTICAL SLICES C.-J. Lee*, B.L. Whitsel, M. Tommerdahl, and C. Wong*, Dept. Physiol., Univ. of North Carolina, Chapel Hill, NC 27599

Evoked potential recordings obtained from layers II-V of cortical slices indicate that the responsivity of local neuronal populations to repetitive low-frequency, low-intensity input drive varies prominently with stimulus repetition. Stimuli (bipolar, squarewave, 0.2 msec duration) were delivered to a single locus at the layer VI/white matter junction. At frequencies between 3-5 Hz, responsivity to this type of stimulation typically modified progressively to attain a new level that was maintained with continued stimulation. Observations obtained from different layers in the same slice under identical stimulus conditions suggest strongly that repetitive stimulation leads to similar changes in responsivity at all levels of the same cortical column, with the largest changes in responsivity occuring in layers II-III and the smallest in layer IV. In addition, the alterations in responsivity observed to accompany such repetitive stimulation (i) were transient (complete recovery routinely occured in less than 1 min after termination of stimulation); and (ii) were either progressive (either incrementing or decrementing), or were complex and non-monotonic. High-density mapping of the responsivity changes evoked by repetitive afferent drive within individual slices clearly revealed a nonrandom spatial distribution of those cortical neuronal populations which exhibit different responsivity alterations to the same repetitive stimulus - based on the available mapping data it appears that repetitive stimulation leads to the formation of column-shaped blocks of cortex whose neurons (on average) exhibit essentially the same alteration in responsivity with stimulus repetition. We view these findings as consistent with the working hypothesis that the intrinsic network of the somatosensory cortex accomplishes a major transformation of its input pattern; and that with repetitive stimulation these interactions dynamically enhance any local differences present in the pattern of excitatory drive reaching neighboring cortical columns. Supported by NIDR Program Project DE07509.

101.3

LAMINAR DISTRIBUTION OF THE EFFECTS OF NOREPINEPHRINE ON SINGLE NEURONS IN THE CAT SOMATOSENSORY CORTEX. R.A. Warren[1] and R.W. Dykes[2]. [1]Dept. Neurol. Neurosurg., McGill Univ., Montréal, Canada, H3A 2B4; [2]Dept. Physiol., Univ. Montréal, Montréal, Canada, H3C 3J7.

Norepinephrine (NE) was administered iontophoretically to 117 neurons in the cat somatosensory cortex. Upon ejection, NE produced both inhibition and excitation (Dykes, R.W. and Warren, R.A., Soc. Neurosci. Abstr., 15:1052, 1989). In several cases, effects were observed that outlasted the ejection of NE for several minutes (Warren, R.A. et al., Soc. Neurosci. Abstr., 15:1053, 1989). The present report describes the laminar distribution of those effects of NE.

At the end of each penetration in anesthetized cat cortex, a dye was ejected iontophoretically from the recording electrode at two sites along the trajectory; those marks were used to identify the penetration in the histology and to correct for shrinkage. The tracks were reconstructed from Nissl-stained sections and each neuron was assigned to a lamina.

Thirty eight (35%) of the neurons located from histological sections were studied with NE. During NE ejection, 80% of the neurons located in the middle layers were inhibited while 78% of the neurons found in the upper and lower layers were excited. Within 5 min of the cessation of NE, larger proportions of neurons in the upper and lower layers as contrasted to the middle layers had their ongoing and evoked activities affected. More than 5 min after the cessation of NE, ongoing and evoked activities of all the neurons found in the upper and lower layers had both their ongoing and evoked activities significantly affected. In contrast, the ongoing and evoked activities were affected in not more than 50% of the cases found in the middle layers. The results were confirmed when the neurons located using the readings on the micromanipulator were analysed in addition to those located in the histology. These results suggest that the role of NE varies as a function of cortical layers. This will be discussed in light of the organization of the somatosensory cortex. (Supported by the Medical Research Council of Canada).

101.4

EFFERENT NEURONS AND SUSPECTED INTERNEURONS IN S-2 OF THE AWAKE RABBIT. H. A. Swadlow. Dept. of Psychology, Univ. of Connecticut, Storrs, CT 06269-1020.

Efferent populations studied in the vibrissa and sinus hair region of S-2 included: callosal (CC) and ipsilateral corticocortical (C-IC) neurons, and descending corticofugal neurons of layer 5 (CF-5) and layer 6 (CF-6). Suspected interneurons (SINs) responded with a hi-frequency (>600 hz) burst of spikes to thalamic stimulation.

Receptive fields were larger in S-2 than previously seen in S-1. SINs had the largest receptive fields and the highest spontaneous firing rates of any population in S-2. CF-5 neurons had high axonal conduction velocities (median > 10 m/s), intermediate spontaneous firing rates (most 4-8/sec), and had the largest receptive fields of any efferent population. In contrast, CC, C-IC and CF-6 populations each had low axonal conduction velocities (medians < 3 m/s), low spontaneous firing rates (medians < 0.5/sec), and had relatively small receptive fields. Whereas all SINs and CF-5 neurons responded to peripheral stimulation, many CC, C-IC, CF-6 neurons did not.

These differences among efferent neurons and SINs of S-2 mirror similar differences among corresponding populations studied in S-1 and V-1 (Swadlow, H. A., J. Neurophysiol., 59:1162, 1988; 62:288, 1989, in press). These data suggest that a common physiological plan underlies the operations of functionally and morphologically diverse neocortical regions.

101.5

IPSILATERAL CORTICOCORTICAL CONNECTIONS OF SI SUBDIVISIONS IN MONKEYS. K.Alloway,M.Fabri and H.Burton. Department of Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110. (Supported by NS22012)

Ipsilateral cortical connections were studied using small amounts (75-400 nl) of different fluorescent tracers that were injected simultaneously, under electrophysiological control, in two or three body representations of areas 1 and/or 3b of SI.

Reciprocal connections exist between one subdivision of SI and numerous areas: 1,2,3a,3b, second somatic sensory area (SII), and retroinsular area (Ri). Local connections within the injected area involve related regions of the body map (i.e., proximal part of a digit with tracer injected into digit tip zone). Connections between injected areas and other parts of SI are mostly topographical and appear to interconnect homotopical zones. Few double labeled cells were seen.

Projections from major body zones in SII and Ri did not display a somatotopy totally consistent with previous views. Each injection labeled multiple, interdigitated patches, especially within adjacent digit tip regions in SII. This interposed pattern of labeled cells and fibers obscured which area served as the primary representation for that body zone.

101.6

MULTIPLE IPSILATERAL CORTICAL CONNECTIONS OF SI IN RATS. M.Fabri, K.Alloway and H.Burton. Department of Anatomy & Neurobiology, Washington University Sch. of Med., St. Louis, MO 63110. (Supported by NS22012)

Ipsilateral cortical connections were studied using small amounts (50-150 nl) of different fluorescent tracers that were injected simultaneously, under electrophysiological control, in two or three zones of the SI body map.

Local connections within SI involve neighboring regions of the body map (i.e., digits and arm or within face areas). Ipsilateral reciprocal connections exist between SI and numerous areas: motor cortex (MI), cortex possibly homologous to area 3a, second somatic sensory area (SII), parietal ventral area (PV), parietal rhinal area (PR), and parietal medial area (PM) (Krubitzer *et al.*, J Comp Neur, 250: 403, 1986). The first four cited areas maintain topographical connections with SI. In PR and PM, overlap was observed between projection zones from different regions of SI. Although such overlap was not extensive in other SI-connected areas, it was greatest amongst infragranular connecting cells. There were almost no double labeled cells.

Finding two maps lateral to SI in which the representations face each other (up-right in SII and upside-down in PV) may explain previous discrepancies regarding orientation of the SII map in rodents.

101.7

NERVE DOMINANCE COLUMNS IN THE PRIMATE PRIMARY SOMATOSENSORY [AREA 3b] CORTEX. J.T. Wall. Department of Anatomy, Medical College of Ohio, Toledo, OH. 43699.

The primary visual cortex of many primates contains column-like aggregates of neurons which are dominantly activated by inputs from one optic nerve (or eye). Does the primary somatosensory cortex contain analogous column-like aggregates which relate to mechanoreceptor inputs from cutaneous nerves? To address this question, estimates of the low threshold mechanoreceptor innervation territories of the three nerves to the hand were derived either from summation of the receptive fields of multifiber recordings made from the nerves, or from summation of residual receptive fields of neurons in the cortical hand map after injury-related loss of one or two nerves. Estimates of the spatial distribution of aggregates of cortical neurons that are activated by low threshold tactile inputs from these nerve territories were then derived from maps of the hand representation by relating multineuronal receptive fields at individual cortical sites to the nerve territories. The results indicate that low threshold inputs from hand nerves activate column or patch-like aggregates of area 3b neurons. In addition, it appears that nerve injury alters the size of these aggregates. Supported by NS21105.

101.8

INTRINSIC CORTICOCORTICAL CONNECTIONS OF CAT PRIMARY SOMATOSENSORY CORTEX. H. Esteky* and H.D. Schwark. Dept. Biological Sciences, Univ. North Texas, Denton, TX 76201.

We have examined the intrinsic corticocortical connections of the forepaw representation in primary somatosensory cortex of the cat. Various amounts of HRP, WGA-HRP, or PHA-L were delivered by pressure injection or by iontophoresis into physiologically-defined regions of the posterior sigmoid gyrus of barbiturate-anesthetized cats. Survival times ranged from 24 hours (HRP injections) to 4 days (PHA-L injections). Analyses were based on sections cut in frontal, sagittal, or tangential planes.

Injections into area 3b resulted in retrograde and anterograde labeling within area 3b and in nearby cortical regions, including areas 3a and 1. These projections were often quite widespread, spanning more than 2 mm in the longest dimension. The 3b injections also resulted in more focal labeling in the anterior wall of the ansate sulcus in a region which appears to be area 2.

Injections into the anterior wall of the ansate sulcus resulted in retrograde and anterograde labeling in areas 1, 3b, 3a and motor cortex. In some cases, such as following a large injection of WGA-HRP (0.5%, 360 nl) into area 2, anterograde and retrograde labeling appeared in distinct patches in each of these regions. Some of these patches were quite restricted in the anterior-posterior dimension (0.5 - 1 mm), but spread 2-3 mm in the mediolateral dimension. In every case analyzed so far, the corticocortical projections originate from neurons in layer III.

These widespread corticocortical connections may be involved in the SI somatotopic reorganization which follows peripheral input restriction. Supported by NS 25729.

101.9

CONNECTIVITY PATTERNS OF PRIMARY SOMATOSENSORY CORTEX (SI) IN AN OLD WORLD PRIMATE, Cercopithecus aethiops. **Susan Warren. Dept. of Anatomy, U. of Mississippi Med. Ctr., Jackson, MS 39216.**

Although four diverse cytoarchitectural areas have been identified within the primary somatosensory cortex (SI) of C. aethiops, the function of each area remains undefined. Application of micromapping techniques to study the topography of the SI hand area in this species (Warren & Carlson, '86) has demonstrated the occurance of complex and variable receptive field (rf) properties which disregard individual SI subdivision boundaries. Furthermore, the topographic organization of thalamocortical inputs to the SI hand area in C. aethiops (Warren, '90) reveals an apparent lack of submodality segregation, and no obvious morphological segregation of cutaneous and proprioceptive inputs into a 'core' and 'shell' arrangement. Identifying connections between individual areas of SI, as well as, SI connections with other cortical targets, including SII cortex, is essential for defining the functional role of each cortical subdivision. Patterns of SI cortical connectivity were defined by making separate injections of different retrograde neuroanatomical tracers into functionally defined SI hand areas for ten animals. Tissue was processed for fluorescence and WGA-HRP histochemistry. Corticocortical labelling in SII cortex, resulting from injections in either area 3b or area 1, was usually restricted to the inner face of the frontoparietal operculum, with the majority of labelled neurons principally located in Layers V and VI. Intracortical connections between subdivisions of SI were noted. Organization of thalamocortical and corticocortical connectivity patterns in this species appears to differ from other Old World Monkeys implying that many of the fine details of the somatosensory system in C. aethiops are species specific. **Support:BRSG #2SORR5386 and NIH #NS27996.**

101.10

THE ORGANIZATION AND CONNECTIONS OF SOMATOSENSORY CORTEX IN THE MEGACHIROPTERA BAT (*PTEROPUS POLIOCEPHALUS*). Mike B. Calford and Leah A. Krubitzer. Dept. Physiol. and Pharm. University of Queensland, QLD. 4072 Australia

In this study, the areal pattern of connections of the primary somatosensory area (S-I) was investigated in in the flying fox. The flying fox was chosen for several reasons. First, because of its close relationship to primates, we expect some features of cortical organization to be similar to that of primates, and some features to be similar to Toketherian mammals in general. Thus, the flying fox could serve as an important link between primates and non primate mammals. Second, the flying fox has a smooth neocortex so that all somatosensory areas are on the cortical surface and readily accessible for microelectrode mapping and connectional studies. Finally, the flying fox is a flying mammal and may possess cortical specializations related to flight, some of which have been reported previously by our laboratory. In these experiments, receptive fields were localized in S-I, lesions were placed at functional boundaries, and different anatomical tracers were injected into separate body part representations within S-I. In all cases, cortex was completely flattened and sectioned parallel to the cortical surface. By relating sections containing transported tracer to adjacent sections stained for myelin or reacted for cytochrome oxidase, the total pattern of connections could be related to architectonically defined fields. Since multiple tracers were used in the same animal, the topographic pattern of interconnected somatosensory areas could be readily assessed. Topographic connections were noted with areas 3a, 1, SII and two other areas rostral and caudal to S-II. We provide evidence that the somatosensory cortex of the flying fox has primate-like features as well as general mammalian features. In addition our results demonstrate at least six somatotopically organized cortical fields in the flying fox.

102.1

NEURAL CONTRAST SENSITIVITY. Andrew B. Watson. Vision Group, NASA Ames Research Center, Moffett Field, CA 94035-1000.

Contrast sensitivity is a useful measure of the ability of an observer to distinguish contrast signals from noise. For an individual linear neuron, it is given by

$$C(\mathbf{u},w) = \frac{\sqrt{T}\, G(\mathbf{u},w)}{\sqrt{2}\eta \sqrt{M(w)}}$$

where $\mathbf{u}$ and w are spatial and temporal frequencies, G is contrast gain, M is the noise power spectrum, T is the measurement duration, and η is the performance criterion. This principle is used to show how the contrast sensitivity of a given neuron depends upon that of neurons earlier in the visual pathway. The general theory is then applied to the specific problem of relating the sensitivities of parvocellular geniculate neurons to their target cortical neurons.

102.2

NONLINEAR MODEL OF CAT STRIATE PHYSIOLOGY. David J Heeger. NASA-Ames Research Center, ms 239-3, Moffett Field, CA 94035 and Stanford Psychology Dept, Stanford, CA 94305

The goal of modeling striate physiology is to predict the response of a cell to any stimulus, based on a limited number of measurements. Simple-cells are often depicted as halfwave-rectified linear-operators and complex-cells as energy-mechanisms, constructed from the squared-sum of the outputs of quadrature-pairs of linear-operators. However, this linear/energy model falls short of a complete account of striate physiology. One of several major objections to this model is the fact that striate cells saturate at high contrasts, whereas linear- and energy-mechanisms do not.

I modified the linear/energy model in two ways to account for a significantly larger body of physiological data. The first modification is to include a feedback, contrast-normalization nonlinearity, and the second is to replace halfwave-rectification with half-squaring (half-squaring is halfwave-rectification followed by squaring) at the output of the model simple-cells. With these modifications, model cells respond similarly to real cells for measurements (using drifting and counterphase grating stimuli) of response vs. contrast (Albrecht and Hamilton, J Neurophys, 48:217-237, 1984), contrast adaptation (Ohzawa et al, J Neurophys, 54:651-667, 1985), cross-orientation and cross-frequency inhibition (Bonds, Visual Neurosci, 2:41-55, 1989), and direction selectivity (Reid et al, Proc Nat Acad Sci, 84:8740-8744, 1987).

102.3

NETWORK MODELING OF SPATIAL PROPERTIES OF UNITS IN STRIATE CORTEX. Sidney R. Lehky[1], Robert Desimone[1], and Terrence Sejnowski[2] [1]NIMH Bethesda, MD [2]Salk Institute La Jolla, CA

We have used the back-propagation learning algorithm to model single unit data from macaque V1 cortex. Stimuli were 400 spatial patterns, including bars, sinusoidal gratings, textures, and 3-D shaded objects. The algorithm was used to integrate these data to produce a model of the cell's response properties. To create the network, the same images used as stimuli were repeatedly presented to the network's input layer, which consisted of several hundred units with receptive fields similar to those of retinal ganglion cells. The output layer had one unit, representing the neuron being modeled, and the middle layer had from 4-10 units. The network was trained so that its output in response to each image reproduced the activity of the actual neuron, using average firing rate as a measure of activity.

The resulting network was able to reproduce responses of the cortical neuron with a correlation of 0.8-0.85, when tested with images that were not included in the training set but for which we had data. This ability of the network to generalize well to images not part of the training set shows that it has captured most of the spatial properties of the cell, including nonlinear ones. We believe that this is the first use of back-propagation to directly model neurophysiological data. This technique may be useful as a more comprehensive and objective means of determining neural response properties, especially in extrastriate cortex, where receptive fields are particularly complex.

102.4

A SIMPLE QUANTITATIVE MODEL FOR SPATIO-TEMPORAL INTERACTIONS IN RECEPTIVE FIELDS OF VISUAL CORTICAL CELLS G. Holt* and F. Wörgötter Computation and Neural Systems Program, CALTECH, 216-76, Pasadena, CA, 91125.

Most single cell models of visual cortical receptive fields (RFs) neglect spatio-temporal mechanisms. We present an analytical model of such interactions in which activity from a stimulated receptive field region is distributed into adjacent parts of the RF (adjacent regions of visual cortex), which were not directly stimulated. The receptive field is treated as a continuum because of the large number of converging inputs to cortical cells.

The model involves two stages of cortical cells. The cells of the first stage have Gabor-type receptive fields (modeling orientation and spatial frequency tuning), and their output is smoothed temporally by a low pass filter. They show weak directional tuning to bar stimuli (direction asymmetry) and velocity low pass behavior. The output from these cells is thresholded and fed into the second stage neuron, which has a similar low pass filter and threshold. Activity distribution is implemented by determining the pattern of connections from the first to the second stage. The second stage neuron shows strong directional tuning (direction selectivity) and velocity tuned behavior. The response of both types of cells to dot stimuli is similar to responses recorded from cortical cells (Wörgötter and Eysel, Exp. Brain Res., 76:307-314, 1989). Also, the model predicts that the optimal velocity for dot stimuli should be higher than that for bar stimuli, and the direction tuning strength should increase with velocity for a dot and decrease with velocity for a bar.

In conclusion: Our model is simple, thus, analytically tractable and implements a neurophysiological plausible mechanism. Most aspects of spatial frequency, orientation, direction and velocity tuning of simple cells are reproduced with this approach.

102.5

NEURAL CONNECTIVITY AND PHASE COHERENCE IN TWO DIMENSIONAL SIMULATIONS OF STRIATE CORTICAL NEURONS. D. M. Kammen, E. Niebur and C. Koch, CNS Program, Division of Biology, 216-76, California Institute of Technology Pasadena, CA, 91125.

Stimulus specific collective oscillations have been reported in olfactory (Freeman, W. J., Elect. Clin. Neurophys., 44:169, 1979) and more recently in visual cortical (Gray, C. M., Nature, 338:334, 1989) areas. The oscillations observed in the striate cortex of anaesthetised cats (Area 17) are remarkable in that cells tuned to similar orientations but spatially widely separated on the cortical surface (up to 7mm) exhibit stimulus dependent frequency locking with no apparent phase delay.

We have extended our previous integrate and fire-type model (Kammen, D. M. *et al.*, In *Models of Brain Function*, R. M. J. Cotterill (ed.), Cambridge University Press, 273, 1989) to a two dimensional array of 8192 neurons. The array models a neural population such as a patch of cortical cells receiving coherent visual input from an associated population of geniculate neurons.

Our aim is to determine the patterns of axonal and dendritic connectivities necessary to subserve the frequency and phase coherence observed in populations of visual cortical cells. We present data for a wide variety of arborization radii and connectivity densities. A simple next-neighbor model is inconsistent with the data of Gray *et al.* 1989. Networks with sparse connectivity (typically 5%) do not exhibit long-range order while fully connected local networks exhibit synchronized activity over areas 2 to 3 times larger than the arborization radius.

102.6

CIRCULAR INHIBITION: ISOTROPIC CONNECTIONS THAT GENERATE ANISOTROPIC BEHAVIOR IN THE VISUAL CORTEX. F. Wörgötter, E. Niebur, & C. Koch, Computation and Neural Systems Program, CALTECH, 216-76, Pasadena, CA, 91125.

Structural cross-orientation inhibition is defined as inhibition that arises from cells with preferred orientation orthogonal to their target cell. Our theoretical analysis shows that, due to the 2-dimensional organization of the orientation columns, this mechanism leads to unequal orientation tuning strengths for populations of differently oriented receptive fields. This situation is inconsistent with the experimental data. To resolve this problem we propose an isotropic arrangement of connections arising from a circle of cells at a distance of half a hypercolumn to the target cell (*Circular Inhibition*).

Circular inhibition is unspecific, thus, no Hebb type enhancement of connections is required. It is developmentally advantageous because only distance information is necessary to establish a connection.

We present an analytical model for the description of the cortical orientation column system and demonstrate the effects of circular inhibition within this model. We show that circular inhibition results in a weak net functional cross-orientation inhibition which corresponds to recents reports indicating the tuning strength of cross-orientation inhibition (Bonds, A.B., Vis. Neurosci., 2:41-55, 1989). Furthermore a significant directional bias is generated from this completely isotropic mechanism. The directional bias results from the location of the circle of connected cells in the column structure relative to the target cell which leads to unequal amounts of inhibition for both direction associated with the preferred axis of stimulus motion.

Thus, an isotropic connection scheme (circular inhibition) leads to two anisotropies: orientation tuning and even more surprisingly a directional bias.

102.7

CHAOS AND THE SINGLE NEURON IN AREA V1 OF THE CAT. R.M. Siegel. IBM Research Center, Yorktown Heights, New York 10598.

Visual perception can be described as the activation of sets of neurons in the cortex with properties that correspond to the qualia of the visual input (Edelman, 1990). Indeed, neurons appear to fire in synchrony after visual stimulation (Gray and Singer, 1989; Grinvald et al., 1989). What constitutes "sets of neurons" and how can the interactions in systems of large numbers of neurons be described? Non-linear dynamical theory (i.e. chaos theory) analyzes physical systems of similar complexity. We apply chaos theory to single neuron activity recorded in V1 of the anesthetized, paralyzed cat. The receptive field of a cell was mapped; optimal oriented rectangular light bars, modulated by a square wave with a 50% duty cycle, were used to probe for patterns of activity. With the stimulus period in the range of 100 to 2000 msec, temporal rhythms in the interspike intervals (ISIs) could be heard. Phase plots are used in chaos theory to extract predictive representations for temporal patterns (Glass and Mackey, 1988). The *neural phase plot*, generated by graphing each ISI against the next ISI, had clusters of points indicating preferred sequences of ISIs (c.f. Strehler and Lestienne, 1986). Some of these clusters were simply, and others more complexly related to the stimulus period (Siegel, 1990). These temporal patterns were found to scale with the stimulus period. No such patterns were seen with spontaneous activity.

A neural model was used to test hypothetical physiological mechanisms for these experimental temporal patterns in V1. The neuron was modeled by a single variable for membrane potential. Poisson distributed spontaneous ISIs were simulated by an integrated random fluctuation of the membrane potential (Gerstein and Mandlebrot, 1964). A "spike" would occur when the membrane potential reached threshold. The membrane potential then rapidly returned to rest. The sum of the synaptic dendritic inputs to the cell was simulated by adding periodically varying values to the membrane potential. The phase plots of ISIs for the model resembled some of the simplest temporal patterns observed in the experimental data, but did not exhibit the full complexities of the data. In order to simulate these, it was necessary to modulate the model's synaptic input using a periodically driven chaotic oscillator (i.e. deterministic and unpredictable). Thus we propose that the periodic visual stimulus is transformed into a chaotic dendritic synaptic input. This synaptic input is then expressed as temporal patterns in the ISIs which impinge on other cells. Therefore the chaotic dynamics may prove to be an emergent property of the interactions between large numbers of neurons.

102.8

HOW THE UTILITY OF NEURAL MAPS INFLUENCES THEIR DEVELOPMENT IN A SELF-ORGANIZING MODEL OF VISUAL PROCESSING. R. Linsker. IBM T. J. Watson Research Center, Yorktown Heights, NY 10598.

To what extent can we derive the functional properties and organization of a perceptual system from partial knowledge of (a) biological design constraints and (b) the adaptive value (to the animal) of discriminating among various environmental situations? To explore this question, I construct and analyze multilayered networks in which signaling activity and synaptic modification arise in a novel and biologically plausible way from feedforward, lateral (intralayer), and feedback interactions.

In the absence of feedback connections, it has been shown that Hebbian modification, combined with cooperative and competitive lateral interactions among cells, can generate networks whose output responses capture statistically and informationally salient properties of the sensed input environment [R. Linsker, Annu. Rev. Neurosci. 13:257-81 (1990)]. However, statistical saliency is not synonymous with utility to the animal.

I propose a way in which network development and modification processes that are local to each layer (or processing stage) can favor the emergence of useful cell response properties and neural maps. A 'reward' (or 'penalty') value is ascribed to the final output layer of a simplified network model, in a way that depends upon the output signals and the state of the environment at that time. (There is no need for a 'desired' or 'correct' output to be specified to the network.) The 'utility' of the final layer is defined in terms of the time-averaged value of the reward. In each intermediate layer, the response properties of each cell (or functional group of cells) are incrementally modified according to three factors: (a) the extent to which the output provided by the cell to other layers contributes to the utility of the cells that receive this output; (b) a Hebbian rule acting on feedforward connections; and (c) lateral interactions that influence the extent to which nearby cells of the same layer have similar response properties. Factor (a) in turn determines the 'utility' of the cell or group being modified. The process is carried out for many network layers at once. A distinctive feature is that no detailed signal values need to be 'back-propagated' to earlier processing layers. Cell response properties and organization generated by the method are compared with observations in visual cortex.

102.9

DETERMINING THE INDEPENDENCE OF MESSAGES CARRIED BY MULTIPLE SIMULTANEOUS NEURONS T. J. Gawne and B. J. Richmond. Lab of Neuropsychology, National Institute of Mental Health, Bethesda, MD 20892.

Why are there so many neurons in each layer of a cortical column? If all the neurons carry redundant messages, then the signal-to-noise ratio (SNR) of that layer would increase. If all the neurons carry independent messages, then the layer would carry more information, but individual messages would have a low SNR. One way to increase the SNR of multiple messages would be to have a redundant sub-population for each message. When recording from multiple neurons simultaneously, how can we determine whether they are carrying redundant or independent messages?

We have used information theory to determine the independence of the messages carried by multiple neurons recorded simultaneously. If N neurons are redundant, then the influence of noise on the transmitted information will decrease by $\sqrt{N}$. If, however, the neurons transmit independent messages, then the information transmitted jointly by the neurons would be the sum of the information transmitted by the neurons individually.

We inserted a microelectrode into the superficial layers of striate cortex in an awake behaving rhesus monkey, and isolated pairs of neurons from the electrode using a template-matching technique. The set of 64 B&W 2-dimensional Walsh patterns were presented a minimum of six repetitions per stimulus in an area covering the common receptive field of both neurons in the pair. We computed the information transmitted by the magnitude of the response for each neuron separately, and for both neurons jointly. Preliminary results show pairs of neurons that are redundant, and other pairs that are independent. Therefore, this technique can indicate how information is coded by multiple neurons, and can be used to determine the functional groupings of the many neurons in cortical layers.

102.10

CORTICAL DYNAMICS OF MOTION SEGMENTATION: DIRECTION FIELDS, APERTURES, AND RESONANT GROUPING. Stephen Grossberg † and Ennio Mingolla‡. Center for Adaptive Systems. Boston University, 111 Cummington Street, Boston, MA 02215.

A neural model, called a motion Boundary Contour System, explains data about visual motion perception, and clarifies computational differences between parallel cortical streams for processing static visual forms ($V1 \rightarrow V2 \rightarrow V4$) and moving visual forms ($V1 \rightarrow MT, V2 \rightarrow MT$). Simulations clarify how ambiguous local movements (the aperture problem) on a complex moving shape are reorganized into a global motion representation. Properties of induced motion, motion capture, and apparent motion of illusory boundaries are clarified. The work analyses how a coherent motion signal is imparted throughout a moving figure, not only at locations where unambiguous motion signals exist. Preprocessed inputs from concentric receptive fields (LGN) go to oriented receptive fields (simple cells) which are then endstopped and rectified. These outputs are temporally averaged over neighboring orientations and position. They are gated by the rectified outputs of transient cells, then spatially averaged again to generate signals that are insensitive to direction-of-contrast but sensitive to direction-of-motion. These signals are then fed into a competitive-cooperative feedback network (CC Loop) whose interactions choose and complete the most globally consistent motion signals across the figure data.

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102.11

GLOBAL PROCESSING OF VISUAL STIMULI IN A NEURAL NETWORK OF COUPLED OSCILLATORS. D. Kleinfeld, D. Golomb* and H. Sompolinsky*. AT&T Bell Laboratories, Murray Hill, NJ 07974 and The Hebrew University, Jerusalem, Israel 91904.

A model neural network is presented that is capable of linking activity in disparate visual receptive fields in a manner that depends on extended features of the stimulus. The network is comprised of neurons that act as oscillators. Local features in the stimulus are encoded in the average firing rate of the neurons while the relationships between these features can modulate the temporal structure of the neuronal output. The model incorporates three aspects of the architecture of primary visual cortex: [1] The average firing rate of neurons is a strong function of the orientation of a bar that passes through the receptive field of the cell. [2] Nearby neurons have overlapping fields and appear to be highly interconnected. [3] Neurons with non-overlapping fields form a sparse set of long-range connections.

The model will be discussed in the context of processing visual stimuli that are coded for orientation. Our results suggest that the recent experimental evidence on coherent oscillatory activity in the cat visual cortex (†) has important implications regarding the underlying pattern of neuronal connectivity: [1] The effective interaction between the phases of the neuronal output depends on the level of activity in both the pre- and post-synaptic cell. [2] Cells with substantially overlapping receptive fields form connections that do not depend strongly on the orientation preference of the pre- and post-synaptic cell. Connections between cells with non-overlapping fields have a strong dependence on their respective orientational and directional preference. [3] The connections formed by cells with overlapping receptive fields are significantly stronger than those between cells with non-overlapping fields.

† Eckhorn, Bauer, Jordan, Brosch, Kruse, Munk, Reitboek *Biol. Cyber.* 60 (1988). Gray, Konig, Engel, Singer *Nature* 338 (1989), *Eur. J. Neuro.* in press.

102.12

A COMPUTATIONAL MODEL OF SPACE-TIME SIGNAL PROPAGATION IN THE VISUAL CORTEX. •P.Patton,E.Thomas,and R. Wyatt. Dept. of Chemistry, Univ. of Texas, Austin, TX 78712.

• In order to simulate the vertical spread of evoked activity through the laminae of the visual cortex, a computational model of a slab of active tissue in the mammalian primary visual cortex has been constructed using anatomical and physiological data derived from the cat. The model will be used to investigate the dynamics of cortical activity flow and in planned studies of cortical self-organization. Up to 1000 model neurons are distributed through the 3-D slab in accordance with realistic cell densities and assigned schematic arbors corresponding to 17 cortical cell classes. Connectivity is determined based on this morphology and on literature data. Each neuron is treated as a single physiological compartment whose activity is describable by a first order, non-linear differential equation. Preliminary results show that our connection algorithm generates connection densities closely matching that reported for cat area 17. We have compared the spatial pattern and time course of activity following afferent stimulation in the model with the behavior of a dummy slab containing only a single cell type. In both the model and dummy slabs, oscillatory behavior was observed. In the later case, oscillations appeared when a fraction of the cells were made inhibitory.

103.1

EFFECTS OF CAPSAICIN AND RESINIFERATOXIN ON ANTINOCICEPTION AND MICTURITION THRESHOLD IN RATS. Antonia Mattia and Frank Porreca, Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724

Capsaicin has been shown to cause desensitization of primary afferent neurons producing long-term biological effects on sensory systems including antinociceptive and micturition reflexes in rats. This study compared the actions of capsaicin (CAP) with resiniferatoxin (RTX), a potent diterpene with desensitizing properties, in adult female Sprague-Dawley rats. Groups of rats (n=8) were injected *s.c.* with graded doses of CAP (12.5, 25 and 50 mg/kg), RTX (12.5, 25 and 50 µg/kg) or vehicle. Antinociception was assessed after 4 d using the 55°C warm-water tail flick and hot plate tests (cut-off latencies of 15 and 60 sec, respectively). Effects on micturition were studied using transvesicle perfusion of the bladder in the urethane anesthetized rat. After a 15 min equilibration, the bladder was continuously infused with saline at 50 µl/min and the detrusor response was recorded. As indicators of toxicity, body weight and bladder weight were recorded. Hot plate, but not tail flick, latencies were elevated by approximately 10% after CAP at 25 and 50 mg/kg in agreement with previous reports. Similarly, RTX elevated hot-plate, but not tail-flick, latencies at all doses tested, showing an approximately 60% increase. Changes in micturition threshold were noted only at the highest doses of CAP or RTX. At these doses, the number of contractions per min significantly decreased by approximately 30% without alteration of contraction amplitude. The volume threshold to micturition was increased by approximately 100% at the highest doses of CAP and RTX. Body and bladder weights were not significantly altered by CAP or RTX. These results indicate that RTX is approximately 1000-fold more potent than CAP in producing changes in sensory function and that additionally, the magnitude of the RTX effect on antinociception is substantially greater than that seen with CAP.

103.2

PHARMACOLOGICAL CHARACTERIZATION OF THE RESINIFERATOXIN/CAPSAICIN RECEPTOR. A. Szallasi* and P.M. Blumberg. Molecular Mechanisms of Tumor Promotion Section, Lab. of Cellular Carcinogenesis and Tumor Promotion, National Cancer Institute, Bethesda, MD 20892.

Our demonstration of specific binding of resiniferatoxin (RTX), an ultrapotent analog of capsaicin, provided a new opportunity for the pharmacological characterization of the RTX/capsaicin receptor (Szallasi & Blumberg, Brain Res., in press). Competition between [^{3}H]RTX and capsaicinoids, RTX-analogs and other vanilloids to binding sites in rat dorsal root ganglion membranes was examined. Both the homovanillyl substituent and the diterpene moiety seemed to play an important role in the high binding affinity of RTX. If the homovanillyl substituent was missing (resiniferonol 9,13,14-orthophenylacetate, the 20-deacylated parent structure of RTX) the affinity for the RTX/capsaicin receptor was lost. Modification of the diterpene moiety (e.g. removal of the orthoester group, such as found in 12-deoxyphorbol 13-phenylacetate 20-homovanillate) also dramatically reduced the affinity. Replacing the homovanillyl (4-hydroxy-3-methoxyphenyl) substituent with a 4-hydroxyphenyl group, such as found in tinyatoxin, resulted in a 3-fold decrease in the K_i value. The use of direct analysis of receptor binding and novel insights provided by the resiniferatoxin class of vanilloids may promote rapid progress in our understanding of vanilloid structure-activity requirements.

103.3

CAPSAICIN, OR THE ANALOGUE NE-21610, APPLIED TO THE TRANSECTED PRIMATE NERVE MARKEDLY INHIBITS HEAT SENSITIVITY OF THE DEVELOPING NEUROMA. R. H. Cohen, and J. N. Campbell. Dept. of Neurosurgery, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

These studies were conducted to determine if fibers in the developing neuroma are sensitive to heat and whether heat sensitivity can be blocked by pretreatment with capsaicin or the analogue, NE-21610. In anesthetized monkeys, the superficial peroneal nerve was crushed, ligated, and cut. Three to 14 days later, action potential activity was recorded from a site proximal to the ligature. Repeatable, short latency (<5s), short duration (≤1 min) responses to heated oil (40°C - 75°C) were observed in 27 of 35 multiunit recordings from the 3 untreated neuromas. Responses were observed in both Aδ- and C-fibers. To determine the effects of vanilloids, the naturally-occurring fascicles in the transected nerve were exposed and soaked for 1 hr in a small cup containing 1% capsaicin in the inactive vehicle, olive oil, or 1% NE-21610 in an active vehicle (10% Tween 80, 10% ethanol, and acetate buffer). When examined 3 to 14 days later, short latency, repeatable, responses to heat in neuromas pretreated with capsaicin (n=2) were found in only 2 of 20 strands and none of the 18 strands responded when neuromas (n=2) had been pretreated with NE-21610. We conclude, therefore, that application of vanilloid to the transected nerve profoundly reduces the heat sensitivity of fibers in the developing neuroma. Another type of heat reaction was also observed in the untreated neuromas, but only after repeated applications of noxious heat. The inability to re-evoke the response, its delayed onset (10-30 s) and longer duration (1 to 5 min) support our interpretation that these reactions were mediated by the release of algesic chemicals. Responses of this nature were also evident in vanilloid pretreated neuromas and, thus, some chemical sensitivity probably remains.

103.4

A CORRELATION BETWEEN CAPSAICIN EVOKED INWARD CURRENT, VOLTAGE-ACTIVATED Ca-CURRENTS AND CELL BODY SIZE IN SENSORY NEURONS.

M. Petersen and R.H. LaMotte, Dept. of Anesthesiology, Yale University School of Medicine, New Haven, CT 06510

The somata with C- and Aδ-fibers are restricted to the small cells within the dorsal root ganglia. Capsaicin, which excites nociceptors, evokes an inward current in a subpopulation of these sensory neurons.

We examined the correlation between cell body size, capsaicin evoked inward current and voltage activated Ca-currents in cultured DRG neurons of adult rat and guinea-pig. The currents were recorded using the whole cell patch clamp technique in solutions containing 10 mM Ba++. Capsaicin (10 uM) was applied for 15-30 sec.

The cross-sectional area of the cells which responded to capsaicin with an inward current varied widely and ranged from about 400 um^2 to about 3000 um^2. The peak of the distribution of areas was below 1000 um^2, for both species. The mean amplitude of the capsaicin evoked inward current was 0.67 pA/um^2, in cells maintained in culture up to 3 days. There was no difference between small and large cells, but in rat cells, we showed that the amplitude of the inward current decreased with time in culture. In guinea-pigs all recorded cells exhibited the high-threshold, L-type Ca-current while only 30% exhibited the low-threshold, T-type Ca-current. A correlation with cell size showed that the T-type Ca-current seemed to be restricted to those with larger diameters. Although capsaicin induced an inward current in about 26% of the cells with diameter greater than 1000 um^2, no cell with a capsaicin evoked inward current showed a T-type Ca-current. Supported by P.H.S. Grant NS 14624

103.5

MULTIPLE ACTIONS OF CAPSAICIN ON SPINAL SENSORY SYSTEMS. L. Urban and A. Dray. Sandoz Institute for Medical Research, 5 Gower Place, London WC1E 6BN, England.

An action on primary afferent neurons at the spinal cord level is important for the acute antinociception produced by capsaicin (Ashwood et al J.Physiol. 1990, 423, 77P). We now show, using a mouse spinal cord-dorsal root ganglion (DRG) preparation maintained in vitro, that several mechanisms may contribute to the capsaicin induced antinociception.

Bath applied capsaicin (100-400nM) depolarized and enhanced synaptic activity in deep spinal dorsal horn neurons. These effects were abolished by removal of extracellular calcium but activation of primary afferent fibres persisted. In the DRG, capsaicin (100nM) depolarized and evoked action potentials in C-neurons. Higher concentrations also depolarized Aδ neurons without evoking APs. Larger A-neurons were generally insensitive, though 2-10µM capsaicin sometimes produced a brief hyperpolarization followed by a depolarization. Electrical stimulation of dorsal roots often failed to evoke APs during the depolarization of small or large cells.

These observations confirm that capsaicin acts on primary afferent elements and not directly on intrinsic spinal neurons. The propagation of nociceptive signals generated from the periphery may therefore be impaired by a) capsaicin-induced depolarization of afferent nerve terminals b) possible conduction block in dorsal root fibres c) block of spike generation due to the slow depolarization of small DRG neurons.

103.6

RESPONSES OF DORSAL HORN NEURONS IN CATS TO INTRACUTANEOUS INJECTIONS OF HISTAMINE AND CAPSAICIN. H. Hirata, H. Uchida*, K. Kishikawa, J.G. Collins, and R.H. LaMotte. Dept. of Anesthesiology., Yale Univ. Sch. of Med., New Haven, CT 06510

Previous psychophysical studies demonstrated that histamine injected intradermally in humans produced a pure sensation of itch that can last several minutes and is not contaminated by other sensory qualities such as pain. Thus, by the use of this chemical we hoped to find central neurons mediating itch.

Seventeen dorsal horn neurons (DHNs) (11 wide dynamic range:WDR; 6 high threshold:HT) were studied in pentobarbital anesthetized cats via a chronically implanted lumbar spinal cord recording chamber. Two HT neurons responded to intracutaneous injections of histamine and subsequently to capsaicin for at least 3 min after injection. None of the other 13 nociceptive neurons (9 WDR, 4 HT) tested responded to histamine, although all but 2 responded vigorously to capsaicin. Lightly stroking the skin surrounding the injection, known to produce itch in humans, did not evoke enhanced responses in most neurons. However, one HT neuron that responded to histamine had unusually long and vigorous afterdischages that outlasted each stimulus. The depth of recording sites from the surface of the spinal cord for units responding to histamine was shallow (730 µm for one unit) while the average depth for the units not responding to histamine was deeper (1457 µm; n=6).

These results suggest the existence of a subpopulation of nociceptive DHNs responsive to intracutaneous injection of "itch" producing chemicals. How activity in these nociceptive DHNs contributes to itch sensation is yet to be determined. (Supported by PHS grant 14624 and NIH grant GM29065).

103.7

INFLAMMATORY MEDIATORS ENHANCE THE EFFECTS OF CAPSAICIN AND OLVANIL ON PERIPHERAL NOCICEPTORS. A.Dray, L.Urban, I. Patel*, A. Rueff* and M. Perkins. Sandoz Institute for Medical Research, Gower Place, London, England.

Capsaicin and its analogue olvanil (NE-19550) have similar antinociceptive potency in vivo (Campbell et al, Br. J. Pharmac. 1989, 98, 907P). However capsaicin activates peripheral nociceptors (EC_{50} 300nM) and at 20-50μM concentrations reduces their responsiveness to noxious stimuli whereas similar concentrations of olvanil are inneffective (Bettaney et al, J. Physiol. 1990, press). We now show that inflammatory mediators enhance the effects of both substances.

In a preparation of the neonatal rat spinal cord with the functionally connected tail maintained in vitro, activation of peripheral fibres by noxious stimuli (capsaicin, bradykinin, heat) was assessed by recording a spinal ventral root response. In the presence of sensitizing concentrations of PGE_2 (0.5-5μM), capsaicin-induced activation of peripheral fibres was enhanced and low concentrations of capsaicin (2μM) now reduced responses to other noxious stimuli. Under these conditions olvanil (100-500nM) activated peripheral fibres and at 50μM, produced a prolonged attenuation of responses to other noxious stimuli. PGI_2 produced similar but less consistent effects whereas PGD_2 was inactive.

These data suggest that specific products of inflammation enhance the actions of capsaicin and olvanil. This may contribute to the efficacy of capsaicin-like compounds as antinociceptive and antiinflammatory agents.

103.8

THE CONSEQUENCES OF LONG TERM TOPICAL CAPSAICIN APPLICATION IN THE RAT. S McMahon*, G Lewin. St Thomas' Hospital Medical School (UMDS), London, UK. (SPON: BRA)

Capsaicin has long been used as an experimental tool and more recently as a clinical treatment for several pain disorders where it is usually administered topically for periods of several weeks. It is the consequences of such treatment that we have studied here in the rat. Capsaicin cream (0.075% or 0.75%), or vehicle, was applied twice daily to the hindpaws of rats for ten weeks. The hindpaws treated with 0.75% capsaicin (but not 0.075%) became transiently hyperalgesic, but there were no signs of discomfort or distress associated with the treatment. After ten weeks of treatment, C-fibres produced significantly less neurogenic extravasation. This fully returned in 0.075% capsaicin treated animals after 4 weeks recovery, but only partially returned in the 0.75% group after 12 weeks. The levels of substance P and CGRP in the sural nerve supplying the treated skin area were unchanged after the 0.075% capsaicin treatment. One interpretation of these results is that topical capsaicin application produces a reversible block of the terminals of C-fibres in the skin, but at low concentrations does not have actions on the cell soma.

The number of afferent neurones in the L5 dorsal root ganglion projecting through the sural nerve was unchanged after 0.75% capsaicin treatment, suggesting that the topical capsaicin treatment does not produce any cell death in the adult animal.

103.9

NEUROPEPTIDE DEPLETION BY NEONATAL CAPSAICIN CORRELATES WITH ALTERATIONS IN VISCERAL AND CUTANEOUS NOCICEPTION. T.J.Ness, V.Kumar* and G.F.Gebhart. Depts. Anesthes. and Pharmacol., College of Medicine, Univ. of Iowa, Iowa City, IA 52242.

Newborn (48 hrs) Sprague-Dawley rats were injected s.c. with vehicle (VEH) or capsaicin (CAP; 50 mg/kg in VEH). At 10 weeks of age the tail flick latency (TF_L; time (s) from onset of tail heating to withdrawal reflex), hot plate latency (HP_L; time (s) from placement on 55°C hotplate to licking of hindpaw), visceromotor threshold (VM_T; minimal pressure (mm Hg) in colorectal distending balloon evoking abdominal contracture) and cardiovascular responses to colorectal distension (80 mm Hg, 20 s; change in mean arterial pressure (Δ MAP in mm Hg); change in heart rate (Δ HR in bpm) measured via chronic femoral arterial catheters) were determined. Following testing, the content of substance P (sP), calcitonin gene-related peptide (CGRP) and vasoactive intestinal polypeptide (VIP) of the lumbar spinal cord was determined using radioimmunoassay (expressed as pg/mg spinal cord tissue). The results are as follows:

	sP	CGRP	VIP	TF_L	HP_L	VM_T	Δ MAP	Δ HR
CAP	38±4*	301±28*	10.7±.5	4.5±.4*	17±3*	45±5*	36±3	76±7
VEH	76±6	614±29	10.9±.7	3.4±.2	12±1	24±2	38±2	69±8

Neonatal capsaicin leads to significant (*$p<0.05$) sP and CGRP depletion and increased thresholds for motor responses to noxious visceral and cutaneous stimuli.

103.10

THE ENDOTHELIUM-DERIVED RELAXING FACTOR (EDRF) CANDIDATE S-NITROSOCYSTEINE (S-NC) ACTIVATES CAPSAICIN SENSITIVE AFFERENTS IN CONSCIOUS RATS. M.J. Brody, S.J. Lewis, S.T. Meller, T.J. Ness, J.N. Bates* and G.F. Gebhart. Dept. of Pharmacology & Cardiovascular Ctr., Univ. of Iowa, Iowa City, IA 52242.

Recent evidence suggests that S-NC may be an endogenous EDRF. The aim of the present study was to examine the cardiovascular effects of S-NC in conscious rats. The injection of S-NC (25-1000 μg/kg, iv) produced reductions in arterial pressure (AP) via reductions in peripheral vascular resistance which were accompanied by tachycardia. These responses were essentially identical to those produced by equimolar doses of sodium nitroprusside (SNP), an agent whose actions may involve release of nitric oxide. Higher doses of S-NC (1500-2500 μg/kg, iv), but not SNP, produced profound atropine-sensitive rapid reductions in AP, heart rate and regional blood flows which were similar to the cardiopulmonary afferent mediated responses produced by serotonin. The transient S-NC-induced cardiovascular responses were markedly diminished in rats treated neonatally with capsaicin. These results suggest that S-NC but not SNP produces a reflex reduction in arterial pressure via stimulation of small diameter unmyelinated C-fibre afferents originating from cardiopulmonary regions.

BASAL GANGLIA AND THALAMUS I

104.1

LOCATION OF KAINIC ACID LESION WITHIN THE BASAL GANGLIA DETERMINES THE DIRECTION OF ROTATION IN RESPONSE TO APOMORPHINE. L.M. Wyatt, P.R. Sanberg, J.P. Hildebrand*, and A.B. Norman. Division of Neuroscience, Departments of Psychiatry and Anatomy, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Quantification of drug-induced rotation behavior after unilateral lesions is a commonly used means of assessing dysfunction and recovery of the basal ganglia. Kainic acid (KA) destroys intrinsic neurons of the striatum that express dopamine receptors. Consequently, after unilateral lesion, only the intact striatum is capable of responding to dopamine agonists such as apomorphine. This unequal response leads to an asymmetry in locomotion which is usually manifested as rotation behavior towards the side of the lesion. However, we have previously reported that rats given KA lesions rotated contralateral to the side of the lesion. Close histological examination of the lesion site indicated that these rats had lesions of the anterior striatum and within the nucleus accumbens. We, therefore, determined if the KA lesion site could influence the direction of rotation subsequent to apomorphine challenge. Male Sprague-Dawley rats received unilateral KA lesions at either 1.5 or 0.3 mm anterior to bregma. There was a significant difference in the direction of apomorphine (1 mg/kg s.c.)-induced rotations with respect to A/P location of the lesion ($p=0.037$) and also in the total number of rotations for rats rotating in each respective direction ($p<0.001$). The more anterior lesions produced contralateral rotations while the posterior lesions produced ipsilateral rotations. Thus, the site of the lesion within the basal ganglia determines the direction of apomorphine-induced rotation behavior. [supported by NINDS]

104.2

CHANGES IN CIRCLING PERFORMANCE AND STRIATAL UNIT ACTIVITY IN FREELY MOVING RATS LESIONED WITH 6-HYDROXYDOPAMINE AND TRANSPLANTED WITH FETAL DOPAMINE CELLS. P. Patino, M. Garcia-Munoz, C.J. Hutt*, E.H. Kriek, and C.R. Freed. Depts. of Med. and Pharm., U. Colo. Sch. Med., Denver, CO.

Studies in our laboratory have shown that rats trained to run on a circular treadmill show increased firing of ventromedial striatal cells particularly on the side contralateral to the circling direction. We have now studied the effect of dopamine depletion on behavioral performance and cell firing. Male Sprague-Dawley rats were unilaterally lesioned in the nigrostriatal bundle with 6-hydroxydopamine (6-OHDA). Normal animals circled at 31 + 2 rpm by the third day of training. Lesioned animals circled at 14 + 4 rpm ipsilateral and 13 + 4 rpm contralateral to the lesion only after 11 days of training. Animals lesioned then transplanted circled at 21 + 2 rpm ipsilateral and 29 + 3 rpm contralateral after 6 days of training. Unit recording from ventromedial striatum showed a basal firing rate of 0.7 hz which increased to 3.4 hz during contralateral circling and 2.2 hz with ipsilateral circling. By contrast, lesioned animals showed increased basal firing of 5.0 hz which fell to 1.3 hz when animals circled either contralateral or ipsilateral to the side of the recording. We conclude that unilateral 6-OHDA lesions change behavioral performance and striatal firing which is partly corrected by fetal dopamine cell implants.

104.3

EFFECTS OF STRIATAL, TECTAL, AND THALAMIC LESIONS ON CIRCLING ELICITED BY INTRANIGRAL INJECTION OF THE KAPPA OPIATE U50,488. L. A. Thompson and J. M. Walker. Dept. of Psychology, Brown University, Providence, RI 02912.

Striatonigral prodynorphin opioids may influence movement through actions on kappa receptors in the substantia nigra pars reticulata (SNR). Previous experiments indicate that intranigral microinjection of the kappa opiate U50,488 produces dose-dependent contralateral rotation due to inhibition of non-dopaminergic nigral efferents. In this study, the relationship between movement induced by intranigral U50,488 and output stations of the SNR was examined. Unilateral ibotenic acid lesions of the superior colliculus (SC), ventromedial thalamus (VM), or striatum (STR) were made in the hemisphere ipsilateral to the SNR injected with U50,488. No lesions were made in control animals.

Tectal, thalamic, and striatal lesions differentially influenced kappa opiate-induced motor activation. Lesions of the SC and VM tended to decrease circling compared to control animals, while striatal lesions tended to increase circling. These results suggest that nigral kappa opioids may influence movement through actions on the nigrotectal and nigrothalamic pathways. Striatal lesions may increase circling due to kappa receptor supersensitivity in the SNR or due to interference with the inhibitory effect of kappa opioids on the nigral dopamine system.

104.4

GRAFT-INDUCED RECOVERY OF FUNCTION FOLLOWING EXCITOTOXIC LESIONS OF THE STRIATUM, IN A VISUAL REACTION TIME TASK IN THE RAT. E. Mayer*, V.J. Brown, S.B. Dunnett & T.W. Robbins. Department of Experimental Psychology, University of Cambridge, Cambridge, CB2 3EB, U.K.

Rats were trained to move their heads away from an eccentrically presented visual stimulus, before receiving unilateral excitotoxic lesions of the striatum governing their preferred side. Testing recommenced six months later when profound deficits in response bias and stimulus control of responding were evident in animals having received lesions alone. They also exhibited attentional changes secondary to their tendency to over respond towards the side of their lesion. By contrast rats that had received implants of E15 embryonic striatal cell suspensions, subsequent to lesioning, showed recovery in certain aspects of their performance, which also precluded the attentional changes found in the lesion group. However the striatal graft group did show an initial lengthening of time taken to complete the head movement away from the side of the lesion.

The results indicate the utility of employing a complex reaction time procedure, with poor stimulus-response compatibility, not only for the study of normal striatal function, but also for assessing the function of grafted striatum and its functional integration into the host.

104.5

PLASTICITY OF FORELIMB-RELATED STRIATAL ACTIVITY DURING ACQUISITION AND MAINTENANCE OF A TONE-DISCRIMINATION TASK IN THE RAT. R.M. Carelli, Wolske, M. and M.O. West. Dept. Psychology, Rutgers Univ., New Brunswick, NJ 08903.

The objective of the present study was to determine the relationships among right forelimb-related unit activity, biceps EMG activity and lever pressing behavior during the acquisition and maintenance of a tone-discrimination task involving lever pressing with the right forelimb. Long-Evans male rats (300g) were chronically implanted with a detachable microdrive for recording in the lateral striatum (+2.0 to 0 mm A-P, 3.6 to 4.5 mm M-L, and 3.0 to 6.0 mm D-V from bregma). Water-deprived rats were trained to obtain water reinforcement by lever pressing with the right (contralateral) forelimb during tone presentation (one reinforcement/tone presentation). To reduce trial-to-trial variability in reaching toward the lever, rats were trained to stand facing the lever, with the right forepaw on the floor prior to initiation of each trial. Each day, forelimb-related cells (n=114) were identified by means of passive examination. Increased discharge time-locked to lever pressing (relative to background firing) was termed "Signal:Noise" (S:N). Results indicate that forelimb-related striatal activity varied as a function of number of days on the task. S:N was large (range 2-16) on days 1-4, during early acquisition, but decreased substantially and remained low (range 0.5-1.5) on days 5-14, during maintenance of stable responding. In contrast, biceps EMG remained related to reaching toward the lever throughout acquisition and maintenance. These findings suggest that striatal activity may participate in movement execution during the acquisition of skilled movements, but not during their routine maintenance when they are repeated in the same manner across days of overtraining. Supported by NSF BNS-8708523, DA 04551 and PHS RR 07058-21.

104.6

ANTERIOR LESIONS OF THE CORPUS STRIATUM PRODUCE A DISRUPTION OF STEREOTYPED GROOMING SEQUENCES IN THE RAT. H.C. Cromwell and K.C. Berridge. The University of Michigan, Department of Psychology, Ann Arbor, MI., 48109.

The corpus striatum (caudate nucleus, putamen, and globus pallidus) has been suggested to be crucial for the sequencing of actions into motor programs (Marsden, 1984, Cools, 1980). Even natural grooming sequences become sequentially disordered after large striatal lesions (Berridge and Fentress, 1987). Small bilateral lesions were made into the corpus striatum to localize the subregion involved in producing the disruption.

Microinjections of quisqualic acid or kainic acid were made in the anterior portion of the caudate nucleus and putamen in the dorsolateral region, and into the posterior caudate nucleus, putamen, and globus pallidus in the dorsolateral, ventromedial, and dorsomedial regions. Control animals had surgical procedures identical to lesioned animals with only vehicle injected. Grooming was videotaped. The focus of the evaluation was upon syntatic sequential transition pattern between face and body grooming that organizes up to 25 actions in to 4 distinct phases (Berridge and Fentress, 1987).

Rats with anterolateral lesions completed the four phases of the grooming only 48% of the time while controls had a 80% completion rate. Rats with posterior lesions did not differ from controls. Nonsyntatic transitions from face to body grooming were preserved. This significant decrease in the ability to sequence the 4 phases of the grooming pattern is consistent with the findings that lateral lesions of the rostral striatum impair coordinated forelimb patterns, (Pisa, 1988) and supports a special role for this region in controlling sequences.

104.7

SINGLE UNIT ACTIVITY IN THE RAT CAUDATE-PUTAMEN DURING STEREOTYPICAL GROOMING SEQUENCES. J.W. Aldridge, K.C. Berridge, M. Herman. Univ. Michigan, Dept. Neurology, Dept. Psychology, 1103 E. Huron, Ann Arbor, MI 48014.

The purpose of this study was to investigate the neural correlates of neostriatal activity and instinctive behavior. In rodents, a syntactical chain of movements connects up to 25 actions in a stereotypical transition between face and body grooming. Single unit activity was recorded from the neostriatum (Bregma AP 1.0) in 13 rats while behavior was videotaped. Unit activity was compared during 1) syntactic grooming chains, 2) isolated grooming movements, 3) body-orienting movements (stepping, rearing, head-turning) and 4) cutaneous and visual sensory tests. Of 25 units, 14 (56%) had unit activity related to at least one behavior studied. The most frequent response (9 units, 64%) was to grooming during the syntactic chain. These units generally did not respond in the same way to similar grooming actions that occurred outside of the grooming chain. Most commonly, chain grooming units had a slow decreased tonic discharge at the onset of grooming and a phasic increase in activity at the end of chain grooming. Five units (36%) had responses to grooming movements that occurred apart from syntactic chain grooming. Body orienting and sensory stimulation each activated 50% (7/14) of the responsive units. In contrast to syntactic chain units, units with non-chain grooming, body orienting and sensory responses typically had responses in more than one behavioral category. In summary, a significant proportion of neostriatal units are functionally related to a particular sequential pattern of instinctive movements. These units do not have a functional overlap with other striatal units that related to elemental actions or sensory events. Supported by the University of Michigan Biomedical Research Council and Office of the Vice President for Research.

104.8

BILATERAL LOCAL METABOLIC EFFECTS INDUCED BY HIGH CONCENTRATION OF MUSCIMOL INJECTED INTRANIGRALLY. H.E. Savaki, V. Raos* and C.R. Dermon*. Lab. of Physiology, Div. of Medicine, Univ. of Crete, 71409 Iraklion, Greece.

The bilateral effects of unilateral intranigral muscimol (1 M) injection on local cerebral glucose utilization were investigated, by means of the autoradiographic 14C-deoxyglucose method, in conscious awake rats. Intranigral injections of 1 M muscimol induced: [1] increased glucose consumption within the injected substantia nigra reticulata, [2] bilateral metabolic decrease within collicular, thalamo-cortical and striatal relays, with the ipsilateral side displaying more pronounced depression. Previous studies have demonstrated that lower concentrations (10-6, 10-3M) of muscimol applied intranigrally induce disinhibition of nigral efferents through presynaptic GABAergic autoreceptors on the striato-nigral terminals (Dermon, C.R., et al., J. Neurosci. in press). It is presently suggested that muscimol applied intranigrally in higher concentration (1 M) induces inhibition of nigro-thalamic and nigro-collicular efferent pathways through postsynaptic GABAergic receptors on the nigral cells.

104.9

CHRONIC LEVODOPA ALTERS BASAL AND DOPAMINE AGONIST STIMULATED CEREBRAL GLUCOSE UTILIZATION. T.M. Engber, Z. Susel*, S. Kuo* and T.N. Chase. Exptl. Therapeutics Branch, NINDS, Bethesda, MD 20892.

The effect of chronic levodopa administration on the functional activity of the basal ganglia and its output regions was evaluated by means of the 2-deoxyglucose (2-DG) autoradiographic technique in rats with a unilateral 6-hydroxydopamine lesion of the nigrostriatal pathway. The rates of local cerebral glucose utilization were studied under basal conditions as well as in response to challenge with a selective D1 or D2 dopamine receptor agonist. Levodopa (100 mg/kg/day, i.p.) was administered for 19 days either continuously via infusion with an osmotic pump or intermittently by twice daily injections. Following a 3 day washout, glucose utilization was decreased by both levodopa regimens in the nucleus accumbens; intermittent levodopa also decreased glucose utilization in subthalamic nucleus, ventrolateral thalamus, ventromedial thalamus and lateral habenula. In control rats (lesioned and treated with saline), the D1 agonist SKF 38393 (5 mg/kg, i.v.) increased 2-DG uptake in the ipsilateral substantia nigra pars reticulata and entopeduncular nucleus by 84% and 56%, respectively. Both continuous and intermittent levodopa blunted the SKF 38393 induced elevation in glucose metabolism in the substantia nigra pars reticulata, while intermittent levodopa also attenuated the increase in the entopeduncular nucleus. The D2 agonist quinpirole (0.4 mg/kg, i.v.) did not increase glucose utilization in any brain region in controls; following intermittent levodopa treatment, however, quinpirole increased 2-DG uptake by 64% in the subthalamic nucleus and by 39% in the deep layers of the superior colliculus on the ipsilateral side. These findings indicate that chronic levodopa administration has long lasting effects on the functional activity of brain regions within the basal ganglia as well as in regions which are targets of basal ganglia output and that the effects of chronic levodopa are dependent on the treatment regimen. Our results further suggest that chronic levodopa treatment differentially alters D1 and D2 receptor mediated striatal output, decreasing D1 output through the striatonigral and striatoentopeduncular pathways and increasing D2 output through the striatopallidal pathway.

104.10

MICRODIALYSIS IN AWAKE RATS SUGGESTS THAT THE SUBSTANTIA NIGRA IS THE MAJOR SITE OF ACTION OF L-DOPA IN A RAT MODEL OF PRECLINICAL HEMIPARKINSON'S DISEASE. Dora Oroscz (1) and James P. Bennett, Jr. (1,2,3). Departments of (1) Neurology, (2) Behavioral Medicine and Psychiatry (2) and Pharmacology (3). University of Virginia School of Medicine, Charlottesville, VA 22908.

Idiopathic Parkinson's disease (PD) in humans arises from progressive, marked loss of midbrain dopamine (DA) neurons in the substantia nigra compacta (SNC), whose axons form the nigrostriatal pathway (NSP) and innervate the corpus striatum (caudate-putamen). These nigral neurons also release DA from their dendrites into the nigra reticulata (SNR) where DA interacts with D1 receptors on terminals of striatonigral neurons. L-DOPA therapy of PD seeks to replace lost DA. In earlier microdialysis work in urethane anesthetized rats, we showed that pharmacological L-DOPA doses did not lead to increased DA in striatal extracellular fluid (ecf) in either control striata or striata ipsilateral to a 6-hydroxydopamine (6-OHDA) lesion of the NSP. Because L-DOPA treatment of rats with unilateral 6-OHDA NSP lesions markedly increases glucose utilization in SNR, we sought to determine if L-DOPA selectively raised ecf DA in the nigra compared to striatum.

Adult male rats had either sham or 6-OHDA lesions of the NSP, followed one week later by implantation of dialysis probe holders above ipsilateral dorsolateral striatum and SNC. After recovery, simultaneous dialysis of striatum and SNC was performed while the animal's activity was electronically monitored, before and after carbidopa 25 mg/kg i.p./L-DOPA methylester 25 mg/kg i.p. Baseline DA ecf levels in striatum were several fold higher than in SNC. Whereas striatal ecf DA levels at most doubled after L-DOPA, nigral ecf DA increased 15-20 fold, reaching peak levels twice those of striatum. After L-DOPA, nigral DA receptors may be exposed to DA levels higher than in striatum and may be the preferential site of action of L-DOPA in this animal model of PD.(Supported by NINDS NS26581, RCDA to JPB and the American Parkinson Disease Association)

104.11

THE EFFECT OF MEDIAL FOREBRAIN BUNDLE (MFB) STIMULATION ON STRIATAL DOPAMINE AND SEROTONIN RELEASE AS MEASURED BY MICRODIALYSIS. L. D. Manley, R. Kuczenski, S. J. Young, D. S. Segal and P. M. Groves. Dept. of Psychiatry, University of California, San Diego, Sch. of Med, La Jolla, CA 92093.

Intracerebral microdialysis was used to examine the effect of MFB stimulation on extracellular concentrations of dopamine (DA), serotonin (5HT) in rat striatum. Urethane-anesthetized Sprague-Dawley rats were implanted with a 4mm concentric dialysis probe and a bipolar stimulating electrode. Ninety minutes after implantation, baseline concentrations were assessed by HPLC-EC at ten minute intervals for an additional hour before stimulation. Equal numbers of stimuli (1mA, 0.5ms; 5-20 per 1.2 sec: 4-33 Hz) were presented continuously or in equally spaced bursts of 75-600ms duration over the ten minute period. Measurements were made both in the absence and presence of cocaine in the perfusion buffer, so as to achieve an extracellular cocaine concentration of 2.5 mM. Cocaine increased basal extracellular DA levels from approximately 15 to 75nM and 5HT from below levels of quantification to approximately 7nM. MFB stimulation linearly increased DA concentrations, both in the presence and absence of cocaine, in a frequency dependent manner. 5HT concentration was also linearly dependent on the frequency of stimulation. The concentrations of both neurotransmitters rapidly returned to baseline levels upon cessation of stimulation. Stimuli delivered as a burst caused enhanced DA, but not 5HT, release as compared to stimuli presented in a continuous fashion. This work was partially supported by NIDA grant numbers DA02854, DA04151, DA01568.

104.12

CONTRASTING TISSUE FACTORS PREDICT HETEROGENEOUS STRIATAL DOPAMINE (DA) NEUROTOXICITY AFTER MPTP OR METHAMPHETAMINE TREATMENT. J.F. Marshall and R.J. Navarrete*. Dept. of Psychobiology, Univ. California, Irvine, CA 92717.

Recent studies have demonstrated the heterogeneous striatal distribution of markers (e.g.[^{3}H]mazindol binding) for the high-affinity DA transport (HADT) in several species. In idiopathic Parkinson's disease, the extent of the DAergic degeneration varies greatly between striatal regions; similar differences in susceptibility to the neurotoxic effects of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) have been described in treated primates, mice, and carnivores. The present work examines whether the regional variations in dopaminergic injury relate to those of the HADT. In male Swiss-Webster mice given four injections of MPTP HCl (20 mg/kg, i.p.), the extent of the DA depletion, as measured in 11 micropunch regions of the striatal complex, corresponded closely ($r = +0.82$, $P < 0.005$) to the specific [^{3}H]mazindol binding and less well to the DA content ($r = +0.50$, N.S.) of these regions in intact mice. In contrast, the striatal DA depletions of a separate group of mice treated with a neurotoxic regimen of methamphetamine HCl (2.5 mg/kg, i.p., 4 times) corresponded to the regional DA content ($r = +0.81$, $P < 0.005$) but not to the [^{3}H]mazindol binding values ($r = +0.50$, N.S.) in intact mice. Thus, the concentration of HADT may determine the susceptibility of mouse striatal DA terminal populations to MPTP but not to methamphetamine.

104.13

ENHANCED POSTSYNAPTIC DOPAMINE EFFECTS AFTER CHRONIC COCAINE. S.P. Banerjee, E. Alter*, M. Nathan, T.I. Lidsky CUNY Med and NYS IBR.

Chronic use of cocaine (Coc) alters the pharmacology and physiology of dopamine (Da) systems. The present study investigated the nature of some of these changes.

After 7-9 days of Coc (10 mg/kg/day, i.p.), striatal somatosensory field potentials were recorded in urethane anesthetized rats. Rats that received Coc showed enhanced sensitivity to the post-synaptic effects of Da as evidenced by suppression of striatal field potentials at lower than normal intensities of substantia nigra (SN) stimulation. In addition, haldol, which mainly acts presynaptically in controls (1), had strong postsynaptic effects in Coc exposed rats. Specifically, i.v. haldol (0.1 mg/kg) in controls attenuated striatal potentials (as did Da release from SN stimulation) and failed to block the inhibitory effects of SN stimulation. In Coc rats, haldol alone had no effect on striatal fields and attenuated the inhibitory influence of SN stimulation. These changes were functional; increased receptor binding was not detected.

1. Lidsky, Cosentino & Banerjee; Neurosci. Abst. 1989, 15.

104.14

NEURONAL ACTIVITY RELATED TO EXPECTATION OF EXTERNAL SIGNALS AND REWARD IN DORSAL AND VENTRAL STRIATUM OF THE MONKEY. P. Apicella*, E. Scarnati*, W. Schultz* and T. Ljungberg* (SPON: European Neuroscience Association). Institut de Physiologie, Univ. Fribourg, CH-1700 Fribourg, Switzerland.

The striatum receives afferents from cortical association areas and limbic structures, suggesting its involvement in cognitive and motivational processes. We investigated neuronal activity in dorsal striatum (caudate nucleus and putamen) and ventral striatum (anteroventral putamen and nucleus accumbens) in relation to predictable environmental events.

Three monkeys were trained in a delayed go-nogo task in which they were required to make a visually-triggered arm movement (go) or to withhold it (nogo) according to the color of a preceding instruction light. Correct go and nogo responses were rewarded with liquid. Impulses were recorded from striatal neurons together with electromyograms of mouth and arm muscles.

Two types of anticipatory neuronal changes were observed: 1) Activations preceding the onset of trigger signal or movement were more frequently found in dorsal (82/542, 15%) than in ventral striatum (10/213, 5%). This activity could be related to expectation of trigger signal or preparation of limb movement. 2) Activations preceding the reward in both go and nogo trials were seen in both dorsal (78/542, 14%) and ventral (38/213, 18%) striatum. They may reflect the expectation of the appetitive consequence of the behavioral response.

Thus, neurons in dorsal and ventral striatum are activated in advance of predictable, specific events. The fact that most neurons with anticipatory activity in ventral striatum are related to reward may suggest that this region is particularly involved in the expectation of events with motivational properties.

104.15

POSSIBLE ROLE OF NIGRAL GABA-T IN STRAIN DIFFERENCES IN VACUOUS CHEWING MOVEMENTS. S.E. Bachus, A. Summerfelt* & C.A. Tamminga, Maryland Psychiat. Res. Ctr., P.O. Box 21247, Baltimore, MD, 21228.

Rat strains differ[1] in incidence of chronic neuroleptic-induced vacuous chewing movements (VCM), which have been proposed as an animal model for tardive dyskinesia[2]. Since we have implicated a GABAergic deficit in VCM[2,3], we have compared levels of activity of the GABA synthetic enzyme glutamate decarboxylase (GAD), and the GABA degradative enzyme GABA transaminase (GABA-T) in basal ganglia regions among rat strains that vary in VCM rates: Long-Evans (LE) (high), Wistar (W) (variable) and Sprague-Dawley (SD) (low).

Samples were dissected bilaterally from striatum, globus pallidus, substantia nigra (SN) and the nigrotegmental target area, and assayed radioenzymatically for GAD[4]. SN was large enough to also enable us to use an enzymatic-fluorometric assay for GABA-T[5]. Statistics were by MANOVA.

For GAD across brain regions, there was a strain effect ($p \leq .001$), and also a strain by region interaction ($p \leq .001$), indicating different profiles of GAD across regions for the 3 strains. Within SN, there was also a strain effect ($p \leq .001$) and a strain by enzyme interaction ($p \leq .05$) for GAD and GABA-T. The SD rats, with the lowest VCM, for which we had predicted protection of nigral GABA by high GAD or low GABA-T, were lowest in both nigral GAD (81% LE, 89% W) and GABA-T (74% LE, 79% W). There was a significant correlation between VCM and nigral GABA-T ($p \leq .05$), suggesting that the latter may be relevant for development of VCM. To assess the net effect on synaptic GABA of changes in GAD and GABA-T, we are now using in vivo intracerebral microdialysis to estimate nigral synaptic GABA.

[1] Dale et al., Soc Neurosci Abst 15:271, 1989. [2] Gunne et al., Exp Neurol 100:459, 1988. [3] Shirakawa et al., Soc Neurosci Abst 15:272, 1989. [4] Sims & Pitts, J Neurochem 17:1607, 1970. [5] DeBoer & Bruinvels J Neurochem 28:471, 1977.

104.16

WHY DO CATS RECOVER FROM THE EFFECTS OF MPTP? A NEUROCHEMICAL ASSESSMENT. D.S. Rothblat, G.V. Smith*, L. Cerone*, and J.S. Schneider. Dept. of Neurology, Hahnemann University School of Medicine, Philadelphia, PA. 19102.

Administration of MPTP to cats results in a parkinsonian syndrome characterized by rigidity, akinesia, extensive loss of striatal dopamine (DA), and permanent loss of substantia nigra pars compacta neurons. Interestingly, cats show significant motor recovery by 4-6 weeks after MPTP. The present study was performed to assess the neurochemistry of the acute and recovery phases of the MPTP-induced syndrome in the cat in greater detail. Cats with severe parkinsonian symptoms and motor impairment (as assessed by several behavioral/motor and reflex tests) and sacrificed 7 days after 1 wk. of daily MPTP (5-7.5 mg/kg, s.c.) had DA losses of 96% in dorsal medial caudate (DM CD), 99% in dorsal lateral caudate (DL CD), and similar losses in ventral striatal regions. HVA levels were decreased to a slightly lesser degree in all striatal regions (ex., 94% and 93% decreases in DL and DM CD, respectively). By 6 wks. after MPTP, most animals had recovered motor function to a near normal level. In these animals, DA was decreased 87% in DM CD, aand 94% in DL CD, while HVA levels had recovered to a greater degree (ex., decreases of only 56% and 41% decreases in DL and DM CD). In contrast to the decreases in DA, serotonin (5-HT) levels were increased in dorsal striatal regions in both parkinsonian and recovered cats. 5-HIAA levels were decreased in parkinsonian animals but were normal in recovered animals. Analysis of amine metabolites in cisternal CSF samples taken during normal, parkinsonian, and recovered phases of the syndrome did not reflect the changes observed in striatal tissue samples. CSF metabolites seemed to reflect more the status of brainstem catecholamine activity. The results suggest that recovery from MPTP-induced parkinsonism in the cat may be due to a small increase in striatal DA with a significant increase in turnover and/or an increase in striatal 5-HT levels also accompanied by an increase in turnover. The apparent discrepancy between striatal tissue and CSF data will also be discussed.
Supported by NIH grant NS23980.

104.17

EFFECTS OF MPTP-INDUCED DOPAMINE DEPLETION ON STRIATAL AND PALLIDAL ELECTROPHYSIOLOGY IN PARKINSONIAN AND RECOVERED CATS. D.W. Duffield, D.S. Rothblat and J.S. Schneider, Dept. of Neurology, Hahnemann University School of Medicine, Philadelphia, PA. 19102.

Administration of MPTP to cats results in greater than 90% loss of dorsal striatal dopamine (DA), extensive loss of substantia nigra pars compacta neurons, and behavioral/motor impairment characterized by akinesia, rigidity, and decreased responsiveness to environmental stimuli. These behavioral deficits, while initially very severe, recover to a great extent by 6 wks. after MPTP exposure. The present study examines the electrophysiological correlates of the parkinsonian and recovery conditions in the dorsolateral caudate (DL CD) nucleus and the globus pallidus (GP) in awake cats. Two adult cats have been recorded from under normal and parkinsonian conditions and three cats have been recorded from under normal, parkinsonian, and recovered conditions. In the DL CD, mean spontaneous firing rates increased 47% while animals exhibited parkinsonian symptoms (with 99% DL CD DA loss) and reverted to near-normal rates during motor recovery (94% DL CD DA loss). In contrast to DL CD neurons, spontaneous firing rates of GP neurons decreased 62% during parkinsonism. In the normal DL CD, approximately 20% of neurons sampled responded to somatosensory or proprioceptive stimuli. In parkinsonian cats, approximately 5% of sampled neurons were responsive to such stimuli, while during recovery, this increased to approximately 15%. Responses to cortical and thalamic inputs during the MPTP syndrome have also been recorded and will be discussed as will pallidal responses to somatosensory and proprioceptive stimuli. Thus far, the data indicate that distinct changes in striatal and pallidal spontaneous firing rates and in responsiveness to external stimuli occur accompanying parkinsonian symptoms. As striatal neurochemistry changes during motor recovery, apparently so does the electrophysiology of the DL CD and GP. Supported by NIH grant NS 23980.

104.18

DEFICITS IN SPONTANEOUS EYE MOVEMENTS INDUCED BY UNILATERAL INFUSION OF MPTP IN THE MONKEY CAUDATE NUCLEUS. **S. Usui*, M. Kato*, A. Kori*, M. Matsumura*, N. Miyashita* and O. Hikosaka, National Institute for Physiological Sciences, Okazaki 444, Japan.**

To study the role of dopamine in the control of saccadic eye movements we injected MPTP unilaterally into the central part of the caudate nucleus of two Japanese monkeys. MPTP was infused using an osmotic mini-pump for a period of 1-2 weeks. Using the scleral search coil method, eye movements were recorded in three conditions: in light, dim and total darkness. The monkey sat in a chair facing a tangent screen.

After MPTP injection the frequency of saccades decreased and the range of eye movements decreased. The mean eye position shifted toward the side ipsilateral to the injection by 10-20 degrees. Saccades toward the side contralateral to the injection (contra-saccades) were fewer in number than ipsilateral saccades. Contra-saccades became significantly shorter in amplitude, slower, and longer in duration; changes in ispi-saccades were less pronounced. Immunohistochemistry of tyrosine hydroxylase (TH) showed that activity of the enzyme decreased locally around the injection site. The contralateral side was not affected. In the substantia nigra, TH activity was decreased in its rostral-dorsal part.

The present study indicates that unilateral dopamine deficiency produced suppression of spontaneous saccades toward the contralateral side. This could result from a change in the level of tonic GABAergic inhibition of the superior colliculus by the substantia nigra pars reticulata.

104.19

DEFICITS IN TASK-RELATED EYE MOVEMENTS INDUCED BY UNILATERAL INFUSION OF MPTP IN THE MONKEY CAUDATE NUCLEUS. **N. Miyashita*, M. Matsumura*, S. Usui*, M. Kato*, A. Kori*, T.W. Gardiner, O. Hikosaka National Institute for Physiological Sciences, Okazaki 444, Japan.**

In two monkeys treated unilaterally with MPTP in the caudate nucleus, we examined two types of saccadic eye movements: visually guided saccades (visual-saccade) and memory-guided saccades (memory-saccade). The target was turned on at the same time (visual-saccade) or 600 ms after (memory-saccade) the central fixation point went off. In the memory-saccade task, the position of the future target was flashed while the monkey was fixating. The position of the target was chosen randomly out of 32 possible locations on the screen.

Changes in saccades during these tasks began to appear 2-3 days after starting MPTP injection, along with changes in spontaneous eye movements. Saccades to targets contralateral to the injection became delayed in latency, slower in peak velocity, and hypometric. The changes were more marked for memory-saccades than for visual-saccades. Memory-saccades to contralateral targets were sometimes misdirected toward the ipsilateral side.

These results suggest that dopamine plays an important role in the initiation of task-specific saccades, especially those guided by the memory of target position. This effect could be explained by a blockade of saccade-related signals in the caudate that would normally be transmitted to the superior colliculus through the substantia nigra pars reticulata.

104.20

RESPONSES OF MONKEY DOPAMINE NEURONS TO EXTERNAL STIMULI DEVELOP DURING LEARNING AND DECREASE AFTER OVERTRAINING. W. Schultz*, T. Ljungberg* and P. Apicella* (SPON: European Brain and Behavior Society). Institut de Physiologie, Univ. Fribourg, CH-1700 Fribourg, Switzerland

Previous studies have shown that dopamine (DA) neurons respond to external stimuli with known behavioral significance. This study investigated how responses of DA neurons change when the behavioral significance of stimuli was modified with task acquisition and overtraining.

We recorded impulses from DA neurons with typical electrophysiological characteristics from areas A8, A9 and A10, in 2 awake Macaca fascicularis monkeys, and obtained the following results: (1) Before behavioral reactions were demanded, DA neurons (n=130) typically lacked responses to opening of a small door and to illumination of a light located ahead of them at eye level and at reaching distance. (2) When a small food morsel was presented behind the opening door, monkeys reached out for it, and most of 76 further DA neurons responded with a short burst of impulses to door opening, but lacked responses to the still neutral light. (3) Then the light was used to trigger an arm movement for touching a lever located immediately below the light for liquid reward. Most of 67 DA neurons responded to light illumination in a similar way as to door opening. (4) During acquisition of this task, half of 21 DA neurons responded to the delivery of liquid reward. These responses were lost after task acquisition. (5) After extensive training with 30 000 trials, the task was performed faster, but only 34 % of 82 DA neurons showed significant responses, which also had lower magnitudes.

The present data show that DA neurons respond predominantly to salient external stimuli that are associated with behavioral reactions and require the attention of the animal. Responsiveness is reduced with more automatic task performance, suggesting that DA neurons are less activated during routine or habitual behavioral acts.

105.1

SUBDIVISIONS OF THE SUBSTANTIA NIGRA PARS RETICULATA (SNr) REVEALED BY CYTOCHROME OXIDASE (CO) HISTOCHEMISTRY: CORRELATION WITH LEVELS OF GLUTAMIC ACID DECARBOXYLASE (GAD) GENE EXPRESSION. L.T. Weiss-Wunder, M. Mercugliano, C. Gonzales and M-F. Chesselet
Dept of Pharmacology, U. of Pennsylvania, Philadelphia, PA 19104

The activity of CO, an endogenous marker of neuronal activity, was examined in the SNr of the adult rat at the light microscopic level. The pattern of histochemical staining observed for CO activity was correlated with immunohistochemistry for tyrosine hydroxylase (TOH, a marker of dopaminergic neurons) and for dynorphin (a peptide present in afferents from the striatum). In addition, the level of GAD immunoreactivity detected with an antibody labelling GAD-containing cell bodies (A. Tobin) and the level of GAD messenger RNA (mRNA) detected with a 35S-radiolabelled cRNA probe (A. Tobin) were measured in neurons of the SNr. The SNr showed a heterogeneous distribution of CO activity that was characterized by denser staining in the ventrolateral than the dorsomedial part of the nucleus throughout its rostrocaudal extent, with the exception of the most rostral levels. This pattern of CO activity was inversely correlated with the density of ventrally descending TOH -positive dendrites arising from the medial portion of the SNc, as well as with the density of dynorphin immunoreactivity. The region of dense CO activity contained neurons that expressed higher levels of GAD mRNA and GAD immunoreactivity than those neurons located in the region of low CO activity. The results suggest that, within the SNr, different levels of CO activity characterize subpopulations of neurons which may be differentially regulated by both striatal and dopaminergic influences. Supp. by BNS86-07645, MH44894, and MH 14654 (MM).

105.2

NEUROTRANSMITTER(S) OF THE AMYGDALO-NIGRAL PATHWAY: AN IMMUNOHISTOCHEMICAL AND IN SITU HYBRIDIZATION STUDY. C. Gonzales, W.R. Patterson* and M.-F. Chesselet, Dept. Pharmacology, University of Pennsylvania School of Medicine, Phila. PA 19104.

We have previously shown (Gonzales and Chesselet, J. Comp. Neurol. 297:2-20, '90) that the amygdalo-nigral pathway originates in a restricted region located both within and dorsal to the central nucleus of the amygdala (CeN). Previous studies did not detect a significant number of peptidergic neurons in this area.(Veening et al. Brain Res. 303:337-357 '84; Cassell et al. J. Comp. Neurol. 246:478-499 '86) In an effort to identify the neurotransmitter(s) present in this pathway, sections of rat brain were processed either for immunohistochemistry (IH) using antibodies against glutamic acid decarboxylase (GAD, from A. Tobin, UCLA) and somatostatin 28 (SOM, from Incstar) or in situ hybridization histochemistry (ISHH) using 35-S radiolabelled cRNA probes for GAD (A. Tobin, UCLA) and SOM (R. Goodman, Tufts). Numerous weakly labelled cells were detected in the entire CeN by ISHH with the GAD probe. In contrast, more densely labelled GAD-cells were found in the area of origin of the amygdalo-nigral pathway both by IH and ISHH. Fewer cells labelled for SOM were found in this area using either technique. Preliminary experiments combining retrograde tracing with fluorogold and ISHH for GAD have shown that GAD cells in this region project to the brainstem. The evidence is compatible with the hypothesis that GABA may function as the neurotransmitter of the amygdalo-nigral pathway. Supported by BNS 86-07645 and MH 44894.

105.3

NEUROTRANSMITTER STATUS OF ELECTROPHYSIOLOGICAL SUBTYPES IN THE SUBSTANTIA NIGRA PARS COMPACTA. W.H Yung* and M.A. Häusser* (SPON: Brain Research Association). University Lab. of Physiology, Parks Rd., OXFORD OX1 3PT, U.K.

Recent studies have demonstrated the existence of at least two distinct classes of neurons in the substantia nigra pars compacta (SNC; Grace and Onn, J. Neurosci. 9, 3463; Lacey et al., J. Neurosci. 9, 1233; Häusser and Yung, J. Physiol. 420, 27P). The cells were classified based on electrophysiological properties as non-bursting (principal) and bursting (secondary). The aim of the present study was to determine the transmitter status of these two classes of neurons in the guinea pig SNC *in vitro*. Double labelling of identified SNC neurons combining biocytin labelling with tyrosine hydroxylase (TH) immunofluorescence revealed that non-bursters (n=24) were consistently double-labelled while none of the bursters (n=12) was TH-positive. Retrograde labelling of SNC neurons with striatally-injected Fluorogold followed by intracellular recording and labelling confirms that non-bursters project to the striatum and indicates that they represent the classical dopaminergic nigrostriatal neurons. Dual color TH and GAD immunostaining was performed on midbrain sections and revealed that GAD-positive neurons were located largely in the dorsal and rostral SNC, which corresponded closely with the distribution pattern of the bursting neurons. This result strongly suggests that the bursting neurons are GABAergic, although the possibility that they use other transmitters such as CCK cannot be excluded.

105.4

AN EM STUDY OF THE RELATIONSHIP BETWEEN ENK+ BOUTONS AND DA+ NIGRAL NEURONS IN PIGEONS. K.D. Anderson, E.J. Karle and A. Reiner. Dept. Anatomy and Neurobiology, Univ. of TN, Memphis.

The substantia nigra (SN) in birds and mammals contains abundant enkephalinergic (ENK+) fibers and terminals. Although various studies indicate ENK is involved in regulating the activity of nigral dopaminergic (DA+) neurons, the specific role of ENK+ nigral afferents in the regulation of DA neurons is unknown. To help clarify this role, we used an EM double-label immunohistochemical technique to determine the relationship between ENK+ terminals (visualized with anti-Leu-ENK using PAP/DAB) and DA+ neurons (visualized with anti-tyrosine hydroxylase, TH, using silver-intensified immunogold). We observed numerous contacts between LENK+ terminals and both TH+ perikarya and TH+ dendrites. Synaptic membrane specializations, some of which were symmetric and others of which were asymmetric, were often observed at the axo-dendritic contacts. LENK+ terminals contacting TH+ neurons contained clear, round, densely-packed vesicles, and also typically contained one or more dense-core vesicles. Segments of TH+ neurons contacted by LENK+ terminals were also contacted by many unlabeled terminals of various morphologically distinct types. LENK+ terminals (with features similar to those of LENK+ terminals contacting TH+ neurons) also contacted unlabeled perikarya and dendrites in regions containing both TH and LENK labeling. These results indicate that ENK+ nigral afferents have a direct influence on the activity of DA+ nigral neurons, and that these ENK+ afferents together with other types of nigral afferents regulate the activity of individual DA+ neurons. Supported by NS-19620, 3P01NS-26473 (A.R.), and University of Tennessee Neuroscience Center of Excellence (K.D.A.)

105.5

CONVERGENCE OF PALLIDAL AND STRIATAL INPUTS TO NEURONES IN THE ENTOPEDUNCULAR NUCLEUS AND SUBSTANTIA NIGRA OF THE RAT: APPLICATION OF A NEW DOUBLE ANTEROGRADE LABELING METHOD AT ELECTRON MICROSCOPIC LEVEL. Y. Smith & J.P. Bolam, MRC Anatomical Neuropharmacology Unit, Dept of Pharmacology, OXFORD, U.K.

The aim of this experiment was to determine whether single cells in the entopeduncular nucleus (EP) or the substantia nigra reticulata (SNr) receive convergent inputs from the globus pallidus (GP) and the striatum (STR). The approach was to combine the anterograde transport of *Phaseolus-vulgaris* Leucoagglutinin (PHA-L) and of biocytin (BIO). The PHA-L was injected in GP and the BIO injected into STR. After perfuse-fixation the PHA-L was revealed by immunocytochemical means using benzidine dihydrochloride as chromogen for the peroxidase reaction whereas the BIO was revealed by incubation in avidin-biotin-peroxidase complex using diaminobenzidine as chromogen. Neurones of the EP and SNr were then selected and examined in the electron microscope for synaptic inputs from PHA-L- and BIO-positive boutons. In the SNr, the cells were further characterized by retrograde transport of lectin-conjugated horseradish peroxidase (WGA-HRP) from the superior colliculus (SC), revealed using tetramethylbenzidine as chromogen.

In the EP and SNr the PHA-L-labeled varicosities (pallidal) were very large and formed pericellular baskets whereas the BIO-labeled terminals (striatal) were much smaller and formed a rich plexus without any apparent neuronal association. In the electron microscope EP and SNr cells were identified that received symmetrical synapses from both pallidal (PHA-L-labeled) and striatal (BIO-labeled) boutons. Some of the nigral cells that received convergent inputs were identified as nigrocollicular neurones by retrograde labeling.

In conclusion, the present findings: (1) suggest the existence of a prominent projection from the GP to the EP and the SNr and (2) reveal that there is a convergence of GP and STR terminals onto neurones in EP and SN. (Supported by the MRC of Canada).

105.6

PROJECTIONS FROM PEDUNCULOPONTINE NUCLEUS (PPN) TO BASAL GANGLIA IN PRIMATE. B. Lavoie, C. Desjardins* and A. Parent. Neurobiol. Lab., Laval Univ., Québec, Canada.

Tritiated amino acids (AA) and the lectin *Phaseolus vulgaris*-leucoagglutinin (PHA-L) were used as anterograde tracers to study the innervation of the basal ganglia from the PPN in the squirrel monkey (*Saimiri sciureus*). After small unilateral pressure injections of AA (2 animals) or iontophoretic injections of PHA-L (4 animals) in PPN, anterogradely-labeled fibers were found in several basal ganglia components, both ipsi- and contralaterally. The ascending fibers first appeared as a diffuse bundle along the dorsal surface of the substantia nigra pars compacta (SNc), but collected as a more compact fascicle as they run within the lateral hypothalamic area. At midbrain level numerous labeled fibers occurred in SNc and, in sections immunostained for both tyrosine hydroxylase and PHA-L, these fibers were found to arborize principally around the cell bodies and primary dendrites of dopaminergic neurons. More rostrally, labeled fibers were noted in the subthalamic nucleus, particularly in its dorsomedial portion, and in ansa lenticularis, and internal and external medullary laminae. In dorsal pallidum fibers arborized much more profusely in the internal than external segment. The ventral pallidum was devoid of labeled fibers whereas the underlying substantia innominata was densely innervated. In the striatum a small number of labeled fibers occurred especially in the rostroventral part of the caudate nucleus where they terminated in the form of patches. Current studies involving NADPH-diaphorase histochemistry and ChAT immunostaining will serve to evaluate the possible contribution of cholinergic neurons in PPN to the basal ganglia innervation in primates. [Supported by MRC].

105.7

AN *IN VITRO* INTRACELLULAR ANALYSIS OF PEDUNCULOPONTINE INPUTS TO SUBSTANTIA NIGRA PARS COMPACTA NEURONS. J. A. Whittaker and S. T. Kitai. Department of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, TN 38163.

Response characteristics of substantia nigra pars compacta (SNc) neurons to electrical stimulation of the Pedunculopontine (PPN) area were investigated *in vitro* using conventional intracellular recording techniques. In parasagittal slices (400-500μm) containing both SNc and PPN, stimulation (10-300μA) of the PPN area produced depolarizing potentials in SNc neurons at latencies of 2.0-3.0msec. with amplitudes over 10mV. Stimulus was applied through bipolar stainless steel electrodes insulated to within 0.2mm from the tips and separated by a distance of 0.3mm. The synaptic responses were identified as excitatory postsynaptic potentials (EPSPs) by intracellular current injection and were considered monosynaptic since their latencies remained constant in spite of changes in stimulus intensities. The morphology and location of recorded neurons were identified via intracellular biocytin injection and their transmitter phenotype revealed by tyrosine hydroxylase (TH) immunoreactivity. The results demonstrate PPN has predominant excitatory synaptic inputs to both Type I and Type II SNc dopaminergic neurons and may play a role in modulating SNc function. (Supported by NIH grants NS 20702 and NS 23886 to S. T. Kitai).

105.8

UNIT RESPONSES RECORDED IN THE SUBSTANTIA NIGRA AFTER STIMULATION OF THE FRONTAL CORTEX. K. Fujimoto and H. Kita. Dept. of Anatomy and Neurobiology, College of Medicine, Univ. of Tennessee Memphis, Memphis, TN 38163.

The subthalamic nucleus (STH) receives excitatory input from the frontal cortex (Cx) and sends excitatory output to the substantia nigra (SN). The neostriatum (Str) also receives excitatory input from the Cx. However the Str sends inhibitory output to the SN. In order to study the function of these pallarel circuits, we assessed unit responses of the SN to stimulation of the Cx. Male Sprague-Dawly rats were anesthetized with urethane (1.2 g/kg, i.p.). Stimulus electrodes were placed in the Cx. Single unit responses of the SN to the stimulation of the Cx were recorded using glass electrodes filled with 2M NaCl. Some rats were anesthesized with Ketamine (30 mg/kg) and Xylazine (0.9 mg/kg), and received ibotenic acid (60M) lesion of the STH 7-10 days before recording. In some other rats, the Str was lesioned similarly by injection of quinolinic acid (200 nmol). In normal rats, 10-20% of the units recorded in the SN showed excitation followed by inhibition after the stimulation of the Cx, while the rest showed only inhibition. After lesion of the Str, the proportion of the inhibited units in the SN decreased. The proportion of the excited units was not affected by Str lesion. After lesion of the STH, excited units were not observed in the SN and the proportion of inhibited units was also decreased. Therefore evidence suggests that the two pallarel circuits converge upon single SN neurons, and the excitatory response, via the STH, precedes the inhibitory response, through the Str. The reason why lesion of the STH decreased inhibitory responses is not clear. (Supported by NIH Grant NS-25783)

105.9

MULTI-CHANNEL SINGLE-UNIT RECORDINGS FROM AWAKE, FREELY MOVING RATS WITH 6-OHDA-INDUCED DAMAGE TO THE DOPAMINERGIC NIGROSTRIATAL PROJECTION. S.F. Sawyer, C.D. Myre, B.N. Maddux and D.J. Woodward. *Department of Cell Biology and Neuroscience, University of Texas Southwestern Medical Center, Dallas, TX 75235*

Damage to the dopaminergic nigrostriatal projection from intracerebral injection of 6-hydroxydopamine (6-OHDA) has proven to be a useful animal model for Parkinson's disease. The behavioral and neurochemical consequences of this lesion have been studied extensively, providing insights into the cellular basis for Parkinsonian symptomatology, but little is known concerning the electrophysiology of neurons in the dopamine-depleted neostriatum of awake, freely moving animals. We report here our preliminary findings using chronic extracellular single unit recording of neostriatal neuronal activity in 6-OHDA treated rats. Male Long-Evans rats received injections of 6-OHDA (8 μg in 4 μl) into the left medial forebrain bundle and substantia nigra pars compacta, and an array of 16 microwires was implanted in the left neostriatum. Damage to the dopaminergic nigrostriatal projection was assessed by orienting deficits to tactile stimuli applied to the contralateral body surface and from contralateral circling to apomorphine (0.05 mg/kg, s.c.). The position of microwires was determined from X-rays and histological reconstruction. Neostriatal neurons in dopamine-depleted rats tended to exhibit higher firing rates than neostriatal neurons in intact animals. In addition, neurons in 6-OHDA-treated animals often exhibited an altered firing pattern consisting of an increased tendency for action potential burst firing with intra-burst rates approaching 500 Hz. In addition, the activity of many neostriatal neurons in 6-OHDA treated rats was episodic to a degree not observed in intact animals. The disruption of the modulatory effects of dopamine on neostriatal synaptic circuits may underlie the observed alterations in firing rates and patterns, and may contribute to the motor impairments observed in Parkinson's disease. *Supported by NIDA Grant DA-05352, MH-44337, AFOSR 90-0416 and Biological Humanics Foundation.*

105.10

SELECTIVE SUBREGIONAL DOPAMINE DEPLETIONS IN THE RAT CAUDATE-PUTAMEN FOLLOWING NIGROSTRIATAL LESIONS. E.A. Pehek and B.K. Yamamoto. Dept. of Psychiatry, Case West. Reserve Univ. Sch. of Med., Cleveland, Ohio 44106

The rat striatum is heterogenous with respect to dopamine (DA) content, degree and pattern of innervation, and responsiveness to the DA-releasing drug amphetamine. The present study examined whether subregions of the striatum would be differentially sensitive to destruction of the nigrostriatal pathway by the DA neurotoxin 6-OHDA. 6-OHDA was injected into the substantia nigra pars compacta. After one month, brains were assayed by HPLC/EC for tissue levels of DA, DOPAC, HVA, 5-HT, and 5-HIAA. Four striatal subregions (anterodorsomedial, anterodorsolateral, posterodorsomedial, and posteroventrolateral) and the nucleus accumbens were examined. DA and DA metabolite levels, but not 5-HT or 5-HIAA levels, were reduced in all four striatal subregions but not in the nucleus accumbens. However, compared to other subregions, this depletion was greatest in the anterodorsolateral area. This relative difference between subregions was most apparent in those animals with partial lesions (40-89% DA depletions). These findings may reflect differences in the pattern of innervation of striatal subregions by nigrostriatal afferents. Furthermore, this research may have implications for the pathogenesis of Parkinson's disease. Supported by PHS NS24814.

105.11

ORGANIZATION WITHIN THE RAT SUBSTANTIA NIGRA OF VENTRAL STRIATAL EFFERENT PATHWAYS AND DOPAMINERGIC CELLS PROJECTING TO MOTOR- OR LIMBIC-RELATED STRIATUM. L. Majumdar, S. Haber. Departments of Neurology and Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

Integration of motor- and limbic-related parallel circuits through the rodent basal ganglia may occur within the substantia nigra. This study was undertaken to investigate the relationship between limbic-related ventral striatal efferent terminals and the dopaminergic cells within the substantia nigra which protect to either motor-related dorsolateral striatum or association-and limbic-related dorsomedial striatum.

The anterograde tracer, PHAL, was iontophoretically injected into the ventral striatum and the retrograde tracer, cholera toxin (subunit B), was pressure-injected into dorsolateral or dorsomedial striatum in the same animal. Sections were double stained using antibodies to PHAL and cholera toxin B. Dopaminergic cells within the nigra were identified using a antibody against tyrosine hydroxylase and a flourescent second antibody.

PHAL-labelled fibers from the ventral striatum entered the medial substantia nigra pars compacta rostrally and spread laterally in the caudal nigra. Fibers entered the ventral tegmental area caudally. Dorsolateral injections labelled the middle third of the substantia nigra with a tendency toward medial and lateral clumping of labelled cells within this region. Double label studies demonstrated significant intermingling throughout the nigra and ventral tegmental area of PHAL-positive ventral striatal efferent terminals and dopaminergic cells which project to association- and limbic-related dorsomedial striatum. PHAL-positive terminals and dopaminergic cells which project to the motor-related dorsolateral striatum were separate in their distributions except within the medial clump of labelled nigral dopaminergic cells. In this region PHAL-positive terminals intermingled extensively with the dopaminergic cell bodies. These results suggest that limbic-related striatal afferent systems may influence the motor-related efferent projection systems of the nigra by virtue of their anatomic interactions within the substantia nigra.

105.12

CIRCLING INDUCED CHANGES IN GABA RELEASE IN THE SUBSTANTIA NIGRA RETICULATA AND GLOBUS PALLIDUS. R.E. Maloney, C.R. Freed. Dept. of Med., Div. of Clin. Pharm. Univ. of Co. Health Sci Center, Denver, Co 80262.

In our ongoing efforts to understand the relationship between movement and the release of neurotransmitters, we have used in-vivo microdialysis to measure GABA release from the substantia nigra pars reticulata(SNR) and globus pallidus(GP) of rats trained to run on a circular treadmill for a water reward. Male Sprague Dawley rats were trained to an FR10 schedule in both directions. After subjects reached criteria they were surgically implanted with guide tube cannulae in either the SNR(Paxinos & Watson coordinates AP:-5.3, LAT:2.78, VENT:-7.4) or GP(AP:-9.5, LAT:3.2, VENT:-5.15). 3 days after surgery animals were retrained and then a 300 um x 1mm dialysis probe was inserted which extended into either the SNR or GP. Animals were perfused with Ringers solution at a flow rate of 1.5ul/min. Twenty min. samples were taken. After a 3 hr. baseline period animals were placed on the circular treadmill for 80 mins and allowed to run in one direction only for a water reward. One week later animals were tested in the opposite direction. Perfusate was analyzed by HPLC/EC with precolumn derivatization with orthophthalaldehyde. There was a 249% increase in GABA release from the SNR when animals circled contralateral to the dialyzed side. There were no significant changes in SNR GABA release ipsilateral or in either side of the GP.

105.13

MOTOR EFFECTS OF SUBSTANTIA NIGRA PARS RETICULATA (SNR) STIMULATION IN THE RAT: LESIONS OF SNR EFFERENT TARGETS, CORTICOFUGAL AND RUBROFUGAL PATHWAYS DO NOT ATTENUATE THE MOTOR EFFECTS. S.I. Lentz and D. Asdourian, Dept. of Psychology, Wayne State Univ., Detroit, MI 48202.

Unilateral lesions disrupting substantia nigra pars reticulata (SNr) efferent targets, and fibers of passage coursing through SNr were made to determine the source of neck and shoulder muscle activity observed during unilateral electrical stimulation of SNr in anesthetized adult male rats. Muscle activity in trapezius, biventer cervicis, and rectus capitis was recorded electrically. In the intact animal, unilateral stimulation of SNr (twin square wave pulses, 1 pulse/sec, duration 1.0 msec, interpulse interval 1.0 msec, at 0.5 mA) drove bilateral muscle activity in all 3 muscles with ipsilateral muscle activity always greater in amplitude than contralateral activity. Lesions to SNr efferent targets: Unilateral lesions in the superior colliculus (SC) and pedunculopontine tegmental nucleus (PPtg) did not have a significant effect on muscle activity driven with SNr stimulation. Lesions to fibers of passage through SNr: In separate groups, unilateral lesions to: (1) ipsilateral motor cortex, (2) ipsilateral pyramidal tract and (3) contralateral red nucleus, to the stimulation did not have a significant effect on muscle activity driven by SNr stimulation. The inability to block muscle activity with lesions of SC, PPtg, corticofugal and rubrofugal systems leaves unanswered the question as to which pathway(s) is involved in driving muscle activity with SNr stimulation.

105.14

PATTERNS OF CALCIUM CHANNEL ANTAGONIST BINDING IN RAT CNS. L.P. Fortier*, B. Lavoie and J.P. Tremblay. Lab. of Neurobiol., Fac. of Med., Laval Univ., Quebec, Canada.

The term voltage-sensitive calcium channel (VSCC) refers to a family of ionic channels characterized by individual kinetic properties. Their multiple roles, level of cell excitability, neurotransmitter release and growth probably derive from their respective characteristics. Using as a probe the omega-conotoxin (ω-CgTX), a specific antagonist of the VSCC, we revealed populations of neurons in coronal sections of rat brain. A population of particular interest for us was located in the substantia nigra. Labelling of the probe binding sites with our monoclonal antibody mab anti-CgTX was found mainly on cell bodies and proximal processes. The labelled cells were not uniformly distributed throughout the substantia nigra. Whether these cells represent a subpopulation remains to be determined. Current studies combining the use of retrograde fluorescent tracers injected into the thalamus and/or superior colliculus with immunohistochemistry should help to better characterized this subpopulation of nigral neurons expressing ω-CgTX immunoreactivity.

105.15

ULTRASTRUCTURE OF CHOLINERGIC NEURONS OF THE NUCLEUS TEGMENTI PEDUNCULOPONTINUS IN THE RAT. B. Spann and I. Grofova. Dept. of Anat., Mich. State Univ., E. Lansing, MI 48824

Choline acetyltransferase (ChAT) immunohistochemistry studies have indicated the nucleus tegmenti pedunculopontinus (PPN) as a center of the Ch 5 cell group in human and experimental animal species (Mesulam, M.-M. et al., J. Comp. Neurol., 281:611-633, 1989). The present immunocytochemical study describes light and electron microscopic features of ChAT-positive neurons and synaptic arrangements of both cholinergic and non-cholinergic cells of the PPN.

Both subdivisions of the PPN, the subnucleus compactus (PPNc) and dissipatus (PPNd) contain cholinergic neurons that are either fusiform or multipolar in shape and vary in size from 20 μm to 60 μm along the longest axis. While the ChAT-positive somata are most numerous in the PPNc, the ChAT-positive dendrites are present in both subnuclei with a particularly dense concentration in the medial portion of the PPNd. In a previous study on the normal ultrastructure of the PPN, we have identified three morphologically distinct types (I-III) of terminal boutons. Electron microscopic examination of the present material reveal all three types synapsing on the ChAT-positive dendrites. Type III terminals are, at least partially, of nigral origin and we have previously reported that they terminate mostly on dendrites within the PPNd. Consequently the present observations suggest, indirectly, that nigral afferents may terminate on the dendrites of PPN cholinergic neurons. (Supported by N.I.H. grant NS 25744).

105.16

PREFRONTAL CORTICAL TERMINALS SYNAPSE WITH DOPAMINERGIC NEURONS IN THE VENTRAL TEGMENTAL AREA AND WITH UNLABELED NEURONAL TARGETS OF DOPAMINERGIC TERMINALS IN THE NUCLEUS ACCUMBENS SEPTI. S.R. Sesack and V.M. Pickel. Dept. Neurology and Neuroscience, Cornell Univ. Med. Coll., New York, NY 10021.

We sought to determine possible ultrastructural bases for known modulatory interactions between the prefrontal cortex (PFC) and the mesolimbic dopaminergic (DA) system, either in the ventral tegmental area (VTA) or in the nucleus accumbens septi (Acb). Terminals from the PFC were identified in rats either by anterograde degeneration following electrolytic lesions or by immunoperoxidase labeling of anterogradely transported *Phaseolus vulgaris* leucoagglutinin. In single sections processed for degeneration or transport, presumed DA neurons and processes were identified by immunoperoxidase or immunogold labeling, respectively, for the catecholamine synthesizing enzyme, tyrosine-hydroxylase (TH). In the VTA, PFC-terminals made asymmetric synaptic junctions with TH-labeled or unlabeled dendrites. In the Acb, PFC-terminals formed asymmetric synapses with dendritic spines, some of which also received symmetric synaptic contact from TH-labeled terminals. PFC and TH-labeled terminals were also directly apposed to each other, but did not form conventional axoaxonic synapses. We conclude that efferents from the PFC monosynaptically modulate, and most likely excite, DA neurons in the VTA and spiny targets of DA terminals in the Acb. Additionally, PFC and DA terminals are anatomically positioned to facilitate presynaptic modulation in the Acb. These findings have important implications for understanding the mechanisms linking cortical and mesolimbic DA pathophysiology in schizophrenia. (Supported by NS08193, MH40342).

105.17

GABA NEURONS IN THE VENTRAL TEGMENTAL AREA PROJECT TO THE MEDIAL PREFRONTAL CORTEX: A NON-DOPAMINERGIC MESOCORTICAL SYSTEM. M. H. Gillham[1], L. Jennes[2], and A. Y. Deutch[1]. [1]Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06510 and [2]Department of Anatomy, Wright State University, Dayton, OH 45435.

The dopaminergic projection from the ventral tegmental area (VTA) to the medial prefrontal cortex (mPFC) has been extensively studied. However, less than half of the VTA neurons which project to the mPFC are dopaminergic. We now report a GABAergic projection from the VTA to the mPFC.

Combined retrograde tracer-immunohistochemical methods revealed GABA-like immunoreactive (GABA-li) neurons in the VTA which were retrogradely labeled with fluorogold (FG); these were most often observed in the nucleus parabrachialis pigmentosus. FG-labeled GABA-li neurons were typically small round cells and contrasted with FG-positive non-GABA-li neurons which were medium-sized. GABA-li axonal pericellular arrays were frequently observed to surround non-GABA-li FG-positive neurons, but were rarely seen in apposition to the GABA-li mesocortical cells.

These data suggest that GABA may regulate mPFC function in two ways: through direct GABAergic projections from the VTA to the mPFC, and via local GABAergic regulation of dopaminergic mesocortical neurons.

Supported by grants MH-45124 and HD-24697 and by grants from the Scottish Rite Schizophrenia Research Program and the American Parkinson Disease Association.

105.18

DO ENTOPEDUNCULAR NEURONS PROJECTING TO THE HABENULA RECEIVE A SUBSTANCE P INPUT FROM THE STRIATUM? N. Rajakumar, K. Elisevich and B.A. Flumerfelt. Depts. of Anatomy and Clinical Neurological Sciences, University of Western Ontario, London, Canada, N6A 5C1.

The entopeduncular nucleus (EPN) of the rat is homologous to the internal segment of the primate globus pallidus and forms an important output pathway of the basal ganglia. Anatomical studies have demonstrated a large projection from the rostral two-thirds of the EPN to the ipsilateral habenular nucleus. Unlike other neostriatal output systems, these fibres terminate in the lateral habenula (LHB) which is known to receive its afferents mainly from limbic centres. The EPN receives its main input from the neostriatum via a projection that contains substance P (SP). However, it remains to be determined if the SP containing synaptic input terminates in relation to the EPN-LHB projection neurons. In this study, the WGA-HRP retrograde tracing method was employed to label the EPN-LHB neurons, and immunocytochemical methods were used to investigate the presence of SP containing synaptic profiles on the labeled cells. Reactive profiles were identified with the PAP technique using the chromogens TMB, DAB, and BDHC in various combinations. SP immunoreactive terminal axons were frequently observed with the L.M. in apposition to EPN neurons retrogradely labeled from the LHB. These results suggest that the LHB receives a large projection from EPN neurons which in turn receive an SP-containing synaptic input, probably from the neostriatum.

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105.19

THE PRIMATE NUCLEUS OF THE FIELD OF FOREL: NEURONS TYPES AND AFFERENT FIBERS. J.A. Rafols, Y. Anavi* and S. Mintz*. Dept. of Anatomy, Wayne State Univ. Sch. of Med. Detroit, MI 48201

The neuronal organization of the nucleus of the Field of Forel(nFF) in the monkey (Macaca mulatta) was examined in Golgi impregnated brains(N=50) sectioned in the frontal, horizontal, and sagittal planes. Four(types I-IV) different neuronal types were distinguished based on morphological features of the cell body, dendrites, and the axon. Type I was the most commonly impregnated neuron. It had large(24-63μm), fusiform or polygonal cell bodies and long, tapering dendrites which radiated preferentially in the dorsoventral and mediolateral planes. Only the hillock and initial segment of the axon were impregnated suggesting that it may be of the long variety. Type II neurons had medium(13-21μm), fusiform or polygonal cell bodies and thin, varicose dendrites. The axon was of the long type and gave rise to a few intrinsic collaterals. Type III neurons were local interneurons with small (8-17μm), round somata and long, non-tapering dendrites which gave rise to numerous bulbous appendages and axon-like processes. The rarely observed type IV neurons had small(8-12μm), round somata, few tightly coiled dendrites and a short axon. In addition, five different types of afferent fibers, some of which were fibers of passage, gave rise to synaptic boutons which terminated in nFF. In horizontal and sagittal sections some of these fibers were followed into the internal capsule, dorsal and ventral midbrain tegmentum, Forel's H2 Field and hypothalamus. The present results show, for the first time, evidence of cell type heterogeneity, as well as diversity of extrinsic and intrinsic neural networks in nFF.

105.20

RELEASE OF THE SUBTHALAMIC NUCLEUS (STN) FROM GABAERGIC INHIBITION IS AN ELEMENT IN THE DEVELOPMENT OF PARKINSONIAN SIGNS. T. Wichmann', H. Bergman', M.R. DeLong, Dept. of Neurology, The Johns Hopkins Hospital, Baltimore, MD, USA.

Studies in MPTP treated primates suggest that parkinsonian signs may be linked to increased activity of the STN, due to release from GABAergic inhibition from the external pallidal segment. The present study was designed to further investigate the role of STN activity and its control by GABAergic mechanisms in the development of parkinsonian signs.

Two African green monkeys were rendered parkinsonian by the injection of MPTP (i.m., 4 - 10 mg/kg). After the development of severe parkinsonian signs, the subthalamic area was mapped first with microelectrode recordings and later with a combined injection/recording device. With this, the GABA receptor agonist muscimol and the GABA receptor antagonist bicuculline were injected into the STN to transiently influence STN activity in two monkeys. Injections of normal saline served as a control.

Injection of 0.6 - 1 ul muscimol (1 mg/ml) into the "arm area" of the STN decreased the neuronal activity, reduced rigidity and tremor, and led to the appearance of voluntary and involuntary movements of the contralateral arm, which then spread to the contralateral leg. Injection into the "leg area" induced the same effects, starting in the leg and spreading to the arm. Injection of 0.6 - 1.0 ul bicuculline (0.25 - 1 mg/ml) increased the neuronal activity. Bicuculline injections did not elicit dyskinetic movements and the parkinsonian signs remained largely unchanged. In both animals, saline had no effect.

These findings support further the notion that increased STN activity due to decreased GABAergic inhibition is important in the development of parkinsonian signs.

105.21

THE PROPORTION OF THE STRIATUM STAINED POSITIVE FOR CHOLINE ACETYLTRANSFERASE (CAT) INCREASES IN A UNILATERAL RODENT MODEL OF PERINATAL HYPOXIA-ISCHEMIA. RE Burke and N Kenyon*, Dept of Neurology, Columbia University, NY, NY 10032.

Little is known of the effect of hypoxic-ischemic injury during development on the neurochemical anatomy of the brain. We have focused on effects on the striatum, because it often shows the predominant pathology in human perinatal asphyxial injury, and it plays a major role in motor control. We and others have previously shown that striatal cholinergic neurons are relatively spared following hypoxic-ischemic injury; their density (neurons/mm^3 striatum) increases. We have here investigated what changes occur in the density and distribution of CAT-positive striatal neuropil staining. Rat pups underwent left carotid ligation and exposure to 8% O_2 at 7 days of age, and brains were prepared for immunohistochemical staining of CAT at 3-4 or 8-12 weeks. Cryostat cut sections were stained with a monoclonal ant-CAT Ab (Boehringer). Representative sections were taken from Paxinos-Watson 10.2, 9.7, 9.2, and 8.2. The area of the striatum stained CAT-positive was determined by segmented field analysis; specific staining was defined as density values between a maximum specific OD and 75% of that value. At 3-4 weeks there was a trend towards an increase in the absolute value of CAT-positive area on the injured side: C:3.4±0.5; E:4.7±0.8 (mm^2±SEM)(p=0.1). There was a significant increase in percent striatal area stained: C:8.6±1.3; E:13.7±2.1 (%)(p=.05, N=11). The effect was uniform in the rostro-caudal dimension. Similar changes were observed in adults; there was a significant increase in the percent area stained CAT-positive: C:14±2.2; E:20.2±3.5 (%)(p=0.5, N=9). These results support our earlier finding that striatal cholinergic systems are resistant to hypoxic-ischemic injury. NINDS NS26836.

105.22

EFFECTS OF UNILATERAL PERINATAL HYPOXIA-ISCHEMIA ON DOPAMINE (DA) D1-and D2-RECEPTORS AND UPTAKE SITES IN RAT. V. Jackson-Lewis, S. Przedboski, V. Kostic, J.L. Cadet, R.E. Burke, Columbia University, New York, New York 10032

The striatum is a major site of pathology following perinatal hypoxic-ischemic (HI) brain injury. Little is known of alterations in neurochemical anatomy. Johnston (1983) had previously shown that pre-synaptic biochemical indices of striatal dopaminergic systems are relatively spared. We have confirmed this morphologically by finding an increased density of tyrosine hydroxylase-positive neuropil in the injured striatum (Soc Neurosci, 1989). However, little is known of effects on post-synaptic dopaminergic elements. We therefore studied effects of perinatal HI on striatal DA-receptors, as well as uptake sites, in rats using quantitative autoradiography. In 7-day-old rat pups, HI was induced by unilateral carotid ligation with subsequent exposure to 8% oxygen. In animals sacrificed at 3-4 weeks of age, radioligand binding saturation experiments revealed significant decreases in Bmax for ^{3}H-SCH 23390-labeled D1 (-23%; P<0.001) and ^{3}H-spiperone labeled D2 receptors (-28%; P<0.01) on the side of the injury. In addition, a significant increase in the Kd for D1 receptors was observed(P<0.05). Mapping experiments showed that these changes were present along the rostro-caudal axis of the striatum and predominated in the dorsal aspect. On the other hand, no changes in either Bmax or Kd were observed in ^{3}H-mazindol labeled DA uptake sites. In 8 to 12-week-old rats, similar changes in D2 receptors were observed; however, both the Bmax and the Kd for D1 receptors had returned to normal. NINDS NSZ6836, PDF.

105.23

EFFECT OF UNILATERAL PERINATAL HYPOXIA-ISCHEMIA ON CHOLINERGIC RECEPTORS IN THE RAT STRIATUM. V. Kostic, S. Przedborski, V. Jackson-Lewis, J.L. Cadet R.E. Burke. Department of Neurology, Columbia University, New York, N.Y. 10032

Little is known of the effect of hypoxic-ischemic (HI) injury during development on the neurochemical anatomy of the brain. We have previously shown in a unilateral rodent model that perinatal HI injury results in an increased density of morphologically-defined cholinergic pre-synaptic elements, both neurons (Ann Neurol, 1990) and neuropil. Little is known of effects on post-synaptic cholinergic markers. We therefore used quantitative autoradiography to investigate effects on total (^{3}HQNB) and M1 (^{3}H Pirenzepine [PZ]) muscarinic receptors. In one-week old rat pups, unilateral HI was induced by carotid ligation followed by exposure to 8% oxygen. Pups were sacrificed at 3-4 weeks. ^{3}H-QNB autoradiography revealed no changes in Bmax or Kd on the HI-injured side. In contrast, saturation experiments using ^{3}H-PZ showed a significant decrease in Bmax in the injured striatum ipsilateral to the ligation, compared to the contralateral side (134.5 ± 4.9 vs 153.9 ± 8.2 fmol/mg tissue; p<.05). No change was observed in Kd. M1 mapping experiments revealed that the observed decrease occurred mainly in the dorsal part of the lesioned striatum (-23%; p<.05).These findings suggest that HI injury may result in an increase in striatal M2 sites, a possibility to be explored with selective M2 ligands. NINDS NS26836, PDF.

106.1

GABA$_A$ RECEPTOR IMMUNOREACTIVITY IN ADULT AND DEVELOPING MONKEY SENSORY-MOTOR CORTEX. G.W.Huntley, A.L.de Blas and E.G.Jones, Dept. of Anatomy and Neurobiology, Univ. of California, Irvine,CA.92717 and Division of Molecular Biology, Univ. of Missouri, Kansas City,MO. 64110.

The areal and laminar distribution of GABA$_A$ receptor immunoreactivity was examined in fetal, early postnatal and adult monkey sensory-motor cortex by using a monoclonal antibody to the purified GABA$_A$ receptor complex (Vitorica et al., J.Neurosci.8,1988). In adult cortex, immunoreactivity was distributed throughout the neuropil and in each area layers I-IIIA exhibited the most intense immunostaining. In deeper layers of SI, layers IIIB and V were lightly stained and alternated with more intensely stained layers IV and VI. In area 4, staining in layers IIIA-VA was light, but was more intense in layers VB-VI. In areas 4,1,2, parallel, radially oriented chains of densely stained puncta resembling small fasciculi were present. Each of the developing sensory-motor cortices examined (E110-PND125) possessed receptor immunoreactivity. At early stages, staining was present in a diffuse band occupying the middle layers of all areas and in the subplate. With increasing age, changes in the distribution of staining occurred resulting in an adult-like pattern by early postnatal ages. It is suggested that laminar changes seen in development are associated with the establishment of afferent connections and inhibitory circuits in the sensory-motor cortex. Supported by Grant NS 21377 from the National Institutes of Health and a predoctoral fellowship from the National Institute of Mental Health.

106.2

A MICROCOLUMNAR STRUCTURE OF MONKEY CEREBRAL CORTEX REVEALED BY IMMUNO-CYTOCHEMICAL STUDIES OF DOUBLE BOUQUET CELL AXONS. J. DeFelipe, S.H.C. Hendry, and E.G. Jones. Dept. of Anatomy and Neurobiology, Univ. of California, Irvine, CA 92717.

Immunocytochemical methods were used to study 28 Kd calbindin and tachykinin immunoreactivity in the monkey cerebral cortex. The staining of long, vertically-oriented bundles of processes - identical to classical double bouquet cell axonal arborizations - is the most prominent feature of both calbindin and tachykinin immunoreactive staining. These bundles form a widespread and regular columnar system descending from layer II through layers III-V. The bundles are most evident in layer III where, in tangential sections, they have a density of 7 to 15 bundles per 10,000 μm^2 with a center-to-center spacing of 15 to 30 μm.

Somatic sensory, auditory, and visual areas display a large number of calbindin immunoreactive bundles but tachykinin immunoreactive bundles are numerous only in the auditory areas and in area 18 of the visual cortex. In the precentral motor cortex (area 4) few or no immunoreactive bundles are visualized with either antibody.

Tachykinin-positive axons of the bundles form symmetrical synaptic contacts with dendritic shafts (57%) and spines (43%). Despite the virtually identical morphological features of tachykinin and calbindin immunoreactive bundles, colocalization studies demonstrate little coexistence of the two antigens in somata and none in the axonal bundles of double bouquet cells.

These data suggest that the double bouquet cell is a chemically heterogeneous, but ubiquitous morphological type of cortical interneuron. Its uniquely bundled axonal system, which is probably GABAergic, imposes a fundamental microcolumnar organization upon the cerebral cortex. Supported by NIH grant no. NS21377.

106.3

MOLECULAR SPECIFICATION OF PRIMATE CORTICAL NEURONS REVEALED BY MONOCLONAL ANTIBODIES. A. Guimaraes, P.L. Strick and P. Levitt. Dept. of Anatomy. Med Coll of PA, Philadelphia, PA 19129 and VA Med. Ctr./Depts of Neurosurg.& Physiol, SUNY-HSC @ Syracuse, Syracuse, NY 13210.

Monoclonal antibodies have been useful in demonstrating rare antigenic determinants expressed by select subsets of neurons in the central nervous system. A new monoclonal antibody was generated by immunizing Balb/c mice with homogenized monkey motor cortex. These mice were immunotolerized neonatally with monkey hippocampus and subsequently treated with cyclophosphamide. Monoclonal antibody 8B3 is an immunoglobulin of the IgM subclass and crossreacts with an epitope in rodent, cat and monkey. Immunocytochemical analysis in the rhesus monkey brain revealed an immunoreactive subpopulation of neurons in all cortical areas except the hippocampus. The distribution of staining is characterized by a single row of neurons on the border of layers I/II, a scattered population in deep aspects of layer V and thoughout layer VI, and a large number of interstitial neurons located in the subcortical white matter. Very few positive neurons are seen in layers II through upper V. The immunohistochemical staining pattern suggests that 8B3 recognizes a surface epitope on the somata and proximal dendrites of a population of cells that include stellate neurons. The distribution of labelled cell classes that are immunoreactive are identical between cortical areas, but the number of 8B3-stained neurons varies substantially depending upon the region examined. This might reflect the relative contribution of this neuron population to distinct functional regions of the primate cerebral cortex. Supported by NIMH grant MH45507 and March of Dimes Basic Research Grant 1-919.

106.4

ORGANIZATION OF NEURAL ELEMENTS IN MOTOR SPEECH AREAS. T.L. Hayes and D.A. Lewis. Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15216.

Motor speech functions are believed to be localized to the region known as Broca's area, located within the left inferior frontal gyrus rostral to the motor region. However, little is known about the intrinsic organization of this area, or about how that organization compares to the homologous region in the right hemisphere. Three control human brains were examined immunohistochemically using Nissl stains and antibodies directed against non-phosphorylated neurofilament proteins (NFP) and calbindin. Nissl stains revealed considerable heterogeneity within this area. The rostral portion of the area was granular, with evenly sized and distributed pyramidal neurons in layers III and V. A distinct population of large layer III pyramidal neurons appeared more caudally, concomitant with a narrowing of layer V. The region was dysgranular at its most caudal extent. Different populations of neurons were NFP- and calbindin-immunoreactive (IR). The soma and proximal apical and basilar dendrites of a subpopulation of pyramidal neurons in layers III and V were NFP-IR. This labelling was particularly intense in the large layer III pyramidal neurons. In contrast, calbindin was present in the soma and apical dendrites of the smaller pyramids in layer III, as well as in nonpyramidal neurons of layers II, III and VI. Numerous processes in layers II and superficial III were calbindin-IR, as were bundles of radial fibers in layers III-VI. These findings provide a basis for comparing the organization of Broca's area with that of its homologue in the right hemisphere.

106.5

CORTICOSPINAL PROJECTIONS IN THE INFANT AND MATURE MACAQUE MONKEY. Galea, M.P.* and Darian-Smith, I. Brain Research Laboratory, Univ. of Melbourne, Parkville, 3052, Victoria, Australia.

Postnatal regressive neuronal changes have been observed in callosal (Innocenti et al,'86, J.Neurosci.6, 1384) and thalamocortical projections to the macaque's sensorimotor cortex, (Darian-Smith et al.,'90 in press), and also in corticospinal projections in non-primate mammals (O'Leary and Stanfield,'86, Dev.Brain Res. 27, 87). We describe the changing postnatal corticospinal projections in the macaque monkey, which also reflect regressive changes. Fluorescent, retrogradely transported dyes [Fast Blue, Diamidino Yellow, Rhodamine and Green Latex Microspheres] were injected into the surgically-exposed cervical cord of each macaque, and the cortical distributions of labeled cells mapped and correlated with the cytoarchitecture in serial coronal sections. In each macaque, either newborn or mature, Fast Blue injections extended through much of the dorsal quadrant of C_2-C_3 on one side, whereas smaller injections of the other dyes were made at the level of C_7-T_1 on the other side.

In mature monkeys, Fast-Blue-labeled cells were found almost exclusively in Lamina V and mainly in the contralateral prefrontal, premotor, supplementary area, motor area 4; areas 3a, 3b, 1, 2, 5, 7a and 7b, the cortical folds of the cingulate sulcus through much of its length, SII and adjacent insular cortex. With injection sites mainly in the dorsal horn, labeled neurons were concentrated in postcentral cortex, while injections centered on the spinal intermediate zone also labeled many precentral cells.

In the newborn macaque additional corticospinal neurons were regularly in the lateral and orbital parts of the inferior frontal gyrus, and the lower lip of the lateral sulcus, with spread into the superior temporal gyrus. The cingulate projection was also denser than that observed in the mature brain, and while corticospinal neuron somas were mainly within lamina V, this was not always so. How these different corticospinal projections in newborn and mature macaques relate to sensorimotor performance is not clear.

106.6

CONNECTIONS OF ELECTROPHYSIOLOGICALLY DEFINED LOCATIONS IN PRIMARY MOTOR CORTEX, MI, OF OWL MONKEYS (AOTUS TRIVIRGATUS). Iwona Stepniewska, Anne Morel and Jon Kaas. Vanderbilt Univ., Nashville, TN 37240.

Microstimulation and anatomical techniques were combined to reveal connection patterns of MI in owl monkeys. Three or four different tracers (WGA-HRP and fluorescent dyes) were placed in the representations of the hindlimb, trunk, forelimb and face in MI. Labeled connections revealed parallel somatotopic inputs from areas 3a, 3b, and 1, with the input from 3b being sparse, especially for the forelimb. Connections with the hindlimb, trunk, and forelimb regions of MI formed a caudorostral sequence in the supplementary motor area. Dense inputs to MI originated from cortex in the lower bank of the lateral sulcus, and sparse label in cortex of the upper bank may have been in SII and the parietal ventral area. Callosal connections were with matched parts of MI, with the forelimb representation having sparse connections. Thalamic input zones in the ventrolateral complex formed a hindlimb, trunk, forelimb and face dorsolateral to ventromedial sequence. Other labeled structures included intralaminar nuclei, the reticular nucleus, CM, the putamen, claustrum, nucleus basalis, and nucleus of the diagonal band. (Supported by NS 16446.)

106.7

EFFECTS OF EXPERIMENTAL INFANTILE HYDROCEPHALUS AND VP SHUNTS ON MOTOR CORTEX CONNECTIONS. J.S.Way, S.D. Katz*and J.P. McAllister. Depts of Anatomy and Neurosurgery, Temple Univ. School of Medicine, Philadelphia, PA. 19140

Since previous neurotransmitter studies suggest that cortical afferents are altered irreversibly by ventriculomegaly, an axon tracer study was initiated on motor cortex connections. Hydrocephalus was induced in 10-11 day old kittens by intracisternal injections of 25% kaolin and monitored by ultrasound. Some hydrocephalic animals received VP shunts at 11-12 days post-kaolin. Normal age-matched animals served as controls. WGA-HRP injections were made unilaterally in cortical area 4 in: hydrocephalic animals at 9-15 days post-kaolin; shunted animals at 1,2 & 4 weeks post shunt; age-matched control animals. Sections were pro cessed by TMB histochemistry. Post-shunt ventriculomegaly was much reduced and accompanied by marked neurological improvement. Hydrocephalic brains had fewer labeled cells in contralateral cortex, ipsilateral claustrum, nucleus basalis, thalamic nuclei, dorsal raphe, ventral tegmental area and midbrain reticular formation, but no decrease in the locus coeruleus. Axonal label was reduced in thalamus, internal capsule, crus cerebri, pons, dorsal column nuclei, pyramids. All shunted animals exhibited labelling similar to controls. These results indicate damage to cortical pathways occurs during hydrocephalus, but decompression allows some structural restoration of these connections. Supported by HD21527 to JPM

106.8

MESIAL AREA 6 IN MACAQUE MONKEY IS FORMED BY TWO DIFFERENT CYTOARCHITECTONIC AND PHYSIOLOGICAL AREAS. M. Matelli *, G. Luppino*, R. Camarda* and G. Rizzolatti* (SPON:European Brain and Behaviour Society) Istituto di Fisiologia Umana Universita' di Parma Via Gramsci 14 I-43100 Parma Italy.

Although there is no agreement on whether the mesial part of area 6 is a single cytoarchitectonic area, this large cortical region has been generally considered coextensive with the so called supplementary motor area (SMA). Recently we demonstrated that a sector of mesial area 6 (area F3) shows a specific pattern of cytochrome-oxidase staining different from that of the dorsal convexity immediately lateral to it (area F2). Area F3 occupies the caudal two thirds of the whole mesial area 6 (Matelli et al.,Behav. Brain Res. 18:125-137,1985).

In the present experiments we investigated the cytoarchitectonic features of mesial area 6 in celloidin embedded macaque brains. Two regions were identified: a larger region, caudally located, which basically coincided with histochemical area F3; a smaller region (which will be referred to as area F6) , located in the rostral third of mesial area 6. Lateral to area F6, on the dorso-lateral convexity, a third cytoarchitectonic area was identified (area F7). In order to see whether physiological differences do exist among these areas, intracortical microstimulation (ICMS) was performed in three macaque monkeys. Area F3 was found to be easily excitable and movements were obtained in 493 out of 600 tested sites. A complete somatotopic map similar to that described by Mitz and Wise (J.Neurosci. 7:1010-1020,1987) was demonstrated. ICMS of area F6 was also effective although its excitability was lower than that of area F3 (108 out of 214 cortical sites). Slow displacements of the arm, which somehow mimicked natural movements or postural adjustements, were observed in 85% of excitable sites. No clear somatotopic organization was observed. Eye movements were elicited from area F7.

In conclusion, mesial area 6 comprises two different areas.One of these (area F3) coincides with the SMA. The other one (area F6) appears to be involved in the arm control at a rather high level . Cortico-cortical connections of this last area and its single neurons properties (Exp. Brain Res., in press) suggest this conclusion.

106.9

CONNECTIONS OF MEDIAL AGRANULAR CORTEX WITH PRIMARY MOTOR AND SOMATOSENSORY CORTICES IN RATS. J.J. Diehl and M.F. Huerta. Center for Neurological Sciences and Dept. BioStructure & Function, Univ. Conn. Health Ctr., Farmington, CT 06032.

While oculomotor-related functions of the medial agranular cortex (AGm) have been emphasized in recent studies, this region is also known to have strong connections with somatosensory and somatomotor structures. In order to study this aspect of AGm, the connections between AGm and primary somatosensory (S-I) and motor (M-I) cortical areas were determined using the fluorescent tracers fast blue (FB) and diamidino yellow (DY). Injections of FB in the hindlimb representation of M-I and DY in more rostral M-I in the same case revealed the topography of the AGm projection to M-I; these data suggest that the same zone of M-I receives input from two widely separated regions within AGm. Other experiments were designed to reveal the laminar patterns of AGm which project to S-I and M-I; injections of FB and DY in the same animal showed that cells projecting to S-I were concentrated in layer V, but also present in VIb, while cells projecting to M-I were dense in layers II, V and VIb, but also present in layers III and VIa. These preliminary findings indicate that AGm has highly organized connections with somatosensory and somatomotor cortex.

106.10

CHARACTERIZATION OF PARVALBUMIN NEURONS AND AXONS IN THE PRIMATE PREFRONTAL CORTEX. S.M. Williams, P.S. Goldman-Rakic, and C. Leranth . Sec. of Neuroanatomy and Dept. of Obstetrics and Gynecology, Yale Univ. School of Med., New Haven, CT 06510.

Previous studies have demonstrated that the primate neocortex possesses a population of non-pyramidal neurons (chandelier and basket cells) that contain the calcium-binding protein, parvalbumin (PV). We have conducted light and electron microscopic analyses of PV-immunoreactive (PV-IR) neurons in the prefrontal cortex (PFC) of the macaque. With the exception of layer I, all cortical laminae contained PV-IR somata; however, immuoreactive cells were concentrated in layers IIIB and V. Electron microscopy revealed that PV-IR axon terminals heavily innervate the soma and axon hillock of layer II/III pyramidal neurons. Interestingly, PV-IR boutons also established synaptic contact with large spines that emerged from the initial segments of these pyramidal cell axons. Furthermore, PV-IR axons formed dense basket-like networks of terminals on layer V pyramidal somata, occasionally contacting the axon hillock of these neurons.

To further characterize the target cells of parvalbumin-containing axons, horseradish peroxidase injections were made in the dorsolateral prefrontal cortex of monkeys. The contralateral PFC was processed for both the detection of retrograde labeling and PV-IR. EM analysis demonstrated that PV-IR processes densely innervate retrogradely labeled large pyramidal neurons of the contralateral hemisphere (i.e. commissural neurons). Furthermore, myelinated PV-IR axons were often observed in both the cortical grey and white matter. This observation has led to the hypothesis that the PFC receives an extrinsic, possibly commissural, parvalbumin input. Preliminary experiments combining retrograde tracing and PV-immunohistochemistry in the rat strongly suggest that a population of commissural neurons are PV-IR. These studies are currently being conducted in primate PFC. Thus, PV-IR neurons may subserve both local circuit and projection roles in the interhemispheric transfer of cortical information. Supported by MH44866-02 and MH38546-11.

106.11

PREFRONTAL CONNECTIONS WITH THE PREMOTOR AREAS. M.T. Lu and P.L. Strick. VA Med. Ctr. and Depts. of Neurosurg. & Physiol., SUNY-HSC @ Syracuse, Syracuse, NY, 13210.

We placed multiple injections of one tracer (e.g., diamidino yellow) in the arm area of the primary motor cortex in macaques to define the premotor areas in the frontal lobe. Then, in the same monkeys, we placed multiple injections of another tracer (e.g., WGA-HRP) in and around the principal sulcus (Walker's area 46) to determine which premotor areas are interconnected with the prefrontal cortex. Neurons projecting to prefrontal cortex (PF neurons) were unevenly distributed in the premotor areas. In fact, over 62% of these neurons were found in the arm representation of a single premotor area, the arcuate premotor area (APA). A smaller number of PF neurons (22%) were found within specific portions of the rostral and caudal cingulate motor areas (CMAr and CMAc) which are buried in the banks of the cingulate sulcus. Finally, about 13% of the PF neurons were found in the arm representation of the supplementary motor area (SMA). Most of these neurons were confined to a small region of the SMA which lies rostral to the genu of the arcuate sulcus.

These observations suggest that the APA represents a major site for interactions between the prefrontal cortex and the motor system. Our results also suggest that input from the prefrontal cortex is focused on specific portions of the premotor areas on the medial wall of the hemisphere (i.e., the SMA, CMAr and CMAc). Support: VA Med. Res. Serv.; USPHS 2957, 24328.

106.12

PREMOTOR AREAS: CORTICOSPINAL PROJECTIONS TO UPPER AND LOWER CERVICAL SPINAL CORD. S.Q. He, R.P. Dum and P.L. Strick. VA Med. Ctr. and Dept. of Neurosurg. & Physiol., SUNY-HSC @ Syracuse, Syracuse NY, 13210.

We have recently presented evidence that the frontal lobe of macaques contains at least 6 premotor areas which project to both the primary motor cortex and the spinal cord. In this experiment, we have examined the topographic organization of the corticospinal projections from the premotor areas by injecting a fluorescent tracer (e.g. DY) into upper cervical segments (C2-4) and a second tracer (e.g. FB) into lower cervical segments (C7-T1) of the spinal cord.

Five of the six premotor areas contained neurons which project to lower cervical segments and neurons which project to upper cervical segments. Only the arcuate premotor area had a segmental termination limited largely to upper cervical segments. Most neurons projected to either upper or lower segments; only 5% of the sample was 'double labeled.' In the premotor areas, as well as in the primary motor cortex, regions which project to upper cervical segments overlapped those which project to lower cervical segments. However, in each cortical area, the regions with the highest density of neurons projecting to a segmental level were spatially separate. Our results suggest that the topographic organization of arm representation in 5 of the 6 premotor areas has characteristics which are similar to that in the primary motor cortex.
Support: VA Med. Res. Serv. and Rehab. R&D; USPHS 24328.

106.13

EMERGING PATTERNS OF CONNECTIVITY IN CAT SENSORIMOTOR CORTEX. L.L.Porter, Dept. of Anatomy, USUHS, Bethesda, MD 20814

The manner in which information is processed in cat somatosensory cortex is reflected in the network of fiber pathways linking its functionally speciallized subregions, each of which may provide motor cortex with different sensory input. Both area 2, which may integrate input and area 3a, which receives muscle afferents project directly to motor cortex. The distribution of area 3a afferents to MI were studied following injections of PHA in area 3a and compared to that of area 2 afferents. Labeled axons branched in the superficial laminae of discrete regions of motor cortex,similarly to axons arising in area 2. Labeled fibers, evenly distibuted through the cortical laminae were noted in area 2 where their input may be integrated with others before being relayed to motor cortex. To further study the flow of information, 2 fluorescent tracers were injected in ideniti-fied forelimb regions of areas 3a and 4. Clusters of retrogradely labeled neurons were located in adjacent or overlapping regions of area 2 but individual cells were not double labeled, indicating that separate groups of cells project to the target sites. Neurons projecting to area 4 are in superficial laminae; those which project to 3a often exhibit a bilaminar arrangement. Variations seen in laminar distributions of axon terminals (which are being confirmed with EM) and differences in laminar distribution of retrogradely labeled cells may indicate a directional flow of information processing in the sensorimotor cortex.

106.14

HORIZONTAL LAYER I INPUTS TO PRIMARY SOMATOSENSORY (BARREL-FIELD) NEOCORTEX OF RAT. L.J. Cauller and B.W. Connors, Section of Neurobiology, Div. of Biology & Medicine, Brown University, Providence, RI 02912.

SII and MI cortex were injected with rhodamine-dextran (10k MW), and anterogradely labeled axons were followed into SI (identified with cytochrome oxidase staining). Within SI, axons ascended directly to layer I, where they often extended >1 mm horizontally and gave off numerous terminal branches. These projections resemble the backward system in the visual cortex of cats and monkeys (Rockland & Virga, *JCN* 1989). Layer I pathways were isolated in coronal slices *in vitro* with a vertical cut that preserved only layer I. Layer I was then stimulated >0.3 mm lateral to the cut while recording medially. Current-source density analysis revealed current sinks restricted to layer I, with smaller current sources distributed in layers II and III. While the layer I-evoked sinks lasted no more than 20 ms, intracellular recordings from layer Vb pyramidal cells showed long-lasting EPSPs (50-200 ms) without any obvious IPSPs. Layer I-evoked fields and EPSPs were blocked by CNQX (5 μM). Only the long-latency (>50 ms), possibly polysynaptic, EPSPs were blocked by APV (50 μM). We conclude that primary sensory cortex may be strongly activated by backward glutaminergic projections to layer I, and that synapses on the most distal apical dendrites can strongly excite neurons as deep as layer Vb.

Supported by the NIH.

106.15

CORRELATIONS BETWEEN PHYSIOLOGY, MORPHOLOGY AND PARVALBUMIN CONTENT IN RAT NEOCORTICAL NEURONS. J.F.M van Brederode * and A.E. Hendrickson, Depts. of Biological Structure & Ophthalmology, Univ. Washington, Seattle WA 98195.

Neocortical neurons have been classified according to their firing patterns in response to intrasomatic depolarizing current injections into regular-spiking (RS), intrinsic bursting (IB), or fast-spiking (FS). Each correlates to distinct morphological cell types (TINS 13:99-104,1990). Some of these firing properties could be due to intracellular calcium-binding proteins such as parvalbumin (PV). This study examines the correlation between the physiology, morphology, and PV immunoreactivity in single cells of rat neocortex in *in vitro* brain slices. After recording the response to intracellular current injections of succesfully impaled cells, biocytin was injected into the cells. Following fixation biocytin was visualized using avidin/rhodamine (RITC). The tissue was then reacted immunocytochemically for PV using fluorescein (FITC)/IgG. Two-wavelength excitation appropriate for RITC and FITC was used to examine injected cells for the presence of PV. Biocytin was transformed into a permanent label for light-microscopic analysis by binding biocytin to avidin/peroxidase and reacting with diaminobenzidine. Our results indicate that most RS cells were pyramidal cells with varying morphology and laminar localization. FS and IB cells were impaled infrequently. FS cells consisted of smooth stellate and inverted pyramidal cells. Preliminary results suggest that PV immunoreactivity is found in a physiologically and morphologically heterogeneous group of cells. This study was supported by grants EY 01208 and EY07031.

106.16

RELATIVE INTRACORTICAL LOCATION OF SYNAPTICALLY INTERACTING CELLS IN PRIMATE PRECENTRAL MOTOR AREAS.

M. Matsumura*, D.-F. Chen & E.E. Fetz. Regional Primate Research Center and Dept. of Physiology & Biophysics, University of Washington, Seattle, WA 98195.

Synaptic interactions between pairs of neurons in precentral gyrus were documented by spike-triggered averages (STAs) of membrane potentials in 3 monkeys. Extracellular trigger spikes were recorded from "EC" cells with carbon-fiber electrodes, while intracellular recordings were obtained from neighboring "IC" cells with K-methylsulfate electrodes. EC trigger spikes could be evoked in anesthetized monkeys by iontophoretic injection of glutamate and were also obtained in awake monkeys during performance of a step-tracking task. STAs revealed serial EPSPs, IPSPs, common synaptic input or their combination. Successive extracellular recordings demonstrated convergent input from multiple EC cells to the same IC cell. Successive intracellular penetrations showed the divergent connections of single EC cells to multiple neighboring IC cells; for example, one inhibitory neuron produced IPSPs in 7 of 10 IC cells. To date, the relative locations of 94 cell pairs recorded under these conditions were determined by reposition of microelectrodes and their cortical tracks confirmed by histological reconstruction. Common synaptic input was found in 68 pairs; a given EC cell could share common input with IC cells up to 2.4 mm away, and located in all cortical layers. In 19 cases EC cells produced serial EPSPs in IC cells located in more superficial layers (II & III) and/or in deeper layers (V & VI), and up to 2.2 mm away. Similarly, in 17 cases EC cells produced serial IPSPs in IC cells located in more superficial and/or deeper layers, and up to 1.4 mm away. These results elucidate the synaptic interactions between cells in a column and confirm the existence of excitatory and inhibitory connections from deeper to upper layers as well as the reverse.

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106.17

SPIKE AFTERPOTENTIALS OF HUMAN NEOCORTICAL NEURONS. Lorenzon, N.M. and Foehring, R.C., Dept of Anatomy and Neurobiology, Univ. of Tenn., Memphis, TN 38163

Intracellular recordings were made from human cortical neurons in an in vitro slice preparation. Neurons were manipulated pharmacologically in order to study Ca^{2+}-dependence of AHPs, the role of K^+ currents in these potentials, and modulation of AHPs by neurotransmitters.

Three afterhyperpolarizations (AHPs) have been studied. Foliowing single action potentials, fast and medium duration AHPs are produced. Repetitive firing episodes result in AHPs of medium and slow durations. The slow AHP had a reversal potential similar to the predicted Nernstian value for K^+ equilibrium. This AHP was Ca^{2+}-dependent and showed sensitivity to norepinephrine and serotonin. The fast and medium duration AHPs reversed polarity at more depolarized membrane potentials than the K^+ equilibrium potential. Both AHPs were Ca^{2+}-sensitive. Studies in which extracellular Cs^+ (2-3mM) was applied suggest that the medium AHP has contributions from outward K^+ current(s) and an anomolous rectifier (Q) current. Some cells exhibited an afterdepolarization following a single spike which was Ca^+-dependent and overlapped temporally with the medium AHP. The conductances underlying the AHPs affect several aspects of cell excitability; for example, spike repolarization and duration, and spike frequency adaptation and habituation. The effects of several substances on these AHPs have been studied: extracellular Cd^{2+}, Co^+, Cs^+, K^+, NE, and TEA.

Supported by NINDS grant #NS27180 and an Epilepsy Foundation Research grant.

106.18

IONIC BASIS FOR BURST-FIRING BEHAVIOR IN HUMAN NEOCORTEX USING IN-VITRO SLICE PREPARATION: POSSIBLE ROLE OF M AND Q CURRENTS. R.S. Waters, and R.C. Foehring, Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, Col. of Medicine, Memphis, TN 38163.

Two types of firing behavior have been described in human neocortical cells in-vitro. Regular spiking neurons respond to a suprathreshold injection of current with repetitive firing throughout the stimulus, while burst-firing neurons respond with an initial short burst of 2-5 spikes that ride upon a slow wave of depolarization. The burst response is voltage-dependent, occurring only from membrane potentials negative to -60mV. Burst-firing neurons also exhibit afterdepolarizations (ADP) and voltage- and time-dependent post-stimulus rebound. Application of TTX in the bath eliminates action potentials leaving only the slow wave of depolarization. This wave has been shown to be Ca-dependent in a number of species since blockage occurs with inorganic Ca channel blockers. These Ca channel blockers do not appear to totally block the slow wave in human neocortical cells, suggesting that other ionic currents may also contribute.

To test this hypothesis, human neocortical tissue that had been removed during epilepsy surgery was placed in a chamber containing artificial CSF bubbled with carbogen at 36°C. Pharmacological agents were gravity fed into the bath or pressure injected in the vicinity of the recording electrode. Using these techniques the following results were obtained:

1. Co^{2+} blocked the ADP.
2. In the presence of TTX, inorganic Ca-blockers greatly reduced the slow depolarizing wave underlying the burst and rebound following hyperpolarizing stimuli.
3. The remaining wave form could be reduce or eliminated by 2mM extracellular cesium suggesting that a Q-like current may play a role.
4. The possible involvement of M current was also examined. (Supported by grants from NSF, BNS 88-02766; NINDS, NS287180; The Epilepsy Foundation.)

106.19

MORPHOLOGY OF PHYSIOLOGICALLY IDENTIFIED HUMAN CORTICAL NEURONS. P. Herron, R. C. Foehring, and C. J. Wilson. Dept. of Anatomy and Neurobiology, The University of Tennessee, Memphis, Memphis, TN 38163

A primary aim of this study was to determine if physiological classes can be related to particular morphological classes of neurons in the human neocortex. These experiments were conducted on slices of cortical tissue removed from occipital, frontal, and temporal lobes during the course of surgeries for the relief of epilepsy. Data from 27 neurons were used in this study. Glass microelectrodes containing 1M acetate and 2% biocytin were used for the intracellular recording and labeling of neurons. Three-dimensional reconstruction and morphometric analyses of filled neurons were accomplished using an Eutectic Electronics Neuron-Tracing System.

Three physiological classes were identified: burst-firing neurons, regular-spiking neurons, and fast-spiking neurons. Fourteen of the 27 neurons were characterized as regular-spiking neurons. Regular-spiking neurons were almost exclusively pyramidal neurons (12 of 14, 86%) with 72% (10 of 14) of these located in layer V. The two remaining regular-spiking neurons were large pyramidal-like spiny stellate neurons located in layers III and IV. Burst-firing neurons were equally divided between pyramidal and nonpyramidal classes. Three of the nonpyramidal neurons were identified as spiny stellate neurons in layers III through V. Only two of the 27 neurons were fast-spiking neurons. One was a nonpyramidal neuron.

These results suggest that the neuronal substrates for different types of physiological behaviors are differentially distributed amongst the morphological classes of neurons in the human neocortex. Supported by NINCDS #NS27180 and The Epilepsy Foundation (to RCF).

CORTEX II

107.1

TRANSCALLOSAL EFFECTS ON MOTOR CORTICAL EXCITABILITY IN MAN. A. Ferbert, A. Priori, J.C. Rothwell, J.G. Colebatch, B.L. Day and C.D. Marsden. MRC Human Movement & Balance Unit, Institute of Neurology, Queen Square, London WC1N 3BG, U.K.

Electric or magnetic transcranial stimulation over the motor cortex can produce evoked potentials over the contralateral hemisphere. Here we show that this is accompanied by a pronounced inhibition of the excitability of the contralateral cortex. The experiments used two Novametrix Magstim 200 stimulators. One of the stimulators was connected to a figure-of-eight shaped coil (loop diameter 7cm) placed with its mid-region over the motor cortical hand area of one hemisphere. This provided the conditioning stimulus. At intervals from 1-160ms later, a test stimulus was given to the opposite hemisphere using a 9cm diameter round coil, or another figure-of-eight coil. Surface EMG responses were recorded from the slightly pre-activated first dorsal interosseous muscles of each hand. The main effect of the conditioning stimulus was to inhibit the test response. The inhibition began at intervals of 6ms, peaked at 10-15ms, and recovered slowly up to 50-100ms. In some subjects, the inhibition was preceded by a short period of facilitaiton at conditioning-test intervals of 4ms. The average depth of the maximum inhibition was of the order of 50%. In contrast to the pronounced effect on the size of magnetically-evoked EMG responses, the same conditioning shock in three subjects had little or no effect on responses evoked by electrical stimulation of the motor cortex. Assuming that the electrical and magnetic test stimuli activate the same motor output pathways from the cortex, then this differential effect of the conditioning shock indicates that inhibition was occurring at a cortical level. In view of its onset latency, we suggest that this inhibition was mediated via a trans-callosal pathway.

107.2

EFFECTS OF TRANSCRANIAL MAGNETIC STIMULATION ON IPSILATERAL MUSCLES IN HUMANS. E. M. Wassermann, P. Fuhr*, L. G. Cohen and M. Hallett. Human Cortical Physiology Unit, Human Motor Control Section, National Institute of Neurological Disorders and Stroke, NIH, Bethesda, MD 20892.

We studied the effects of focal transcranial magnetic stimulation on ipsilateral muscles in 6 normal volunteers. An "8"-shaped coil was used to deliver stimuli to different scalp positions over one hemisphere. EMG recordings were made from muscles of both upper extremities, at rest and at several degrees of voluntary ipsilateral muscle activation.

No ipsilateral responses were observed with the arm at rest. During muscle activation, stimuli evoked a transient attenuation of ongoing voluntary EMG activity (ipsilateral silent period, ISP). ISPs were highly reproducible and could be evoked in scalp locations which activated corresponding contralateral muscles. The ISP in abductor pollicis brevis had an onset latency of 35±4msec (12 msec longer than the onset of the contralateral excitatory response), and a duration of 46±15msec. At 50% muscle activation, the peak attenuation was 71±18%. In wrist flexors, biceps and deltoid ISPs tended to be less prominent. On some occasions excitatory responses preceded the ISPs.

This study demonstrates the existence of a powerful inhibitory influence of sensorimotor cortex on ipsilateral upper extremity muscles and on distal muscles in particular.

107.3

PHYSIOLOGICAL ANALYSIS OF REORGANIZATION FOLLOWING LOWER LIMB AMPUTATION IN HUMANS. P. Fuhr*, L.G. Cohen, T.W. Findley*, J. Macedo* and M. Hallett. Human Cortical Physiology Unit, Human Motor Control Section, Medical Neurology Branch, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD 20892, and Kessler Institute for Rehabilitation, West Orange, NJ 07052.

We studied the locus of motor reorganization after amputation in 5 subjects who had loss of a lower limb. A Cadwell MES-10 stimulator was used to deliver a magnetic stimulus through a round coil centered on the sagittal line 4 cm anterior to Cz and through an 8-shaped coil positioned over locations 1 cm apart along the coronal axis. Surface EMG was recorded bilaterally from quadriceps femoris. Excitability of the spinal alpha-motoneuron pool to I a afferents was evaluated by comparing the maximal H reflex with the maximal M wave (H:M ratio).

Motor reorganization after lower limb amputation was similar to that previously observed after upper limb amputation (Cohen, L.G. and Hallett, M. Soc. Neurosci. Abstr., 15:1239, 1989). In all 5 subjects, stimuli of equal intensity in both hemispheres recruited a larger percentage of the alpha-motoneuron pool in muscles ipsilateral to the stump than in contralateral muscles (46.8% vs 7.7%, $p \leq 0.05$) and mean onset latencies were 2 ms shorter ($p \leq 0.01$). Muscles ipsilateral to the stump were activated from a larger number of scalp positions than contralateral muscles ($p \leq 0.05$). There was no difference in the H:M ratio between the legs (8.0% ipsilateral vs 11.0% contralateral). The apparent absence of a change in the excitability of the alpha-motoneuron pool after amputation suggests that reorganization occurs at a suprasegmental level.

107.4

ARE LONG LATENCY CUTANEOUS REFLEXES TRANSCORTICAL IN MAN? P. Ashby, E. Palmer. Playfair Neuroscience Unit, Toronto, Canada

We tested the hypothesis that the late reflex activity occurring in small hand muscles following stimulation of cutaneous afferents is a long loop reflex involving the cortex.

Cutaneous afferents of the index finger were stimulated electrically at 1.5-2 x perception threshold. Cortical neurons were excited using magnetic stimulation. Single motor units were recorded with a needle electrode in the first dorsal interosseous. Three simultaneous post-stimulus time histograms were generated following submaximal stimuli to cutaneous afferents, the cortex, and to both sites at a suitably timed interval. We assumed that the short latency facilitation produced by magnetic stimulation is due to monosynaptic activation of spinal motoneurons. Convergence on a common pool of upstream neurons would be suspected if the facilitation produced by both stimuli was different from the sum of the facilitations produced by each stimulus alone.

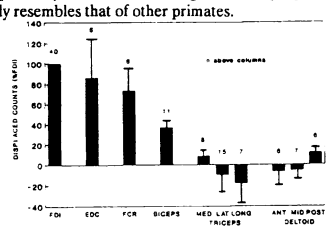
In 7 of 8 experiments the facilitation produced by both stimuli was less than the algebraic sum of each alone. Furthermore the short latency facilitation of motoneuron produced by magnetic stimulation was reduced by a burst of cutaneous stimuli at 50 Hz beginning 500 msec prior to magnetic stimulation. Thus although both stimuli produce facilitation of spinal motoneurons the combination of stimuli results in less facilitation. There are a number of possible explanations for this. 1. Failure to superimpose the facilitations exactly. 2. Collision between the two afferents volleys, 3. Inhibition between the two fiber systems activated.

These data do not support the hypothesis that the late reflex activity from cutaneous stimuli is a transcortical reflex.

107.5

CORTICOSPINAL PROJECTIONS TO UPPER LIMB MOTONEURONS IN MAN E.Palmer , P. Ashby, Playfair Neuroscience Unit, Toronto, Canada

Magnetic stimulation of the motor cortex was used to excite corticospinal pathways in normal human subjects. The characteristics of the shortest latency post synaptic potentials (PSPs) generated in individual motoneurons were derived from PSTHs of single motor units. The number of displaced counts in a period of increased or decreased firing probability was used to estimate the amplitude of the underlying composite EPSPs or IPSPs. The amplitudes of PSPs produced in various motoneurons were expressed as a percentage of the amplitude of the EPSP generated in the first dorsal interosseous muscle (FDI) of that subject,under the same conditions. The means of these percentages were used to determine the overall strength of the short latency corticospinal projections to upper limb motoneurons (shown with 1 SE in fig.). We conclude that the short latency corticospinal pathways have a recognizable projection pattern in man, which closely resembles that of other primates.



107.6

DYNAMICS OF SOMATOSENSORY-MOTOR INTEGRATION IN HUMAN NEOCORTEX. Steven Bressler, Brian Cutillo* and Alan Gevins. EEG Systems Lab., San Francisco, CA 94117. Neurosurgery patients at the UCSF Epilepsy Center, with 64-contact electrode grids implanted subdurally to localize seizure foci, performed several hundred trials of a somatosensory discrimination task. Trials began with a fixation point on a CRT screen. After 1 sec, constant voltage pulses of 3 different durations were presented to the little finger of 1 hand. Subjects responded to the low and medium duration stimuli with index finger flexions of the same hand to produce required pressures of 0.2 and 0.8 kg. They were required not to respond to the high duration stimulus. Visual feedback of the exerted response pressure was presented 0.5 sec after completion of the response in response trials. Somatosensory Evoked Potentials (SEPs) were averaged for each stimulus type and low-pass filtered at 7 Hz. Event-Related Covariance (ERC) functions were computed between all electrode pairs in 187-msec overlapping intervals covering the SEP for 1 sec poststimulus. The maximum ERC value and the time delay to maximum were derived for each electrode pair.

Results from 1 patient are reported. In the first poststimulus interval, the pattern of covariance following all 3 stimulus types was focused on 2 somatosensory sites believed to represent the little finger. The early cortical SEP peaks(N22-P37-P54) were only found at those sites, and P90 was maximal there. The ERC patterns for the 3 stimulus types then diverged. For the 2 conditions requiring a pressure, the patterns went through a similar progression in which ERCs were first established between the little finger area and a group of temporal sites, then between these temporal sites and a motor site, possibly representing the index finger. This sequence was delayed preceding the hard pressure, which also was delayed in reaction time. Possible signs of signalling from the motor to sensory representation of the index finger were also evident. In the no-response condition, the sequence did not progress beyond the temporal sites. These results show the sequencing of sensory-motor integration in the human neocortex with unprecedented spatiotemporal precision, and demonstrate that the ERC method can measure signs of cortical functional relation.

107.7

THE EFFECT OF IMAGINARY MAXIMAL MUSCLE CONTRACTION TRAINING ON THE VOLUNTARY NEURAL DRIVE TO MUSCLE. G. Yue, Dept. of Exercise Science, University of Iowa, Iowa City, IA 52242.

Studies have demonstrated that initial strength gains in a strength training program are primarily due to the changes in the nervous system because muscle hypertrophy cannot take place as rapidly. This finding suggests that neural factors alone may be able to produce strength increases providing that the central neural states can be altered. Other studies have shown that the neural output from the cortex to spinal motor neurons may be altered by mental practicing of skilled movements. This evidence indicates that similar neural changes obtained through strength training may be yielded during mental training. Given that neural changes alone are able to produce voluntary strength gains and such neural changes may be achieved in mental training, it is reasonable to hypothesize that imaginary maximal muscle contraction (IMMC) training can increase voluntary neural drive to the trained muscle and produce strength increases. Subjects were asked to imagine 15 maximal contractions of the left abductor digiti minimi (ADM) in each training session for a total of 20 sessions. EMG recordings during training indicated that the trained muscle was inactive. After training there were increases in maximal strength and integrated EMG of the ADM muscles of both hands. Such increases were not found in the control group and control muscles of the subjects who were trained by the IMMC method. These results suggest that training which utilizes imaginary muscle contractions may yield neural events that are similar to those which yield increased strength in strength training before muscle hypertrophy occurs. Moreover, cross-training effect may also result from these neural events.

107.8

MOTOR AND FRONTAL CORTEX INVOLVEMENT IN PLANNING AND EXECUTION OF MOVEMENT DIRECTION IN HUMANS. K. L. Kerman*, J. Martin*, J. N. Sanes, J. P. Donoghue, Department of Clinical Neuroscience and Center for Neural Science, Brown University, Providence, RI 02912.

Recent studies suggest that the primary motor (MI) and frontal cortex are important in coding movement direction. To explore this issue further, we examined whether MI and frontal cortex have separate roles in planning and executing two-dimensional arm movements by studying humans with distinctive pathology of the two regions.

Arm kinematics were measured in 7 normal subjects and in 6 patients each with pathology, localized on CT scan, in frontal cortical areas. Subjects moved a hand-held stylus across a horizontally oriented digitizing tablet as quickly and as accurately as possible through a three point course displayed on a video monitor. The start and end points had the same X but different Y coordinates. The pattern had a direction reversal point with displaced X and mid Y coordinates relative to the start and end points. The target position was always visually available on the video monitor, and the hand was visible. The movements primarily required elbow and shoulder joint rotations.

Normal subjects moved through the path with a near ideal parabolic trajectory performing a continuous, smoothly executed movement having two velocity peaks and a non-zero-trough at direction reversal. Their trajectories closely fit a minimum jerk profile. With MI lesion, contralateral arm movements had frequent small changes in direction, concomitant multiple peaked velocity profiles, and longer movement times. The frequent direction shifts helped to maintain an overall, but jerky, parabolic trajectory. By contrast, with lesions rostral to MI, movements were decomposed into 3 straight segments, one being parallel to the start and end points, and each with sharp direction changes. Segments executed by these patients had smooth velocity profiles. With both lesions, the ipsilateral hand followed a normal path.

These results suggest that MI and frontal cortex have different roles in coding movement direction. Since MI lesion disrupted movement smoothness, but not the overall movement path, these data suggest that MI controls movement execution, but not movement planning. In contrast, cortical areas anterior to MI seem necessary for organizing optimal movement strategy, while contributing little to execution.

107.9

CORTICAL ACTIVITY PRECEDING SEQUENTIAL HAND MOVEMENTS IN HUMANS. J. Artieda*, S.M. Papa*, J. A. Obeso*. Dpt. of Neurology, University of Navarra, Pamplona-Spain.

We recorded in 10 normal subjects (mean age 27±4) the readiness potential (RP) preceding self-paced and externally induced sequential hand movements. Using the first EMG discharge (wrist extension) to trigger averaging showed a typical RP starting 1 second before self-induced movements but absent RP for externally referenced movements. Using the second EMG burst (wrist supination) for triggering revealed a motor potential timelocked to the externally referenced movement and preceding it by some 200 ms. Brain mapping analysis showed its origin restricted to motor areas. These results indicate that for sequential motor acts the first movement may act as a "go" signal for the second one and that the earlier component of the RP can be dissociated from the actual motor potential.

107.10

EFFECTS OF POSTERIOR ASSOCIATION CORTEX LESIONS ON BRAIN POTENTIALS PRECEDING SELF-INITIATED MOVEMENTS

Jaswinder Singh and Robert T. Knight Dept. of Neurology, University of California, Davis, VAMC; Martinez CA.

Movement Related Potentials (MRPs) were recorded in patients with unilateral lesions centered in temporal-parietal junction (T-PCx, n = 5; male; mean age = 59. 1, SD = 4.6; damaged areas 22, caudal 39 and 40; lesion volume = 38.5cc), lateral parietal (ParCx, n = 5; male; mean age = 52. 1, SD = 15. 7; damaged areas 5, 7, rostral 39 and 40; lesion volume = 35.3cc), posterior association cortex (T-PCx and ParCx; n = 5; 4 male; mean age = 65. 2, SD = 6.9; damaged areas 22, 39, 40, 41 and 42; lesion volume 88.1cc) and normal controls (n = 10; 7 male; mean age = 56. 4, SD = 7. 4). Subjects performed a self-paced button press task. Data epochs beginning 1400 msec prior to and 600 msec after each motor response were extracted from scalp sites over precentral, central and parietal regions. Controls and patients with temporal lesions generated comparable Readiness potentials (RPs, onset 1000 msec), Negative shifts (NS's, 500 msec) and Motor potentials (MPs, onset 100 msec). In contrast, unilateral lesions involving the lateral parietal cortex markedly reduced MRPs. The findings indicate that parietal association cortex contributes to MRP generation.

107.11

DIRECT CORTICAL RECORDINGS FROM HUMAN MOTOR CORTICES: EVIDENCE FOR DIRECTIONAL TUNING IN TWO DIMENSIONAL MOVEMENTS. C. Toro*, C. Cox*, RE. Maxwell*, JR. Gates*, TJ. Ebner. Depts of Neurology, Neurosurgery and Physiology Univ of Minnesota, Minneapolis, Mn 55455.

Chronic unit data suggests that movement direction is represented in premotor, motor and parietal cortices of monkeys. Attempting to extend these findings to humans we analyzed the electroencephalographic activity from 32 subdural electrodes in eight epileptic patients during two dimensional arm movements. The task consisted in displacing a hand controlled cursor from a start position in the middle of a video screen to one of 18 possible targets in six different directions and three different distances. Changes in signal power in the 8-12 Hz band related to the task were calculated for each electrode computing a Fast Fourier Transformation (FFT) on a 1.0 sec moving window shifting over the raw data at increments of 250 msec. The probabilities for power change between the baseline period (start position) and different time cross-sections during the task were determined. Groups of electrodes displayed probabilities for power change broadly "tuned" to movement direction. A pattern of greater power decline when moving into contralateral space (to the hand performing the task) was present in all patients. These findings suggest that kinematic variables are represented in the human motor cortex. Supported by UMHC and MINCEP.

COMPARATIVE NEUROANATOMY: REPTILES, BIRDS, MAMMALS

108.1

CALCIUM BINDING PROTEIN IMMUNOREACTIVITY IN REPTILIAN DORSAL THALAMUS. M.B. Pritz and M.E. Stritzel*. Div. of Neurol. Surg., Univ. Calif. Irvine Med. Ctr., Orange, CA 92668.

Neurons in mammalian thalamus can be characterized by their immunoreactivity for certain calcium binding proteins. We investigated the analagous situation in reptiles, Caiman crocodilus, using antibodies to a variety of calcium related proteins. These included: calbindin, parvalbumin, calmodulin, calcitonin gene-related peptide (CGRP), troponin-T, and caldesmon. Experiments employed both monoclonal (calbindin, parvalbumin, troponin-T, caldesmon) and polyclonal (calbindin, parvalbumin, calmodulin, and CGRP) antibodies. In some cases, colchicine pretreatment was used. Free floating 30μm sections were cut on a sliding microtome and processed utilizing the avidin-biotin complex technique. Preliminary data is the following. Neurons in nucleus rotundus, nucleus diagonalis, and the medialis complex were found to be immunoreactive for parvalbumin, calbindin, and CGRP. By comparison, parvalbumin immunoreactivity in these areas was less intense than either calbindin or CGRP. Cells in nucleus reuniens were immunoreactive for calbindin and CGRP. Neurons in nucleus dorsolateralis anterior expressed immunoreactivity for calbindin. Although immunoreactivity to calmodulin, troponin, and caldesmon was found elsewhere in the brain, neurons immunoreactive for any of these three antibodies have yet to be found in any dorsal thalamic nucleus. Further experiments to complete this analysis are presently underway.

108.2

AXONAL ARBORIZATIONS OF PYRAMIDAL AND NON-PYRAMIDAL NEURONS IN THE CEREBRAL CORTEX OF THE TURTLE. P. H. Desan and A. R. Kriegstein. Dept. of Neurology, Stanford University, Palo Alto, CA, 94305.

We examined the axonal morphology of neurons in the medial areas DM and M of the cerebral cortex of the pond turtle (Pseudemys scripta elegans). Neurons were filled with the tracer biocytin using whole-cell patch-clamp recording, a technique enabling the visualization of axonal arborizations. The axons of pyramidal cells gave rise to a relatively dense arborization within a tangential radius of 500-800 um about the soma. The peak density of axonal varicosities, presumably synapses, per square 100 um of tangential cortical area was as high as 20-40/100 um^2. Long, seldom branching collaterals with infrequent varicosities extended great distances in all directions from the soma. Some were traced as far as 2300 um. Within this area the density of varicosities was remarkably constant in the range of 4-8 varicosities/100 um^2. In addition all pyramidal cells possessed one or more axon collaterals entering the fornix. By contrast, the axons of non-pyramidal cells arborized densely within a radius of 600-800 um about the soma. The density of varicosities reached a maximum of 150-170/100 um^2 close to the cell body and declined smoothly with distance from the soma. These results suggest that intracortical excitation extends over a large area, while intracortical inhibition is limited to a smaller area but may be more powerful.

108.3

COMPARATIVE DISTRIBUTION OF MELATONIN RECEPTORS IN THE AVIAN BRAIN. Teresa A. Kelm* and Vincent M. Cassone. Department of Biology, Texas A&M University, College Station, TX 77843.

The role of the pineal gland and its hormone melatonin in the regulation of circadian rhythmicity and photoperiodism appears to vary among avian species. To determine if there is an anatomical correlate of this diversity, we have employed in vitro binding of 2[^{125}I]iodomelatonin (IMEL) and autoradiography to ascertain an analogous diversity in the distribution of putative brain receptors for melatonin. Brains from Psitticaformes, Columbiformes, Galliformes, and Passeriformes were sectioned on a cryostat at 20 μm, thaw-mounted on gelatin-coated slides and dried. Sections were incubated in either 50 pM IMEL or 50 pM IMEL plus 1μm melatonin. Sections were exposed to X-ray film for 7 days. Film was developed, fixed and dried. Sections were stained with cresyl violet. The identity of structure specifically binding IMEL was determined by computer-aided image analysis. IMEL binding is present in the retinorecipient aspects of the visual system in all species. Secondly, thalamic relay nuclei of the visual system, n. rotundus, and tectofugal integrative areas, ectostriatum, bind IMEL to varying degrees. The brain of the budgerigar bound little else than the above structures and the neostriatum. In addition, Columbiform species indicated a great deal of binding in the telencephalic neostriatum, n. Edinger-Westphal (EW) and n. isthmoopticus (IO). However, there was relatively little binding in the ventrolateral geniculate (GLv) complex. In the chicken, binding was similar to the doves and pigeons, although there was binding in GLv and no binding in IO. In passerine species, all structures, with the exception of EW, described above bound IMEL. In addition, lobus paraolfactorius, n. ovoidalis and n. habenularis bound IMEL. In composite, the data indicate a wide array of sensory and integrative function under melatonin influence. Further work is directed at studying a wider taxonomic transect and at unveiling general anatomical principles of IMEL binding. Supported by NSF Grant 88-96225

108.4

ORIGIN OF PROJECTIONS UPON THE PALEOSTRIATAL COMPLEX IN THE MALLARD, ANAS PLATYRHYNCHOS L. J.L. Dubbeldam* (Spon: European Brain and Behaviour Society). Dept. of Organ. Zoology, Leiden Univ., 2300 RA Leiden, The Netherlands.

In a study on the afferent and efferent connections of n.basalis (Bas) (Dubbeldam, J.L. & Visser, A.M., Neurosci. 21: 487, 1987) also projections from the overlying layers upon the frontal part of the paleostriatal complex (PC) were observed. We analysed the sources of these projections using the combination of HRP-WGA for retrograde tracing and Phaseolus-agglutinin for anterograde tracing. Injections were made in hyperstriatum ventrale (HV), the dorsal and ventral zones of the frontal neostriatum (NF,d and NF,v), n.basalis (Bas), the paleostriatum augmentatum and primitivum (PA and PP) and the n.intrapeduncularis (INP). The anterograde experiments suggest that only Nf,d and Nf,v project upon PA and INP, but not upon PP. Bas projects upon Nf and HV, but not upon parts of PC. PA is a thin lamellar area and it was not possible to place injections exclusively in this area. Injections in either INP or the combination of INP/PP/PA always labeled cells in Nf,d. Injections including Bas, PA and PP labeled a few cells in Nf,v. Only an HRP/WGA injection in the deepest part of INP labeled some cells in HV and the dorsal hyperstriatum.

In conclusion, an intermediate zone of Nf,d is the main source of projections upon the frontal part of the paleostriatal complex in the mallard.

108.5

TELENCEPHALIC AND THALAMIC INPUTS TO THE STRIATAL PORTION OF THE PIGEON BASAL GANGLIA. C.L. Veenman and A. Reiner. Dept. of Anatomy & Neurobiology, Univ. of Tennessee, Memphis, TN 38163.

The little available data on the sources of telencephalic and diencephalic input to the avian striatum are largely based on the use of HRP as a retrograde marker. We have used injections of the highly sensitive retrograde markers fluorogold, fast blue, and rodomine latex beads into the striatum to examine the sources of corticostriatal and thalamostriatal projections in pigeons.

Our results indicate that the connectivity of the striatum in pigeon is similar to that of mammals. In pigeons, the equivalents of the isocortex, namely the Wulst and the dorsal ventricular ridge (DVR) project topographically to the striatum. The rostral Wulst and DVR preferentially project to medial striatum. Lateral parts of the DVR, including the temporo-parieto-occipital area (TPO) and the dorsolateral corticoid area (CDL), project preferentially to the lateral striatum. The caudolateral DVR (caudolateral neostriatum) projects to the entire striatum. The archistriatum also projects heavily to the striatum, with the rostral part of the archistriatum projecting to all of the striatum, the rim of the archistriatum preferentially to the lateral striatum, and the core of the archistriatum to the medial striatum. Within the thalamus, the dorsomedial and dorsolateral thalamic nuclei (which may be comparable to the mammalian intralaminar nuclei) were found to project heavily to the medial striatum, but sparsely to the lateral striatum. Our results indicate that forebrain projections to the avian striatum are more extensive than hitherto shown. Supported by NS-19620 (A.R.)

108.6

AUDITORY PROJECTIONS TO THE ANTERIOR TELENCEPHALON IN THE BUDGERIGAR. S.E. Brauth, W.S. Hall* and P. Cohen* Dept. of Psychology, Univ. of Maryland, College Park, MD. 20742

The connections of a higher order auditory area in the neostriatum intermedium pars ventrolateralis (NIVL) of the budgerigar (Melopsittacus undulatus) were mapped with pathway tracing techniques. Previous research (Brauth and McHale, 1988) has shown that NIVL receives projections from Field "L" as well as adjacent portions of the dorsolateral neostriatum intermedium and may be comparable to the "shelf" nucleus around HVc as described in songbirds.

The results show that NIVL neurons project to the rostromedial archistriatum (RAm) in agreement with prior work and also show that NIVL neurons project to the medial frontal neostriatum and hyperstriatum ventrale. Injections of HRP into this part of the telencephalon labeled many neurons in NIVL, the dorsal and ventral archistriatum, RAm, the core nucleus of the ectostriatum and within a medial crescent shaped area in the nucleus dorsomedialis thalami. HRP injections centered more laterally, within nucleus basalis, labeled neurons within a different auditory nucleus, the ventrolateral nucleus of the lateral lemniscus. This latter pathway may be comparable to that reported in pigeons from the intermediate nucleus of the lateral lemniscus (Arends and Ziegler, 1986). Taken together, these results support the notion that many pathways exist in the budgerigar telencephalon for correlation of auditory and non-auditory information.

Supported by NIMH Grant MH 40698 to S.E. Brauth

108.7

INTRATELENCEPHALIC PROJECTIONS OF THE VISUAL WULST IN BIRDS (*Columba livia*): A phaseolus vulgaris leucoagglutinin study. T. Shimizu, H. J. Karten and K. Cox*. Department of Neurosciences, University of California, San Diego, La Jolla, CA 92093.

The visual wulst of birds is a laminated dorsomedial telencephalic elevation with many similarities to the mammalian visual cortex. The major laminar components of the visual wulst, from dorsal surface inward, include HA, IHA, HIS, and HDI. An anterograde tracer, PHA-L, was used to study the intratelencephalic connections of these laminar components. The following major projection areas were found. The IHA (the major target area of the visual thalamic input) and HIS project to HA. Efferents of HA were observed in the lateral portion of LPO and a restricted region in PA (the avian equivalents of basal ganglia), as well as the visual nuclei in the brainstem via the tractus septo-mesencephalicus. At least some regions of HA, HIS, and possibly HDI were found to project to several areas of the neostriatum, including the neostriatum frontale pars laterale, the periectostriatum, and the lateral edge of the neostriatum caudale (NC). The HDm projects to the medial LPO, area parahippocampalis, and area corticoidea dorsolateralis. When PHA-L was injected into HVdv, immediately ventral to the wulst, labelled fibers and terminals were found in NC and some portions of archistriatum, but not in the wulst. These results indicate that the visual wulst projects upon multiple targets within the telencephalon. These include projections upon the more massive tectofugal pathways, indicating the possibility of interaction of channels of parallel visual information processing within the telencephalon of non-mammals. (Supported by ONR N00014-88-K-0504 and NINCDS PHS NS24560-03).

108.8

The Distribution of Cholinergic Neurons in the Central Nervous System of the North American Opossum, Didelphis Virginia. Lili Huang, R.H. Ho and G.F. Martin. Department of Anatomy and Neuroscience Program, The Ohio State University, Columbus, OH 43210.

The distribution of cholinergic neurons in the central nervous system of the opossum was studied by PAP immunohistochemistry for choline acetyltransferase (ChAT) and acetylcholinesterase (AchE) histochemistry. ChAT-immunoreactive neurons were detected in four major cell groups: (1) striatum, where they were scattered within the caudate, the putamen, the nucleus accumbens, and olfactory tubercle, (2) the magnocellular basal nuclei, which included the medial septal nucleus, the nucleus of diagonal band, the magnocellular preoptic nucleus, the substantia innominata, and the globus pallidus, (3) the pontomecencephalic tegmentum, which included the pedunculopontine tegmental nucleus, the parabrachial complex and the presumptive laterodorsal tegmental nucleus, and (4) all cranial and spinal nerve motor nuclei. In addition, ChAT-immunoreactive cells were seen in the supraoptic nucleus, the medial habenula, the parabigeminal nucleus, the locus ceruleus, the ventral nucleus of lateral lemniscus, the reticular formation, the substantia nigra, the red nucleus and the superior olivary complex, the last three of which were weakly stained. All of the nuclei which contained ChAT-immunoreactive cells also contained AchE-positive cells. However, in the hypothalamus and the thalamus there were many AchE-positive neurons, but none that immunostained for ChAT. AchE-positive cells were also found in the raphe nucleus, the ventral part of gigantocellular reticular nucleus and the mesencephalic nucleus of the trigeminal nerve, where ChAT-immunoreactive cells were rare. Our results suggest that the distribution of cholinergic neurons in this marsupial is similar to that in placental mammals. (Supported by NS-25095).

108.9

"NEUROLIPOMASTOCYTE" OF THE RAT BRAIN: A MISNOMER. R.V.W. Dimlich. Departments of Emergency Medicine and Anatomy/Cell Biology, University of Cincinnati, Cincinnati, OH 45267.

Certain cells associated with the vasculature of the brain were named "neurolipomastocytes" (Ibrahim, M.Z.M., et al., Acta Anat., 104:134, 1979). Although there is evidence to the contrary (Kiernan, J.A., J. Anat., 121:303, 1976; Edvinsson, L., et al., Neurology, 27:878, 1977), these cells are still considered by some investigators to be a special type of mast cell (Dimitriadou V., et al., Neuroscience, 22:621, 1987; Theoharides, Life Sci., 46:607, 1990). The similar vascular association of "neurolipomastocyte" and typical connective tissue mast cells in the brain as well as species variability may account, at least in part, for these opposing views. In this study, immunoreactivity, fluorescence, and routine histochemistry were used to verify that "neurolipomastocytes" in the rat brain do not contain heparin (Kiernan, Ibid.) or histamine (Edvinsson, Ibid.). In addition, this study demonstrated that these cells also do not contain 5-HT. Therefore, these cells have been misidentified as a type of mast cell and for that reason, "neurolipomastocyte" is an inappropriate name for these cells. Supported in part by NIH Grant NS-25635.

108.10

THREE DIMENSIONAL DIGITAL BRAIN ATLAS FOR MACACA NEMESTRINA

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Digital imaging techniques for the three dimensional (3D) reconstruction and display of serial data provide powerful new tools for studying neuroanatomy. Our development of a computerized rat brain atlas provided a proof-of-concept for this approach (1). In the current study we have applied these imaging techniques towards the visualization of monkey brain anatomy.

The data set used to build this atlas consists of 1200 digital images of the specimen blockface captured during cryostat sectioning of a whole head. This method of data collection eliminated the problem of image alignment. In addition, it allows for visualization of bony landmarks and other structures, such as cranial nerves, that are not usually preserved in histological studies. The bony landmarks provided reference to published stereotaxic atlases. Structures were defined by series of contours. Models of the cortical surface, basal ganglia, thalamus, and hippocampal formation were reconstructed and rendered.

These rendered displays enable the viewer to appreciate the shape and locations of anatomic structures within the monkey brain. In addition, the digital atlas provides the opportunity for quantifying a variety of morphometric features of the anatomy.

(1) Toga, A.W., Samaie, M., and Payne, B.A, Brain Res Bull. 22:323-33, 1989.

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108.11

WARPING 3D MODELS FOR INTERBRAIN COMPARISONS

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Three dimensional (3D) reconstructions of whole brain anatomy are routinely derived. The results of these computations are spatially accurate and realistic 3D models of brain. However, these results are based upon the serial sections of a single subject. Because the size, shape and location of various structures can vary between brains, 3D statistical comparisons are difficult or impossible to compute. To facilitate visual and statistical inspections of multiple brains, we have developed a method to warp one 3D volume so that it maps into a second.

The problem was solved in two steps. First a global registration followed by a global warp was performed. Second, local deformations were computed to correct for structure to structure differences. Calculations were performed on voxels using a continuous inverse mapping function. The global registration and warp were restricted to affine transformations. The local deformations, on the other hand, were the results of elastic displacements of grid points under the influence of local forces derived as the negative gradient of the local similarities between the two models. These displacements were obtained by solving the elastic equilibrium equations in three dimensions.

These algorithms have been applied to 3D models of rat brain anatomy and the resulting warped data sets rendered to produce realistic displays.

108.12

MIDSAGITTAL AND CORONAL FEATURES OF THE NORMAL HUMAN BRAIN: A QUANTITATIVE MRI ANALYSIS. M.L. Collaer, C. Karatekin* and J. Beatty. Behavioral Neuroscience Program, Dept. of Psychology, UCLA, Los Angeles, CA 90024.

The resolution of magnetic resonance imaging (MRI), combined with its safety, permit a detailed examination of the healthy human brain for research purposes. Quantification of normal neural features provides a perspective for better understanding the effects of pathological processes. Magnetic resonance images were obtained in normal adults, ranging in age from 19 to 34 years, from a 0.04 tesla Instrumentarium Magnaview MRI using a saturation recovery sequence (TR 600 / TE 60). Morphological measures were made on midsagittal (28 subjects) and coronal (16 subjects) scans of 10 mm thickness. The coronal slice was located at the anterior boundary of the thalamo-fornical junction. Prominent features from the midsagittal analysis include (overall group means, uncorrected for brain size): cerebral area (112.9 cm^2), cerebellar area (16.0 cm^2), maximal pons cross-sectional width (26 mm) and distance from the frontal to occipital poles (171 mm). Selected results from the coronal analysis include: cerebral area (102.4 cm^2), maximal frontal width (122 mm) and maximal temporal widths (right,47.9 mm; left,46.8 mm). Results are analyzed for each gender and presented in absolute and allometric forms.

PSYCHOTHERAPEUTIC DRUGS: ANTIPSYCHOTICS I

109.1

CHRONIC GABA AGONIST EFFECTS ON HALOPERIDOL-INDUCED VACUOUS CHEWING MOVEMENTS AND D1 AND D2 DOPAMINE RECEPTOR DENSITY. F. Tarazi, O. Shirakawa, L. Goodman*, and C.A. Tamminga, Maryland Psychiatric Research Center, University of Maryland at Baltimore, Baltimore, MD 21228

Tardive dyskinesia (TD) is a hyperkinetic movement disorder in humans associated with chronic neuroleptic treatment. Chronic blockade of dopamine (DA) receptors resulting in DA receptor supersensitivity and secondary changes in other brain areas are to be involved in the pathophysiology of TD. We have previously reported that the coadministration of a GABA agonist, progabide with haloperidol significantly reduced the development of vacuous chewing movements in rats (Shirakawa, O., et al., Soc. Neurosci. Abst. 15:1989). To clarify the neurochemical mechanisms of this progabide effect in VCMs, we studied the changes in D2 receptors labeled by ^{3}H-spiperone using in vitro quantitative autoradiography. The rats were perfused and their brains were rapidly removed, frozen by immersion in isopentane, mounted and cut into 20-um-thick coronal sections. ^{3}H-spiperone binding in the caudate-putamen (medial and lateral) was significantly increased after 6 months treatment with haloperidol (H) and with haloperidol plus progabide (H+P) (medial; W: 63.1 ± 10.0 fmol/mg tissue (6), H: 89.4 ± 9.4 (12, H+P: 97.1 ± 12.0; lateral; W: 94.5 ± 8.6 (6), H: 125.5 ± 8.6 (12), H+P: 131.4 ± 12.4 (6); mean ± S.D. (N)). No significant correlations were found between VCMs and ^{3}H-spiperone binding in caudate-putamen. Correlations between VCMs and changes in D1 and D2 receptors in other brain regions will be reported as well as changes in 5HT-2 receptors.

109.2

CHRONIC GABA AGONIST EFFECTS ON HALOPERIDOL-INDUCED VACUOUS CHEWING MOVEMENTS AND GABA-A-RECEPTOR DENSITY. O. Shirakawa, F. Tarazi, L. Goodman* and C.A. Tamminga, Maryland Psychiatric Research Center, University of Maryland at Baltimore, Baltimore, MD 21228

Vacuous chewing movements (VCMs) in rats induced by chronic neuroleptic treatment are thought to be an animal analogue of human tardive dyskinesia. We have previously reported the prophylactic effect of a GABA agonist, progabide in haloperidol-induced VCMs (Shirakawa, O., et al., Soc. Neurosci. Abst. 15:1989). In an attempt to examine the involvement of GABAergic mechanisms in TD, we have studied changes in GABA-A receptors labeled by 3H-muscimol using quantitative in vitro autoradiography. We treated rats orally with water alone (W), haloperidol alone (H), progabide alone (P) and haloperidol plus progabide (H+P) for 6 months. The rats were perfused and their brains were rapidly removed, frozen by immersion in isopentane, mounted and cut into 20-um-thick coronal sections. ^{3}H-muscimol autoradiographic method was based on Palacios, J.M. et al. (Brain Res., 222:285, 1981) and Penney, J.B., Jr. et al. (Science, 214:1036, 1981). ^{3}H-muscimol binding was significantly increased in substantia nigra reticulata after 6 months of treatment with H and H+P (W: 301.9 ± 41.9 fmol/ mg tissue (7), H: 354.9 ± 37.3 (12), H+P: 368.9 ± 37.1 (6): mean ± S.D. (N)). In subthalamic nucleus, ^{3}H-muscimol binding was significantly increased with H, but not significantly increased with H+P. No significant correlations were found between VCMs and ^{3}H-muscimol binding in these two brain areas. Correla-tions between VCMs and changes of GABA-A receptors in the other brain regions will be reported.

109.3

NICOTINE POTENTIATION OF HALOPERIDOL-INDUCED CATALEPSY: STRIATAL MECHANISMS. P.R. Sanberg, D.F. Emerich, S. Lamb*, C. Latkin*, B.J. McConville* and A.B. Norman, Division of Neuroscience, Department of Psychiatry, University of Cincinnati College of Medicine, Cincinnati, Ohio 45267.

Previously it was demonstrated that the co-administration of low doses of nicotine and haloperidol in rats caused an almost ten-fold increase in cataleptic behavior, as compared to haloperidol alone. Subsequently, Tourette's patients treated with haloperidol and nicotine gum showed remarkable improvement of their motor tics. In order to determine if the potentiation by nicotine of haloperidol catalepsy is mediated via the striatum, we tested whether nicotine could potentiate the haloperidol response in animals devoid of intrinsic striatal neurons. Rats were injected bilaterally with quinolinic acid (150 nmols) into the striatum. At least one month later, the rats were tested for catalepsy following administration of nicotine (0.1 mg/kg i.p.), haloperidol (0.3 mg/kg), nicotine plus haloperidol, or vehicle. In control animals nicotine plus haloperidol produced a significantly greater catalepsy than haloperidol alone. In animals with striatal lesions, there were no significant differences between groups. The nicotine alone or vehicle groups did not produce catalepsy in either control or lesioned animals. The amount of catalepsy produced by haloperidol in the lesioned group was significantly reduced, as demonstrated by earlier research. This not only supported our previous research demonstrating that haloperidol catalepsy is reduced by striatal lesions, but more importantly that nicotine could not potentiate cataleptic behavior without an intact striatum. This supported a role for the striatum as the site of interaction for these two drugs on cataleptic behavior. Supported by Smokeless Tobacco Research Foundation, Tourette Syndrome Association and Merrell Dow.

109.4

DIFFERENTIAL EFFECT OF NICOTINE ON D_1 VS D_2 ANTAGONIST-INDUCED CATALEPSY. D.F. Emerich, P.R. Sanberg, P.Z. Manderscheid, B.J. McConville*, J.M. Rich*, M.M. El-Etri*, M.T. Shipley and A.B. Norman. Division of Neuroscience, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Nicotine produces a 10-fold increase in haloperidol-induced catalepsy. Furthermore using nicotine gum as an adjunct to haloperidol produced a remarkable improvement in motor tics in Tourette's patients. In order to determine whether these effects of nicotine interact with D_1 or D_2 dopamine (DA) receptor mechanisms, we examined whether nicotine could potentiate the catalepsy produced by the selective D_1 antagonist SCH23390.

Male Sprague-Dawley rats were randomly assigned to one of four treatment groups: SCH23390 plus nicotine, SCH23390 alone, nicotine alone, or vehicle control. SCH23390 (0.5 mg/kg, ip) was administered 30 minutes prior to nicotine (0.1 mg/kg) and rats were tested for catalepsy using the bar test.

Although nicotine produced approximately a 40% increase in SCH23390-induced catalepsy during the first hour, this did not reach statistical significance. Thus, it appears that striatal D_2 rather than D_1 receptor mechanisms are predominantly involved in nicotine's profound potentiation of neuroleptic-induced catalepsy.

We also investigated whether nicotine, in conjunction with haloperidol, synergistically increased striatal DA turnover. Nicotine alone produced a small increase while haloperidol produced a marked increase in DA turnover. However, the combination of nicotine and haloperidol did not produce any significantly greater effect on DA turnover. Thus, it is unlikely that the remarkable behavioral effect of nicotine and haloperidol co-treatment was due to any change at the level of DA turnover. Supported by Smokeless Tobacco Research Foundation, Tourette Syndrome Association and Merrell Dow.

109.5

Topographical Imaging of the Rabbit Electroencephalogram: Differential classification of major CNS drug classes. G. P. ALBERICI, J.-L. GASKILL, L. COOK, AND G. F. STEINFELS. E. I. DuPont de Nemours & Co., Med. Prod. Dept., Wilmington, DE 19880-0400 USA.

Topographical analysis of EEG provides a 2 dimensional image of electrical brain activity. This study sampled 16 electrodes , 8 per hemisphere, placed symmetrically across the skull of the rabbit. EEG activity was collected online (60, 4 sec intervals) and then transformed into power spectra via a fast Fourier transform (FFT). The results were then grouped into EEG bandwidths, (*delta* 1-5 Hz, *theta* 5-9 Hz, *alpha* 9-13 Hz, and *beta* 13-35 Hz), averaged and then compared to control for each subject. The change from control values for relative power (RP) for each subject where then averaged and processed, using TMAP (Nicolet Instruments Corp) , into topographical images. The drugs tested included; morphine, chlorpromazine, diazepam, and 3 putative cognitive enhancers; physostigmine, THA (tetrahydroacridine, Cognex, Warner Lambert) and DuP996. Morphine and chlorpromazine both increased frontal *alpha* and *beta* RP. However, morphine tended to increase parietal and occipital *delta* RP whereas chlorpromazine tended to decrease frontal *delta* RP. Diazepam increased *alpha* RP across all cortical regions with minimal effects on other bandwidths. Finally the cognitive enhancers showed an increase in occipital *beta* RP and a decrease in frontal *alpha* RP. This model has thus far distinguished several classes of pharmacologic agents and in some cases these changes parallel those reported in clinical topographical EEG studies.

109.6

ACUTE HALOPERIDOL-INDUCED DEPOLARIZATION BLOCK OF DOPAMINERGIC REWARD NEURONS IN PARTIALLY 6-OHDA-LESIONED RATS. M. D. Doherty and A. Gratton, Douglas Hosp. Res. Ctr., McGill Univ., Montréal, CANADA, H4H 1R3.

The present study was designed to examine the behavioral significance of depolarization block (DB) of dopamine (DA) cells and, in particular, how partial loss of DA neurons affects the inhibitory effects of acute haloperidol (HAL) on brain stimulation reward (BSR). Male rats were each implanted with a chronic stimulating electrode in the medial forebrain bundle and with bilateral guide cannulae in the lateral ventricles. A psychophysical method was used to measure changes in the animals' responsiveness to BSR following HAL (50-150 ug/kg, i.p.) and apomorphine (APO, 25-100 ug/kg, s.c.) administered either alone or during HAL blockade. The animals then received bilateral intraventricular infusions of the DA neurotoxin, 6-OHDA (80-100 ug/ventricle). In animals exhibiting normal baseline BSR 21-28 days post-lesion, HAL, at doses that caused only a moderate decrease in responsiveness to BSR before the lesion, now caused a complete cessation of BSR. Normal responsiveness to BSR could be reinstated during HAL blockade with APO, at doses that caused an inhibition of BSR before the lesion. These behavioral data are consistent with electrophysiological data (Grace et al., 1989) showing that; 1) DA cells that survive 6-OHDA lesions are more active and thus more likely to be driven into DB by acute HAL treatment, 2) APO, a hyperpolarizing agent of DA cells at low, autoreceptor selective doses, can repolarize DA cells driven into DB. The present data also suggest that although partial DA depletion has, by itself, little effect on reward functions, the surviving reward-relevant DA cells may be more susceptible to inactivation by a variety of stimuli that increase DA cell firing. Our preliminary data suggest that acute stress may also cause DB in partially DA-depleted animals. Funded by the MRC of Canada.

109.7

BRIEF MATERNAL SEPARATION INTERACTS WITH PARENTAL DRUG SENSITIVITY TO INFLUENCE AKINESIA IN ADULT RATS. H. Schreiber.Dept. Psychol., Univ. Texas at Tyler, Tyler, TX 75707. M. Coffman*, C. Maes* and M. Juarez-Turner*. Dept. Behav. Sci., Highlands Univ., Las Vegas, NM 87701.

Briefly removing the dam from the nest in the neonatal period can alter haloperidol induced akinesia in rats and mice (Gallegos,et al., Behav Neural Biol,53,172-183,1990).

Akinesia was assessed in male and female hooded rats. Matched pairs of highly sensitive females were mated with highly sensitive males, less sensitive females with less sensitive males, etc. For 7 days after birth, one member of a matched pair was removed from her nestcage (30 min) while the control dam was not. The offspring received forepaw on dowel testing 90 min after haloperidol (2mg/kg,IP) or saline injection on days 56 and 58.

The offspring of highly sensitive parents showed greatly enhanced akinesia at their second exposure to the drug in comparison with the others. The effect of maternal separation was to reverse this increase among only the offspring of the highly sensitive parents, while increasing the akinesia of the offspring of the least sensitive parents. While this experiment does not separate innate from other parental influences, it suggests that neonatal manipulations of the dam may influence powerfully the adult response to neuroleptic drugs.

Supported by NIH-MBRS grant RR08066-13.

109.8

CHLOPROMAZINE INDUCED WEIGHT GAIN. W.B. Lawson Ida Michele Williams, and J. Hill. Department of Psychiatry, Vanderbilt University School of Medicine, Nashville, TN 37232.

Obesity related to neuroleptic use is a common problem among the chronically mentally ill. Animal research has shown that many neuroleptics will cause increased eating and weight gain by D_2 blockade. Chlopromazine (CPZ) consistently fails to cause weight gain in rats in contrast to clinical observation in humans. We studied food intake and body weight in adult male and female Sprague Dawley albino rats on a diet of 50% chow and water for 42 days. Groups of 7 rats were injected i.p. with either vehicle or chlopromazine 2,4, or 8 mg/kg. Female rats showed increased weight gain past the tenth day for all doses. Male rats gained proportionally less weight with higher doses. Total food intake and energy utilization were significantly higher for females on CPZ. Amount of adipose tissue increased with CPZ dose for both males and females. CPZ may cause weight gain in females by increasing food intake and adiposity.

109.9

SERTINDOLE - A NEW ATYPICAL NEUROLEPTIC

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In a study of neuroleptic activity within a series of 1-aryl-3-(4-piperidyl) substituted indoles[1] sertindole (Lu 23-174), 1-[2-[4-[5-chloro-1-(4-fluorophenyl)-1H-indol-3-yl]-1-piperidinyl]ethyl]-2-imidazolidinone, was found to be a potent neuroleptic with prominent selectivity for brain limbic areas. *In vitro* sertindole has a very high affinity for central 5-HT_2 receptors (IC_{50} = 0.72 nM, displacement of [^{3}H]ketanserin), but also high affinity for central DA D-2 receptors (IC_{50} = 4.1 nM, displacement of [^{3}H]spiperone) and central α_1 adrenoreceptors (IC_{50} = 3.4 nM, displacement of [^{3}H]prazosin). The compound is non-cataleptic and does not antagonize dopaminergic induced stereotypies (methylphenidate, amphetamine) in proposed clinical doses. Potent 5-HT_2 antagonism with long duration of action was found in rats (antagonism of LSD cue and quipazine-induced head twitches). A selective and dose dependent decrease in the number of spontaneously active DA neurones in A10 versus A9 area was induced by sertindole after 21 days po treatment (ED_{50}'s = 0.015 and 1.6 µmol/kg/day, respectively) [2]. These results will be compared to data obtained for haloperidol and clozapine.

1) J.Perregaard, *US Patent No 4.710.500 (1987)*
2) T.Skarsfeldt and J.Perregaard, *Eur.J.Pharmacol.* (submitted)

109.10

DISCRIMINATIVE STIMULUS PROPERTIES OF CLOZAPINE. **H.F. Villanueva, S. Arezo*, J.A. Rosecrans* & J.H. Porter, Depts. of Pharm./Tox. and Psych., Va. Commonwealth Univ., Richmond, VA 23298.**

Due to the lack of extra-pyramidal side effects of the atypical antipsychotics and therefore the lack of adequate screening procedures for new atypical antipsychotic compounds, a two-lever operant drug discrimination paradigm was investigated as a possible screening technique for atypical antipsychotic compounds. Also, the drug discrimination paradigm offers an opportunity to investigate the mechanisms of action of the atypical antipsychotic clozapine. Male Sprague-Dawley rats were trained to discriminate 5 or 10 mg/kg clozapine from vehicle using a VI-15 second schedule of food reinforcement. Following drug discrimination training, the following compounds were substituted to test for generalization to clozapine: thioridazine, an atypical antipsychotic, haloperidol, a D2 antagonist and SCH 23390, a D1 antagonist. Rats trained to discriminate 5 mg/kg clozapine from vehicle demonstrated partial generalization to thioridazine, while those trained at 10 mg/kg showed no generalization. Neither haloperidol nor SCH 23390 substituted for clozapine, suggesting that the discriminative cue is not mediated through D1 or D2 receptors.

109.11

EFFECTS OF DOPAMINE PARTIAL AGONISTS ON FIRING RATES OF DOPAMINE NEURONS IN SUBSTANTIA NIGRA PARS COMPACTA. W.E.Hoffmann, J.T.Lum, and M.F.Piercey. CNS Research, The Upjohn Company, Kalamazoo, MI 49001 USA.

The aminoergolines, SDZ 208-911 (S911) and SDZ 208-912 (S912), have been characterized as potent D2 receptor partial agonists that, because of low intrinsic activities, act as dopamine (DA) receptor antagonists in behavioral assays (D.M.Coward *et al.*, JPET 252:279, 1990). The dopamine autoreceptor, because of spare receptors (Meller *et al.*, Eur. J. Pharmacol, 123:311, 1986), is very sensitive to dopamine agonist effects. We have examined S911 and S912 on DA neuron firing rates and antagonism of DA agonists amphetamine (AMPH) and apomorphine (APO). Using dye-filled glass microelectrodes, DA neurons in SNPC were identified by classical electrophysiological/neuroanatomical criteria (Bunney *et al.*, JPET 155:560, 1973). S911 depressed DA neuron firing, but only partially (maximum effect = 38%, ED_{50} = 38 μg/kg). AMPH and APO agonists were partially reversed by S911. S912 was much weaker as an agonist. Maximal depression was 15%. In contrast, TDHL (Piercey and Hoffmann, JPET 243:391,1987), (-)-3-PPP and APO depressed firing by 50%, 80% and 100%, respectively. S912 completely reversed inhibition by AMPH, but only partially reversed APO. It is concluded that S912 is a partial agonist at the DA autoreceptor with an intrinsic activity near the level of detectability.

109.12

THE NEUROCHEMICAL PROFILE OF HP 873, A POTENTIAL ATYPICAL ANTIPSYCHOTIC WITH D_2/$5HT_2$ RECEPTOR ANTAGONIST ACTIVITY. C.A.Wilmot, A.M. Szczepanik, L.R.Brougham*, J.E.Roehr*, G. Bores*, H.B. Hartman*, P.G. Conway, J. Chernack*, J.T.Strupczewski*, E.J. Glamkowski*, D.B. Ellis* and G.C. Helsley*. Dept. Biol. and Chem. Res. Hoechst-Roussel Pharmaceuticals Inc., Somerville NJ 08876.

HP 873, 1-[4-[3-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl]-propoxy]-3-methoxyphenyl]ethanone, has a preclinical behavioral and electrophysiological profile of an atypical antipsychotic. *In vitro*, HP 873 had moderate to high affinity for $5HT_2$, α_2 and α_1 receptors (IC_{50}= 9.3, 60, 0.4 nM, respectively) with lower affinity for D_2, $5HT_{1A}$, σ and D_1 (IC_{50}= 109, 210, 180, 750 nM, respectively). *Ex vivo*, HP 873, 2.5 to 20 mg/kg, ip, inhibited cortical and subcortical $5HT_2$ receptor binding by 50-94% with a duration greater than 4 hrs, in contrast to a peak effect of 10-20% inhibition of D_2 receptor binding at 1 hr. *In vivo*, HP 873 inhibited 5HTP-induced head twitch in rats at doses below those active against apomorphine-induced stereotypy. HP 873 increased DA turnover in rats at 0.3 to 10 mg/kg, ip, and partially reversed the apomorphine inhibition of GBL-induced DOPA synthesis at 3 and 10 mg/kg. Chronic treatments with 10 mg/kg, ip, for 19 days produced no change in the Bmax for D_2 receptors in the n. accumbens or 6 regions of the striatum, however the Bmax for $5HT_2$ receptors was reduced to 41% of control in the frontal cortex. These studies suggest that potent $5HT_2$ receptor antagonism may be an important component in the profile of HP 873 as an atypical antipsychotic.

109.13

THE *IN VIVO* PHARMACOLOGY OF THE NOVEL ANTIPSYCHOTIC HP 873. M.R. Szewczak, R.W. Dunn*, R. Corbett*, H.M. Geyer*, D.K. Rush*, J.C. Wilker*, J.T. Strupczewski*, G.C. Helsley*, and M.L. Cornfeldt. Department of Biological Research, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ 08876.

HP 873, 1-[4-[3-[4-(6-fluoro-1,2-benzisoxazol-3-yl)-1-piperidinyl] propoxy]-3-methoxyphenyl] ethanone, an antagonist with potent $5HT_2$ receptor binding activity, displays an atypical antipsychotic profile in *in vivo* assays predictive of clinical efficacy. It was effective in blocking apomorphine-induced climbing behavior in mice (ED_{50} = 0.10 mg/kg, ip). HP 873 also potently blocked intracranial self-stimulation behavior in rats (ED_{50} = 0.15 mg/kg, ip), pole-climb avoidance in rats (avoidance ED_{50} = 1.4 mg/kg, ip, escape failure ED_{50} = 6.0 mg/kg ip), and continuous Sidman avoidance in monkeys (ED_{50} = 3.6 mg/kg, po). Acute administration of HP 873 to rats caused an increase in the number of active dopamine neurons sampled in both the ventral tegmental area (A10) and substantia nigra (A9), whereas chronic administration resulted in significantly fewer active dopamine neurons in the A10 with increased numbers until observed in the A9. In a social interaction test in rats, HP 873 significantly increased social interaction time between rats at 1.0 mg/kg ip, without significantly reducing motor activity. HP 873 displays an *in vivo* profile consistent with that of an atypical antipsychotic agent and is predicted to be clinically effective with fewer neurological side effects than classical antipsychotic agents.

109.14

COMPARISON OF ACUTE EFFECTS OF HALOPERIDOL AND CHLORPROMAZINE ON EEG WITH BRAIN WAVE FACTOR SCANS

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Various compounds of different structural classes are used to treat psychoses. In this study the electrophysiological effects of haloperidol, a butyrophenone, and chlorpromazine, a phenothiazine, were examined. Female Sprague-Dawley rats were prepared with chronic EEG recording electrodes and permanent indwelling i.v. cannulae. EEG data were analyzed with a Nicolet Pathfinder 1 computer. Animals were administered haloperidol (0.1 mg/kg) and chlorpromazine (0.5 mg/kg). Multivariate analyses of EEG spectral parameters demonstrated differences between these antipsychotic compounds. After haloperidol administration, one bipolar factor emerged which described decreases in slower EEG frequencies and increases in faster EEG frequencies and the edge frequency. Chlorpromazine, however, contained a comparable factor which was monopolar and described increases in faster EEG frequencies and the edge frequency. The differential EEG effects may reflect differences in chemical structure or mechanism of action. (Supported by NIDA DA01050).

109.15

REGIONAL SPECIFIC EFFECTS OF CLOZAPINE AND HALOPERIDOL ON GABA RELEASE IN RAT BASAL GANGLIA. K.L. Drew, W.T. O'Connor*, J. Kehr* and U. Ungerstedt*. Department of Pharmacology, Karolinska Institute, Stockholm, Sweden.

The release of ɣ-aminobutyric acid (GABA) from dorsolateral striatum, fundus striati (a ventral region of the striatum) and globus pallidus was measured following subcutaneous administration of haloperidol or clozapine using in vivo microdialysis in halothane anaesthetized rats. Clozapine (5.0 mg/kg) increased GABA release in fundus striati while haloperidol (0.5 mg/kg) increased GABA release in globus pallidus. In contrast, clozapine (2.5 to 40 mg/kg) failed to increase GABA release in globus pallidus and haloperidol (0.1 to 2.0 mg/kg) failed to increase GABA release in the fundus. Thus, haloperidol and clozapine are clearly distinguished by their effects on GABA release in fundus striati and globus pallidus. Both drugs increased GABA release in dorsolateral striatum. Drug-induced increases in GABA release were reversed by the addition of 1 μM tetrodotoxin to the perfusion medium. These data suggest that regional differences in the effects on GABA release in the basal ganglia may parallel the unique clinical profiles of haloperidol and clozapine.

109.16

CLOZAPINE AND THIORIDAZINE, BUT NOT MOST OTHER ANTIPSYCHOTIC DRUGS, PRODUCE LARGE ELEVATIONS IN BRAIN GLUCOSE CONCENTRATIONS. L.D. Kreamer* and C.F. Saller. ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

Previous studies indicated that the stimulation of brain dopamine receptors can increase brain glucose concentrations which can in turn suppress dopaminergic activity. Thus, brain glucose was measured after a variety of antipsychotic drugs, which block DA receptors. In addition, the effects of several antipsychotic drugs with low affinities for DA receptors were also measured. Only clozapine and thioridazine, antipsychotic drugs with atypical properties, produce very large increases (i.e., >100% at 20 mg/kg, i.p.) in brain glucose. None of a variety of compounds, such as: d-butaclamol, chlorpromazine, fluphenazine, haloperidol, pimozide, remoxipride, rimcazole, SCH 23390, and setoperone produce more than a 30% change in brain glucose. Loxapine, a typical antipsychotic, was intermediate between clozapine and thioridazine and the other drugs tested. Depletion of catecholamines completely prevented clozapine-induced increases in brain glucose. These data are discussed with regard to how increases in brain glucose might contribute to the therapeutic effects of clozapine and thioridazine.

109.17

EFFECTS OF CI-943, A POTENTIAL ANTIPSYCHOTIC AGENT, ON CEREBRAL GLUCOSE UTILIZATION IN RATS. L.P. Raymon, A.S. Kimes, L.T. Meltzer, T.G. Heffner, and E.D. London. NIDA Addiction Research Center, Baltimore, MD 21224 and Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

CI-943 is a unique antipsychotic drug candidate in that it is not a dopamine (DA) receptor antagonist and does not bind to a variety of other neurotransmitter receptors. However, CI-943 has modulatory effects on dopaminergic and serotonergic neural systems, and exhibits an antipsychotic profile in a variety of preclinical behavior tests in rodents and primates (Heffner, T. et. al., JPET, 251:105, 1989). We used the 2-deoxy-D-[1-^{14}C]-glucose method (Sokoloff, L. et al., J. Neurochem., 28:897, 1977) to study the effects of CI-943 on local cerebral glucose utilization (LCGU) in 22 male Sprague Dawley rats. CI-943 (40 mg/kg, i.p.) was administered 10 or 60 min before the radiotracer. Compared to saline-treated controls, CI-943 increased LCGU in the lateral habenula at both time points, and in the globus pallidus and substantia nigra, pars reticulata at 60 min. CI-943 decreased LCGU in dopamine projection areas (e.g., caudate-putamen, accumbens n., frontal and anterior cingulate cortical areas, subthalamus) and several additional areas; but the drug had no effect on LCGU in areas of dopamine cell bodies (e.g., substantia nigra, pars compacta, ventral tegmental area). Thus, CI-943 produces haloperidol-like effects on LCGU in some brain areas (e.g., lateral habenula), but not in others (e.g., globus pallidus). The results support the view that CI-943 has a different profile of CNS effects than clinically available antipsychotics.

Supported in part by a grant from the Warner-Lambert Co.

109.18

EFFECTS OF CI-943, A POTENTIAL ANTIPSYCHOTIC DRUG, AND HALOPERIDOL ON REGIONAL BRAIN NEUROTENSIN CONCENTRATIONS. B. Levant, G. Bissette, M.D. Davis, T.G. Heffner, and C.B. Nemeroff. Depts. of Pharmacology and Psychiatry, Duke Univ. Med. Ctr., Durham, NC 27710 and Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

Treatment with efficacious antipsychotic drugs, such as haloperidol, selectively increases the concentrations of neurotensin (NT) in the nucleus accumbens and caudate nucleus of the rat. These increases in NT concentrations may be associated with the therapeutic and side effects of antipsychotic drugs. CI-943, a novel antipsychotic clinical candidate, produces behavioral effects in animals which suggest that it may possess antipsychotic activity but is not a dopamine antagonist. This study evaluated the effects of CI-943 and haloperidol on regional brain NT concentrations. Adult, male, S-D rats were treated with CI-943 (40 mg/kg, ip) or haloperidol (1 mg/kg, ip) for 23 days. NT concentrations of discrete brain regions were determined by a sensitive and specific RIA. NT concentrations in the nucleus accumbens, anterior caudate and posterior caudate were significantly increased by both drugs. Unlike haloperidol, CI-943 also increased the concentrations of NT in the substantia nigra/ventral tegmental area and hypothalamus. These data provide further evidence that putative antipsychotic drugs increase NT concentrations in the nucleus accumbens and other brain regions. (Supported by NIMH MH-39415 and Parke-Davis).

109.19

THE EFFECT OF ICI 204,636, A POTENTIAL ANTIPSYCHOTIC AGENT, IN A BATTERY OF BEHAVIORAL TESTS PREDICTIVE OF ANTIPSYCHOTIC ACTIVITY AND TARDIVE DYSKINESIA. B.M. Migler, J.B. Malick, and E. J. Waraya. ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

ICI 204,636, a dibenzothiazepine, was tested in a variety of behavioral tests predictive of antipsychotic activity, including a) conditioned avoidance in squirrel monkeys; b) antagonism of apomorphine-induced blinking in squirrel monkeys; c) antagonism of apomorphine-induced visual searching in cats; d) antagonism of apormorphine-induced climbing in mice; e) antagonism of amphetamine-induced hyperactivity in rats; f) restoration (normalization) of apomorphine-induced disruption of swimming in mice; and g) restoration (normalization) of amphetamine-induced disruption of swimming in mice. The results with ICI 204,636 were compared with the effects of standard antipsychotic agents and suggest that ICI 204,636 is a potential antipsychotic agent. In addition, ICI 204,636 was tested in haloperidol-sensitized cebus monkeys and in drug-naive cebus monkeys for the induction of dyskinetic reactions. The results were compared with the results obtained with standard agents. The data suggest that ICI 204,636 has a markedly reduced potential to produce dyskinetic reactions in cebus monkeys. We conclude that ICI 204,636 is a potential antipsychotic agent with a marked reduction in the potential to produce EPS and tardive dyskinesia in humans.

109.20

ELECTROPHYSIOLOGICAL PROFILE OF ICI 204,636: A NEW AND NOVEL ANTIPSYCHOTIC DRUG. J. M. Goldstein, L. C. Litwin, E. B. Sutton and J. B. Malick. ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897 USA.

Extracellular single unit recording techniques were used to compare the effects of ICI 204,636 (ICI) with the reference antipsychotic (AP) agents clozapine (CLZ) and haloperidol (HAL) in electrophysiological tests considered predictive of either AP activity or extrapyramidal side effects (EPS). ICI and CLZ were differentially more active in reversing d-amphetamine inhibition of mesolimbic (A10) than nigrostriatal (A9) DA-containing neurons, whereas HAL exhibited the opposite selectivity. In cell population studies, acute administration of ICI and CLZ produced selective increases in the number of spontaneously active A10 DA cells, which correlated with the ability of both these drugs to cause depolarization inactivation (DI) of only A10 DA cells following chronic administration. This profile of activity was unlike that of HAL, which acutely caused a nonselective increase in the number of actively firing A9 and A10 DA cells, associated with its ability to cause DI of both A9 and A10 DA cells after chronic administration. Inasmuch as DI of A10 DA cells may be correlated with AP efficacy whereas DI of A9 DA cells may predict EPS, ICI may be a potential AP with a reduced likelihood for producing EPS.

109.21

ICI 204,636, A POTENTIAL ANTIPSYCHOTIC WITH AN "ATYPICAL" BIOCHEMICAL PROFILE. C.F. Saller and A.I. Salama. ICI Pharmaceuticals Group, ICI Americas Inc., Wilmington, DE 19897.

ICI 204,636 and the atypical antipsychotic clozapine (CLZ) were compared using measures in rats which may indicate antipsychotic activity and extrapyramidal side-effect (EPS) liability. These compounds are low potency D-2 dopamine (DA) antagonists and are much more potent 5-HT_2 antagonists. Both produce similar elevations in DA metabolites. Like CLZ, ICI 204,636 produces a short-lasting increase in plasma prolactin. Unlike CLZ, ICI 204,636 lacks activity at D-1 DA and muscarinic receptors. After chronic administration, at doses which produce large increases in DA metabolites, both fail, unlike typical antipsychotics, to increase striatal D-2 receptors, but do decrease 5-HT_2 receptors in frontal cortex. These data are discussed with regard to the hypothesis that 5-HT_2/D-2 receptor interactions may account for the low propensity of ICI 204,636 to produce behavioral and electrophysiological indices of EPS.

110.1

REPEATED ADMINISTRATION OF COCAINE DECREASES DOPAMINE SYNTHESIS AND DIALYSATE DOPAC BUT NOT TISSUE DOPAMINE OR DOPAC IN THE NUCLEUS ACCUMBENS. J.W.Brock, J.P. Ng, and J.B. Justice, Jr. Department of Chemistry, Emory University, Atlanta, GA 30322.

Using microdialysis, reduced steady state formation of dihydroxyphenylalanine (DOPA) (47%) was found in the nucleus accumbens (N ACC) of rats treated with ten days of repeated cocaine injections (10 mg/kg i.p.) as compared to saline controls after NSD 1015 perfusion (0.1 mM). In addition, prior to NSD 1015 perfusion, basal extracellular levels of the dopamine (DA) metabolite, dihydroxyphenylacetic acid (DOPAC) in the N ACC were 33% lower in the chronic cocaine treated animals. The serotonin metabolite, 5-hydroxyindole acetic acid (5-HIAA) examined under the same conditions as DOPAC was found to be unaffected by repeated cocaine administration. The results indicate the rate of DA synthesis in the N ACC is significantly reduced by repeated cocaine administration.

Preliminary results also indicate a challenge injection of cocaine (20 mg/kg i.p.) further reduced the DOPA levels from steady state in both saline and cocaine treated animals.

In an additional experiment, tissue levels of DA, DOPAC, 5-HIAA were measured in the N ACC, striatum (STR), and prefrontal cortex (PFC) of rats treated for 10 days with either saline or cocaine (10 mg/kg i.p.). Tissue levels of norepinephrine were also examined in the PFC. No statistically significant difference was found for any of the analytes in the N ACC, STR, or PFC between cocaine and saline treated groups.

110.2

REPEATED COCAINE ADMINISTRATION INCREASES STIMULATED RELEASE AND UPTAKE OF DOPAMINE IN NUCLEUS ACCUMBENS AS MEASURED BY IN VIVO VOLTAMMETRY. J.P. Ng, G.W. Hubert, and J.B. Justice Jr.; Emory University; Dept. of Chemistry; Atlanta, Georgia 30322.

Electrically-stimulated dopamine (DA) release (overflow) and reuptake were measured with in vivo voltammetry in nucleus accumbens of anaesthetized rats given repeated administration of cocaine. Electrically-stimulated DA release was induced by a 10 sec stimulation in the medial forebrain bundle (2-ms, 200uA biphasic, 100 Hz). DA overflow and uptake were quantified with fast chronoamperometry using Nafion-plated carbon fiber electrodes. Animals (N=7/grp) given repeated cocaine (10 mg/kg s.c. from day 1-5, 20 mg/kg s.c. from day 6-10) showed a 185% increase in uptake rates compared to saline controls (6.1±1.0 uM/s vs. 3.3±0.8 uM/s) and a 151% increase in stimulated overflow over 10 day controls (27.3±2.9 uM vs. 18.1±3.2 uM). This effect was found to be temporary as cocaine-treated animals showed no difference in uptake or release vs. controls following a10 day abstinence period (N=7/grp). GBL (300 mg/kg) administered to controls (N=5) showed a 200% augmented DA release with no change in uptake indicating that enhanced uptake rates observed in cocaine treated animals were independent of augmented stimulated release of DA. These results suggest that alterations produced by repeated cocaine adminstration may be a compensatory response to prolonged uptake blockade of synaptic DA. These alterations of the dopaminergic nerve terminal may be important during withdrawal from repeated cocaine use.

110.3

ENHANCED RESPONSE OF MESOLIMBIC DOPAMINE SYSTEM TO COCAINE FOLLOWING PREEXPOSURE TO CAFFEINE: AN IN VIVO MICRODIALYSIS STUDY P.J. Wellman, B. Davies* and S. Schenk, Texas A&M Univ., Dept. Psychol., College Station, TX, 77843.

Rats were preexposed with 9 daily injections of either caffeine (20 mg/kg, IP) or the saline vehicle. On the tenth day, the rats were injected with cocaine (10 mg/kg, IP) and the response of the mesolimbic dopamine system was measured through the use of in vivo microdialysis. Cocaine-induced increases in extracellular dopamine levels were apparent in both saline and caffeine pretreated rats. However the caffeine pretreated animals demonstrated greater increases in accumbens dopamine. This enhanced response of the mesolimbic dopamine system to cocaine following caffeine preexposure parallels behavioral indices of sensitization to cocaine observed under identical preexposure parameters.

110.4

EFFECTS OF COCAINE ON DOPAMINE AND ITS METABOLITES IN THE STRIATUM BETWEEN WKY AND SHR: A MICROLIALYSIS STUDY. T. Inada, R.W. Rockhold, K. Polk, C. Jin, B. Hoskins and I.K. Ho, Dept. Pharmacol. and Toxicol., Univ. of Mississippi Med. Ctr., Jackson, MS 39216

The present study examined effects of intraperitoneal injections of cocaine (20 and 40 mg/kg) on the extracellular levels of dopamine (DA) and its metabolites in the striatum of halothane-anesthetized 20-30 weeks WKY and SHR using an *in vivo* microdialysis technique. The baseline levels (pg/20μl) of DA and its metabolites were as follows:

	DA	DOPAC	HVA
SHR (n=14)	19.3±4.6	1638±418	2119±404
WKY (n=12)	22.9±6.3	1985±542	2498±592

There were no significant differences in the baseline levels of DA and its metabolites between WKY and SHR. The extracellular DA levels increased significantly following injections of cocaine (46% WKY, 52% SHR for 20mg/kg, $p<0.05$; 74% WKY, 78% SHR for 40mg/kg, $p<0.01$). However, there were no significant differences between strains. No significant changes were observed after cocaine administration in levels of DOPAC and HVA. Our previous findings showed significant differences in binding characteristics of both D-1 and D-2 receptors in the striatum between SHR and WKY. The present results indicate that acute challenge with cocaine does not differentially alter striatal DA release between anesthetized SHR and WKY. (Supported by DA04264)

110.5

EVALUATION OF CHANGES IN DOPAMINERGIC FUNCTION FOLLOWING CHRONIC COCAINE: AN *IN VIVO* MICRODIALYSIS STUDY. A. Mele, P. Glue, D.J.Fontana and A. Pert, BPB/NIMH and LCS/NIAAA, Bethesda, Md. 20892.

Some of the behavioral effects of cocaine increase in intensity with repeated administrations. A variety of neurochemical mechanisms have been postulated to underlie this behavioral sensitization. The purpose of this study was to evaluate alterations in dopaminergic processes with *in vivo* microdialysis following exposure to chronic cocaine. Rats were administered 30 mg/kg cocaine or saline daily for 7 days. On day 8 all animals were anaesthetized with chloral hydrate and challenged with cocaine administered either systemically or through microdialysis probes situated in the striatum or nucleus accumbens. A cocaine (10 mg/kg) challenge administered systemically on day 8 produced a somewhat greater increase in DA overflow in both the striatum and nucleus accumbens of rats pretreated chronically with cocaine. On the other hand, when rats were challenged with cocaine (0.01 mM or 1 mM) through the dialysis probe, there was no difference in DA overflow between the two groups in either structure. We have found that injections of apomorphine into the substantia nigra decrease levels of DA in the striatum, presumably through interaction with somatodendritic autoreceptors. No differences were found in apomorphine-induced decreases in striatal DA between groups of rats treated chronically with saline or cocaine. These findings suggest that the enhancement in DA overflow induced by systemic cocaine in cocaine-pretreated rats is not due to alterations in presynaptic DA functions or to DA autoreceptor subsensitivity. Experiment are in progress to determine whether the differences between the two groups can be attributed to differences in the disposition of cocaine and cocaine metabolite in the brain.

110.6

BEHAVIORAL AND NEUROCHEMICAL INTERACTIONS BETWEEN COCAINE AND BUPRENORPHINE: IMPLICATIONS FOR PHARMACOTHERAPY OF COCAINE ABUSE. E.E. BROWN, J.T.F. WONG*, J.M. FINLAY, C. WILSON* and H.C. FIBIGER. Div. of Neurol. Sci., Dept. of Psychiatry, Univ. of British Columbia, Vancouver B.C., V6T 1W5 Canada.

Buprenorphine (BUP) is a synthetic opiate that attenuates opiate self-administration. Recently, it has been reported that BUP also suppresses cocaine self-administration, suggesting that BUP might be useful in the pharmacotherapy of cocaine abuse. However, BUP is a drug of abuse as shown by epidemiological and self-administration studies. The conditioned place preference (CPP) paradigm was used to address two specific questions. First, does BUP induce CPP? Second, is cocaine-induced CPP affected by BUP?

Cocaine-induced CPP was linearly related to the dose administered. BUP also elicited CPP in a dose-related manner; an inverted-U shaped function was obtained. Subthreshold doses of BUP and cocaine, individually incapable of eliciting CPP, produced significant CPP's when given together. Moderate doses of BUP and cocaine individually capable of eliciting a CPP, produced a significantly larger effect in combination.

A large body of evidence suggests that mesolimbic dopamine (DA) is implicated in the rewarding properties of drugs of abuse. The effects of BUP, cocaine, and a combination of both drugs on DA and its metabolites in the nucleus accumbens were measured using *in vivo* microdialysis. BUP (10 μg/kg) caused a progressive increase in DA, reaching 200% of basal levels at five hr. A rapid increase in DA (180% of basal values) was observed following cocaine (5.0 mg/kg), which returned to baseline in 2-3 hr. BUP potentiated the effects of cocaine on extracellular DA (260% of basal values), providing neurochemical corroboration of the CPP results. Taken together, these data suggest that BUP can interact synergistically with cocaine.

110.7

MARKEDLY REDUCED STRIATAL DOPAMINE LEVELS IN BRAIN OF A CHRONIC COCAINE ABUSER by J.M. Wilson, J. Deck*, K. Shannak*, L.J. Chang*, L.M. DiStefano* and S.J. Kish, Clarke Institute of Psychiatry, Rotman Research Institute of Baycrest Centre and Toronto General Hospital, Toronto, Canada.

To our knowledge, no information is available concerning the behaviour of the major neurotransmitter systems in brain of human chronic cocaine abusers. We measured the levels of dopamine (DA) and dopamine D_2 receptor binding in autopsied basal ganglia of a chronic cocaine abuser (age 26). DA levels were markedly decreased outside of the normal control (n=8) range in subdivisions of the caudate (-51 % to -59%), putamen (-53% to -70%), and nucleus accumbens (-67%), but were normal in substantia nigra. DA (D_2) receptor binding density and affinity were within the normal control range in caudate and putamen. We suggest that the marked reduction of striatal DA levels may be due to either a reversible cocaine-induced inhibition of DA synthesis, or may represent an actual loss of dopaminergic nerve terminals. We emphasize the preliminary nature of our data and the importance of extending our findings in a greater number of cases.

110.8

EFFECTS OF CHRONIC COCAINE TREATMENT, WITHDRAWAL AND RECHALLENGE ON ANIMAL BEHAVIOR AND SYNAPTIC DOPAMINE METABOLISM IN NUCLEUS ACCUMBENS. Matthew W. Robertson, III (1,2), Catherine A. Leslie (2), Eric W. Lothman (1) and James P. Bennett, Jr. (1,2,3). Departments of Neurology (1), Behavioral Medicine and Psychiatry (2) and Pharmacology (3), University of Virginia School of Medicine, Charlottesville, VA 22908.

We studied the time course of development and duration of behavioral sensitization to cocaine in adult rats, and the effects of chronic cocaine administration and withdrawal upon subsequent cocaine-induced behavior and synaptic dopamine metabolism in vivo, measured with microdialysis. Adult male rats were implanted with microdialysis probe holders (Carnegie-Medicin) aimed to terminate 3 mm above ventromedial striatum/nucleus accumbens. After recovery, two groups of animals received either daily saline i.p. (SAL) or daily cocaine HCl 30 mg/kg i.p. (COC) for 18 days. On every third day, each animal's activity was monitored for 60 minutes before and after saline (SAL group) or cocaine injection (COC group). Animals received no injections from days 19-24 (washout). On day 25 a 3 mm microdialysis probe (Carnegie-Medicin) was inserted into the holder and perfused at 1 uL/min with artificial csf; 2 hours later activity monitoring was begun and consecutive 20 min dialysis samples were collected. After 60 min of baseline monitoring and dialysate collection, each animal received 30 mg/kg i.p. cocaine; monitoring and dialysis continued for 240 min.

Behavioral sensitization to cocaine was present by Day 13, persisted through Day 18, and was not present on Day 25. Day 25 baseline DA and HVA levels in extracellular fluid (ecf) were reduced 30% in the COC group, suggesting synaptic DA deficiency. Ecf DA levels rose to 340-360 % of baseline in both SAL and COC groups; ecf HVA fell less in the COC group. Total activity after cocaine in the SAL group was greater than in the COC group.

Sensitization to cocaine occurs in rats and does not persist after one week of drug withdrawal. 18 days of cocaine followed by 7 days of withdrawal:1) reduced baseline ecf DA and HVA levels, suggesting synaptic DA deficiency; 2) decreased behavioral response with similar increase in ecf DA after cocaine rechallenge, suggesting decreased DA receptor function, and blunted the fall in ecf HVA, suggesting autoreceptor desensitization. (Supported by NIMH-PSA to CAL and NINDS-RCDA and NS26581 to JPB).

110.9

EFFECTS OF INTRAVENOUS COCAINE ON SINGLE UNIT ACTIVITY IN THE NUCLEUS ACCUMBENS OF FREELY-MOVING RATS. L.L. Peoples* and M.O. West. Dept. Psychol, Rutgers U., New Brunswick, NJ.

This study characterized the effects of intravenous cocaine on single unit activity in the nucleus accumbens of freely-moving rats. Male Long-Evans rats were chronically implanted with either microwires or a detachable microdrive, positioned for accumbens recording; a stimulating wire in the fimbria; and a jugular catheter. That recorded units were accumbens neurons was verified by stimulating the fimbria to evoke unit discharges at monosynaptic latencies and by histological analysis. During self-administration training, a 0.2 ml infusion of 1.65 mg/kg cocaine followed each lever-press. To control for the potential effects of cocaine-induced motor behaviors on accumbens unit activity, pre- vs post-cocaine comparisons were made with behavior held constant across conditions. Two approaches were taken to create stable behavioral baselines: 1) periodically, rats were forced to locomote at a constant rate on a moving treadmill pre- and post-drug; and 2) neural activity was measured in relation to particular behavioral events with a computer-synchronized videotape analysis. In all cells thus far tested (i.e., 11 cells in four rats), mean firing rate increased during the pre-drug period of treadmill-walking and following the initial cocaine infusions. In nine cells, the cocaine-induced increase in mean firing rate exceeded the increase induced by treadmill-walking. For the majority of cells characterized across the entire self-administration training session (n = 8): 1) the initial cocaine-induced increase in unit activity dissipated with continued cocaine infusions so that unit activity at the end of the session approached pre-drug levels; and 2) unit activity appeared related to number of cocaine infusions but not to either the timing of lever-presses, or the occurrence of cocaine-induced stereotypies. Supported by DA04551.

110.10

ACTIVITY OF NEURONS IN NUCLEUS ACCUMBENS DURING COCAINE SELF-ADMINISTRATION IN FREELY MOVING RATS. J.Y. Chang, S.F. Sawyer, R.-S. Lee, B.N. Maddux and D.J. Woodward. *Dept. of Cell Biology and Neuroscience, Univ. of Texas Southwestern Medical Center, Dallas, TX 75235.*

A consensus has evolved that the nucleus accumbens (NAc) plays an important role in rewarding behaviors, including drug self-administration. Most of the experimental studies to date have focused on pharmacological and behavioral aspects of this issue. The present study employed chronic extracellular recording techniques in a cocaine self-administration paradigm in rats to explore the physiological mechanisms that underlie cocaine self-administration. Two bundles of eight stainless steel teflon-insulated microwires were chronically implanted in the NAc of male Long-Evans rats (275-300 g) and silastic tubing was inserted in the right jugular vein. Single bar presses activated a pump that infused 1 mg/kg cocaine (in 0.1 ml PBS) into the jugular vein. Daily 3 hour experimental sessions were conducted over periods of 7 to 28 days. Approximately 30% of the neurons recorded in NAc responded to drug self-administration, with most neurons exhibiting a decrease in firing rate following cocaine infusion. In some animals, simultaneous recordings obtained from 3-4 NAc neurons revealed heterogenous but reciprocal anticipatory responses, with some neurons increasing impulse activity 10-20 seconds prior to bar pressing and other neurons showing decreased activity. Post-drug inhibitory responses of these neurons were also present when drug infusion was given randomly under computer control. The increase in firing rate observed during approach to the bar may be unrelated to locomotion since increased unit activity was not observed during treadmill-enslaved locomotion. The dopamine D_2 receptor antagonist, pimozide (0.25 mg/kg, i.p.), blocked the decreased firing in some NAc neurons after bar pressing, but had no effect on anticipatory responses that preceded bar pressing. These preliminary results suggest that the NAc may be involved in the processes that initiate or control cocaine self-administration and that dopamine receptor activation appears to play a role in the responses that follow cocaine infusion. *Supported by DA2338, MH44337 and Biological Humanics Foundation.*

110.11

THE D1 ANTAGONIST SCH 23390 AND THE D2 ANTAGONIST RACLOPRIDE SELECTIVELY DECREASE BEHAVIOR MAINTAINED BY COCAINE OR FOOD IN THE RAT. S.B. Caine, M. Berhow*, M. Amalric and G.F. Koob. Department of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037.

The purpose of this study was to determine whether a specific D1 or D2 dopamine receptor antagonist selectively alters cocaine self- administration in the rat. Male Sprague-Dawley rats equipped with chronic jugular catheters were food-deprived and placed on an FR 15/ 2min. time-out schedule. Half-hour components of the two-hour session were alternately reinforced with food or cocaine (.075 mg/kg/inj.). Once baselined, animals were injected with SCH 23390 (2.5, 5, 10, 20 ug/kg sc) or raclopride (50, 100, 200, 400 ug/kg sc) 30 min. prior to the session.

At lower doses SCH 23390 dose-dependently decreased responding for cocaine down to 41% of baseline, yet had no effect on responding maintained by food. Higher doses of SCH 23390 reduced responding for both reinforcers, but more potently inhibited responding for cocaine. SCH 23390 is also reported to decrease responding for cocaine in the rhesus monkey (Woolverton and Virus, Pharm. Biochem. & Behav. 32:691, 1989) at doses which minimally affect responding for food (Kleven and Woolverton, Soc. Neurosci. Abs. 322.8, 1989). In contrast, the D2 antagonist raclopride failed to inhibit responding for cocaine at doses which decreased responding for food. Other studies in our laboratory have shown that raclopride in this dose range disrupts motor performance on a reaction time task, while SCH 23390 fails to alter performance in this task at doses which suppress cocaine self- administration. Taken together these results may suggest that at low doses D1 antagonists selectively block the mesocorticolimbic dopamine system and D2 antagonists selectively block the nigrostriatal system.

110.12

LOCOMOTOR ACTIVITY INDUCED BY INFUSIONS OF COCAINE INTO THE CA3 REGION OF THE HIPPOCAMPUS: MEDIATION BY DOPAMINERGIC SUBSTRATES. H.O. Pettit, M.S. Hooks and J.B. Justice Jr.; Emory University, Department of Chemistry, Atlanta, Georgia 30322.

Studies have revealed that behavioral effects can be produced by infusions of cocaine into the nucleus accumbens, the medial prefrontal cortex, and (more recently) the CA3 region of the hippocampus. In a series of four experiments locomotor activity was examined following bilateral infusions of cocaine directly into the CA3 region of the hippocampus. In the first experiment infusions of cocaine into the hippocampus were found to significantly increase locomotor activity (0.0, 6.8, 13.6 and 27.2 μg/0.5 μL/cannula, F=13.5, df=3/24, P<0.0001). Significant effects were primarily produced by the 27.2 μg dose. In a second experiment the increase in locomotor activity produced by cocaine was not affected by bilateral 6-hydroxydopamine lesions of the nucleus accumbens (F=1.0, df=1/25, NS). The third experiment revealed that infusions of higher doses of cocaine into the CA3 region of the hippocampus can increase locomotor activity in a dose dependent manner (0.0, 25, 50 and 100 μg/0.5 μL/cannula, F=6.1, df=3/32, P<0.003). In the last experiment the administration of a dopamine antagonist (*cis*-flupentixol, 0.125 and 0.25 mg/kg, I.P.) blocked the hyperlocomotion produced by infusions of cocaine into the hippocampus in a dose dependent manner (F=15.5, df= 2/24, P<0.0001). Present findings indicate that the effects of cocaine in the CA3 region of the hippocampus can produce locomotor activity that is mediated by dopaminergic mechanisms. The parallel relationship between drug-induced locomotor activity and reinforcement indicates that reinforcing effects produced by infusions of cocaine directly into the hippocampus are possible and warrant further study.

110.13

ASSESSMENT OF CHRONIC COCAINE-INDUCED CHANGES IN DOPAMINE MEDIATED BEHAVIORS DURING AND AFTER COCAINE TREATMENT. C.M. Joyner*, T.H. Lee*, E.H. Ellinwood, S.T. Cain. Dept. of Psychiatry, Duke University Medical Center, Durham, NC

Using electrophysiological techniques we have in the past demonstrated that the dopamine systems undergo time dependent changes in sensitivity following chronic stimulant (amphetamine) administration. It is important to characterize these time dependent changes behaviorally; therefore, we have initiated an examination of various behaviors which allow distinguishing different groups of animals.

Male Sprague Dawley rats weighing 100 to 125 grams are pretreated with either continuous cocaine (40 mg/kg/day) via Alzet osmotic minipumps, daily cocaine injections (40 mg/kg/day s.c.), or chronic saline. Following 14 days of pretreatment, these animals were behaviorally examined based on a number of rating scales developed in this lab as well as others.To assess development of sensitization or tolerance their response to cocaine was tested (20 mg/kg i.p.) the day after pretreatment and with apomorphine (80 μg/kg s.c.) seven days after withdrawal.

The daily injection group exhibits sensitization and the infusion group tolerance as shown by us and others in past studies. Our preliminary results show there are certain behaviors which may correlate with functional changes following differential modes of pretreatment. We will be presenting optimal drug regimes for producing sensitization versus tolerance as well as other distinguishing behavioral measures.

110.14

EFFECTS OF HALOPERIDOL PRETREATMENT DURATION ON COCAINE INDUCED ACTIVITY. P.A. LeDuc and A.G. Golden. Dept. of Psychology, Austin Peay State Univ., Clarksville, TN 37040.

Three haloperidol (HAL) pretreatment durations produced different effects on cocaine induced locomotor activity measured in photocell cages. Rats were given IP injections of haloperidol (0.2 mg/kg) or bacteriostatic water (BW) for 6, 12, or 18 days prior to the start of testing with cocaine hydrochloride. All rats received cocaine (0.0, 7.5, 15.0 mg/kg) in a semi-randomized order, every other day for 36 days. Daily injections of HAL or BW were continued during testing. The animals that received 18 days of HAL pretreatment were significantly more active than the 18 day controls at the 7.5 and 15.0 mg/kg levels of cocaine. Neither 6 or 12 days of HAL pretreatment produced significant increases in activity. HAL injections were continued throughout cocaine testing. When the 6 and 12 day pretreatment groups reached a total of 18 days of HAL injections their cocaine induced activity levels were significantly lower than the group pretreated for 18 days prior to cocaine exposure. These findings suggest that the critical factor in the modification of cocaine induced activity with haloperidol is the pretreatment duration prior to but not following cocaine exposure.

110.15

EFFECTS OF SELECTIVE DOPAMINE ANTAGONISTS ON THE DEVELOPMENT OF BEHAVIORAL SENSITIZATION TO APOMORPHINE. B.A.Mattingly, J.K.Rowlett, J.Graff*, and G.Lovell*. Department of Psychology, Morehead State University, Morehead, KY 40351.

The purpose of the present study was to determine the involvement of specific dopamine receptor subtypes (D1, D2) in the development of behavioral sensitization to apomorphine. In 3 experiments, male rats received 10-21 daily injections of a selective D1 (SCH23390;0 or .5 mg/kg IP) or D2 (sulpiride; 0, 30, or 100 mg/kg IP) antagonist followed by an apomorphine (APO; 0 or 1.0 mg/kg SC) injection. In 2 experiments, the rats were tested for locomotor activity in photocell arenas after the daily injections. In all experiments, the rats were tested for sensitization to APO following the subchronic pretreatments. The results indicated that APO produced a progressively greater increase in activity with each injection, and this APO-induced increase in activity was completely blocked by both sulpiride and SCH23390 treatments. However, although both sulpiride and SCH23390 blocked APO-induced activity, only SCH23390 injections blocked the development of behavioral sensitization to APO. That is, rats treated with APO and sulpiride displayed significant sensitization when subsequently tested with APO-alone. These findings suggest that the development of behavioral sensitization to apomorphine is related specifically to the stimulation dopamine D1 receptors.

110.16

Behavioral and cardiovascular effects of cocaine alone and in combination with the novel D1 antagonist SCH 39166. V. L. Coffin, L. C. Iorio, R. W. Watkins and A. Barnett. Schering Research, Dept. of Pharmacol., Bloomfield, NJ 07003.

Cocaine was studied alone and in combination with SCH 39166 in mice, rats and cebus monkeys in several test procedures. Cocaine produced stimulant effects in mice (10-30 mg/kg sc), rats (100-300 mg/kg sc) and monkeys (3 mg/kg sc) using either a locomotor activity monitor or observational measures. These effects were blocked by relatively low oral doses of SCH 39166 (0.3 - 3.0 mg/kg po), doses which had little if any behavioral effects on locomotor activity. Cocaine also produced dose-related increases in mydriasis, exophthalmus and lethality in rats (100-300 mg/kg sc), effects which could be blocked or delayed by SCH 39166. Cocaine given iv (0.02 -1.25 mg/kg) produced dose-related increases in diastolic pressure in conscious rats. Acute or chronic pretreatment up to two weeks with SCH 39166 did not potentiate or alter the pressor effect of cocaine. Potentiation of cocaine's effects or the emergence of new adverse actions were never observed under any of the different paradigms or species studied. Instead, SCH 39166 attentuated a number of behavioral and neurological functions produced by cocaine including lethality.

110.17

EVIDENCE FOR INVOLVEMENT OF BOTH D1 AND D2 RECEPTORS IN MAINTAINING COCAINE SELF-ADMINISTRATION. D.R. Britton, P. Curzon and J.W. Kebabian, Neuroscience Research, 47U, Pharmaceutical Discovery, Abbott Laboratories, Abbott Park, IL 60064.

We have extended testing of the hypothesis that D1 receptors are involved in cocaine reward (Koob et al. 1987) by using the D1 antagonist A69024 which has high affinity for the D1 site and is highly selective relative to D2 as well as to 5HT1c sites (Kirkman et al., 1989). We have also extended the testing of D2 antagonists with YM-09151-2. Rats trained to self-administer cocaine (0.75 mg/Kg/infusion) on an FR-5 schedule were treated with selective D1 and D2 antagonists. The D1 antagonist, A69024 (2.5, 5.0 or 10.0 umol/Kg, sc) increased cocaine self-administration by 47, 71 and 56 % respectively. SCH-23390 (.007, .015 and .030 umol/Kg) increased self-administration by 16, 47 and 65%, respectively. Both D1 antagonists decreased responding in some animals at the highest dose tested. The D2 antagonist, YM-09151-2, showed a similar profile, increasing cocaine self-administration at doses of .003 and .01 umol/Kg and producing either further increases or suppression of responding at the dose of .03 umol/Kg. These data give further support to the hypothesis that both D1 and D2 receptors are involved in maintaining cocaine self-administration.

110.18

EFFECTS OF DOPAMINE RECEPTOR-SELECTIVE DRUGS IN RATS TRAINED TO DISCRIMINATE COCAINE FROM SALINE. J.L. Katz, D.E. Nichols and J.M. Witkin. NIDA Addiction Research Ctr., Baltimore, MD 21224 and Purdue Univ. West Lafayette, IN.

The involvement of dopamine receptor subtypes in the behavioral effects of (-)-cocaine was evaluated by the ability of a series of compounds selective for D1 or D2 dopamine receptors to produce behavioral responses comparable to cocaine. Male, Sprague-Dawley rats were trained to discriminate 10 mg/kg (-)-cocaine HCl from saline (IP, 15 min prior) in a two-lever discrimination procedure. Both D1 and D2-selective agonists with diverse structures partially generalized to cocaine; these compounds produced responding that was not significantly different from that produced by either cocaine or saline. The D1 agonists studied were SKF 38393, SKF 75760, and CY 208-243. Dihydrexidine, a full agonist for induction of adenylate cyclase activity, also partially generalized to cocaine. The peripherally-acting D1 agonist, fenoldopam, produced only minimal cocaine-like responding which was unrelated to dose. The D2 agonists tested were pergolide, quinpirole, (-)-NPA, N0434, and N0437. Neither the D2 antagonist haloperidol nor the D1 antagonist SCH 23390 consistently blocked the discriminative stimulus effects of cocaine. These results suggest that both D1 and D2 receptors may play a role in the discriminative stimulus effects of cocaine but that stimulation of either dopamine receptor subtype alone is not sufficient.

110.19

THE EFFECTS OF INTRA-A10 PERTUSSIS TOXIN INJECTIONS ON COCAINE-INDUCED MOTOR ACTIVITY. J.D. Steketee and P.W. Kalivas. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Behavioral sensitization to psychostimulants, such as cocaine or amphetamine, has been suggested to result from an alteration of the inhibitory control of A10 dopamine neurons. The inhibitory mechanisms consist mainly of dopamine D_2 autoreceptors and $GABA_B$ receptors which are coupled via G proteins to K^+ channels. Activation of the D_2 and $GABA_B$ receptors leads to a hyperpolarization of the neuron. The G protein which couples the D_2 autoreceptors to K^+ channels is reported to be pertussis toxin (PTX)-sensitive. In order to investigate the potential role of G proteins in behavioral sensitization, we injected PTX (1.0 $\mu g/\mu l$, 0.5 μl/side) into the A10 region 2 weeks before testing the behavioral response to cocaine. Both saline control and PTX rats showed similar motor activity responses to saline. However, PTX rats exhibited a significantly greater motor activity response to cocaine relative to control animals receiving cocaine. In vivo microdialysis revealed that PTX rats had a significantly augmented release of dopamine in the nucleus accumbens which paralleled the augmented behavioral response to cocaine. Intra-A10 pretreatment with the $GABA_B$ agonist baclofen, which has been shown to block the acute response to cocaine, was unable to block the cocaine response of PTX rats. These data suggest that PTX-sensitive G proteins may play an important role in the development of behavioral sensitization by in part uncoupling the $GABA_B$ receptor from its G protein.

110.20

SUPERSENSITIVITY TO THE INITIAL REWARD PROPERTIES OF COCAINE FOLLOWING 6-OHDA LESIONS TO THE MEDIAL PREFRONTAL CORTEX IN RATS. S. Schenk, B.A. Horger, R. Peltier, K. Shelton* and H. Vercesi-Bonavera*. Dept. Psychol., Texas A&M Univ., College Station, TX, 77843.

Acquisition of intravenous cocaine self-administration (0.25, 0.5 or 1.0 mg/kg/infusion) was measured in separate groups of rats 14 days following either a sham or 6-Hydroxydopamine lesion to the medial prefrontal cortex. For sham rats, the dose/response curve was an inverted U with the 1.0 and 0.5 mg/kg doses supporting reliable self-administration. Reinforced response rates were reduced when 0.25 mg/kg was the available dose and there was a loss of discriminative responding suggesting that it was subthreshold for acquisition of self-administration. For rats that sustained a 70% depletion of dopamine in the medial prefrontal cortex, the dose/response curve was an inverse function across the entire dose range tested. They demonstrated good discrimination for all doses including 0.25 mg/kg/infusion, suggesting a supersensitive response to the initial reward effect of cocaine.

DRUGS OF ABUSE: COCAINE, SEROTONIN

111.1

TROPANE ANALOGUES INHIBIT COCAINE TOXICITY TO RAT FETAL 5-HT NEURONS IN CULTURE. **V. Williams, E.C. Azmitia, X. Hou, D.I. Schuster, and R.B. Murphy. Depts. Biol. and Chem., New York University, New York, NY 10003.**

Cocaine is toxic to fetal rat 5-HT neurons in primary dissociated tissue culture. Structural analogues of cocaine were studied as antagonists of its neurotoxicity. The selective $5\text{-}HT_3$ antagonist ICS-205-930 at 10^{-9} M provided protection against cocaine toxicity (10^{-6} and 10^{-7} M) in cells treated 3 days in culture, whereas various other dopaminergic, adrenergic, and serotonergic antagonists did not. We synthesized a series of tropane analogues from tropanone and evaluated their activity in this system. Equivalent protection to ICS-205-930 was provided by 2-thienyl-tropan-2-ol and several indolyltropanones, which were potent at nanomolar concentrations. These results suggest that further refinement of structure-activity relationships within this series of compounds will produce even more highly potent antagonists of cocaine neurotoxicity. Supported by NIDA contract 271-87-8144 (ECA) and NIDA RO1 DA 05728 (RBM)

111.2

ALLOSTERIC MODULATION BY PAROXETINE OF THE [^{3}H]COCAINE BINDING SITE ASSOCIATED WITH THE SEROTONIN TRANSPORTER IN GUINEA PIG BRAIN

H. C. Akunne[1], B. R. de Costa*[2], A. E. Jacobson*[2], K. C. Rice[2] and R. B. Rothman[1]. [1]Unit on Receptor Studies,LCS, NIMH, Bldg 10, 3D41 Bethesda, MD 20892; [2]Laboratory. of Medicinal Chemistry, NIDDK, Bethesda, MD 20892.

We studied the characteristics of [^{3}H]cocaine binding in membranes prepared from whole guinea pig brain. Cocaine binding was specific and saturable. Binding surface analysis demonstrated that a two-site binding model fit better than a one-site binding model. The Kd values for [^{3}H]cocaine were 15.2 and 650 nM with Bmax values of 183 and 1880 fmol/mg protein. In competition studies, serotonergic reuptake inhibitors (paroxetine, clomipramine, fluoxetine) were more potent than dopaminergic (GBR12909, benztropine, mazindol) or noradrenergic (desipramine) reuptake inhibitors. Both high affinity serotonin and dopamine uptake inhibitors produced dose-dependent wash-resistant (pseudoirreversible) inhibition of [^{3}H]cocaine binding. However, serotonerigc uptake blockers were more potent than dopaminergic uptake inhibitors The pseudoirreversible inhibition produced by paroxetine was due to an increase in the Kd of [^{3}H]cocaine, accompanied by an increase in the dissociation rate of [^{3}H]cocaine. These studies suggest that using membranes prepared from whole guinea pig brain, [^{3}H]cocaine labels two binding sites associated with serotonin transporter and that paroxetine binds to a site which allosterically controls the conformation of the [^{3}H]cocaine binding site.

111.3

EFFECTS OF REPEATED COCAINE ON MONOAMINE TRANSPORTERS: STUDIES IN VITRO WITH SEROTONIN AND DOPAMINE TRANSPORTER SUBSTRATES AND INHIBITORS R.S. Salah, M.J. Keegan*, B.N. Mathews*, E. A. Novak*, and M.P. Galloway, Lafayette Clinic, Cell. & Clin. Neurobiol. Prog., Dept. Psychiatry, Wayne State Univ Sch Med, Detroit MI

Repeated use of psychomotor stimulants such as cocaine (COC) or amphetamine (AMPH) produces a behavioral and neurochemical sensitization of DA systems to subsequent stimulant challenges. Additionally, recent behavioral studies suggest that increased 5HT neuronal tone decreases the rate of drug self-administration in rats. We sought to determine if repeated COC treatment in vivo (20 mg/kg/ip/day, for 7 days, 7 days off) altered either the 5HT or DA transporter by examining 1) the ability of transporter substrates (eg, (+)MDMA, AMPH) to release 5HT or DA from brain slices in vitro, and 2) the ability of transporter inhibitors (eg. COC) to block these effects. Expressed as percent control, striatal DA released by AMPH (0.3-10 μM) was greater in treated animals, whereas striatal 5HT released by AMPH was not altered. Similarly, AMPH induced 5HT release from cortical slices was not different. However, (+)MDMA induced 5HT release was enhanced in cortical slices obtained from treated subjects. The ability of cocaine to reverse either AMPH or TFMPP (a 5HT releaser) did not appear to be altered by the above treatment protocol. Although further studies are currently in progress, these preliminary data suggest that transporter function (ie the releasing effect of substrates) and the inhibition afforded by cocaine at its receptor may be differentially regulated by repeated treatment with cocaine. Further, enhanced releasability of 5HT may be related to development of either tolerance or behavioral sensitization associated with DA systems. Support: MH-41227 DA-04120 State of Michigan, Dept of Mental Health.

111.4

COCAINE EFFECTS ON SEROTONIN AND DOPAMINE RECOGNITION SITES IN RAT BRAIN: IN VITRO AND IN VIVO STUDIES F. Tung, A.M. Bonadonna, P.A. Rittenhouse, E. Bakkum, L.D. Van de Kar, and G. Battaglia. Department of Pharmacology, Loyola Univ. of Chicago, Stritch School of Med., Maywood, IL 60153.

This study focuses on the *in vitro* and *in vivo* effects of cocaine at brain 5-HT and DA recognition sites. An *in vitro* pharmacological profile at these sites demonstrated that cocaine had an extremely low affinity (Ki > 100 uM) for striatal D-1 and D-2 dopamine receptors and cerebral cortical $5\text{-}HT_1$ and $5\text{-}HT_2$ serotonin receptors. In contrast, cocaine exhibited relatively high affinity for the uptake sites for DA (Ki=0.75 uM) and 5-HT (Ki=0.14 uM). To assess the effects of cocaine on the modulation of DA and 5-HT receptors, presumably via indirect actions on uptake blockade, rats were treated with cocaine (15 mg/kg, i.p. b.i.d) or saline for 7 days and sacrificed 36 hours after the last injection. With respect to dopaminergic systems, cocaine caused no change in D-1 receptors in cerebral cortex or striatum but slightly reduced the binding to D-2 receptors in striatum. DA uptake sites in the same brain region were unaffected by cocaine. Similarly, cocaine had no affect on $5\text{-}HT_1$ or $5\text{-}HT_2$ receptors in cerebral cortex or hippocampus. In contrast, cocaine significantly decreased 3H-paroxetine-labeled 5-HT reuptake sites, but only in striatum; no changes were observed in cerebral cortex, hippocampus or hypothalamus. These results provide preliminary data which suggest that cocaine may "down regulate" 5-HT transporters or may be toxic to striatal 5-HT axons and/or terminals. (Supported by NIDA DA 04865)

111.5

AUTORADIOGRAPHIC AND BEHAVIORAL ANALYSIS OF LONG-TERM EFFECTS OF CHRONIC COCAINE AND AMPHETAMINE. Steve Zeigler, Jack Lipton, and Gaylord Ellison, Dept of Psychology, UCLA, Los Angeles, CA, 90024. Rats were given continuous amphetamine(AMPH), or cocaine(COC) via subcutaneous pellets for 5 days, or were given five days of once-daily COC injections. 40 days after drug discontinuation, rats were observed in open field and then sacrificed for autoradiography. Coronal brain sections were prepared for autoradiography using the following receptor ligands: [3H]spiperone for D-2, [3H]SCH23390 for D-1, [3H]QNB for ACH, [3H]ketanserin for 5-HT2, [3H]5-HT for 5-HT1, and [3H] flunitrazepam for benzodiazepine. While AMPH rats did not differ from controls in open field behavior, they showed large reductions in S-2 binding in most brain regions.Chronic COC rats were the most fearful in open field, yet showed less dramatic reductions in 5-HT2 binding than AMPH rats, and only in selected regions.COC injection rats showed the least fear and highest activity in open field but did not differ from controls in 5-HT2 binding. No differences between groups were found for 5-HT1 receptor binding.Analysis of results for the other 4 ligands will be discussed.

111.6

LACK OF PROTECTIVE EFFECTS OF CHLORPROMAZINE (CPZ) ON 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) INDUCED-NEUROTOXICITY ON BRAIN SEROTONIN (5-HT) NEURONS IN RATS. S.Y. Yeh, Neurobiology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224.

MDMA, a ring-substituted derivative of methamphetamine (MA), is a potent neurotoxin. Mechanisms of MDMA-induced neurotoxicity are not clear. CPZ has been shown to decrease the lethality induced by amphetamine (A) and stress, to prevent MA-induced depression of striatal tyrosine hydroxylase activity. Furthermore, CPZ has been used effectively to treat the poisoning of A, MA, and the interaction of meperidine and MAO inhibitors. The present study examined whether CPZ antagonized the toxic effects of MDMA in rats on brain serotonin neurons. Rats were injected with CPZ (10 mg/kg, i.p), or MDMA (10 mg/kg, s.c.), CPZ + MDMA (CPZ was given 30 min prior to the injection of MDMA) and saline every 12 h for 5 doses. The rats were killed at 24 h after the last injection and brain tissues were dissected. Monoamine levels in the brain tissues were assayed by HPLC-EC. 5-HT and 5-hydroxyindoleacetic acid concentrations in the frontal cortex and hippocampus were comparably decreased in the MDMA and MDMA + CPZ treated rats (60% of the saline or CPZ control group). No significant differences in brain indolamine levels were noted between CPZ- and saline-treated rats. These data suggest a lack of neuroprotective effects of CPZ on neurotoxic effect of MDMA treatment on brain 5-HT neurons in rats.

111.7

COCAINE: BEHAVIORAL SENSITIZATION AND SEROTONIN (5-HT) IMMUNOREACTIVITY IN THE RAT FOREBRAIN. *J.M. Paris, P.M. Callahan, J.M. Lee*[1] *and K.A. Cunningham,* Dept. Pharmacol., Univ. Texas Med. Branch, Galveston, TX 77550; [1]Dept. Pathol., Mass. General Hosp., Boston, MA 02114

With repeated exposure, rodents become sensitized to the motor-activating properties of cocaine and amphetamine derivatives while humans develop psychoses and sensory hallucinations. *Behavioral sensitization* to cocaine appears to be associated with an enhanced sensitivity of 5-HT systems in the absence of any overt alterations in central 5-HT and metabolite levels. This is in contrast to some amphetamine derivatives which are 5-HT neurotoxins. The purpose of the present study was to assess whether behavioral sensitization is associated with perturbations of 5-HT-immunoreactive processes in the rat forebrain. Male Sprague-Dawley rats were administered (ip) either saline (1 ml/kg;N=10) or cocaine (15 mg/kg;N=11) twice daily for 7 days. Following the last injection, behavioral sensitization was expressed in cocaine (median:6, range:6-8) but not saline-treated rats (median:3, range:2-6; Kilby-Ellinwood scale; $p<0.0002$). Twenty-four hours following the final injection, rats were treated with pargyline (30 mg/kg, ip) 60 min prior to sacrifice and perfusion. 5-HT immunoreactivity was visualized in brain sections using a rabbit anti-5-HT antibody and an avidin-biotin peroxidase system. Behaviors and intensity of 5-HT immunoreactivity were scored by a rater blind to the animals' treatment. No consistent alterations in 5-HT fibers in cortex (frontal, cingulate, parietal), septum, hippocampus and striatum were observed in cocaine-treated rats. The data lend further support to the hypothesis that, although psychomotor stimulants share similar behavioral profiles, behavioral sensitization to cocaine is not associated with neurotoxicity of 5-HT neurons. Furthermore, we will show data to indicate that the variability of 5-HT fiber staining in control animals justifies the need for careful interpretation of drug-induced 5-HT fiber loss. (Supported by DA05708 to K.A.C. & DA05381 to J.M.P.).

111.8

COCAINE INHIBITION OF SEROTONIN (5-HT) DORSAL RAPHE (DR) NEURONS: EFFECTS OF HABENULAR LESIONS. *K. A. Cunningham, J. M. Paris, P. M. Callahan.* Department of Pharmacology and Toxicology, University of Texas Medical Branch, Galveston, TX 77550.

Pathways which originate in and pass through the habenular nuclei provide a major source of regulatory afferents to mesencephalic 5-HT systems. Cocaine potently inhibits the activity of 5-HT DR neurons probably as a consequence of local reuptake blockade and potentiation of 5-HT somatodendritic autoregulatory processes. The purpose of the present study was to ascertain whether the suppressive effect of cocaine on the activity of 5-HT DR neurons may also be dependent upon the integrity of the habenula. Bilateral radiofrequency lesions of habenula were made in chloral hydrate-anesthetized rats. Conventional single-unit extracellular recording techniques were used to identify spontaneously-active 5-HT DR neurons 1-4 hrs post-lesion. Cocaine (0.125-2 mg/kg,IV) rapidly and reversibly suppressed cell firing ($n=7$; ID_{50}=0.54 mg/kg) in sham-lesioned and unoperated controls. In rats with habenular lesions and partial damage of the dorsomedial thalamus, the cocaine-induced depression ($n=6$; ID_{50}=3.75 mg/kg) was significantly attenuated [$F(4,27)=2.99$, $p=0.036$]. For example, cocaine at a dose of 2 mg/kg completely inhibited cell firing in control rats but elicited an average 40% depression in lesioned rats. The firing rate (# spikes/sec) observed in lesioned rats (1.33±0.55) did not differ from that of control rats (1.13±0.14). Electrical stimulation of habenula suppresses the spontaneous activity of 5-HT DR neurons. Thus, the present data suggest that the observed suppressive effects of cocaine may be mediated in part by a local action in habenula or via feedback pathways from limbic forebrain that relay or course through the habenula.

Supported by NIDA grants DA05708 (KAC) and DA05381 (JMP).

111.9

DEVELOPMENT OF TOLERANCE TO ACUTE ADMINISTRATION OF COCAINE IN THE DORSAL RAPHE SEROTONERGIC NEURONS OF SUB-CHRONIC COCAINE-TREATED RATS. **E.W. Black and J.M. Lakoski. Dept. of Pharmacology and Toxicology, Univ. of Texas Medical Branch, Galveston TX 77550**

The effects of subchronic cocaine administration on serotonergic cell firing in the dorsal raphe nucleus (DR) was examined using an in vivo, extracellular slice preparation. Male Sprague-Dawley rats were administered either cocaine or saline (30 mg/kg, ip) for a period of 7 days prior to experimentation. Following a washout period of 24 h, the rats were anesthetized and decapitated. DR slices (400 μm) were obtained using a vibrotome and were allowed to equilibrate in oxygenated ACSF (37°) for a period of at least 1 h. Recorded slices were superfused with ACSF containing 1 μM phenylephrine and 1 μM 5-hydroxytryptamine (5-HT). This concentration of 5-HT was found to inhibit basal firing rates of both cocaine and saline treated animals by 10.5% ± 2.23. In addition, inhibition responses to acute applications of 5-HT showed no differences between treatment groups and there were also no indications of a developed tolerance to 5-HT application. However, animals treated subchronically with cocaine do show an apparent tolerance to acute cocaine application as compared to saline controls. Animals treated subchronically with cocaine required an acute cocaine treatment of 5.5 ± 0.5 μM to inhibit firing by 50% versus 2.4 ± 0.4 μM for saline controls. In both treatment groups, repeated superfusion of the slice with equal concentrations of cocaine produced a dose-dependent inhibition which remained constant with each application indicating no development of an acute tolerance to cocaine application. Finally, ligand binding studies using hippocampal tissue and the 5-HT_{1A} specific ligand 8-OH-DPAT indicated that there were no differences in either the K_d or B_{max} between the treatment groups. In summary, these results indicate that the inhibitory effects of cocaine are not due to direct effects upon the presynaptic autoreceptor but are instead produced by its interactions with the 5-HT reuptake system. Supported by DA04296 and a Research Career Development Award from NIA to JML.

111.10

SELF-ADMINISTRATION & NEUROTOXICITY OF 3,4-METHYLENEDIOXY-AMPHETAMINE IN RATS. L.E. Markert, D.C.S. Roberts. Department of Psychology, Carleton University, Ottawa, Ontario, K1S 5B6.

The reinforcing properties of 3,4-methylenedioxy-amphetamine (MDA), a drug known to be toxic to the central serotonergic system of rats, have rarely been examined experimentally. The present study investigated whether rats would self-administer MDA and whether MDA would be neurotoxic at self-administered doses. Rats were permitted access to MDA at doses of 0.25, 0.50, or 1.0 mg/injection on an FR 1 schedule of reinforcement for 5 hrs/day. Results showed that animals would self-administer MDA in a dose-dependent manner and that after access to the drug for 5-7 days, 5-HT was depleted in the hippocampus but not the cortex. The data indicate that neurotoxicity is associated with MDA self-administration in rats.

(Supported by NSERC Scholarship to LEM and MRC Grant to DCSR).

111.11

LOW DOSE EFFECTS OF 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) ON BRAIN AMINE LEVELS. TH Champney and D Dischler*. Dept Anat, Coll Med, Texas A&M Univ, College Station, TX 77843-1114.

Acute and chronic injection of high doses of MDMA in rats markedly depress serotonin (5HT) and 5-hydroxyindoleacetic acid (5HIAA) levels and transiently alter dopamine (DA) and 3,4 dihydroxyphenylacetic acid (DOPAC) levels in numerous brain regions. Low dose, discontinuous administration of MDMA to rats has not been reported even though this is the primary type of administration utilized by humans. Singly housed male rats were injected at 1000 hr twice per week (on Mondays and Tuesdays) with saline or 2 mg/kg of ± MDMA (sc) for up to 8 weeks. Body weight was determined weekly and overt behavior after injection was noted. After two weeks, groups of saline- and MDMA-injected rats were killed 24 hr after the last injection (n=5/group). After eight weeks, groups of saline- and MDMA-injected rats were killed 3 hr, 12 hr, 24 hr and 48 hr after the last injection (n=5/group). Cortical, hippocampal, hypothalamic and striatal brain regions were collected and assayed for amine content by HPLC-EC. No differences in cortical and striatal 5HT levels were observed between the MDMA and saline treated rats. DA and DOPAC levels were depressed in the striatum 48 hr after the last injection (32 and 54%, $p<0.01$). 5HT levels were increased ($p<0.05$) in the hypothalamus (26%) and the hippocampus (33%) at the two week time point, while 5HIAA levels were depressed (34%, $p<0.05$) in the hippocampus 12 hr after the last injection. Hypothalamic 5HIAA levels were increased at 12 and 24 hr after the last injection (40 and 29%, $p<0.05$). Body weight was decreased slightly ($F=6.2$, $p<0.05$) in MDMA-treated rats with no major behavioral effects observed at this dosage. The observed changes in amine levels in this study are small compared to the major declines in 5HT content observed in previous studies utilizing high doses of MDMA. These results indicate that even small discontinuous doses of MDMA are capable of altering brain amine levels in rats.

111.12

EFFECT OF MDMA ANALOGUES ON THE EXTRACELLULAR CONCENTRATION OF DOPAMINE (DA) IN THE STRIATUM AS MEASURED BY IN VIVO MICRODIALYSIS. J.F. Nash and D.E. Nichols. Dept. of Psychiatry, Case Western Reserve Univ., Cleveland, OH 44106 & Dept. of Med. Chemistry & Pharmacognosy, Purdue Univ., W. Lafayete, IN 47907.

The effect of the racemic mixture of MDMA (3,4-methylenedioxymethamphetamine) and its alpha- and N-ethyl analogues, MBDB and MDE, respectively, on the extracellular concentration of DA and its major metabolites DOPAC and HVA was measured in the rat striatum using in vivo microdialysis. Acute administration of these compounds increased the extracellular concentration of DA in the striatum. The rank order of potency of these drugs to increase DA concentrations was: MDMA > MDE > MBDB. The concentrations of DOPAC and HVA were reduced following the administration of these drugs. These data are consistent with in vitro studies which demonstrate that extending the alpha-carbon and amine substitutions from methyl to ethyl groups decreases the effect of these phenethylamines on dopaminergic systems. The rank order of potency of these drugs to decrease brain serotonin content 7 days following a single administration was the same as that to increase the extracellular concentration of DA. These data are supportive of the hypothesis that DA may play a role in the serotonin depleting effects of these drugs. Suported by Ohio Board of Regents RIF(OBR).

111.13

EFFECTS OF CHRONIC METHYLENEDIOXYMETHAMPHETAMINE (MDMA) ON LOCOMOTOR ACTIVITY, LEARNING AND MEMORY, PAIN PERCEPTION AND MAINTENANCE OF BODY WEIGHT. D.B. Miller, K.F. Jensen and J.P. O'Callaghan. U.S. Environmental Protection Agency, Res. Tri. Pk., NC 27711

Acute and chronic treatment with MDMA produces serotonin (5-HT) depletions that last many months. 5-HT is involved in cognition, pain perception and feeding. Locomotor activity in home-cage monitors and figure-8 mazes, Morris-water maze learning, colonic temperature, tail-flick response, as well as body weight and associated food and water consumption were measured to determine if chronic treatment with MDMA produces long-lasting functional alterations. Long- Evans male rats were administered MDMA by a chronic regimen (5 - 30 mg/kg, s.c., twice daily for 7 days). MDMA caused the development of dose-related hyperactivity during dosing but resulted in no permanent activity alterations. Morris-water maze acquistion and performance were not affected by prior exposure to MDMA. MDMA did not affect the tail-flick response during or after dosing. Body weight as well as food and water consumption were reduced during the period of dosing. Food and water consumption recovered after the cessation of dosing but no rebound feeding occurred. Thus, food intake and rate of weight gain were equivalent in MDMA and control rats in the period following dosing but body weights of MDMA rats were still below those of the controls 60 days after dosing. MDMA, like the known anorectic - fenfluramine, may alter metabolic set point. Supported by NIDA IAG ND89-4

111.14

SEROTONIN DEPLETIONS ARE NOT PREDICTIVE OF NEUROTOXICITY: EVIDENCE FROM INCREASES IN GLIAL FIBRILLARY ACIDIC PROTEIN INDUCED BY METHYLENDIOXYMETHAMPHETAMINE (MDMA) AND 5,7-DIHYDROXYTRYPTAMINE (5,7-DHT). J.P. O'Callaghan, D.B. Miller, K.F. Jensen and C.J. Schmidt[1]. U.S. Environmental Protection Agency, Res. Tri. Pk., NC 27711 and [1]Merrell Dow Research Inst., Cincinnati, OH 45215.

Astrocytes hypertrophy following chemical damage to the CNS and the hallmark of this response is enhanced expression of the intermediate filament protein, glial fibrillary acidic protein (GFAP). In this study we used assays of GFAP to determine if MDMA is neurotoxic. Rats were administered MDMA by acute (20 mg/kg, s.c.) or chronic (5-30 mg/kg, s.c., twice daily for 7 days or 80 mg/kg, s.c., twice daily for 2 days) regimens. Another group of rats received the serotonergic neurotoxicant, 5,7-DHT (200 µg, i.c.v.) as a positive control. Rats were killed at intervals between 2 hours and 2 months post dosing. Brain halves were dissected into 4 - 6 regions and assayed for GFAP or serotonin (5-HT). Acute or chronic MDMA resulted in 5-HT depletions in all areas with decrements as great as 95% (striatum). GFAP was increased (50%) only in cortex and only after 4 x 80 mg/kg MDMA; 5-HT levels in cortex from the same rats were decreased by 60%. 5,7-DHT caused brain-wide decreases in 5-HT that were less than those seen with MDMA but it increased GFAP in several regions by as much as 50%. Long-lasting 5-HT depletions due to MDMA may not be indicative of neurotoxicity. Supported by NIDA IAG ND-89-4.

111.15

EVIDENCE FOR THE NEUROTOXICITY OF METHYLENEDIOXYMETHAMPHETAMINE (MDMA) USING A CUPRIC SILVER STAIN FOR NEURONAL DEGENERATION. K.F. Jensen, D.B. Miller, J.K. Olin*, and J.P. O'Callaghan. Neurotoxicology Division, U.S. Environmental Protection Agency and *NSI Technology Services Co., RTP, NC 27711

MDMA (80 mg/kg, s.c.) was administered to rats twice a day for 2 days and the animals were sacrificed by transcardial perfusion with fixative under deep pentobarbital anesthesia 48 hours after the first dose. Frozen sections of the brains were stained for degenerating terminals, axons and cell bodies with a recent modification of the DeOlmos cupric silver stain for neuronal degeneration. Evidence of neuronal damage was observed in frontal and parietal regions of the neocortex and included terminal and perikaryl degeneration in upper layers as well as axonal degeneration in both the upper and lower layers. These observations support the hypothesis that MDMA induces neuronal damage. This work was supported by NIDA IAG ND-89-4.

111.16

MDMA'S EFFECT ON THE FIRING RATES OF MEDIAL PREFRONTAL CORTICAL (mPFc) NEURONS IS MEDIATED THROUGH THE SEROTONERGIC (5-HT) SYSTEM. H.S. Pan and R.Y. Wang. Dept. of Psychiatry and Behavioral Science, SUNY, Stony Brook, N.Y. 11794.

The designer drug MDMA (3,4-methylenedioxymethamphetamine; "Ecstasy") enhances emotion and cognition but is nonhallucinogenic. It releases 5-HT and blocks its reuptake; it has a similar but less potent effect on the dopamine system. We used standard single unit recording and microiontophoretic techniques to study the mode of action of MDMA on mPFc cells of chloral hydrate anesthetized male Sprague-Dawley rats. When MDMA was given intravenously, it dose-dependently inhibited the majority (86%) of the mPFc cells (IC_{50} = 5.3 ± 1.8 mg/kg, n = 12). A minority (14%) of the cells were modestly excited. The coadministration of 5-HT receptor antagonists such as metergoline and granisetron reversed the effect of MDMA. When administered directly onto the cells by microiontophoresis, MDMA inhibited the firing of mPFc cells (IC_{50} = 27 nA, n = 10). In PCPA (*p*-chlorophenylalanine, 400 mg/kg, i.p., 24 hr before recording)-pretreated animals whose brain 5-HT levels had presumably been depleted, MDMA, either given intravenously or by microiontophoresis, failed to inhibit the neurons. In these PCPA-treated rats, the i.v. administration of 5-hydroxytryptophan (5-HTP, the immediate precursor of 5-HT; 50-200 mg/kg, n=4) but not L-DOPA (the immediate precursor of dopamine, 50-100 mg/kg, n=4) reinstated the inhibitory action of MDMA. In contrast, in AMPT (α-methyl-*p*-tyrosine, 250 mg/kg, i.p., 4 and 2 hr before recording)-pretreated animals whose brain dopamine content had presumably been depleted, MDMA inhibited the cells with an IC_{50} of 2.2 ± 1.0 mg/kg (n = 5), which is not statistically different from that seen in control rats. The data suggest that MDMA affects the firing rates of spontaneously active mPFc cells indirectly via the release of endogenous 5-HT, not dopamine.

(Supported by USPHS Grants MH-41440 and MH-00378.)

111.17

THE MECHANISMS OF NEUROCHEMICAL CHANGES INDUCED BY 4-METHYLAMINOREX (4-MAX)-A NEW STIMULANT OF ABUSE. C.F. Bunker, M. Johnson, G.R. Hanson, and J.W.GIBB. Dept. Pharmacology and Toxicology, University of Utah, Salt Lake City, Utah 84112.

4-MAX is a new stimulant of abuse which has recently gained significant attention. Although classified as a Schedule I drug, little is known about its neurochemical or neurotoxic properties. We have reported that 4-MAX is an extremely potent convulsant in rats and mice; a single dose (10 mg/kg) of 4-MAX elicits an immediate dramatic rise in the dopamine metabolite dihydroxyphenyl acetic acid, and a decline in tryptophan hydroxylase (TPH) activity in striatal tissues 3 h after treatment. In order to determine the mechanism of 4-MAX-induced neurochemical changes, this drug was given as single or multiple (5 doses; 6-h interval) administrations. TPH activity which declined 3 h following a single dose (20 mg/kg) was completely reactivated to control enzyme activity by a 20-h preincubation in vitro under strong reducing conditions as has been reported for other amphetamine analogs. The neurotoxic properties of 4-MAX were assessed by evaluating TPH and tyrosine hydroxylase (TH) activity 7 d after both single and multiple doses. Following both treatment paradigms, striatal TPH activity declined significantly to approximately 80% of control but TH activity was unchanged. This long-term decrease in TPH activity likely reflects selective damage to serotonergic neurons; however, after single and multiple doses, 4-MAX does not appear to be as neurotoxic as MDMA or methamphetamine but may be comparable to MDE. (Supported by grants DA 00869 and DA 04222).

111.18

CHRONIC COCAINE MODIFIES GROWTH HORMONE RELEASE AFTER 5-HYDROXYTRYPTOPHAN IN MALE RATS. NS Pilotte, LG Sharpe, EM Dax*. Addiction Res. Ctr., NIDA, Baltimore, MD 21224.

More growth hormone (GH) is released between 0500-0900 hr in human males who voluntarily abstain from cocaine use than in males who do not use cocaine habitually. To determine if the chronic administration of cocaine could alter GH release, we infused male Lweis rats with cocaine (1 mg/kg IV) or 0.15 M NaCl (0.1 ml/kg IV) every 12 min for 2 hr for 10 days. The rats were also injected with 5-hydroxytryptophan (10 mg/kg ip) 30 min after the last infusion on days 1, 5 and 10. Blood was removed from an indwelling jugular catheter before and after the session on these days and its GH content was determined.

A wide range of concentrations of GH were measured at each time, reflecting the operation of an independent circadian rhythm that in part governs its release. The pre-session concentrations of GH were similar in both groups of rats on each day. Post-session GH was reduced and less variable in cocaine-treated rats by Day 5, but did not differ from that of the 0.15M NaCl-treated rats. Although the administration of 5-hydroxytryptophan always markedly increased GH in both groups of rats, this response was significantly blunted ($P < 0.03$) by Day 5 in rats given cocaine. These results suggest that an alteration in responsiveness to serotonergic agents occurs in cocaine-treated rats.

111.19

DIFFERING EFFECTS OF ACUTE AND CHRONIC COCAINE TREATMENT ON 5-HT_2- MEDIATED HEAD-TWITCH RESPONSE IN MICE. N.A. Darmani[1], M.G. Hadfield[2] and B.R. Martin*[1]. Departments of Phamacol/Toxicol[1]. and Pathology[2], MCV/VCU, Richmond VA 23298.

Cocaine inhibits the uptake of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) and is reported to be a more potent inhibitor at the 5-HT uptake sites than at the DA or NE sites. Radioligand binding studies have identified at least four types of 5-HT receptors (5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4). Recently, we reported that acute cocaine administration inhibited DOI-induced 5-HT_2 receptor mediated head-twitch response(HTR). This inhibition appeared to result from the stimulation of the inhibitory α_2 and 5-HT_{1A} receptors.

5-MeO DMT was used to induce HTR in mice and the behavior was scored for 10 minutes post injection. 5-MeO DMT produced HTR in a dose-dependent manner up to 16 mg/kg (i.p.). Higher doses did not further increase the behavior. For drug interaction studies a dose of 8 mg/kg 5-MeO DMT was used to induce HTR. After acute pretreatment, cocaine (1.2-20 mg/kg, i.p.) dose dependently inhibited HTR. Another group of mice was chronically treated with either cocaine (10, 20 and 40 mg/kg, i.p.) or saline twice daily for 12 days and once at 9:00 am on day 13. On day 13 (2-5 pm), the animals were injected with 8 mg/kg 5-MeO DMT, and the HTR was scored as above. The 10 and 20 mg/kg cocaine treated mice exhibited 2.6 and 1.7 times ($p<0.05$) more HTR than saline treated control. Although cocaine lacks affinity for 5-HT_2 receptors, these data suggest that it does modify 5-HT_2 receptor function differentially after acute and chronic administration. NIDA grants DA-02396 and DA-05274.

111.20

BETA-ADRENERGIC ADAPTATION TO COCAINE: ROLE OF SEROTONIN. A.C. Swann. Dept. of Psychiatry, Univ. Texas Medical Sch., Houston, TX 77225.

There is little information about monoamine effects of repeated cocaine. Cocaine blocks reuptake of norepinephrine (NE), dopamine (DA), and serotonin (5HT), initially increasing synaptic concentrations of these amines. Cocaine increases synthesis of DA and NE but decreases that of 5HT. Adaptation of NE or DA to repeated cocaine may be impaired by 5HT depletion. Repeated cocaine has been reported to increase beta-NE receptor (DHA) binding (24 h later) in brain, either from adaptation to NE depletion during cocaine treatment, from NE decrease during cocaine withdrawal, or from 5HT depletion, which has been reported to increase, and to prevent adaptive decreases in, NE receptor binding. We have investigated the role of 5HT in effects of cocaine on NE receptors. Cocaine (9 mg/kg bid for 2 weeks) resulted (2 h later) in decreased metabolites of NE, DA, and 5HT, and increased beta-receptor binding in several brain regions. Treatment with the 5HT precursor 5-hydroxytryptophan prevented the cocaine-induced increase in DHA binding. There was no effect on MHPG, showing that the ability of cocaine to block NE uptake was not impaired. These data suggest that potentially increased sensitivity to NE develops during cocaine treatment and is prevented by treatments that enhance 5HT synthesis.

111.21

EFFECTS OF THE HALLUCINOGEN, DOI, UPON REGIONAL BRAIN ACTIVITIES OF RATS. B.E. Morton and F.M. Fujimoto*, Univ. of Hawaii School of Medicine Honolulu, HI 96822

Determining the mechanism of classic hallucinogens, such as LSD, has been difficult because they have affinities for several neurotransmitter sites. Recently, hallucinogens, such as DOB and DOI have been synthesized which have high affinity for only one neurotransmitter receptor, the 5HT2-5HT1c class, appearing to act as agonists, although there is conflict regarding this point.

In an attempt to determine the site of action of the potent hallucinogen, (+/-)-1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane HCl (DOI), we have used 2-deoxyglucose-1-14C (2DG) to measure its effects upon regional brain activities in rats. Ten min. after rats were injected, i.p., with 0.3 or 3.0 mg/kg DOI, they were injected with 125 uCi/kg 2DG, i.p. Both DOI concentrations caused back twitches. After 45 minutes, brains were removed and frozen. Autoradiograms of 20 um sections were prepared and film densities were quantitated at 30 brain sites.

As reported for LSD and 5OH-O-Methyl DMT, DOI (0.3 mg/kg), caused a marked generalized reduction of brain glucose uptake, which was more pronounced at 3.0 mg/kg. Densitometric analysis of regional activity ratios is underway. Assuming the reductions of regional glucose uptake prove to be as globally uniform for DOI as for classic hallucinogens, one conclusion is that it is a common element in hallucinosis. The brain site causing this overall depression remains to be identified.

112.1

THE HYPOTHERMIC AND ATAXIC EFFECTS OF ETHANOL: EVIDENCE FOR MODULATION BY CENTRAL ALPHA$_2$-ADRENOCEPTORS.

M.J. Durcan, R.G. Lister and M. Linnoila. Laboratory of Clinical Studies, NIAAA, Blg10 3C102, 9000 Rockville Pike, Bethesda, MD 20892, USA.

The hypothermic and ataxic effects of ethanol can be attenuated by alpha$_2$-adrenoceptor antagonists in mice. The present study examined whether these effects are mediated by central or peripheral alpha$_2$-adrenoceptors. The administration of 1-10 mg/kg L 659,066, a selective alpha$_2$-adrenoceptor antagonist which poorly penetrates the brain, had no effect on the hypothermia induced by a 2 g/kg dose of ethanol or the ataxia induced by a 2.4 g/kg dose of ethanol. Whereas the centrally active antagonist atipamezole (1 mg/kg) significantly attenuated both ethanol-induced hypothermia and ataxia.

Many alpha$_2$-adrenoceptor ligands have affinity for an imidazoline binding site in addition to the alpha$_2$-adrenoceptor itself. In order to investigate the role the imidazoline binding site might play in attenuating ethanol's hypothermic and ataxic effects RX 821002, an alpha$_2$-adrenoceptor with low affinity for the imidazoline binding site was compared with atipamezole. Both atipamezole (1 mg/kg) and RX 821002 (0.06-0.2 mg/kg) significantly attenuated the hypothermic and ataxic effects of ethanol. These results suggest that central alpha$_2$-adrenoceptors and not the imidazoline binding site are implicated in the ethanol attenuating properties of alpha$_2$-adrenoceptor antagonists.

112.2

IN VIVO MEASUREMENTS OF DOPAMINERGIC ACTIVATION IN BRAINS OF ALCOHOL PREFERING (P) AND NON-PREFERING (NP) RATS FOLLOWING ORAL SELF ADMINISTRATION OF ETHANOL. C.A. Cohen, E. Vavrousek-Jakuba, W.J. Shoemaker. Alcohol Res. Ctr, U. of Conn. Health Center, Farmington, CT 06032.

Dopaminergic activation has been shown to play a role in response of the organism to various drugs of abuse, including alcohol. In the present investigation we assessed in vivo dopamine D2 receptor binding in the nucleus accumbens (NA) and caudate-putamen (CP) of P and NP rats that have been trained to drink alcohol during a single daily 30 min. session. The experiment was divided into two phases. Phase one compared animals that received either water or a 10% ethanol solution. In order to increase the amount of alcohol consumed, a solution of 10% sucrose and 20% alcohol was presented to the animals receiving alcohol during phase two. To assess dopaminergic activation after presentation and consumption of the experimental drink, 3H-raclopride was injected via the tail vein 10 minutes after the presentation of the drink. Rats were sacrificed 35 min later and receptor binding determined by liquid scintillation counting of tissue digests of the aforementioned brain regions. Binding in the cerebellum was used as a measure of nonspecific binding.

A significant decrease in binding (reflecting a release and binding of the endogenous transmitter in response to the presentation and/or consumption of the ethanol drink) was observed in the NP rats when both phases were combined (NA: -23%; CP: -35%, $p<0.05$), but not in either phase in the P rat. While these data suggest that the NP rats were more responsive to the daily presentation of the ethanol drink (regardless of the presence or absence of sucrose), a 15% decrease in nonspecific binding found in the ethanol receiving NP rats may be a potential confound to this observation. To help clarify this issue, in vivo microdialysis experiments are currently underway.

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112.3

INHIBITION OF GABA-STIMULATED $^{36}Cl^-$ FLUX BY PICROTOXIN AND DMCM IS NOT ALTERED IN CHRONIC ETHANOL RATS. D.W.Sapp, N.Kokka and R.W.Olsen. Dept. of Pharmacology, UCLA, Los Angeles, CA 90024.

Chronic exposure to ethanol increases the binding of benzodiazepine inverse agonists in cultured spinal cord neurons (Mhatre and Ticku, JPET,251:164, 1989). We examined whether the inhibition of GABA-stimulated $^{36}Cl^-$ flux by inverse agonists was also increased in the brains of rats chronically exposed to ethanol. In our model rats were intubated with 6 g/kg ethanol on an alternate day schedule for 3 months. GABA-stimulated $^{36}Cl^-$ flux was measured in brain microsacs of both control and chronic-ethanol rats. GABA (100 μM)-stimulated flux was inhibited with the beta-carboline inverse agonist DMCM or the antagonist picrotoxin, both at concentrations of 0.1-100 μM. The inhibitory concentration-dependence curves were similar in control and chronic ethanol rats, with IC50 of 1.0 μM for DMCM and 5.0 μM for picrotoxin. GABA stimulated $^{36}Cl^-$ flux by itself was also unchanged in the ethanol rats. These results indicate that sensitivity to picrotoxin or benzodiazepine inverse agonists was not increased in chronic ethanol rats when measured by Cl^- flux. Supported by Grant AA07680.

112.4

ETHANOL STIMULATES [^{3}H]5-HT ACCUMULATION BY RAT FOREBRAIN SYNAPTOSOMES: ROLE OF 5-HT RECEPTORS AND VOLTAGE CHANNEL BLOCKERS.

T. Alexi and E.C. Azmitia, Dept. of Biology, New York University.

Serotonin (5-HT) has long been associated with alcohol consumption. We proposed that ethanol might have specific effects on serotonin accumulation in vitro.

We report that 54 mM ethanol enhanced the accumulation of [^{3}H]5-HT by rat forebrain synaptosomes by 15-54% when applied acutely for 5-10 minutes at 37C. Increasing intracellular Ca^{++} levels ($[Ca^{++}]_i$) with ryanodine and the 5-HT$_2$ receptor agonist, DOI, also stimulated 5-HT accumulation. In combination with etOH, these substances did not supplement the etOH-induced accumulation of 5-HT. The 5-HT$_3$ antagonist, ICS 205930, as well as lidocaine and tetrodotoxin (TTX), which all block voltage-dependent Na^+ channels, were shown to have no effect on the accumulation of 5-HT. Tetraethylammonium (TEA), which blocks voltage-dependent K^+ channels, also did not alter 5-HT accumulation. ICS 205930 and TEA did, however, block ethanol's action on accumulation. These results indicate that etOH's stimulation of 5-HT accumulation is due to increased $[Ca^{++}]_i$ and not inhibition of voltage Na^+ and K^+ fluxes, although it is sensitive to these Na^+ and K^+ fluxes.

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112.5

5-HT3 BUT NOT 5-HT2 RECEPTORS IN THE NUCLEUS ACCUMBENS MAY MEDIATE ALCOHOL DRINKING IN ALCOHOL-PREFERRING (P) RATS. **A.D. Levy, J.M. Murphy, W.J. McBride, L. Lumeng*, & T.-K. Li*. Psychiatric Res. & Regenstrief Insts., Indiana Univ. Sch. Med., VAMC, & Psychology Dept., Purdue Sch. Science, Indianapolis, IN 46202.**

The serotonin (5-HT) system in the nucleus accumbens (ACC) has been implicated in ethanol (EtOH) drinking of P rats. To determine if 5-HT2 and/or 5-HT3 receptors in the ACC might be involved in regulating oral EtOH intake, adult female P rats (N=13-19/group) received bilateral microinjections into the ACC of ICS205-930 (5-HT3 antagonist; 0.1-0.5 ug/side), DOI (5-HT2 agonist; 0.1-1.0 ug), ketanserin (5-HT2 antagonist; 0.05-0.5 ug) or vehicle prior to scheduled 1-hr access to 10% EtOH. Food and water were always available. EtOH intake averaged 1.9 g/kg following vehicle injections. ICS205-930 (0.25 ug) increased EtOH drinking by 28% ($p<.05$), while the 0.5 ug dose did not alter EtOH intake, but reduced general motor activity. DOI (1 ug) reduced EtOH intake by 50% ($p<.05$), but also disrupted ongoing activity. Lower doses did not alter drinking. Ketanserin did not alter EtOH consumption at any dose tested. The findings implicate ACC 5-HT3 but not 5-HT2 receptors in EtOH drinking of the P rat.

(AA03243, AA07611, & AA07462)

112.6

QUANTITATIVE AUTORADIOGRAPHIC ANALYSIS OF BRAIN DOPAMINE RECEPTORS IN ETHANOL-PREFERRING RATS. S. Sigala*, C. Missale, P. Rizzonelli*, E. Zanelli*, M. Memo, P.F. Spano. Inst of Pharmacol Exp Ther, Sch of Med, Univ of Brescia, Italy.

Activation of brain dopamine (DA) neurons is considered to mediate the reinforcing properties of drugs of abuse, including ethanol. An ethanol-preferring line of rats was obtained through selective breeding (20th progeny). This progeny of alcohol-preferring rats, which never consumed ethanol during their life-time, were killed at the age of six months together with water-preferring rats from the same progeny. The distribution and the density of brain DA receptors was evaluated in binding studies on brain slices followed by quantitative autoradiography. 3H-SCH 23390 was used to label D-1 receptors and 3H-spiroperidol to study D-2 receptors. The density of D-1 receptors was similar in the two rat populations. In contrast, the analysis of 3H-spiroperidol binding revealed a decreased density of D-2 receptors in the hippocampus, nucleus accumbens and amygdala of ethanol-preferring rats; no changes were found in striatal D-2 receptor density. The selective decrease of D-2 receptors in the areas associated with the rewarding mechanisms of ethanol-preferring rats could reflect an altered activation of DA neurons leading to compulsive alcohol consumption.

112.7

THE EFFECT OF GLYCINE ON THE ETHANOL INHIBITION OF N-METHYL-D-ASPARTATE INDUCED TRANSMITTER RELEASE IN THE CORTEX, HIPPOCAMPUS AND STRIATUM. L. M. Brown, R. A. Gonzales and S. W. Leslie, Institute for Neuroscience, University of Texas, Austin, Texas 78712.

The ability of glycine to reverse the inhibitory effects of ethanol on N-methyl-D-aspartate (NMDA) stimulated catecholamine release was examined. Brain slices from the rat cortex and hippocampus were prepared and labelled with ^{3}H-norepinephrine and the striatum was labelled with ^{3}H-dopamine. The slices were washed extensively with Mg^{2+} free Krebs-Ringers bicarbonate buffer and the release of ^{3}H transmitter examined. NMDA induced a concentration-dependent increase in ^{3}H-transmitter release. The maximal responses (NMDA 1000μM) were 5.42±0.27, 7.72±0.18 and 1.61±0.20 percent fractional tritium released from the cortex, hippocampus, and striatum, respectively. The maximal responses were proportional to the number of NMDA receptors reported for each brain region. Ethanol (30-60mM) caused a concentration-dependent inhibition of NMDA-stimulated transmitter release. Ethanol (30mM) inhibited release by 10-12% and at 60mM inhibited release by 17-22% in all brain regions. Glycine, an NMDA coagonist, caused a concentration-dependent increase in ^{3}H-catecholamine fractional release in the slice preparation. Glycine (3μM) enhanced release by 17-21% in the cortex and hippocampus and by 47% in the striatum. The ethanol-induced inhibition of NMDA-stimulated release was reversed by glycine in all brain regions. However, no apparent statistical interaction of ethanol and glycine was observed. These results indicate that ethanol was a potent inhibitor of NMDA-stimulated neurotransmitter release in several brain regions. The glycine reversal of ethanol was confounded by the enhancing properties of glycine on NMDA-stimulated release of transmitter. (Supported by NIAAA grants, AA07297, AA05809, AA08104, RSDA AA00044 to S.W.L., L.M.B. supported by training grant AA07471).

112.8

EFFECTS OF CHRONIC ETHANOL ADMINISTRATION ON MUSCARINIC ACETYLCHOLINE RECEPTORS ASSESSED BY QUANTITATIVE LIGAND AUTORADIOGRAPHY AND IN SITU HYBRIDIZATION HISTOCHEMISTRY IN LONG- AND SHORT-SLEEP MICE. S. Vincent, L. Tsiokas, S.C. Zhang*, S.P. Aiken, J.J. McArdle and M. Watson. U. of Med. and Dent. of N.J.- N.J. Med. Sch., Newark, N.J. 07103

Ethanol (ET) alters muscarinic function and differences in muscarine acetylcholine receptors (mAChR) have been reported in long- and short-sleep (LS/SS) mice that are differentially sensitive to hypnotic effects of ET. Study of a specific nonsubtype selective antagonist [^{3}H](-)quinuclidinylbenzilate, an M1 selective antagonist [^{3}H]pirenzepine, a partial M2 selective antagonist [^{3}H]AF-DX 116, an agonist [^{3}H](+)cis-methyldioxolane (that selectively labels a super high affinity agonist state), and a blocker of sodium-dependent high affinity choline uptake [^{3}H]hemicholinium-3, were done as previously described. In situ hybridization histochemistry (ISSH) was done with oligonucleotide probe complementary to 4-48 or 4-51 base sequence of m1-m5 mAChR mRNA by 3'-end labeling by ^{35}S-dATP (specific activity>2.4x10^{9}dpm/ug) by terminal deoxynucleotidyl transferase. Slices were hybridized (25°C;36h), washed (T_m=55°C;2xSSC), air-dried and apposed to Hyperfilm-βmax (4wk;0-4°C). Autoradiograms were quantified by DUMAS image analysis. ET-induced sleep time (LS;4g/kg,ip=131m v SS;5g/kg=78m & 3g/kg=0m) varied. By 7d ET (3.8%v/v;Liquidiet) Rx ~50% tolerance was seen in LS and SS by respective 4 or 5g challenge dose. Low SS m3 mRNA and 14d ET Rx data on LS/SS mice may relate to differential ET sensitivity. MH-43024.

112.9

RESPONSES OF CEREBELLAR GRANULE CELLS SHOW DIFFERENTIAL SENSITIVITY TO ETHANOL. G.LIU*, R.HUANG* And C.HUANG. Div. Structural & Systems Biol., Sch. Basic Life Sci., Univ. Missouri-Kansas City, Kansas City, MO 64110.

The cerebellum is very sensitive to acute intoxication of ethanol (ETOH). ETOH increases dramatically the discharge rate of climbing fibers, however, its effect on the mossy fiber system has not been fully elucidated. We have recorded granule cells (GC) from the cerebellum of cats under low (0.3g/Kg, IV) and high (1.2g/Kg, IV) dosage of ETOH. ETOH severely inhibited GC's responses to auditory and visual stimuli. Complete or maximal inhibition occurred in 5-8 min. Recovery followed a near exponential time course, reaching a plateau in 60-90 min. ETOH had a more severe inhibition on long-latency responses (>40 ms) than short-latency ones (<25 ms). Within a given animal, patches of GC's with long latencies were more resistant to ETOH than patches with shorter latencies. Acute tolerance to ETOH caused marked reduction and a faster recovery in the inhibitory effect of a second injection if the second injection was given within 4 hrs of the first one. Such acute tolerance was clearly seen in short-latency responses. (Supported by PHS grant AA07643.)

112.10

CHRONIC EXPOSURE OF CULTURED SEROTONERGIC NEURONS TO ETHANOL INCREASES SEROTONIN UPTAKE. D. K. Lokhorst and M. J. Druse The Neuroscience Program, Loyola U. of Chicago, Stritch Sch. of Medicine, Maywood, IL. 60153.

In order to determine how ethanol influences the development of serotonergic neurons, we cultured rhombencephalic neurons which were obtained from rats on the 15th embryonic day. After 2 days, ethanol was added to the culture media at a concentration of 50, 150 or 300 mg/dl. Following 4 days in the presence of ethanol, the uptake of [^{3}H]-serotonin (5-HT) was determined in cultured cells. The effect of acute ethanol exposure on [^{3}H]-5-HT uptake was also determined. In the latter studies [^{3}H]-5-HT uptake was measured in ethanol-naive cells, exposed to ethanol only during the uptake experiment.

The results of these experiments demonstrate that the presence of 50, 100 and 150 mg/dl ethanol in the culture media for 4 days significantly increased [^{3}H]-5-HT uptake in developing rhombencephalic cells. The stimulation in [^{3}H]-5-HT uptake occurred in a dose-dependent manner. In contrast, acute exposure of cultured rhombencephalic cells to 50, 100, or 150 mg/dl of ethanol had no effect on [^{3}H]-5-HT uptake. Thus, it appears that exposure of embryonic rhombencephalic cells to ethanol for 4 days significantly alters the development of 5-HT containing neurons.

112.11

LITHIUM-INDUCED INOSITOL MONOPHOSPHATE ACCUMULATION AS SENSITIVE MARKER FOR ACUTE AND CHRONIC ETHANOL IN BRAIN. Navidi M.*, Lin T-N.*, Sun G.Y. Dept. Biochem., Univ. Missouri, Columbia, MO, 65203.

It has been well demonstrated by Sherman (1985) that systemic injection of lithium results in a large increase in inositol monophosphates (IP), namely Ins(1)P and Ins(4)P. In this study, a radiotracer technique together with lithium treatment was used to assess acute and chronic effects of ethanol on poly-phosphoinositide (PI) turnover in brain. Typically, inositol metabolites in brains of control and ethanol treated C57/Bl mice were labeled with ^{3}H-inositol for 16 hr. This was followed by injection i.p. of lithium (6 meq/kg) 4 hr prior to analysis. In some experiments, isomers of inositol phosphates were quantitatively analyzed by ion chromatography. Using this experimental protocol, the lithium-induced accumulation of labeled IP was suppressed in mice given an intoxicating dose of ethanol (6 gm/kg) via intragastric intubation. On the other hand, mice that were administered ethanol chronically and subsequently lithium 4 hr before analysis showed a higher level of labeled IP as compared to the pair-fed controls. Analysis of the inositol phosphates in lithium-treated ethanol and control brain samples by ion chromatography indicated large increases in Ins(1)P, Ins(4)P and Ins(1,4)P_2 with respect to lithium treatment and furthermore, with respect to chronic ethanol treatment. Results thus revealed a sensitive procedure for assessing the effects of ethanol in brain.

112.12

CHRONIC ETHANOL EFFECTS ON PROTEIN KINASE C IN MOUSE BRAIN CYTOSOL AND MICROSOMES. P.M. Wixom and G.Y. Sun. Dept. of Biochem., Univ. Missouri, Columbia, MO 65203.

C57/Bl mice were maintained on an ad lib liquid diet of flavored Sustacal containing 5% ethanol for 8 weeks. A pair-fed control group was given an isocaloric amount of sucrose. After 12 hr withdrawal, the cerebrum was homogenized and centrifuged to isolate the cytosol and microsome fractions. The samples were partially purified on a DE-52 anion exchange column prior to phosphorylation with ^{32}P-ATP. Lipid activation was assessed by adding oleoylacetylglycerol (OAG, 3 μg/tube) and phosphatidylserine (PS, 4 μg/tube) to the incubation mixture. Using this procedure for assay of protein kinase C (PKC) activity on endogenous substrates, we observed that chronic ethanol group had specific and different effects on the lipid activated kinase activity in cytosol and in microsomes. In the cytosol fraction, the total phosphorylation was significantly less in the ethanol-treated mice as compared to pair-fed controls. This effect was observed regardless of the presence or absence of Ca^{2+}. Under this condition, OAG activation of kinase activity was unchanged but the PS enhanced kinase activation in cytosol was less in the ethanol group as compared to controls. On the other hand, ethanol administration did not alter the microsomal PKC activity. It is concluded that chronic ethanol administration results in specific alterations on the activation of PKC activity in brain.

112.13

DIFFERENCES IN GABA-A RECEPTORS STUDIED BY EXPRESSION OF LS/SS MOUSE BRAIN MESSENGER RNA IN XENOPUS OOCYTES. D.M. Burnett, T.V. Dunwiddie, J.M. Sikela, and R.A. Harris. Dept. of Pharmacology, Univ. of Colorado Hlth. Sci. Cent. and V.A. Med. Cent., Denver, Co 80262.

Alterations in the functioning of the GABA-A receptor/ chloride channel complex have been implicated as a mechanism of action for ethanol (EtOH) and benzodiazepine intoxication. The genetically selected LS and SS lines of mice differ markedly in their sensitivity to EtOH. We used Xenopus oocytes to express whole brain mRNA from LS and SS mice in order to study genetic differences in the sensitivity of the GABA-A receptors to modulation by EtOH, diazepam and pentobarbital. Oocytes were injected with 100 ng of either LS, SS, or a 50/50 mixture of LS and SS whole brain mRNA. Following a 2-3 day incubation, GABA-stimulated chloride currents were recorded under voltage-clamp conditions (-90 mV). Drugs were applied by perfusion. Application of GABA (10-100 uM) elicited inward currents of similar magnitude in LS-, SS- and LS/SS-expressing oocytes. Pentobarbital (100 uM) potentiated GABA-stimulated currents to the same extent (500-600% of control) across oocyte groups, as did diazepam at 10 nM (140%) and 100 nM (250%). With EtOH, however, genetic differences were seen. In LS oocytes, EtOH (20 mM) potentiated GABA-stimulated currents to 230% of control, whereas it attenuated them to 50% of control in SS oocytes. In oocytes expressing a 50/50 mixture of LS and SS mRNA, the effect of EtOH was not a summation of the opposing phenomena; rather, LS/SS oocytes responded in SS fashion (50% inhibition). These results suggest that protein(s) translated from SS mRNA alters the effects of EtOH on GABA-A receptors and that this protein can convert LS GABA-A receptors to "SS-like" when co-expressed. Supported by the VA and Grant AA06399.

112.14

ANTAGONISM OF ETHANOL ACTIONS BY $GABA_B$ ANTAGONISTS. S.J. McQuilkin*, D.M. Burnett, A.M. Allan, R.A. Harris, Denver VAMC, Denver, CO 80222; Dept. Pharmacol., UCHSC, Denver, CO 80262; Washington Univ. Med. Sch., St. Louis, MO 63110.

The ability of the $GABA_B$ receptor antagonist, phaclofen, to block some behavioral effects of ethanol (motor activity, hypothermia), has been shown (Life Sci. 45:1771,1989). Saclofen and phaclofen were tested to see whether these $GABA_B$ antagonists could block the effects of ethanol on the $GABA_A$ receptor/chloride channel complex in brain membranes and Xenopus oocytes expressing whole brain mRNA. GABA-dependent $^{36}Cl^-$ uptake measurements were performed as previously described (Life Sci. 46:527,1990) using ICR mouse cortical microsacs. Long-sleep mouse whole brain mRNA was injected into defolliculated oocytes and GABA responses were measured using 2 electrode voltage clamp conditions. In microsacs, saclofen (100μM) and phaclofen (100μM), significantly blocked ethanol (15mM) augmentation of GABA(5μM)-dependent chloride flux, without having any effect on the GABA response alone. In oocytes expressing brain mRNA, ethanol (20mM) stimulated GABA(10μM)-mediated currents 5 fold. This effect of ethanol was reduced by 100μM saclofen. Although saclofen and phaclofen completely antagonized the ethanol effects in microsacs, the same concentration of saclofen (100μM), antagonized ethanol by only 50% using oocytes. Saclofen did not antagonize the enhancement of GABA actions on oocytes produced by diazepam or pentobarbital. These studies suggest that activation of $GABA_B$ receptors is necessary for ethanol potentiation of chloride conductance through $GABA_A$ operated channels. The mechanism responsible for this interaction remains to be defined. Supported by the VA and grant AA06399.

112.15

CHRONIC ETHANOL EXPOSURE OF XENOPUS OOCYTES EXPRESSING MOUSE BRAIN mRNA REDUCES GABA RECEPTOR-ACTIVATED CURRENT AND BENZODIAZEPINE RECEPTOR LIGAND MODULATION. K.J. Buck and R.A. Harris. Dept. of Pharmacology, UCHSC, Denver, CO, 80262; Veterans Admin. Med. Res. Serv., Denver, CO, 80222.

Xenopus oocytes microinjected with exogenous total messenger RNA isolated from mouse cerebral cortex express a variety of neurotransmitter-activated channels. We found that exposure of Xenopus oocytes to ethanol (50 mM) for 2 to 4 days during expression of mouse brain mRNA reduced the amplitude of current activated by the $GABA_a$ receptor-selective agonist, muscimol (100 uM). Muscimol-activated currents were reduced from 164 $\pm$ 21 nA (control) to 61 $\pm$ 10 nA after ethanol exposure. Allosteric modulation of muscimol-activated current by flunitrazepam (benzodiazepine agonist) and DMCM (benzodiazepine inverse agonist) was also reduced in ethanol treated oocytes. Flunitrazepam (1 uM) enhanced muscimol-activated current by 195 $\pm$ 23% vs. 95 $\pm$ 24% in control and ethanol exposed oocytes, respectively ($p < 0.01$); DMCM (100 nM) reduced muscimol-activated current by 61 $\pm$ 5% vs. 30 $\pm$ 8%, respectively ($p < 0.01$). Currents activated by kainate, NMDA, acetylcholine, serotonin and glycine were not significantly reduced by ethanol exposure. These results indicate that ethanol exposure may selectively reduce expression of the GABA/benzodiazepine/ chloride channel complex. Supported by the VA and grant AA06399.

112.16

ETHANOL REDUCES RESPONSES TO DENDRITICALLY APPLIED NMDA IN CA1 PYRAMIDAL NEURONS IN RAT HIPPOCAMPAL BRAIN SLICES. W.R. Proctor and T.V. Dunwiddie, Veterans Administration Medical Services and University of Colorado Health Science Center, Denver, CO 80262

Ethanol has a biphasic excitatory/inhibitory effect upon the amplitude of the population spike response recorded in the CA1 pyramidal cell layer of hippocampal brain slices. The mechanism(s) underlying this effect are unclear: intracellular recordings from CA1 pyramidal neurons show minimal or no changes in passive membrane characteristics during superfusion with 50-100 mM ethanol. Ethanol also had no significant effect on EPSPs or GABAergic IPSPs. Interneurons were similarly unaffected by ethanol superfusion. Since inhibition of NMDA receptor function has recently been implicated in the depressant effects of ethanol on extracellularly recorded responses (Lovinger et al. J. Neurosci. 10:1372, 1990), we studied the effects of superfused ethanol on responses to local pressure application of NMDA (5-10 msec, 30-60 psi) onto pyramidal cell dendrites. In medium containing 0-0.1 mM Mg^{++}, the amplitude and duration of the depolarizing response to pressure ejection of NMDA in stratum radiatum were reduced by 80 mM ethanol (28$\pm$7% and 24$\pm$11%, respectively). When the Mg^{++} concentration was raised (1.3 mM), the NMDA response was smaller, but was still depressed by ethanol. These results suggest that synaptically evoked EPSPs and IPSPs are not affected by ethanol, but that responses mediated by NMDA receptors are reduced in this preparation.
Supported by AA03527 & VA Med. Res. Service.

112.17

ARE ETHANOL-INDUCED DEPRESSIONS OF NEURONAL ACTIVITY MEDITATED BY A $GABA_a$ MECHANISM OF ACTION? Ronald K. Freund, Maan Yuh Lin*, Craig G. van Horne, J. Timothy Harlan*, and Michael R. Palmer, Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262

Biochemical and behavioral studies indicate that ethanol (ETOH) will facilitate the activation of the $GABA_a$ Cl^- channel. However, in the present study, we found that ETOH, when locally-applied by pressure ejection from micropipettes, caused only modest and infrequent potentiations of the depressant effects of locally-applied GABA on cerebellar Purkinje neurons and that local ethanol applications required very specific conditions in order to observe this effect. Longer, low-dose applications of ethanol, which caused small depressions of neuronal activity, appeared to antagonize rather than to augment the GABA-induced depressions. Even so, bicuculline, a $GABA_a$ receptor antagonist, reversibly blocked the depressant effects of pressure-ejected ETOH when locally-applied from the same micropipette, and this antagonism appears competitive with our techniques. These data might suggest that GABA mechanisms play a permissive role in the expression of ETOH-induced depressions of single-neuron firing rate in the cerebellum. In apparent contrast to this hypothesis, a preliminary investigation indicates that local applications of picrotoxin, an agent which blocks the chloride channel response to $GABA_a$ activation, did not consistently antagonize ETOH-induced depressions on cerebellar Purkinje neurons. We are currently investigating the depressant vs more subtle effects of ETOH which may alter GABA mechanisms. (Supported by USPHS grants AA 05915 and AA 00102. JTH is currently in the Department of Pharmacology, Univ. of Arizona Col. Med., Tucson, AZ. MRP is supported by an ADAMHA Research Scientist Development Award.)

112.18

LOW CONCENTRATIONS OF ETHANOL INHIBIT LONG-TERM POTENTIATION IN RAT HIPPOCAMPUS. R.D. Blitzer, O. Gil and E.M. Landau. Psychiatry Svce., Bronx VAMC, Bronx NY 10468

In humans, ethanol (EtOH) impairs memory at concentrations associated with mild intoxication. A possible mechanism for this effect is the suppression of long-term potentiation (LTP) by EtOH. EtOH blocks responses mediated by NMDA-type glutamate receptors, and intact NMDA receptors are required for LTP in various brain regions. We tested the effects of EtOH (5-100 mM) on LTP in the Schaffer collateral - CA1 pathway using extracellular techniques. EtOH reduced LTP with an IC_{50} of 13 mM, and even 5 mM EtOH had a significant depressant effect. In voltage-clamped CA1 neurons, EtOH blocked NMDA-induced currents with a similar potency (IC_{50} = 23 mM). EtOH was also found to block a component of the tetanic field potential which is sensitive to the NMDA antagonist APV. The inhibition of LTP by EtOH was intact in picrotoxin, indicating that the effect does not depend upon GABAergic transmission. The results indicate that EtOH inhibits LTP at concentrations in the low clinical range, probably by interfering with NMDA receptor-mediated processes. Supported by NIAA Grant AA06659 and the VA Merit Program.

112.19

ANTAGONISM OF 5-HT3 RECEPTORS ATTENUATES THE EFFECTS OF ETHANOL ON DOPAMINE. K.M. Wozniak, A. Pert and M. Linnoila. LCS/NIAAA and BPB/NIMH Bethesda, MD 20892.

Serotonin has been implicated as a modulator of dopaminergic neurotransmission (Cheeselet, 1984). Recently, the identification of 5-HT3 binding sites in brain (Kilpatrick et al. 1987) has prompted a number of interesting studies, the results of which suggest a role for this receptor subtype in modulating dopamine (DA) release (Blandina et al.1988). 5-HT is thought to have a role in alcoholism and the motor effects of ethanol (Rockman et al. 1982,Liljequist et al.1981). Interestingly, Grant et al.(1990) have recently reported that 5-HT3 antagonists attenuate the discriminative properties of ethanol. Moreover, morphine and nicotine induced place preference has been attenuated by 5-HT3 antagonists (Carboni et al. 1988) as has morphine-induced dopamine increase (Imperato and Angelucci 1989). Ethanol has been shown to increase extracellular DA (Di Chiara and Imperato 1988,Wozniak et al.in press). We therefore chose to investigate the effect of pretreatment with 5-HT3 antagonists on this ethanol-induced DA increase. Animals were anesthetized and placed into a stereotaxic frame. A microdialysis probe was then positioned in the striatum or accumbens. Following the attainment of stable DA values (2-3 h), ICS 205-930 (500 µg/kg) or saline was administered s.c. After 1 h, a 3% v/v solution of ethanol was perfused for 20 mins through the probe. Pretreatment with ICS attenuated the subsequent ethanol-induced DA response when compared to saline treated animals. ICS alone had little or no effect on DA.These data provide evidence that 5-HT may be involved in the effects of ethanol on DA. Furthermore, they suggest a possible application for the use of 5-HT3 antagonists in alcoholism and substance abuse.

112.20

EFFECT OF CHRONIC ETHANOL CONSUMPTION ON SYNAPSES OF THE DENTATE FASCIA. P. Schaner*, E. Fifkova, P. McMechen*, J. Markham, T. McIntyre. Department of Psychology, University of Colorado, Boulder, CO 80309.

Long- and Short-Sleep mice (LS_{IBG}; SS_{IBG}) were divided in groups and fed for 4 months with either a control isocaloric liquid diet or with isocaloric liquid diet containing ethanol . They were withdrawn from this diet for 1 month. Subsequently, the mice were prepared for electron microscopy. The GABA terminals were labeled with antibodies against GABA using a gold probe in a postembedding protocol. Two types of synapses which are essential for transmission through the hippocampus were studied: the cross sectional area of dendritic spines in the dentate molecular layer (DML) and the number of GABAergic synapses on dentate granule cell bodies.

The population of dendritic spines in the DML was divided into groups of simple and concave spines, and the average cross sectional area was computed for each group separately. The cross sectional area of concave dendritic spines in the distal third of the DML, projection zone of the perforant path (PP), of alcohol treated mice was significantly smaller (by 21%) than in controls. This change could be caused by ethanol effect on the actin based cytoskeleton of dendritic spines. This possibility is supported by the observation in cultured neurons that a low concentration of ethanol disrupts actin filaments and causes cells to shrink (Hassler and Moran, Experientia, 42:575, 1986). Shrinkage of dendritic spines could adversely affect synaptic transmission in these spines in the DML. Given that concave spines represent 25% of the entire spine population in the DML and given that synapses in the projection zone of PP represent the first synapse in the trisynaptic hippocampal circuitry, this change, resulting from alcohol consumption, could seriously affect the flow of information into the hippocampus.

The density of GABAergic terminals was not affected by ethanol exposure. However, comparison between the LS and SS controls revealed significantly fewer (by 23%) GABAergic contacts on dentate granule cell bodies in the SS line. This observation is in line with the suggestion that the LS and SS lines differ not only in their sensitivity to ethanol but also in their respective GABAergic systems. Supported by NIAAA #AA06196.

112.21

CHRONIC ETHANOL TREATMENT DIFFERENTIALLY EFFECTS AFFERENT RESPONSE PROPERTIES IN RAT HIPPOCAMPUS. B.S. Rothberg, C.T. Smothers and B.E. Hunter. Dept. of Neuroscience, University of Florida, Gainesville, FL 32610.

We compared afferent fiber response properties in stratum radiatum(SR) and stratum oriens(SO) of the rat hippocampus after chronic ethanol treatment(CET). Ethanol-treated(Group E) and sucrose control(Group S) groups were fed a liquid diet containing ethanol or sucrose for 20 weeks. An 8 week abstinence period was introduced prior to experimentation. Slices from the dorsal and ventral hippocampus were maintained in a slice chamber. Stimulating electrodes were placed in either SR or SO and a recording electrode was placed in the synaptic layer(afferent volley[AV]; dendritic EPSP) or in the pyramidal cell layer(population spike[PS]) of CA1. Responses to afferent stimulation were studied by systematically varying electrode distance at 100, 250 and 500 microns. CET increased the size of the AV with stimulation of SR but not SO. Plotting the EPSP as a function of AV showed that CET significantly reduced synaptic transmission in a regionally specific manner confined to the ventral hippocampus. Finally, CET increased PS amplitude but this was also dependent upon source of afferents. These results suggest that CET produces regionally specific alterations in afferent volley magnitude and in synaptic response strength which varies as a function of the afferent source and position in the hippocampus. The results demonstrate a selectivity in the toxic actions of ethanol and emphasize the potential differences in the adaptive properties of the hippocampal formation.
(Supported by the Veterans Administration and NIAAA #AA00200)

LEARNING AND MEMORY: PHYSIOLOGY I

113.1

OPPOSITE ACTION OF DOPAMINE AND GLUTAMATE IN OPERANT CONDITIONING OF HIPPOCAMPAL CA1 CELLS. X. B. Gang* and L. Stein. Department of Pharmacology, UCI School of Medicine, Irvine, CA 92717.

Previous work indicates that hippocampal CA1 bursting activity may be reinforced by local micropressure application of dopamine (1mM) or cocaine (0.5mM) (Neurosci. & Biobehav. Rev. 13:69-80, 1989). However, there is concern that these agents may act merely by direct or indirect pharmacological stimulation of bursting. One approach is to attempt to reinforce hippocampal bursting with a relatively nonspecific depolarizing agent, such as glutamate. Unlike dopamine and cocaine, burst-contingent applications of glutamate (0.1 and 0.2mM) did not produce selective facilitation of cellular bursting when compared to random presentations; indeed, both contingent and random glutamate applications reduced the likelihood of bursts, while at the same time greatly increasing the frequency of individual spikes. In a second approach, excellent reinforcement was demonstrated with the highly specific dopamine D2 receptor agonist N-0923 (3 and 6mM), but not with its much lower affinity optical isomer N-0924.

These results are consistent with the idea that dopamine's reinforcing action on hippocampal bursting cannot be attributed to nonspecific stimulation and is exerted at dopamine D2 receptors.

(Supported by AFOSR 89-0213 and NIDA 05107).

113.2

DISSOCIATION OF BEHAVIORAL STATE - DEPENDENT SYNAPTIC GATING AND EXPLORATION RELATED SYNAPTIC EFFICACY CHANGES IN RAT FASCIA DENTATA. E. J. Green. Department of Psychology, University of Miami, Coral Gables, FL 33124.

Environmental exploration is associated with a number of moderately persistent alterations in perforant path - evoked population responses, including a large increase in evoked EPSP slope, and a substantial reduction in both the onset latency and area of the population spike. These changes have a *gradual* onset, and decay relatively slowly following exploration. The magnitude of perforant path evoked responses also vary as a function of the animal's behavioral state at the time of the stimulus - a phenomenon originally described by Winson and colleagues. The present experiments were designed to evaluate possible interactions between these two types of synaptic efficacy change.

Baseline evoked responses, hippocampal EEG, and EMG from the dorsal neck muscles were recorded for 3 - 12 hours from rats in a soundproof chamber, which served as the animals home cage. Following the baseline period, animals were allowed to explore an open field containing junk objects for 20 minutes, and were then returned to their home cage for an additional 3 - 12 hours of recording. Baseline responses fluctuated in accordance with the animal's behavioral state at the time of the stimulus. Exploration resulted in gradual, though substantial (30 - 50%), increases in evoked epsp slope and decreases in the onset latency and area of the evoked population spike. Epsp and spike values observed following exploration were larger and smaller, respectively, than any observed over the 3 -12 hour period preceeding exploration. During the post-exploration period in the home cage, the momentary alterations associated with sleep/waking states were *superimposed* upon the exploration related changes in evoked response values. This was particularly evident for the evoked spike, which remained relatively depressed for hours, but continued to vary in amplitude according to the sleep/wake cycle. The results of these experiments further dissociate the synaptic alterations associated with exploration from the gating of synaptic transmission observed during various sleep and attentional states.

113.3

EFFECT OF REVERSIBLE INACTIVATION OF THE HIPPOCAMPUS ON A DELAYED NONMATCHING TO SAMPLE TASK IN THE RAT: EVIDENCE FAVORING THE CONSOLIDATION OF WORKING MEMORY. Edwin J. Barea and Douglas C. Smith, Dept. Psychology. Southern Illinois University, Carbondale, IL 62901.

While evidence has been accumulating in favor of Olton's theory that the hippocampal system in the rat plays an important role in working memory (WM), the persistence of WM in a spatial memory task remains controversial. Indeed, Knowlton, McGowan, and Olton (1985) reported disruption of WM even after an 8 hr delay in rats tested in a DNMTS task in a radial arm maze following seizure stimulation of the hippocampi (HPC) and concluded that the time course for consolidation of WM is very long or that it never occurs.

We now report the within animal effects of reversible inactivation of the HPC via bilateral microinfusion of 2% lidocaine HCl administered at various delays in a DNMTS task in an 8 arm radial maze. Eleven rats were allowed to freely choose the first four arms of the maze and then removed from the apparatus for 3, 5, 10 or 20 min prior to choice 5. Once criterion level performance had been reached at each delay, Ss were tested with HPC inactivation beginning immediately (3 min delay), 2 min (5 min delay), 7 min (10 min delay), or 17 min (20 min delay) following the completion of choice 4.

Our results demonstrate a temporal gradient of disruption of WM. In choices 5-8 the mean number of errors was 4.00 (3 min delay), 3.27 (5 min), 2.18 (10 min) and 1.18 (20 min) indicating that the greater the amount of the time between the choices to be remembered and the disruption of the HPC, the better the memory is preserved. Such results are most consistent with the idea that WM consolidates over a much more rapid time course than has been previously been suggested. (Support in part by NIMH 1 T32 MH-18882 to E.J.B.)

113.4

HIPPOCAMPUS AND WORKING MEMORY: CONTINUOUS CONDITIONAL DISCRIMINATION AND T-MAZE ALTERNATION. R.Q.Wan, K.Pang and D.Olton, Dept. of Psychology, The Johns Hopkins University, Baltimore, MD. 21218.

Hippocampal function in working memory was tested in two tasks. (1) In the continuous conditional discrimination (CCD), rats were trained to press one lever when the current stimulus (a tone or light) was same as the previous one (match trial), and another lever when those two stimuli were different (nonmatch trial). Three delays, 2.5, 10 and 20 sec, were imposed between stimuli. (2) In the T-maze discrete trial alternation task, rats were trained to alternate choices between arms of a T-maze. After preoperative training in both tasks, rats were given either a hippocampal (HPC) lesion by radio frequency current or a control operation. In the CCD task, during the first few weeks, a severe impairment was found in HPC group. The size of impairment was dependent on the delays and the ratio of match to nonmatch trials. In the CCD task, performance in HPC group recovered to normal within 4 weeks. In the T-maze task, a severe impairment endured during all 7 weeks of postoperative testing. These data indicate that the hippocampus is involved in spatial and nonspatial working memory. The dissociation of performance in CCD and T-maze alternation tasks suggests that the hippocampus may have a different role in these two tasks.

113.5

A NEW ROLE OF INHIBITORY HILAR NEURONS IN FEEDBACK CIRCUITRY IN THE DENTATE GYRUS/HILUS/CA4/CA3 AREA IN THE GUINEA PIG HIPPOCAMPAL SLICE U. Misgeld and W. Müller, Dept. of Neurophysiology, Max-Planck-Institut für Psychatrie, Am Klopferspitz 18a, D-8033 Martinsried F.R.G.

In paired extra- and intracellular recording from the guinea pig hippocampal slice, picrotoxin (ptx) induced time-locked rhythmically ocurring bursts of repetitive population spikes in the CA3, CA4 and hilar region but no extracellularly recorded activity in the granule cell layer. 4-aminopyridine (4-AP) induced rhythmically occurring positive field potential waves in the CA3, CA4 and granular layer coincident to negative field potentials in the hilus. Under intracellular recording, ptx induced bursts in CA3, CA4 and hilar neurons, but only K-dependent slow IPSPs in granule cells. 4-AP induced rhythmically occurring bursts in hilar neurons or Cl- and K-dependent IPSPs in CA3, CA4 and granule cells. These events were time-locked to field potential activity in the CA3 layer. Ptx-induced activity originated in the CA3 area and subsequently appeared in the CA4 and hilar region, whereas 4-AP-induced activity appeared simultaneously in all subfields. Blockade of fast glutamatergic excitation by CNQX blocked the ptx-induced activity, but was ineffective on 4-AP-induced activity. Focal application of tetrodotoxin between CA3 and CA4 blocked ptx-induced activity in the CA4 and hilar region, but reduced 4-AP-induced activity in the CA3 area. This study reveals that hilar neurons are excited by CA3/CA4 pyramidal neurons in addition to the well known excitation by granule cells and perforant path fibers, and, in turn, hilar neurons inhibit CA3, CA4 and granule cells. Supported by the DFG, SFB 220-C4.

113.6

BEHAVIORAL CORRELATES OF HIPPOCAMPAL UNIT ACTIVITY IN AN ODOR-GUIDED DELAYED NONMATCH TO SAMPLE TASK. T. Otto, H. Eichenbaum, & C.G. Wible, Dept. Biological Science, Wellesley College, Wellesley, MA 02181

The delayed nonmatch to sample (DNM) task has been used extensively in studies identifying the neural pathways critical to visual memory in primates. Continuing efforts toward designing for rats tasks similar to those commonly used with primates have resulted in our development of an odor-guided continuous delayed nonmatch to sample (cDNM) task that is suited ideally to both behavioral and electrophysiological studies of memory in rats.

Successful performance in the cDNM task required the rat to remember across a variable intertrial interval the odor presented on the preceding trial, and respond for water reinforcement only if the odor presented on the current trial was different, i.e. a *nonmatch*. Individual odors were selected randomly from a finite set, and were presented equally often on match and nonmatch trials. Performance in this task decayed with increases in either delay (intertrial interval) or interference imposed by reducing the size of the odor set from which samples were chosen, demonstrating that performance in this task is sensitive to manipulations that increase memory demand.

Of 22 CA1 pyramidal cells recorded to date from rats performing cDNM, 4 exhibited differential responses during odor sampling on match versus nonmatch trials. Three cells increased in firing rate during correct but not incorrect responses. The remaining cells had no identifiable behavioral correlate. Additional unit data from this study-in-progress will be reported; these preliminary data suggest that hippocampal pyramidal cells exhibit striking nonspatial mnemonic correlates, and further that the hippocampus participates in the comparison among representations of previous and present experiences.

113.7

HIPPOCAMPAL PYRAMIDAL CELLS "THETA-BURST" DURING BEHAVIORAL EVENTS CRITICAL TO OLFACTORY LEARNING. H. Eichenbaum, T. Otto, S. Wiener, & C.G. Wible, Dept. of Biological Science, Wellesley College, Wellesley, MA 02181.

Long-term potentiation (LTP), a lasting enhancement of synaptic efficacy which can be induced in many monosynaptic pathways, has been proposed as a candidate mnemonic device due to its similarity in induction and maintenance characteristics to those of memory. LTP in the hippocampus is preferentially induced by high-frequency bursts of stimuli repeated at 5-10 Hz, the theta frequency (Larson et al, 1986), and can be induced by a single burst if a single "priming" stimulus precedes that burst by 130-170ms (i.e. at a frequency of 6-7 Hz; Rose et al, 1986). We now report that these optimal activation patterns, called "theta-bursting", occur naturally in the hippocampi of rats, time-locked to behavioral events associated with mnemonic processing.

The firing patterns of a subset of hippocampal cells previously found to have significant nonspatial behavioral correlates in a simultaneous odor discrimination paradigm (Eichenbaum et al. 1989) were reanalyzed for evidence of theta-bursting. A burst was defined as the occurrence of at least 2 spikes with an interspike interval of no more than 10 ms; a cell was considered to theta-burst if it had a predominance of interburst-intervals between 85 and 140 ms (i.e. between 12 and 7 Hz). Of 16 cells reanalyzed, all exhibited significant bursting; 11 of these burst repetitively within the specified theta range. In all instances, the bursts were well time-locked to periods of odor sampling or to discriminative responses.

These data provide compelling evidence that the conditions optimal for inducing hippocampal LTP are indeed occurring in the hippocampus of behaving rats during behavioral events critical to mnemonic processing.

113.8

MEMORY-RELATED RESPONSES IN RAT HIPPOCAMPUS DURING LEARNING PLACE ASSOCIATED WITH REWARDING ICSS. M. Fukuda, T. Ono, T. Kobayashi* and E. Tabuchi*. Dept. Physiol., Fac. Med., Toyama Medical and Pharmaceutical Univ., Sugitani, Toyama 930-01, Japan

Single unit activity in the rat hippocampal CA1 field was recorded in a cylindrical field (diameter, 1.5m; depth, 0.7m) to study its function in place learning and memory. If the rat alternately visited one of a pair of particular hidden areas (reward areas), brain electrical stimulation was delivered to the lateral hypothalamus as reward. Some hippocampal cells fired when the rat was in a particular part of the field. When the positions of the reward areas, or illumination in the experimental room were changed, some of these cells indicated a similar place field, but others fired more irregularly. When colored paper covered part of the wall in the apparatus, the place field and behavior were disturbed. The behavior of ICSS recovered after a few trials. Reconstruction of the place field was well-correlated to recovery of behavior. The results suggest that hippocampal neurons might code some particular positions within the environment depending on visual information, and formation of a place field might be correlated with place learning.

113.9

FUNCTION OF MONKEY HIPPOCAMPAL NEURONS IN MEMORY AND RECOGNITION. T. Ono, R. Tamura*, K. Nakamura* and M. Fukuda. Dept. Physiol., Fac. Med., Toyama Medical and Pharmaceutical Univ., Sugitani, Toyama 930-01, Japan

Neuronal activity in the hippocampus of conscious monkeys was recorded during performance of operant feeding, drinking, and active avoidance tasks coupled to the presentation of objects having positive (reward), negative (aversion), or unfamiliar implication.

Of 837 neurons recorded in the hippocampus, 155 responded to the sight of certain object(s) and 82/155 neurons responded differentially to different objects. Some differential neurons responded predominantly to rewarding, aversive, or unfamiliar objects. Some differential neurons responded selectively to only one object or one category of objects. Fourteen neurons that responded predominantly to rewarding or aversive objects were tested in reversal or extinction learning. Even after reversal or extinction was behaviorally evident, 12/14 neurons maintained their original responsiveness to the rewarding or aversive objects.

The results suggest involvement of the hippocampus in the preservation and recognition of past information of objects.

113.10

A TOPOLOGICAL REPRESENTATION OF SPACE BY A REALISTIC NEURAL NETWORK. R.U. Muller and J.L. Kubie Depts. of Physiology and Anatomy. SUNY-Brooklyn, Brooklyn, NY 11203

Place cells are hippocampal pyramidal cells that show location-specific firing. Each cell has a firing field; it fires only if a rat's head is in the field. Computer models have focused mainly on how location-specific firing arises and less on how the set of place cells represents local space. We are using a model that takes place cells as given to ask instead how the structure of space is represented in CA3. The model uses 2 properties of CA3 pyramids. 1) CA3 cells make direct excitatory synapses with each other (Miles and Wong, 1986). Contact probability is low for random pairs but constant over large areas of CA3. 2) Synaptic strength grows when the pre- and post-synaptic cells fire in closely in time (Miles et al, 1987). The modifiable synapses do not affect firing; they only integrate conjoint firings of pre- and post-synaptic units.

A first aim is to show that the synaptic strengths can reflect the connectivity of local space to give a topological representation. Synaptic strengths are zeroed and the rat's position is initialized. A probability of firing (P) is calculated from the spatial firing pattern of each cell. A cell fires if P is higher than a random number. The rat's position is updated from a record of real motions and firing is again determined. Synapses between cells that both fire within 250 msec are strengthened.

Synapses between cells with over-lapping fields should increase in strength, whereas synapses between cells with distant fields will stay weak. The encoding of distance by synaptic strength arises because rats cannot move fast enough to make cells with distant fields fire in close temporal order. We will show how synaptic strength varies with field distance and running speed. Our next step is to investigate how the connections can be used to guide the rat's behavior.

113.11

PLACE FIELDS ARE ALTERED BY NMDA ANTAGONIST MK-801 DURING SPATIAL LEARNING. K.B. Austin, W.F. Fortin*, and M.L. Shapiro, Department of Psychology, McGill University, Montreal, Quebec.

Single unit activity recorded from complex-spike cells in the hippocampus correlates with selective locations occupied by rats behaving in spatial environments. These *place cells* may encode and store relationships among spatial stimuli. Hippocampal neurons have synapses which show NMDA-dependent plasticity, and blocking NMDA receptors impairs spatial learning in naive animals, but not performance of the same spatial memory task in trained animals. If place cells encode spatial information during learning via an NMDA-dependent form of plasticity, then NMDA receptor blockade may alter place fields during spatial learning, but not after learning has occurred.

Single unit activity was recorded from CA1 cells in the dorsal hippocampus of rats traversing an enclosed chamber. The rats were either naive to the chamber or had a minimum of 4 prior 20 minute exposures. NMDA antagonist MK-801 (0.0625 mg/kg) or an equivalent volume of normal saline was injected i.p., and the same units were recorded again 30 min, 4h, and 6h later. Both naive and experienced rats given saline had stable place fields during all recording sessions. MK-801 altered place fields in naive rats, changing the location of the firing fields. In some cases, the changes in place fields produced by MK-801 diminished 6 h after drug administration; in other cases, the changes persisted. However, MK-801 did not alter place fields in experienced animals. Thus, spatial encoding in the hippocampus may require NMDA-dependent synaptic changes that establish stable place fields. After these synaptic changes occur, place field stability in a given spatial environment may no longer depend upon NMDA receptors.

113.12

EFFECTS OF THE NMDA RECEPTOR ANTAGONIST, MK-801, ON LTP IN FREELY BEHAVING RATS. E. Bostock, S.D. Croll, and P.E. Sharp. Dept. Psychol., Queens Col., CUNY, Flushing, NY 11367.

The hippocampus has been shown to be involved in learning and memory. Long-term potentiation (LTP), a candidate learning mechanism, can be induced by high frequency stimulation of the perforant path(PP)-dentate gyrus(DG) system of the hippocampus, resulting in LTP of the EPSP and population spike(PS) of the evoked potential. LTP of DG evoked potentials is attenuated by NMDA receptor blockade in both hippocampal slices and anesthetized rats. We tested whether the NMDA antagonist MK-801 would also attenuate LTP in freely behaving rats. Rats were chronically implanted with stimulating electrodes in the PP and recording electrodes in the DG. Thirty min prior to recording sessions rats were injected with vehicle or .1 mg/kg MK-801 s.c. They were then recorded from for 15 min followed by high frequency stimulation of the PP and 40 min of subsequent recordings. Vehicle injected rats exhibited LTP of both the EPSP and PS. MK-801 tended to reduce LTP of the EPSP ($\bar{x}$=13%) and significantly reduced LTP of the PS ($\bar{x}$=47%), indicating that DG LTP in freely behaving rats is dependent on the NMDA receptor.

113.13

EFFECTS OF THE NMDA RECEPTOR ANTAGONIST, MK-801, ON ENVIRONMENTALLY INDUCED PLASTICITY OF DENTATE GYRUS EVOKED POTENTIALS. S.D. Croll, P.E. Sharp, and E. Bostock. Dept. Psychol., Queens Coll., CUNY, Flushing, NY 11367.

Research has demonstrated environmentally induced plasticity (EIP) of hippocampal dentate gyrus evoked potentials (EP). Other research has shown a role of the NMDA receptor in long-term potentiation (LTP). We tested the role of the NMDA receptor in EIP. Rats were chronically implanted with stimulating electrodes in the perforant path and recording electrodes in the dentate gyrus bilaterally. EPs were recorded from freely behaving rats for 20 min/week for 4 weeks; rats received 0, .05, .08, or .10 mg/kg MK-801 s.c. 30 min prior to recording sessions in either an ascending or descending dose series. Results showed that MK-801 produced a significant dose-dependent reduction of EPSP enhancement. The effects of MK-801 on spike depression varied as a function of dose-series and time within a session, suggesting a long-term effect of MK-801 on spike depression. There was no detected effect of MK-801 on behavior. Results suggest a role of the NMDA receptor in EIP, with differential effects of NMDA receptor antagonism on EPSP enhancement and spike depression.

113.14

ASSOCIATIVE LEARNING ENHANCES [^{3}H]MK-801 BINDING IN RABBIT HIPPOCAMPUS. L.T. Thompson, S.N. Murphy, S.G. Brown, D.J. Luchins, and J.F. Disterhoft. Depts. of Cell, Molecular, & Structural Biology, Northwestern University Medical School, and of Radiology and of Psychiatry, University of Chicago, Chicago, IL.

Hippocampal neuronal plasticity in learning may involve specific neurotransmitters or receptor subtypes. Glutamate binding, either to receptors or to uptake sites, has been shown to increase in the hippocampus of blink-conditioned rabbits (Mamounas *et al.*, 1984). The NMDA receptor has been implicated in induction of other forms of hippocampal neuronal plasticity, including LTP. Recently, AMPA binding to quisqualate receptors in hippocampus was reported to be increased in the hippocampi of trained rabbits, but no enhancement of NMDA receptor binding was found (Tocco *et al.*, 1989). The present study tested the effects of learning on binding of the highly specific non-competitive NMDA receptor antagonist MK-801 (dizocilpine).

Young adult male rabbits (n=5) were trained using standard techniques (Disterhoft *et al.*, 1977) in a delay-eyeblink paradigm to a behavioral criterion of 80% conditioned responses per daily block of 80 trials. Conditioned animals were matched with equal numbers of handled control and pseudoconditioned animals. Rabbits were sacrificed 24 hr after completion of training, and whole hippocampi were removed and immediately frozen in liquid nitrogen. All biochemical assays were then performed blind. Membranes were prepared in a manner similar to that previously described (Reynolds *et al.*, 1987), although with only a single freeze-thaw cycle. 100 µM glutamate and 30 µM glycine were added to all samples. Membranes were incubated for 4 hr to assure binding reached equilibrium. All saturated MK-801 binding assay data was pooled within groups and analyzed with the LIGAND analysis program.

No significant differences were found between pseudoconditioned and control animals, so further analysis pooled these 2 groups. The B_{max} for MK-801 binding of 6.24 ± 0.22 pmol/mg protein in the conditioned group was significantly higher ($p < 0.02$) than that in the control groups, 4.79 ± 0.36 pmol/mg protein. In the assay system used, increases in B_{max} may equate directly to increases in the number of NMDA receptor-associated ion channels present in the neuronal membrane. The increase in B_{max} reported may be a potent cellular mechanism for inducing or maintaining the plasticity in hippocampus observed during and consequent to learning.

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113.15

A MONOCLONAL ANTIBODY FACILITATES ASSOCIATIVE LEARNING IN RABBITS. J.F. Disterhoft, L.T. Thompson, S.W. Conroy and J.R. Moskal. Depts. of Cell, Molecular, and Structural Biology, Northwestern Univ. Medical School, Chicago, IL and of Neurosurgery, Albert Einstein College of Medicine, Bronx, NY.

Recent work in other laboratories has demonstrated that glycine allosterically modulates biophysical properties of the NMDA receptor. A monoclonal antibody to dentate gyrus (B6B21) that modulates NMDA receptors in a glycine-like fashion has been described. Like glycine, B6B21 enhances NMDA and glutamate stimulation of [^{3}H]-TCP binding but has no effect on D-AP5 inhibition of binding. B6B21 significantly elevates LTP when applied to CA1 pyramidal cells in rat hippocampal slices (Moskal *et al., Soc. Neurosci. Abst.*, 1989, *15*: 202). It has been suggested that LTP and associative learning may share common cellular mechanisms. We thus evaluated the effects of B6B21 on associative learning. The behavioral task used was long-interval trace eyeblink conditioning in rabbits, which other work from our laboratory has shown is hippocampally-dependent (Moyer *et al., Behav. Neurosci.*, 1990, *104*: 241-250).

Rabbits were surgically implanted with bilateral ventricular cannulae and fitted with restraining headbolts. After recovery and habituation, rabbits received daily 5 µg infusions of B6B21 in ACSF (treated) or of ACSF (controls) delivered by micropump at a rate of 1 µg/µl/min. Trace conditioning [using a 100 ms duration, 6 KHz, 85 db tone CS followed after a 500 ms trace interstimulus interval by a 150 msec corneal air puff US] commenced immediately after infusion for 80 trials/day. Training continued until a criterion of 80% conditioned responses was attained.

Pilot studies on 3 matched pairs of rabbits showed that infusion of B6B21 reduced the time required to reach the behavioral criterion. Ongoing experiments indicate that B6B21 treated rabbits require at least one-third less training trials than controls to acquire the task. These data suggest that glycine modulation of NMDA receptors may be important in associative learning. Pseudoconditioning procedures will be used to evaluate changes in nonspecific behavioral excitability in B6B21 treated rabbits. A goal of these experiments is to determine if monoclonal antibodies which interact with NMDA receptors affect associative learning in intact behaving animals.

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113.16

LOCUS COERULEUS CELL ACTIVITY AND POTENTIATION OF THE PERFORANT PATH EVOKED DENTATE GYRUS POPULATION SPIKE. C. W. Harley and S. J. Sara. LPN2, CNRS, 91190 Gif/Yvette, France

Single and multiunit activity in the locus coeruleus (LC) of urethane-anesthetized rats was monitored concomitantly with the dentate gyrus (DG) potential evoked by perforant path (PP) stimulation at .1 Hz. LC cell firing was decreased by iv injections of clonidine (6-12 ug/kg). The DG evoked potential was unchanged during the LC silence induced by clonidine. LC cell firing was increased by several means. Injection of glutamate (500 mM, 100 nl) characteristically produced a strong increase in firing for 10 sec. followed by loss of action potential height, probably due to depolarization block. Minutes after glutamate injection LC activity appeared to return to baseline levels (1.2 Hz). The abrupt increase in LC activity was invariably correlated with an increase in the DG population spike. Typically a long-lasting increase in the population spike followed these manipulations despite the return to baseline LC activity. Similar effects were seen with iv injections of idazoxan (2 mg/kg). Idazoxan ejected locally in the LC rarely produced increased firing but when it occurred the population spike also increased. Introduction of an iv catheter was seen to produce increased LC firing lasting 10-20 sec. and, again, the cell firing increase was correlated with an increased population spike, however tail pinch which briefly excited LC neurons (<1 sec) did not induce changes in the evoked potential. EPSP slope increases were observed in some experiments but were not invariable. These data suggest (1) that short-term increases in LC firing are correlated with and, probably, initiate potentiation of the DG population spike if a sufficient number of cells are activated for periods of at least several seconds; (2) that long-lasting potentiation does not depend on continued LC activation and (3) that short-term absence of LC firing does not reduce the DG population spike.

113.17

CHOLINERGIC DIAGONAL BAND LESIONS AND LIMBIC CORTICAL AND THALAMIC UNIT ACTIVITY DURING LEARNING IN RABBITS. Y. Kubota, T. Holmbo*, A. Poremba, E. Kang and M. Gabriel. Dept. of Psychol. and Beckman Institute, Univ. of Illinois, Urbana IL 61801.

Previously systemic injection of scopolamine impaired performance of discriminative avoidance behavior and reduced training-induced unit activity in AV thalamic nucleus and posterior cingulate area 29 (Henzi, V., et al. Br. Res., in press). To test whether the neuronal decrement was a source of the behavioral impairment, we attempted to specifically reduce cholinergic function in cingulate cortex by making electrical lesions in the nucleus of diagonal band of broca. Marked reduction of acetylcholinesterase in cingulate cortex later confirmed cholinergic depletion. Ablated rabbits trained to step in an activity wheel to a tone, predictive of footshock US, and to ignore another tone acquired the task at a normal rate, but made significantly fewer CR's than sham lesion controls. Effects of lesions on CR and UR measurements were smaller than but consistent with effects of cholinergic blockade by scopolamine, e.g. unaffected US-sensitivity. The brief-latency (1-100 msec) cingulate cortical unit response to CS was reduced, whereas limbic thalamic units responded more to CS in ablated rabbits than in controls. The lesions had no effect on unit activity in dentate gyrus. These data suggest that reduced cholinergic input disturbs brief-latency cortical and thalamic CS processing. The depletion-related CR impairment suggests that the altered processing interfered with CR-triggering information flow from thalamus to motor system. (Supported by AFOSR and NIH grants to M.G.).

113.18

HIPPOCAMPAL LESIONS, LIMBIC CORTICAL AND THALAMIC TRAINING-INDUCED UNIT ACTIVITY, AVOIDANCE CONDITIONING, AND RESPONSE TO DIFFERENT FORMS OF NOVELTY IN RABBITS. E. Kang, Y.Kubota, A. Poremba and M. Gabriel. Dept. of Psychol. and Beckman Institute, Univ. of Illinois, Urbana IL 61801.

Past studies have implicated cingulate cortex and limbic thalamus in mediation of discriminative avoidance learning, wherein a conditioned response (CR) of stepping in an activity wheel to a tone CS+ prevents a footshock US scheduled to occur 5 sec. after CS+ onset. Lesions of the subicular complex, the hippocampal origin of efferents to cingulate cortex and anterior thalamus (AT), increased the magnitude of AT training-induced multi-unit discharges in response to the CS+ and reduced the cingulate cortical unit discharges. In addition, CR frequency was enhanced in novel training stages (the first sessions of conditioning and extinction, Gabriel, M., et al., Exp. Br. Res., 67:131, 1987). Here we test a hypothesis that hippocampal lesions will mimic the effects of subicular lesions. As after subicular lesions, hippocampal lesions increased AT unit discharges to CS+. However, the hippocampal lesions enhanced the cingulate cortical unit activity and did not increase CR-frequency during acquisition or during extinction with the standard or with a novel CS. Context (illumination and odor) change which suppressed CR performance in intact rabbits failed to do so in rabbits with lesions. These results suggest functional independence of subiculum and hippocampus: subicular neurons deprived of hippocampal inputs are sufficient to mediate CR-suppression in response to CS alterations; hippocampus is however needed for CR-suppression in response to contextual alterations. (Supported by NIH and AFOSR grants to MG).

113.19

CS-RELATED HIPPOCAMPAL UNIT ACTIVITY DURING AVOIDANCE CONDITIONING IN RABBITS. A. Poremba, Y. Kubota, E. Kang and M. Gabriel. Dept. of Psychol. and Beckman Inst., Univ. of Illinois, Urbana IL 61801.

This study tested the hypothesis that hippocampal circuits not essential for conditioned response (CR) acquisition nonetheless engender a mnemonic representation of task events that is used for novelty-induced CR suppression (Gabriel, M. et al., Exp. Br. Res., 67:131-152, 1987). Multi-unit and field potential (FP) activity in dentate gyrus (DG), CA3, CA1 and posterior subiculum (PS) was recorded as the CR (stepping in an activity wheel to a tone CS+ to prevent a shock US 5 sec. later) was acquired. Latencies (ms.) of initial and peak unit discharges respectively were: DG:15,25; CA1:15,95; PS: 15,115; CA3: 15,155. Rapid associative change in all areas except PS was indicated by significant firing increases in the first conditioning session, relative to pretraining with unpaired CSs and US. Firing diminished with further training and virtually ceased as the CR reached asymptote. DG and CA1 units showed modest CS+/CS- discrimination. Brief-latency DG FP components replicated those found in rats (Deadwyler, S.A. et al., Science, 211:1181-1183, 1981). Elicited DG theta amplitude increased with training and discriminated during asymptotic CR performance as a positive 55-ms. discriminative FP component emerged in CA3. These associative changes, consistent with the preliminary hypothesis, provide a basis for analysis of CR suppression mechanisms. (Supported by NIH and AFOSR grants to MG).

114.1

BILATERAL INJECTIONS OF MORPHICEPTIN INTO THE MEDIAL PREOPTIC NUCLEUS PRODUCE A DRAMATIC DELAY IN THE INITIATION OF SEXUAL BEHAVIOR IN THE MALE RAT.

L. Matuszewich* and W.A. Dornan. Dept. of Psychology, Illinois Wesleyan University, Bloomington, IL 61702.

Behavioral experiments examined the role of morphiceptin in male rat copulatory behavior. Male copulatory behavior was recorded subsequent to bilateral injections of either 10, 500, or 1000 ng of morphiceptin aimed at the medial preoptic nucleus (MPN) of the medial preoptic area (MPOA) in sexually vigorous male rats. In the first experiment, all three doses of morphiceptin injected bilaterally into the MPN produced a dramatic delay in the initiation of copulation. Both mount and intromission latencies significantly increased following injections of all three doses of morphiceptin when compared to saline injected controls. No other parameter was affected. In experiment 2, the inhibitory effects of morphiceptin on male copulatory behavior were abolished by pretreatment with specific opioid receptor antagonists 20 minutes prior to intracerebral morphiceptin injections. This study represents an attempt at pharmacological characterization of the inhibitory effects of opioids on male rat sexual behavior. The results of experiment 1 indicate that mu receptors located within the MPN mediate the initiation of male rat copulatory behavior.

114.2

THE EFFECTS OF INTRA-AMYGDALOID β-ENDORPHIN INFUSIONS ON THE SEXUAL BEHAVIOUR OF THE MALE RAT. A. McGregor* and J. Herbert, Department of Anatomy, Cambridge University, Cambridge CB2 3DY, U.K.

β-Endorphin infused into the amygdala of the male rat lengthened the intromission latency of the copulatory series in a dose dependent manner, but had no effect on the ejaculation latency itself or the post ejaculatory interval. This effect was naloxone (5mg/kg i.p.) reversible at the 60 pmol dose. Corticotropin releasing factor (CRF) infused into the amygdala had no effect on either the intromission latency or the ejaculation latency of the copulatory series.

To investigate the role of female specific information processing in the response, the first intromission of the series was allowed to proceed, followed by a 60 pmol β-endorphin infusion before replacing the male with either the original female or a different female. The effect on the intromission latency following replacement was measured. When replaced with the original female β-endorphin had no effect on the intromission latency (i.e. on reinitiation of the copulatory series). However, when β-endorphin was infused prior to the replacement with a different female, the intromission latency was increased. This effect implies that the "newness" of the novel female is registered when β-endorphin is acting within the amygdala, but that the female specific information cannot be employed appropriately to engage the copulatory series immediately.

These results suggest that β-endorphin within the amygdala prohibits the incoming female specific information from gaining access to the neural circuitary responsible for initiating the copulatory series, or prevents the information from being classified appropriately within the amygdala itself. Furthermore, the lack of response following the action of another stress related peptide (CRF) suggests that the effects of β-endorphin on sexual behaviour are not part of a more global stress response.

114.3

EVIDENCE TOWARD A ROLE FOR CENTRAL ANGIOTENSIN II IN THE REGULATION OF SEXUAL BEHAVIOR IN THE MALE RAT. L.S. Myers and M.K. Steele. Psychology Dept. CSUS, Stanislaus, Turlock, CA 95380 and Physiology Dept., Univ. of Calif., San Francisco, CA 94143.

A recent study has shown that intracerebroventricular (ICV) administration of angiotensin II (AII) increased the number of intromissions and intromission latency as well as increasing the length of the postejaculatory interval (Clark, Physio. & Beh., 45:221, 1989). The present study investigated the role of central AII in sexual behavior by using a specific AII receptor blocker, sarthran, and observing sexual behavior. After preliminary mating tests, rats were implanted with third ventricular cannulae. Only those rats that ejaculated in postsurgical mating tests were used. Testing was started an hour and a half intolights on. Using a counterbalanced design, rats received ICV injections of either sarthran (10ug/10ul) or vehicle immediately prior to mating tests. Sarthran injections significantly increased the number of mounts, while significantly decreasing the intromission latency. No other sexual behaviors were affected. These data suggest that the brain renin-angiotensin system may be playing a role in the regulation of sexual behavior in the male rat.

114.4

PREVENTION BY ω-CONOTOXIN GVIA OF PENILE ERECTION AND YAWNING INDUCED BY APOMORPHINE AND OXYTOCIN. M.R. Melis, R. Stancampiano* and A. Argiolas. B.B. Brodie Department of Neurosciences, Univ. of Cagliari, Via Porcell 4, 09124 Cagliari (Italy).

The effect of the administration of ω-conotoxin GVIA on penile erection and yawning induced by oxytocin or by the dopaminergic agonist apomorphine was studied in male rats. Conotoxin, 1-10 ng given ICV 5 min before oxytocin (30 ng ICV) or apomorphine (80 μg/kg SC), but not its carboxymethylated (CM) derivative, prevented the above behavioral responses in a dose-dependent manner. Conotoxin (5 ng) unilaterally injected in the paraventricular nucleus of the hypothalamus (PVN), prevented penile erection and yawning induced by the microinjection of oxytocin (10 ng) or apomorphine (50 ng) in the PVN. ω-Conotoxin injected in the PVN, but not in the preoptic area, prevented also penile erection and yawning induced by systemic apomorphine (80 μg/kg SC). ICV ω-conotoxin was unable to prevent stereotypy induced by apomorphine (500 μg/kg SC). The present results provide further evidence that calcium plays a major role in the expression of penile erection and yawning and that apomorphine and oxytocin induce these behavioral responses by mobilizing calcium through ω-conotoxin-sensitive (N type) calcium channels.

114.5

ROLE OF CALCIUM IN THE EXPRESSION OF ACTH-INDUCED STRETCHING, YAWNING AND PENILE ERECTION. A. Argiolas, M.R. Melis, R. Stancampiano* and G.L. Gessa*. B.B. Brodie Department of Neurosciences, Univ. of Cagliari, 09124 Cagliari (Italy).

The effect of ω-conotoxin GVIA, a potent and selective inhibitor of N-type calcium channels and of the organic calcium channel inhibitors nimodipine, verapamil and flunarizine, on stretching, yawning and penile erection induced by ACTH 1-24 was studied in male rats. Conotoxin (1-10 ng ICV 15 min before ACTH, 10 μg ICV) induced a dose-dependent prevention of all ACTH effects. In contrast, organic calcium channel inhibitors (20 mg/kg IP 30-60 min before ACTH) failed to modify ACTH-induced stretching and yawning but induced a 25% decrease in the number of penile erection episodes induced by the peptide, and prevented, like ICV conotoxin, oxytocin- and apomorphine-induced yawning and penile erection. When injected in the paraventricular nucleus of the hypothalamus, conotoxin prevented the above responses induced by apomorphine and oxytocin but not by ACTH 1-24. The present results suggest that ACTH induces stretching, yawning and penile erection by mobilizing calcium through central ω-conotoxin-sensitive calcium channels in brain sites different from those sensitive to oxytocin and apomorphine.

114.6

NEUROPHYSIN PROJECTIONS FROM THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS TO LOWER LUMBAR SPINAL CORD AND THE DISTRIBUTION OF NEUROPHYSIN FIBERS IN THE REGION OF THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS. C.K. Wagner and L.G. Clemens, Neurosci. Prog. & Dept. Zool., Mich. State Univ., E.Lansing, MI 48824

The paraventricular nucleus (PVN) of the hypothalamus projects to the sexually dimorphic segments of the spinal cord, L5-L6 (*Soc. Neurosci. Abstr.* 15: 1089, 1989), which contain the afferents of the sensory pudendal nerve and the motor nuclei responsible for penile reflexes (*J. Comp. Neurol.* 248: 532, 1980). Neurophysin (NP) is found in fibers and terminals throughout the spinal cord and the PVN is the major source of neurophysin in the CNS. The present study examined the presence of NP in neurons of the PVN that project to L5-L6 segments of spinal cord, as well as the distribution of NP fibers in this region of the cord. Male rats received 0.5μl 4% Fluorogold into segments L5-L6. Following 1-2weeks, animals were perfused and immunohistochemistry for NP was performed on 30μm sections through PVN. Double labelled neurons were found within the dorsal and lateral parvocellular subnuclei of PVN. In other male rats, 30μm sections through the lumbosacral enlargement were reacted for NP immunohistochemistry. NP fibers and terminals appeared to contact motoneurons within the spinal nucleus of the bulbocavernosus (SNB). Fibers were also found in the sympathetic nucleus in the lateral funiculus, at the lateral, medial and dorsal edges of the dorsal gray matter and in the dorsal horn between the central canal and the dorsal funiculus. These results demonstrate that oxytocin and/or vasopressin fibers, thought to arise from the PVN, are found in regions of the dorsal horn known to contain afferents from the sensory pudendal nerve and may directly modulate the activity of SNB motoneurons.

114.7

ELECTROPHYSIOLOGICAL RESPONSES OF PERIAQUEDUCTAL GRAY NEURONS OF FEMALE RATS TO LHRH, SUBSTANCE P, OXYTOCIN, AND TRH *IN VITRO*. Sonoko Ogawa, L.-M. Kow & D.W. Pfaff. The Rockefeller University, New York, NY 10021.

Lordosis behavior of estrogen-primed females rats and other behaviors are modified by certain neuropeptides in the periaqueductal gray (PAG). To study possible roles of neuropeptides in neural circuitry for female reproductive behavior, responses of PAG neurons to LHRH and substance P (SP), which facilitate lordosis in PAG, and also oxytocin (OT) and TRH were assessed in brain tissue slices prepared from estrogen-treated (OVX+E) or nontreated (OVX) ovariectomized females rats. Extracellular single-unit activity was recorded and changes in spikes/sec were analyzed. Responses to all four neuropeptides tested were virtually all excitatory. Both spontaneously firing neurons and silent neurons were affected. SP was most effective for exciting PAG neurons, and effectiveness was similar in OVX+E (29/48 cells) and OVX (25/34 cells) preparations at 10^{-6}M. OT and TRH were less effective than SP, and responses to them appeared to be affected by estrogen. OT tended to excite more neurons in OVX+E (19/45 cells) than in OVX (6/23 cells). In contrast, TRH tended to excite more neurons in OVX (10/13 cells) than in OVX+E (13/29 cells). LHRH was the least effective neuropeptide for direct excitation. It excited only a few PAG neurons even at 10^{-7}M and the magnitude of the changes was small. Orderly dose-response relations in terms of the percent of excited neurons were obtained between the concentrations of 10^{-6}M and 10^{-7}M for SP, OT, and TRH. Cell populations responsive to SP, OT, or TRH overlapped: 23 out of 31 peptide-responsive neurons were excited by more than one. Thus, SP may have direct excitatory effects on lordosis-relevant PAG neurons whereas LHRH may modulate actions of other neurotransmitters on them.

114.8

EFFECTS OF PRENATAL ADMINISTRATION OF ACTH AND NICOTINE ON THE SUBSEQUENT SEXUAL BEHAVIOR OF FEMALE RATS. S.E. Alves and F.L. Strand. Department of Biology and Center for Neural Science, New York University, New York, NY 10003.

Previous studies have shown that prenatal administration of ACTH or nicotine alters the subsequent sexual behavior of adult *male* rats (Segarra and Strand, 1989: a. Br. Res., 480: 151.; b. ICPS XXXI, P3492). This study was undertaken to investigate whether the sexual behavior of the *female* offspring is affected. Sprague-Dawley rats were injected with either ACTH 1-24 (10μg/kg), nicotine hydrogen tartrate (0.25mg/kg) or saline vehicle twice daily from gestation day 14-21. Females were tested as virgins at approximately 110 days of age. Sexual behavior was measured by recording the lordosis quotient and the lordotic quality score. Animals prenatally treated with ACTH had lower lordotic quotients and quality scores when compared to control animals. Prenatal administration of nicotine did not alter the lordotic quotient in these animals, however the quality score was significantly decreased. Based on this study, we suggest that sexual differentiation of the brain in the *female* rat is also susceptible to prenatal manipulation with ACTH or nicotine. We are currently investigating plasma estradiol and progesterone levels. This study was supported by the Council for Tobacco Research.

114.9

HYPOPHYSECTOMY ALTERS THE MAGNOCELLULAR NEUROSECRETORY SYSTEM BUT SPARES VASOPRESSIN-DEPENDENT FLANK-MARKING BEHAVIOR IN GOLDEN HAMSTERS. C.G. Pilapil, E.T. Koh, and C.F. Ferris. Dept of Physiol, U. Mass. Med. Center, Worcester, MA 01655.

There is evidence that subpopulations of magnocellular neurons in and around the anterior hypothalamus (AH) of the hamster do not project to the neurohypophysis. It is hypothesized that these non-neurosecretory neurons provide vasopressin (VP) neurotransmitter to the hypothalamus for the control of flank-marking behavior. Therefore, it is predicted that hypophysectomy should disrupt neurosecretory function but spare flank marking and the magnocellular neurons that control this behavior. HYPOX (n=6) and CONTROL (n=6) animals displayed similar odor-induced flank marking behavior. However, HYPOX animals had 4X the urine flow and water intake of CONTROLS due to the absence of VP neurohormone. The HYPOX group had an overall loss of approximately 50% of the VP neurons in the hypothalamus. However, computer-aided cell counting and mapping showed a 63% reduction in VP immunoreactive neurons in PVN, 52% in the lateral SON, 44% in medial SON and 34% in the nucleus circularis as compared to CONTROL. These results suggest that there may exist functionally and anatomically distinct populations of VP magnocellular neurons in the hypothalamus of the golden hamster. (Work was supported by NIH grant #NS23557).

114.10

MICROINJECTION OF ARGININE-VASOPRESSIN (AVP) INTO THE MIDBRAIN CENTRAL GRAY STIMULATES DOSE-DEPENDENT FLANK MARKING IN SYRIAN HAMSTERS. A.C. Hennessey, D.C. Whitman and H.E. Albers. Lab. Neuroendocrinol. & Behav., Depts. Biol. & Psych., Georgia State Univ., Atlanta, GA 30303.

AVP microinjected into the medial preoptic-anterior hypothalamus induces intense bouts of flank marking in hamsters. Injection of AVP into the midbrain central gray elicits flank marking in female hamsters. The present study investigated whether AVP would stimulate flank marking in males at midbrain central gray sites that induce flank marking in females. Hamsters were implanted with guide cannulas aimed at the central gray and were injected with 0.9 μM, 9.0 μM, 90.0 μM, and 900 μM AVP in 100 nL of saline on 4 consecutive days in a counterbalanced manner. After behavioral testing some animals were microinjected with rhodamine labelled microspheres (50 nL) to examine afferent projections to AVP sensitive sites. AVP stimulated flank marking in a dose-dependent manner for both male ($p<0.01$) and female hamsters ($p<0.05$). These data suggest that the neurochemical control of flank marking may involve vasopressin-sensitive neurons in the midbrain central gray in male and female hamsters. (Supported by NSF BNS-8910863)

114.11

NOREPINEPHRINE (NE) BUT NOT NEUROPEPTIDE Y (NPY) BLOCKS ARGININE VASOPRESSIN (AVP) STIMULATED FLANK MARKING WITHIN THE MEDIAL PREOPTIC ANTERIOR HYPOTHALAMUS (MPOA-AH) OF THE HAMSTER. D.C. Whitman, A.C. Hennessey and H.E. Albers. Lab. of Neuroendocrin. & Behav., Depts. Biol. & Psych., Georgia State Univ., Atlanta, GA 30303.

AVP in the MPOA-AH is critical for the control of flank marking. Since NE and NPY are found within projections to this region, the effects of NE and NPY on AVP stimulated marking were investigated. Female hamsters (n=29) implanted with guide cannulas aimed at the MPOA-AH were tested for marking on 3 consecutive days. On day 1, 9.0 μM AVP was microinjected; on day 2, AVP and NPY (250 μM) or AVP and NE were co-administered; and on day 3, AVP was given alone. Co-administration of AVP with 4, 0.4 or 0.2, but not 0.04 or 0.004 nM NE, into the MPOA-AH, significantly ($P<.05$) reduced AVP induced marking. Co-administration of AVP and NPY did not significantly reduce AVP induced marking. Hamsters were injected with 50nl of rhodamine labelled microspheres to localize the injection site and identify afferent projections. These data indicate that NPY does not block AVP stimulated flank marking and NE may be involved in regulating this behavior. (Supported by NSF BNS-890863)

114.12

DISTRIBUTION OF THYROTROPIN RELEASING HORMONE BINDING SITES IN THE AMPHIBIAN BRAIN. S.K. Boyd and J.A. Taylor*. Dept. of Biological Sciences, Univ. of Notre Dame, Notre Dame, IN 46556.

TRH alters sexual and locomotor behaviors of the frog Xenopus laevis. The precise neural sites where TRH acts are unknown however. We used in vitro autoradiography to locate binding sites for ^{3}H-TRH in the brain of Xenopus. Brains were frozen-sectioned at 50 μm (frontal) and sections incubated with 10 nM ^{3}H-TRH (NEN), with or without 10 μM unlabelled TRH (Sigma) for 1.5 hr. Films were exposed for 4 months at room temperature.

Binding of ^{3}H-TRH to Xenopus brain was displaced by unlabelled TRH and was reversible and saturable. Greatest densities of specific binding sites were observed in the preoptic and infundibular nuclei of the hypothalamus. In the telencephalon, binding sites were found in the striatum and dorsal and lateral pallia. Significant binding was also observed in the thalamus, optic tectum, and brain stem. The distribution of sites was similar in males and females.

Since some of these neural areas have been previously implicated in the control of amphibian sexual behaviors, they may represent the sites of TRH-induced behavioral changes.

114.13

MORPHOLOGY OF THE TERMINAL NERVE SYSTEM IN A TROPICAL FISH, THE DWARF GOURAMI: GNRH IMMUNOCYTOCHEMISTRY AND ELECTRON MICROSCOPY. Y.Oka and M.Ichikawa. Zool. Inst., Fac. Sci., Univ. of Tokyo, Tokyo 113, and Dept. Anat. Embryol., Tokyo Metro. Inst. for Neurosci., Tokyo 183

It has recently been suggested that the terminal nerve (TN) system may be involved in the control of sexual behavior in teleosts and mammals. We have examined the morphological characteristics of the TN system in the dwarf gourami as the basis for a study of its involvement in the control of sexual behavior. The TN cells in the transitional area between the olfactory bulb (OB) and the telencephalon reacted strongly with a monoclonal anti-GnRH (LRH13, Park & Wakabayashi, '86). The GnRH-ir fibers were abundant in the OB, ventral telencephalon (VT) and several other areas. A distinct bundle of axons emanating from the TN cells ran caudally through the VT and the preoptic area (POA). Some of the axons entered the optic nerve (ON) and innervated the retina. Thus, the GnRH-ir TN system may affect both olfactory and visual functions as well as function of the VT and POA (these areas have been suggested to be involved in the control of sexual behavior in teleosts) as a neuromodulator and/or neurotransmitter. We next examined the cytology and synaptology of the TN cells by EM. The TN cell bodies had morphological characteristics similar to those of peptidergic neurons. The frequent occurrence of coated vesicles in close vicinity of the plasma membranes of the cell bodies was suggestive of membrane retrieval of secretory granules after exocytosis. The adjacent TN cells were either in direct juxtaposition or made specialized "glomeruloid" cell-to-cell contacts, which may be relevant for the intercellular communication among TN cells. In the glomeruloid, the somatic processes of the TN cells received inputs from synaptic terminals that have spherical synaptic vesicles and some DCVs.

114.14

CENTRAL INJECTIONS OF GROWTH HORMONE-RELEASING FACTOR (GRF) ANTISERUM ATTENUATE THE INCREASED FEEDING ASSOCIATED WITH DARK ONSET FEEDING IN RATS. F. J. Vaccarino[1], D. Feifel[1], J. Rivier[2], W. Vale[2] [1]Dept. of Psychology, University of Toronto. [2]Clayton Foundation Laboratories for Peptide Biology, The Salk Institute, La Jolla, CA.

Current findings indicate that GRF-induced feeding is photoperiod-sensitive and is mediated by GRF actions in the MPOA and SCN, a critical brain region underlying circadian rhythms. These findings raise the possibility that endogenous GRF represents a feeding-stimulatory signal in the brain which is associated with circadian feeding rhythms.

In order to examine this possibility, the effects of intra-SCN/MPOA injections of GRF antiserum (abGRF) on food intake at four time points across a 12:12 hour light-dark cycle were tested. The four time points tested were: mid-light, dark-onset, mid-dark, and light-onset.

Results indicated that intra-SCN/MPOA abGRF treatment decreased feeding associated with onset of the active dark phase. None of the other time points tested revealed any significant effect of abGRF treatment. These effects appeared to be behaviourally specific as evidenced by the lack of abGRF effect on locomotor activity and the lack of any feeding effect following extra-SCN/MPOA abGRF treatment.

The present results suggest that endogenous GRF actions in the SCN/MPOA region contribute to the naturally increased feeding associated with the onset of the active phase of the circadian feeding cycle. This research was supported by a NSERC grant to F.J. Vaccarino.

114.15

PRETREATMENT WITH CENTRAL SOMATOSTATIN (SS) ANTISERA BLOCKS GRF-INDUCED FEEDING. D. Feifel and F.J. Vaccarino, Dept.'s of Psychology, University of Toronto, Toronto, Canada, M5S 1A1.

Previously we have reported that both GRF and SS, administered icv in picomole doses, increase feeding acutely in sated rats. The current study examined the effects of icv pretreatments with rabbit antisera raised against GRF(abGRF) and SS(abSS) in order to determine whether the feeding effects of either peptide was contingent upon the release of the other.

Male Wistar rats (300-400 gms) were housed in separate cages and given free access to food and water. Each was stereotaxically implanted with a chronic guide-cannulae aimed at the right or left lateral ventricle. Following recovery subjects were given a feeding-effective dose of icv GRF (4 pm) or SS (1 pm) or saline. Injections were preceded by an icv injection of abGRF or abSS or normal serum. Food intake for the following hour was recorded.

Both GRF and SS significantly increased feeding relative to saline injections ($p<.05$). Neither antisera had a significant effect on baseline feeding. abGRF blocked GRF-induced feeding but had no significant effect on SS-induced feeding. abSS, on the other hand, blocked both SS and GRF-induced feeding. These results suggest that somatostatin is involved in the expression of GRF-induced feeding. This conclusion is consistent with evidence that low doses of GRF induce the release of SS from hypothalamic neurons.

114.16

CORTICOTROPIN RELEASING FACTOR AND RESTRAINT STRESS REDUCE PREFERENCE FOR NOVEL FOODS IN SELF-SELECTING RATS. S.C. Heinrichs and G.F. Koob. Dept. of Neuropharmacology, Scripps Clinic and Research Foundation, 10666 N. Torrey Pines Rd., La Jolla CA, 92037.

Exogenous corticotropin releasing factor (CRF) produces behavioral responses in locomotor activity, conditioned suppression, operant conflict and shock-induced fighting tasks which reflect the postulated stress-like action of this peptide. For instance, locomotor/open Field results demonstrate CRF-induced behavioral suppression in an unfamiliar environment and delayed contact with novel stimuli. This reduced response to novelty was examined in the present study of CRF effects on ingestive behavior using a two-choice test of familiar vs. unfamiliar foods.

Fifty three rats were fed either protein-free or protein-replete diets for three days and on the fourth day were given a choice between this familiar maintenance diet and an unfamiliar alternative. Under such conditions, rats consuming protein-imbalanced diets show an increased preference for novel food choices. Neither I.C.V. infusion of 500 ng CRF nor physical restraint 30 min prior to the 1 hr choice test affected consumption of the two diets in protein-replete controls. In the nutritionally deprived group by contrast, both treatments reduced consumption of the novel diet without affecting familiar diet intake. Further, this dose of CRF did not produce a conditioned taste aversion suggesting that the reduction in consumption of a novel diet did not result from malaise. These results suggest a stress-like action of CRF in reducing intake of unfamiliar foods while sparing appetite for familiar foods.

114.17

The Long-term Effects of Neonatal Exposure to Bombesin (BN) and a BN-antagonist.

H. Piggins[1] and Z. Merali [1,2]. [1]School of Psychology, [2] Dept. of Pharmacology University of Ottawa, Ottawa, Canada K1N 9A9

The longterm effects of neonatal exposure to the peptide bombesin (BN) and [D-Phe^6,ΨLeu^{13}-Cpa^{14}]BN(6-14) a BN-antagonist was assessed in all male litters (8 pups/litter) by treating pups subcutaneously (sc) with one of the following 2x/day for postnatal days 1-8: Untreated (handled), saline, BN (5mg/kg -LDB or 10 mg/kg -HDB), and BN-antagonist (5mg/kg -LDA or 10 mg/kg -HDA). At 65 days of age, the animals were implanted with 3rd ventricular cannulae and placed on a water access schedule (4 hrs of water access/day). Grooming and food and water consumed were assessed in the first 30 min following water presentation. No group differences were noted at baseline. Animals were administered BN centrally (0.0-1.0 ug; icv) or systemically (0.0-8 ug; ip) and the above measures noted. The rats neonatally exposed to the 10 mg/kg dose of BN were consistently more sensitive to the central as well as systemic effects of BN. This was reflected by increased grooming and decreased food and water intake at the 0.1 ug (icv) dose and by decreased food consumption at the 4 ug/kg (ip dose). In contrast to the long-lasting effects of of neonatal exposure to the agonist, exposure to the BN antagonist failed to significantly alter the response to central or peripheral BN in adulthood. This lack of effect may have been due to the dosage regimen or the testing intervals employed. However, since BN receptors develop earlier in ontogeny as compared to the synthesis of BN-like peptides, the pups may be more succeptable to effects of a receptor agonist. These data would indicate BN-receptor interactions early development may play a role in the synaptogenesis and/or development of systems utilizing BN-like peptides as neurotransmitters/neuromodulators. (Supported by MRC).

114.18

PERIPHERAL RECEPTOR SUBTYPE SPECIFICITY FOR CHOLECYSTOKININ-INDUCED INHIBITION OF FEEDING.

J.N. Crawley, S.M. Fiske*, C. Durieux*^, M. Derrien*^ and B.P. Roques^*, Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892 and ^Laboratoire de Chimie Organique, INSERM U266, CNRS UA 498, Universite Rene Descartes, Paris, France.

New agonists and antagonists selective for the peripheral-type (CCK-A) and for the brain-type (CCK-B) cholecystokinin receptor were used to determine whether a peripheral or a central type of CCK receptor mediates the action of cholecystokinin (CCK) on reducing food consumption. Rats administered BC 264, a CCK-B receptor agonist, did not decrease consumption of a palatable meal in a 30 minute test session, after an overnight fast, when BC 264 was administered either intraperiteonally (5-50 ug/kg) or into the lateral ventricles (20 ng-5 ug) of the brain. CCK decreased feeding when administered intraperitoneally at the same doses, and when administered intraventricularly at the highest dose (5 ug). L-364,718 (50 ug/kg i.p.), an antagonist selective for the CCK-A receptor, completely blocked the action of centrally administered CCK. L-365,260 (50 ug/kg i.p.), an antagonist selective for the CCK-B receptor, did not affect the ability of centrally administered CCK to inhibit feeding. Propionyl CCK, administered with radiolabelled tracer, intraventricularly at a dose which inhibited feeding (5 ug), was detected in the plasma 30 minutes later at a concentration of 4 nM. CCK appears to inhibit feeding in the rat by a peripheral, not a central, CCK receptor subtype mechanism.

114.19

INTERACTION OF CCK-8 AND STRESS IN FOOD INTAKE IN RATS. M.Shuck, J.Prather, M.Mims, and E.Quinton. Lab. of Psychobiology, University of Louisville, Louisville, KY 40292.

Exogenous administration of the octapeptide fragment of cholecystokinin (CCK 26-33) inhibits feeding; stress that elicits an endogenous opiate response increases feeding in rats. This study compared the effects of CCK-8 on feeding in stressed and non-stressed rats. Male Sprague-Dawley rats were injected ICV with either 10 ul of physiological saline solution or 10 ug of CCK dissolved in the solution. Rats were deprived of food for 4 hrs. and then allowed to feed freely for 1 hr. immediadely prior to injection. On the first day the rats were injected and food intake was measured each hour for 6 hrs. Three days later the rats were injected as before but 5 min. after injection received a 1mA footshock for 90 sec. and food intake was measured as before. Results show a significant increase in feeding during the first hour after stress, and a significant CCK inhibition of feeding. A significant interaction effect supports findings that CCK inhibits stress induced feeding and may provide additional evidence of an opiate antagonist function of CCK.

114.20

EFFECTS OF FMRFamide AND SCP_B ON SPONTANEOUS AND INDUCED CONTRACTIONS OF THE ANTERIOR GIZZARD IN APLYSIA.
N. Ewadinger & K. Lukowiak, Dept. of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Biologically active peptides can function as transmitters, modulators, or hormones in the central nervous systems of both vertebrates and invertebrates. Previous studies on the anterior gizzard of the opisthobranch mollusc Aplysia californica have shown that the spontaneous contractions of this organ can be influenced by various transmitters and peptides. In the present study, we tested the affects of the neuropeptides FMRFamide and small cardioactive peptide B (SCP_B) on longitudinal strips of the isolated anterior gizzard. In addition, because of the possibility that peptides may co-exist with 'classical' neurotransmitters, such as acetylcholine (ACh), the co-perfusion of carbachol (an ACh analog) and SCP_B was also done to test for possible synergistic effects. FMRFamide at concentrations between 10^{-9} to 10^{-8} M inhibited spontaneous gut activity. In addition, carbachol alone produced tonic contractions of the gut at relatively high concentrations (e.g., 10^{-4} M). SCP_B did not significantly effect either the amplitude or the rate of contractions of the gut. When carbachol (10^{-4} M) and SCP_B (10^{-8} M) were co-perfused, the tonic contractions induced were shifted by SCP_B, leading to more rhythmic (phasic) contractions; thus, no synergistic effects were observed. These results suggest that both FMRFamide and SCP_B may function as neuromodulators of gut contractility in Aplysia.

LEARNING AND MEMORY: CONDITIONING

115.1

CEREBELLAR CORTEX LESIONS DISRUPT THE TIMING OF CONDITIONED EYELID RESPONSES. S.P. Perrett, B.P. Ruiz*, and M.D. Mauk. University of Texas Medical School, Houston, TX 77225.

Previous studies suggest that the cerebellum is a necessary component of the pathways that mediate classical conditioning of skeletal muscle responses. However, the precise role of the cerebellum, particularly the cerebellar cortex, remains unresolved. We report here that lesions of the cerebellar cortex have a very specific effect on the performance of conditioned eyelid responses in rabbits; the normal timing of the responses, in which eyelid closure peaks near the onset of the unconditioned stimulus (US), is disrupted in a characteristic manner.

To examine the effects of lesions on conditioned response (CR) timing, we use a differential conditioning paradigm in which individual animals are trained to elicit two differently timed responses. Two distinguishable conditioned stimuli (CS; 0.4 & 8 kHz tones) are paired with the US (air puff to the cornea), each using a different inter-stimulus interval (ISI). For example, CS1 may be paired with the US at an ISI of 150 ms, and CS2 may be paired with the US at a 750 ms ISI. These animals show high rates of conditioned responding (90-100%) and the timing of the responses elicited by the two CSs is significantly different. Rabbits were trained to asymptotic performance and subjected to aspiration lesions of the ipsilateral cerebellar cortex (ansiform and paramedian lobes).

We find that CRs are spared by the lesions (though in some animals there is a decrease in the percentage and amplitude of the responses elicited), however, their timing is significantly affected. Before the lesion the responses to the respective CSs were timed differently, each peaking near the onset of the US. The post-lesion responses, however, were similarly timed for both CSs and displayed an abnormally short latency to onset and to peak. The lesions produced decreases in latency to onset of up to 100 ms and decreases in latency to peak of up to 750 ms. The post-lesion responses showed latencies to onset as short as 60 ms and latencies to peak as short as 95 ms. These data suggest a CR pathway not involving the cerebellar cortex is capable of mediating the expression of responses with short latency. We hypothesize that the cerebellar cortex modulates this pathway to contribute to normal timing of conditioned responses.

Supported by National Down Syndrome Society & The McKnight Foundation.

115.2

RECOVERABLE AND NON-RECOVERABLE DEFICITS IN CONDITIONED RESPONSES (CRs) AFTER CEREBELLAR CORTICAL LESIONS. **J.A. Harvey, C.H. Yeo, J.P. Welsh and A.G. Romano.** The Medical College of Pennsylvania/EPPI, Philadelphia, PA 19129 and University College London, London WC1E 7JG, UK.

This study reexamined the effects of unilateral damage to cerebellar cortical lobule VI (HVI) on the rabbit's conditioned nictitating membrane response. Animals received 15 conditioning sessions. Each session consisted of 60 pairings of a tone CS with a corneal air puff US and 6 tone alone test trials. Animals then underwent surgery and 3 weeks later were given 12 more conditioning sessions. Total removal of HVI along with extensive damage to the medial ansiform cortex in 11 rabbits significantly impaired their ability to perform CRs as compared with 7 sham-operated controls or 14 animals with only partial damage to HVI. CR frequency was reduced by 52% and CR amplitude by 54% on the 1st post-operative session. CR frequency recovered to control levels but CR amplitudes were permanently reduced. Thus, HVI plays an important role in the retention and performance of learned responses, but is not essential for reacquisition. Supp. MH16841.

115.3

HARMALINE BLOCKS ASSOCIATIVE LEARNING IN RABBIT. **A. G. Romano and J. A. Harvey.** Medical College of Pennsylvania at EPPI, Philadelphia, PA 19129.

This study examined the effects of harmaline on acquisition of the conditioned nictitating membrane response. In Phase 1, harmaline (5, 10 or 20 mg/kg) or saline was injected s.c., 30 min prior to a single acquisition session consisting of 120 pairings of a tone conditioned stimulus (CS) with a corneal air puff unconditioned stimulus (US). In Phase 2, two days later, all animals received a second conditioning session except that no drug or saline was injected. Control animals demonstrated a significant acquisition of CRs in Phase 1 and CR retention in Phase 2. However, all three doses of harmaline blocked CR acquisition during Phase 1 and there was no evidence of retention during Phase 2. Control experiments established that harmaline (5 mg/kg): 1) altered the CS threshold for eliciting CRs 2) had no effect on the unconditioned response; and 3) did not alter non-associative responding. Given harmaline's known effects on the inferior olive, our results provide further evidence that associative learning is dependent on normal activity of the inferior olive. Supported by Grant MH16841.

115.4

ASSOCIATIVE LEARNING REQUIRES THE FUNCTION OF THE INFERIOR OLIVE. J.P. Welsh and J.A. Harvey. Univ. Iowa, Iowa City, IA 52242 & Med. Col. of Pa/EPPI, Phila., PA 19129.

This study examined the role of the inferior olive (IO) in the acquisition and retention of conditioned nictitating membrane responses (CRs). Infusions of lidocaine (LID) into the dorsal accessory olive (DAO) immediately and reversibly abolished CRs acquired to either a tone or light CS. In order to determine the reason why CRs were abolished by DAO inactivation, a 3-phase procedure was employed. In Phase 1, rabbits were trained to make CRs to a light CS that was paired with an air-puff UCS. In Phase 2, rabbits received infusions of LID into the DAO during a conditioning session in which a tone CS was paired with the UCS. Interpolated light-CS trials monitored the degree to which performance of CRs was impaired by LID. In Phase 3, two days later, animals received tone-CS alone trials to determine whether learning had occurred in Phase 2 but was not observed due to a performance deficit resulting from inactivation of DAO by LID. Animals that received infusions of LID into the DAO during Phase 2 showed no CRs to the light CS and no evidence of CR acquisition to the tone CS. In Phase 3, when no LID was infused and performance was not impaired, these animals showed no retention of CRs to the tone. Therefore, in contrast to lesions of the cerebellum which abolish CRs by disrupting motor function, lesions of the IO abolish CRs through an action on associative learning. The results are interpreted to support a putative role for the IO in sensory function and/or timing. (Supp. NIMH Grant MH16841)

115.5

Effects of Age on Eyeblink Conditioning in Freely Moving Fischer-344 and F1 Hybrid Rats. C. Weiss, E. Savay, and R. F. Thompson, Neurosciences Program, University of Southern California, Los Angeles, California 90089-2520.

Classical conditioning of the eyeblink response in humans and rabbits shows progressive age related impairments. Contradictions in the literature are likely to be due to the specific paradigm being used. We have implemented a standard "delay" paradigm using a 350ms white noise conditioning stimulus and a 2mA, 60 Hz, 100ms coterminating unconditioned stimulus. Our results indicate that Fischer 344 male rats exhibit severe associative deficits as a result of aging. The two older groups, 18 & 30 months, exhibit significantly fewer conditioned responses (CRs) than the two younger groups, 3 & 12 months, $F(3,20)=3.11$, $p<.05$. In contrast to the F-344s, all ages (9, 18 & 24 months) of the F1 hybrids (F-344 x Brown Norway) exhibited substantial numbers of CRs. In fact, the oldest F1 hybrids exhibited more CRs than the youngest Fischer rats. These data indicate clear strain differences in rate of learning on this paradigm. These data also indicate that the delay paradigm is sufficiently demanding to demonstrate age related deficits in the Fischer rat.

%CRs on Last (4th) Session (Mean±SE)

F-344:	3mth	12mth	18mth	30mth	F1:	9mth	18mth	24mth
	59±13	63±10	43±13	42±10		92±2	85±10	95±2

Support: BNS-8718300 to R.F.T.; NIH: AG00093 & AG05142

115.6

MEDIODORSAL THALAMIC LESIONS AND CLASSICAL CONDITIONING OF HEART RATE AND EYEBLINK RESPONSES. Shirley L. Buchanan and Richard H. Thompson. VA Medical Center and University of South Carolina, Columbia, SC 29201.

Rabbits received bilateral ibotenic acid injections in the mediodorsal nucleus of the thalamus (MD), and were compared to sham control animals in a simple classical conditioning experiment, in which both eyeblink (EB) and heart rate (HR) conditioning were assessed over 4 acquisition and 1 extinction session consisting of 60 trials each. Lesions of MD enhanced the initial bradycardiac component of the HR conditioned response (CR) that appears in unoperated animals at the beginning of conditioning, and completely abolished the tachycardiac component of the HR CR that occurs later, after EB conditioning reaches maximum. In lesioned animals, EB conditioning was delayed during the first session, when most animals begin to show EB CRs. These data suggest that MD's role in classical conditioning may involve somatomotor response selection, based on information processed in other brain areas, e.g., the prefrontal cortex. They also provide additional evidence that MD is involved in mediating sympathetic control. These two effects may be related, since the acquisition of a somatomotor response would be expected to involve engagement of sympathetic mechanisms.

Supported by VA Institutional Research Funds

115.7

IBOTENIC ACID LESIONS OF AREA 32 OF THE MIDLINE PREFRONTAL CORTEX GREATLY ATTENUATE CONDITIONED BRADYCARDIA IN THE RABBIT. D.A. Powell and Karen L. Watson*. Neuroscience Laboratory, VA Medical Center and University of South Carolina, Columbia, SC 29201.

Aspiration lesions of the midline prefrontal cortex (PFC) greatly attenuate the bradycardia elicited by classical conditioning contingencies (Buchanan & Powell, J. Comp. Physiol. Psych., 96, 1982, 755-774). In the present study, bilateral multiple injections of ibotenic acid in the midline PFC selectively destroyed: (a) the dorsomedial precentral agranular portion of the midline PFC (approximately Brodmann's area 24); (b) the more ventral anterior limbic area (approximately Brodmann's area 32); or (c) the infralimbic area (Brodmann's area 25) in separate groups of animals. After recovery from surgery, the rabbits received differential classical conditioning, in which two tones served as a reinforced CS+ or a nonreinforced CS-. A paraorbital electric shock train was the unconditioned stimulus. The more dorsal lesions of area 24 slightly impaired responding to the CS+ in the lesioned animals compared to sham operated animals. More ventral lesions in area 32 appeared to completely abolish conditioned bradycardia in most animals, but area 25 lesions had no effect. These findings thus suggest that conditioned bradycardia in the rabbit is dependent upon the anterior limbic area of the midline PFC.

Supported by VA Institutional Research Funds

115.8

PURKINJE CELL NUMBER RELATED TO RATE OF NM/EYEBLINK CLASSICAL CONDITIONING IN THE TRACE PARADIGM. D.S. Woodruff-Pak & J. F. Cronholm. Department of Psychology, Temple University, Philadelphia, PA 19122

Cerebellar Purkinje cells are likely to play a role in acquisition and retention of the classically conditioned NM response in rabbits. We demonstrated that Purkinje cell number and acquisition in the trace paradigm were highly correlated in a group of aging rabbits. Here we report that in *young* rabbits there is a significant correlation of -.60 ($p<.01$) between Purkinje cell number and trials to learning criterion. Eighteen 3-month-old rabbits were conditioned in the trace paradigm with an 85 dB, 250 msec, 1 KHz tone CS and a 3 psi corneal airpuff US presented 750 msec after the onset of the CS. Animals were trained to learning criterion, sacrificed, and perfused. Brains were kept in formalin and then embedded in an albumin-gelatin mixture. Every fourth 80 um section was mounted and stained with cresyl violet. Purkinje cells were counted on 6 sections (18 micrographs/rabbit). Counts were made of Purkinje cells in the HVI area and in vermis and were limited to a single row of Purkinje cells 0.5 mm wide. Counters were blind to the performance of the rabbits. Inter-rater reliability was .95 ($p<.001$). These data provide additional evidence for the role of Purkinje cells in normal acquisition of the classically conditioned NM response in the trace paradigm. (Supported by NIH grant AG05312 and a grant from the American Federation for Aging Research).

115.9

LESIONS OF THE ROSTRAL FASTIGIAL N. AFFECT BRADYCARDIAC ORIENTING, BUT NOT CONDITIONED, RESPONSES IN RABBITS. C.M. Gibbs, K. Watson*, P. Shah* & A. Gibbs*, VA Med. Center & Univ. of South Carolina, Columbia, SC 29201.

Aversive Pavlovian conditioning procedures lead to the rapid development of bradycardiac adjustments (HR CRs) in rabbits that are dependent upon the integrity of corticolimbic mechanisms (e.g. Kapp et al: Physiol Behav 23:1109). Recent preliminary data (Supple et al: Soc Neurosci Abstr 15:640) have suggested that the anterior cerebellar vermis (Va) may also be involved in this learning process. Accordingly, the present studies were undertaken to determine the effects of lesions of the rostral fastigial n. (FA), the deep nuclear target of Va Purkinje cells, on the expression of bradycardiac orienting responses (ORs) and the subsequent development of discriminative HR CRs.

Following lesion or sham surgical procedures, animals received habituation training to two distinctive 4-sec tones (CSs), followed by three 40-trial sessions of differential conditioning (CS+/eyeshock pairings interspersed with presentations of CS- alone). The results to date indicate that bilateral FA lesions, in contrast to sham operations, markedly enhance HR ORs ($ps<.01$) but have minimal impact on HR CRs. Thus, the rostral FA appears to be involved in the modulation of unconditioned, but not conditioned, cardiac adjustments.

Supported by VA Institutional Research Funds

115.10

LESIONS OF THE AMYGDALA DISRUPT REFLEX FACILITATION OF THE NICTITATING MEMBRANE (NM) RESPONSE IN RABBIT. D.G. Harden, Z. Xiang, J. Davis*, and D.J. Weisz. Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

The presentation of a previously neutral or conditioned stimulus (CS) at an appropriate interval prior to the delivery of a reflex-eliciting or unconditioned stimulus (US) to the cornea of a rabbit can facilitate the amplitude of the NM response that is elicited by the US (e.g. Ison and Leonard, JCPP, 75:157, 1971). In two experiments we investigated the role of the amygdala in reflex facilitation using an auditory CS (85 db) and an airpuff US (2.0 psi). In Experiment 1 animals with lesions that included approximately 70% of the central nucleus of the amygdala exhibited no significant reflex facilitation of NM amplitude at a wide range of interstimulus intervals (ISIs) (125-8000 msec) (12 presentations at each of 8 ISIs), whereas control animals demonstrated robust facilitation ranging from 1.5-3.0 mm. In Experiment 2, we tested the relation between reflex facilitation and the acquisition of conditioned responses (CRs) to CS presentation by using a fixed ISI (250 msec) throughout training. Both control and amygdala lesion groups showed comparable facilitation (2.0-2.5 mm) for the first block of training (5 presentations of each trial type) and comparable CR acquisition rates. Whereas reflex facilitation was maintained in the control group across the first day of training, no facilitation was observed in the lesion group after the first block. As in Experiment 1 approximately 70% of the central nucleus of the amygdala was damaged in the lesion group. The results suggest that the amygdala plays a critical role in the maintainance of reflex facilitation but not in its initial appearance. (Supported by RO1MH42800)

115.11

THE EFFECT OF RUBROSPINAL TRACTOTOMY ON A CONDITIONED LIMB RESPONSE IN THE CAT. **T.J. Voneida, Dept. of Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272.**

Cats were conditioned to perform a right forelimb response to a paired tone CS - shock UCS. Right rubrospinal section was carried out following criterion CR performance. Lesion sites were confirmed histologically and by demonstration of severe chromatolysis in the left red nucleus. Tractotomy resulted in total or near-total loss of the CR. The UCR remained unaffected, as did limb placing, accuracy of striking at moving objects, running and walking. Postoperative scores remained at zero or near-zero for 2000-3000 trials, at which time training of the left limb resulted in scores of 75-80% within 5 sessions. In 1963, Voneida reported right limb CR loss following left sensorimotor cortical lesions, and in 1967 demonstrated that pyramidal lesions had no effect on CR performance. These results, in combination with those reported here and with Tsukahara's findings, ('81), lend support to the contention that a non-pyramidal, corticorubral, rubrospinal pathway represents a critical part of an associative mechanism in conditioned learning. Together with studies demonstrating that cerebellar lesions also severely impair conditioned limb and nictitating membrane responses, these data support a dual ("cooperative") control model recently proposed by Houk ('89), in which motor cortex/red nucleus represent an associative, memory-based system, and the cerebellum represents a motor programming, computational system. Interaction of these two systems, according to the Houk model, is mediated by various cortical and brain stem neuronal loops. Our findings, past and present, lend support to this model, and suggest further that at least in the case of a conditioned limb response, the rubrospinal tract represents a critical path by which the combined effect of these two mechanisms is expressed to final common pathway neuronal pools. (Supported by NINCDS Grant NS26053).

115.12

DISCRIMINATION OF BILATERAL INTER-SESSION UCS SHIFTS DURING NICTITATING MEMBRANE CLASSICAL TRACE CONDITIONING.

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It has been hypothesized that the correlation between shifts in the frequency of conditioned responses (CRs) and bilateral shifts of the unconditioned stimulus (UCS), in the rabbit nictitating membrane (NM) classical delay conditioning paradigm, is representative of some form of spatial learning (Greiner, Wilson & Patterson, *Neurosci. Abst.*, 15:890, 1989). It has also been hypothesized that trace conditioning of the NM preparation involves more hippocampal control than delay conditioning (Weisz, Solomon & Thompson, *Bull. Psychonom. Soc.*, 16, 1980). Various theories of hippocampal function describe it as a spatio-temporal, or cognitive map. The goal of this study is to use a trace conditioning procedure, similar to Greiner's procedure, in order to engage these spatial functions. The resulting trace data could then be compared to the earlier delay data in order to determine any behavioral differences.

Subjects (n=4) received 80 paired stimuli/session on the left eye for odd numbered sessions, and on the right eye for even numbered sessions, for a total of 16 sessions (not including an initial adaptation session). The stimuli consisted of a 100 msec 1 kHz tone conditioned stimulus (CS), and a 100 msec airpuff UCS. CS offset and UCS onset were separated by 400 msec.

A comparison of the delay and preliminary trace data shows no significant difference in the level of discrimination (defined as stimulated eye %CRs minus unstimulated eye %CRs) of the UCS shift. However, there is a significant effect ($p<.05$) due to session. In fact, the level of discrimination unexpectedly fluctuates in a regular manner, with both paradigms apparently synchronized, but not with the direction of the UCS shift.

115.13

INVESTIGATIONS OF THE MODULATION OF THE R1 AND R2 COMPONENTS OF THE REFLEXIVE EYEBLINK IN RABBIT. D.J. Weisz, T. A. Blanpied, and D.G. Harden. Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

In this experiment we began our investigation of the neural sites in the reflex pathways at which an auditory stimulus modulates the amplitude of a subsequently elicited reflexive eyeblink. A prior auditory stimulus can inhibit the amplitude of these reflexes at short interstimulus (ISIs) (20-80 msec) and facilitate the amplitudes at longer ISIs (100 to over 1000 msec). In this study we examined the effects of prior auditory stimulation on the R1 and R2 components of the reflexive eyeblink. The presentation of a brief periorbital shock elicits two discrete EMG responses (R1 and R2) from the orbicularis oculi, which reflect the output of disynaptic and polysynaptic reflex arcs, respectively. The disynaptic arc is comprised of cells whose cell bodies are in the semilunar ganglion, spinal trigeminal nucleus, and the facial nucleus. The polysynaptic arc is probably mediated by structures in the reticular formation in addition to those in the disynaptic loop. We found that both R1 and R2 exhibited significant facilitation at 250-8000 msec ISIs. No significant facilitation of R1 or R2 was seen at 30-125 msec ISIs; however, there was significant inhibition of R2 at a 30 msec ISI. These results indicate that the disynaptic reflex arc is facilitated by the prior presentation of an auditory stimulus and that inhibition of the behavioral eyeblink at short ISIs is probably mediated by the polysynaptic arc. In future studies we will determine which reflex arc is affected by lesions of structures that are critical for reflex facilitation (e.g. amygdala) or inhibition. (Supported by RO1MH42800)

115.14

CS/US PRE-EXPOSURE RETARDS ACQUISITION OF DISCRETE MOTOR RESPONSES USING HVI STIMULATION. A.F. Nordholm, R.A. Swain, and R.F. Thompson. Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

Prior studies have shown that paired presentations of tone CS and HVI stimulation produce robust classical conditioning (Brogden & Gantt, 1937; Shinkman, Swain & Thompson, 1989). Eight New England White rabbits were implanted with bipolar microstimulation electrodes in the white matter of HVI. Following 1 wk recovery, the animals were given five days of explicitly unpaired training prior to a maximum of twenty days of paired training. They were compared with a group of 8 animals that received only paired training.

The results indicated that baseline responding to CS alone presentations in unpaired training was $\leq$ 10%. Acquisition following CS/US pre-exposure was significantly retarded ($t(14)=-2.39$, $p<.05$). These results extend previous work using HVI stimulation as a US and emphasize that learning acquired in this paradigm follows all of the rules of normal associative conditioning.

Supported by NSF BNS8718300, ONR N0001488K0112, & McKnight to R.F. Thompson.

115.15

EFFECTS OF COOLING INTERPOSITUS DURING ACQUISITION OF CLASSICAL CONDITIONING. D.G. Lavond, A.S. Kanzawa*, V. Esquenazi*, R.E. Clark & A.A. Zhang. Dept Psych & Bio Sci/HNB 501, Univ So Cal, Los Angeles, CA 90089-2520.

Rabbits were implanted with a cooling probe near the cerebellar interpositus nucleus (IN) (Antr 0.5, Lat 6.5, Vent 14.0 mm from lambda), and implanted with multiple-unit recording electrodes in IN bilaterally and contralaterally in the red nucleus. After 1 wk recovery, they were adapted, and trained on 5 days for classical conditioning while the probe tip was cooled to +/-10 °C (CS=conditioned stimulus, 1KHz, 85 dB SPL, 352 msec tone; UCS=unconditioned stimulus, 2.1 N/cm^2, 100 msec corneal airpuff; CR/UCR=conditioned/unconditioned responses nictitating membrane extension measured with a Solomon minitorque potentiometer; 12 blocks of CS, CS-UCS, CS-UCS, CS-UCS, UCS, CS-UCS, CS-UCS, CS-UCS, CS-UCS, CS-UCS for a total of 108 trials per day; 20-40 sec intertrial interval). During cooling the rabbits had UCRs but no CRs. They were then trained on 5 days without cooling. There was no evidence for retention, and CRs developed as would be expected for naive rabbits. These results are discussed in relation to the idea that IN is a putative site for learning and memory, and related to lidocaine experiments by Chapman (1988 Stanford dissertation) and by Welsh & Harvey (Neurosci., 15:639, 1989).

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115.16

COOLING CEREBELLEAR HVI LOBULE DOES NOT ABOLISH CONDITIONED RESPONSES. A.A. Zhang, R.E. Clark & D.G. Lavond. Dept Bio Sci & Psych/HNB 501, Univ So Cal, Los Angeles, CA 90089-2520.

A previous study has shown that temporary cooling of the interpositus nucleus reversibly abolishes learned responses (Lavond et al. Neurosci. 15: 889, 1989).

In the present study, rabbits were implanted with a cooling probe next to cerebellar lobule HVI. Multiple unit recording electrodes were implanted in HVI bilaterally and in red nucleus contralaterally. After recovery, the rabbits were overtrained for classical eyeblink conditioning. The conditioned stimulus was 1 KHz, 85 dB SPL, 352 msec tone coterminating with 2.1 N/cm^2, 100 msec corneal airpuff unconditioned stimulus, and nictitating membrane extension was measured as the response. A total of 108 trials were given each day.

We then attempted to inactivate cerebellar cortex HVI by injecting Freon into the probe implanted next to HVI. We tested with 27 normal trials, 54 trials with the cooling probe turned on, and finally 27 more normal trials. This procedure was repeated on 5 days.

Results indicate that learned behaviors are not abolished for the duration of cooling. These results support previous observations that cerebellar cortex is not essential for classical conditioning.

Supported by NSF BNS-8906612.

115.17

REACQUISITION OF CLASSICAL CONDITIONING AFTER REMOVAL OF CEREBELLAR CORTEX IN DUTCH BELTED RABBITS. R.E. Clark, D.J. Brown*, R.F. Thompson & D.G. Lavond. Dept Psych & Bio Sci/HNB 501, Univ So Cal, Los Angeles, CA 90089-2520.

While many agree that cerebellar interpositus lesions abolish or prevent classical conditioning, the role of cerebellar cortex is controversial. Yeo and colleagues report that HVI lesions also abolish conditioning (Beh. Brain Res., 13:261-266, 1984). However, Lavond and colleagues report both retention and acquisition without cerebellar cortex (Exp. Brain Res., 67:569-593, 1987; Beh. Brain Res., 33:113-164, 1989). Of many possible explanations, only strain differences (Yeo's Dutch Belted vs Lavond's New Zealand White) remain untested.

In the present study we used Dutch Belted rabbits (n=3) and replicated Yeo's paradigm: 550 msec CS, either 90 dB SPL white noise or 190 lux light; 50 msec UCS, 2.5 mA, 60 Hz periorbital stimulation; 15 sec intertrial interval; 220 daily trials, 50% light CS and 50% noise CS; 5 days of training, cerebellar aspiration, 1 week recovery, retraining. The rabbits were retrained for 20 days, and then trained for 20 more days using our usual paradigm (Thompson et al. Am. Psych., 31:209-227, 1976). Preliminary evidence indicates that Dutch Belted rabbits relearned following the lesion using both paradigms.

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115.18

MEASUREMENTS OF REFLEXIVE REACTIONS TO DIFFERENT UNCONDITIONED STIMULUS INTENSITIES OVER THE COURSE OF CLASSICAL CONDITIONING. D. Ivkovich, D.G. Lavond, C.G. Logan, & R.F. Thompson, Neurosciences Program, University of Southern California, Los Angeles, CA 90089-2520.

Rabbits were classically conditioned with a 3 psi unconditioned stimulus (UCS) to the cornea, lesioned in the ipsilateral interpositus (IP) nucleus, and subsequently retrained. Reflexive eyeblinks to 4 different UCS intensity levels (1, 2, 3, & 4 psi) were measured over the course of training. These input/output (i/o) tests included amplitude, onset and peak latency, and amplitude-time area of the unconditioned responses (UR). The lesion completely abolished conditioned responses in all animals.

Amplitudes and latencies of URs in i/o tests varied significantly with airpuff intensity. After training, the UR amplitudes to 3 and 4 psi were significantly larger than prior to training. Immediately after the lesion, UR amplitudes at 3 and 4 psi were slightly reduced but returned to prelesion levels after retraining. The lesion did not affect onset or peak latencies.

Preliminary results with animals receiving random unpaired training suggest that there was no training potentiation of responses to 3 and 4 psi.

Supported by NSF BNS8718300, ONR N0001488K0112, & McKnight to R.F. Thompson.

115.19

ANATOMICAL RELATIONSHIPS BETWEEN THE CEREBELLAR VERMIS AND THE PARABRACHIAL NUCLEI IN THE RABBIT. W. F. Supple, Jr. and B. S. Kapp. Department of Psychology, University of Vermont, Burlington, VT 05405.

The cerebellar vermal cortex is importantly involved in aversive classically conditioned bradycardic responses in the rabbit. Lesions block acquisition and retention of bradycardia,and Purkinje cells in this region show electrophysiological changes which accompany the learned response. This study examined the connections between the anterior vermis and the parabrachial nucleus (PBN), a region recriprocally connected with the amygdaloid central n.(ACE) which is also involved in conditioned bradycardia.

New Zealand albino rabbits received 20 - 40 nl injections of WGA-HRP into either the lateral PBN (IPBN), anterior vermis or rostral fastigial nucleus. Following 1 to 2 d survival, the brains were processed using the TMB procedure of Mesulam. Injections into the IPBN resulted in anterogradely labeled fibers in lobules III, IV and V of the anterior vermis. These fibers were unusual as they appeared to be neither mossy nor climbing fibers, and were distributed to each layer of the cortex in a pattern similar to that observed by Dietrichs & Haines (Anat. Embryol.,173: 279, 1985) for hypothalamocerebellar fibers in the cat. These PBN injections also resulted in retrogradely labeled fastigial nucleus neurons. Injections into the vermis produced many retrogradely labeled neurons in the lateral and medial PBN, and there was evidence of anterograde terminal labeling in the IPBN. Fastigial injections resulted in terminal label in the PBN but no retrogradely labeled neurons were observed.

These findings suggest the existence of a neuroanatomical circuit from the PBN to anterior vermis to fastigial n. to PBN in the rabbit. The vermal connections with the PBN, which in turn is reciprocally connected with the ACE, suggests a potential important interrelationship between cerebellar and amygdaloid neurons in the acquisition of conditioned bradycardia in the rabbit.

115.20

RED NUCLEUS PROJECTIONS TO THE ACCESSORY ABDUCENS NUCLEUS IN RABBIT REEXAMINED WITH WGA-HRP. M.E. Rosenfield* and J.W. Moore. Dept of Psychol, Univ of Mass, Amherst, MA 01003.

The accessory abducens nucleus (AAN) is the main pool of motoneurons innervating eye ball retraction and the nictitating membrane response (NMR). Motor commands for the classically conditioned NMR presumably originate in the cerebellum and are relayed to AAN via the red nucleus (RN). We implanted WGA-HRP (Sigma L3892) unilaterally into the region AAN in 5 albino rabbits (method of Mori, J., et al, *Brain Res Bull*, 6:19, 1981). The pipette remained *in situ* for 53 hours before sacrifice. Animals were perfused transcardially (descending aorta clamped) with approximately 2 L of .9% saline followed by 1 L of 10% formalin and then 3 L of 12% sucrose solution at 4 degrees C. Brains were blocked immediately on extraction (saving only the brain stem and cerebellum), placed in 30% sucrose in .1 M phosphate buffer (pH = 7.2), and stored at 4 degrees C for 24 h. Brain stem and cerebellum were embedded in gelatin; frozen sections were cut transversely at 60 μ, mounted on subbed slides, and reacted with tetramethylbenzidine (TMB). All cases showed retrogradely labeled cells in the caudal portions of magnocellular RN, consistent with (a) projections from "eye/face" regions of the cerebellum to RN, (b) Rosenfield and Moore's RN lesion studies of NMR conditioning (*Behav Brain Res*, 17:77, 1985), (c) Desmond, J., et al's HRP implant study of AAN (*Brain Res Bull*, 10:747, 1983).

This work was supported by grant AFOSR 89-0391.

STRESS, HORMONES AND THE AUTONOMIC NERVOUS SYSTEM II

116.1

MICRODIALYSIS OF THE DORSOMEDIAL HYPOTHALAMUS (DMH) WITH NIPECOTIC ACID (NA) ATTENUATES STRESS-INDUCED TACHYCARDIA. J.J. Anderson* and J.A. DiMicco, Dept. of Pharm. & Tox., Indiana Univ. Sch. of Med., Indianapolis, IN 46202-5120.

Injection of muscimol, a $GABA_A$ receptor agonist, into the DMH blocks the increase in heart rate (HR) seen in air stress in rats. This study examined the effect of microdialysis of the DMH with NA, an inhibitor of GABA uptake, on extracellular levels of GABA and on HR and mean blood pressure (MBP) in an air stress paradigm. Rats were instrumented with a microdialysis probe in the DMH on one side (for perfusion with 0.5 mM NA or Ringer's solution starting 2-2.5 hr prior to stress) and a guide cannula (for injection of 88 pmol muscimol or saline 5 min prior to stress) in the contralateral DMH. Below are increases (mean ± SEM, n=6) from baseline HR and MBP 5 min after the start of stress:

	HR (bpm)	MBP (mmHg)
Ringer-saline	132 ± 12*	17 ± 2*
NA-saline	107 ± 8*	14 ± 2
Ringer-muscimol	88 ± 5*	13 ± 2
NA-muscimol	28 ± 8*	5 ± 3*

* different from the other 3 groups by ANOVA ($p<0.05$). Perfusion with NA elevated [GABA] in dialysates 6-8 fold. These results suggest that elevating extracellular levels of endogenous GABA in the DMH reduces the tachycardia induced by stress. (Supported by NS 19883 and American Heart Association, Indiana Affiliate)

116.2

STRESS-INDUCED INCREASE IN GLUTAMATE UPTAKE AND RELEASE. V.H. Gilad, Y. Tizabi, R.J. Wyatt and G.M. Gilad. Neuropsychiatry Branch, NIMH Neuroscience Center at St. Elizabeths Hospital, Washington, DC 20032 and Dept. of Pharmacology, College of Medicine, Howard University, Washington, DC 20059.

We sought to determine if stressful stimuli cause changes in the activity of rat forebrain neurons that use glutamate as their neurotransmitter. Measurements of glutamate high-affinity uptake and release in synaptosomal preparations served as indices. Restraint stress lasting 0.5 to 4 hours, but not shorter, led to a 40-50% increase in both indices in the frontal cortex, hippocampus and septum, but not in the striatum. The increase in uptake was observed with glutamate concentrations of up to 10 μM, but not higher. The results indicate that forebrain glutamatergic neurons are activated by stressful stimuli in a regionally selective manner.

116.3

CARDIOVASCULAR REACTIVITY TO NON-SIGNAL STIMULI: NORMAL AND CATECHOLAMINE-DEPLETED RATS. K.S. Quigley, B.M. Potter*, G.G. Berntson and J.P. Bruno, The Ohio State Univ., Columbus, OH 43210.

We examined the autonomic origins of cardiovascular response to non-signal stimuli in rats. Pharmacological blockade of sympathetic (atenolol) or vagal (scopolamine) innervation to the heart indicated that the acceleratory response to a high intensity tone (75 db) was largely of sympathetic origin, whereas the deceleratory response to a low intensity tone (55 db) appeared to arise from co-activation of both vagal and sympathetic controls. To examine the potential contributions of central catecholamines (CA) to cardiovascular responses, we also tested adult animals that had received neonatal CA depletions. Such animals have been reported to exhibit deficits in the development of sympathetic control. In fact, CA-depleted animals exhibited a more robust acceleratory heart rate response to the high intensity stimulus which included an additional short-latency acceleratory component reminiscent of the startle response. Both components were blocked or attenuated by atenolol, but were unaffected by scopolamine. Contrary to previous speculation, these animals may evidence increased sympathetic lability.

116.4

AGE-RELATED DIFFERENCES IN STRESS-INDUCED ADRENAL HORMONE RESPONSES. B.Buwalda*, S.M.Korte*, G.Bouws*, P.G.M.-Luiten, J.M.Koolhaas* and B.Bohus*, Dept. of Animal Physiology, University of Groningen, P.O. Box 14, 9750 AA HAREN, The Netherlands.

There is no general agreement on the nature of sympatho-adrenal stress responses in aged mammals. The type and intensity of stressors and the behavioural responses they evoke may be the intervening factor. Mild stress of sudden silence imposed on background noise does not evoke overt behavioural changes and therefore seems suitable to compare catecholamine and corticosterone responses in young (3 mo. old) and aged (24 mo. old) male Wistar rats equiped with permanent venous catheters. Blood samples were taken in the home cage before and after exposure to the mild stress in another environment. Post-test samples were also taken with delay in order to investigate recovery rate to baseline.

Basal levels of norepinephrine (NE) and corticosterone (CORT) were elevated in the aged rats while no differences in epinephrine (E) levels were found. Stress-induced increases of E and NE were similar but CORT response was blunted in the aged animals. Finally, recovery of all three adrenal hormones were impaired in the aged rats. Although young and aged rats exhibit comparable increments in plasma levels of catecholamines, the blunted CORT response and the slower recovery rates suggest an age-related impairment in the regulation of adrenal function in relation to mild stress.

116.5

TEMPORAL CHARACTERISTICS OF STRESS-INDUCED SECRETION OF PITUITARY-ADRENAL HORMONES AND BRAIN CATECHOLAMINES IN PRENATALLY STRESSED MALE RAT PUPS. L. K. Takahashi, N. Cai,* J. G. Turner,* and N. H. Kalin. Dept. Psychiatry, Univ. Wisconsin Med. Sch., Madison, WI 53792 and Middleton Veterans Hospital, Madison, WI 53705.

We showed that stressed pregnant rats produce pups with heightened stress-induced secretion of pituitary-adrenal hormones. We now report on the temporal characteristics of this potentiated stress-induced hormone release. We also measured brain NE and DA because they mediate stress-induced hormonal and behavioral responses.

14-day-old prenatally stressed (PS) and control male Sprague-Dawley rats received 5 foot shocks (0.5 mA, 1 s) in a 10-min period. Plasma ACTH and corticosterone were measured by RIA 0, 0.5, 1.0, 2.0, and 4.0 h after shock. A no-shock control group provided basal values. NE and DA levels were determined by HPLC using EC detection.

Plasma ACTH was elevated ($p<.001$) in PS pups only at 0 h; plasma corticosterone was elevated not only at 0 h but also at 0.5 and 4.0 h ($p<.01$), suggesting increased sensitivity of the adrenal to ACTH. In PS pups, basal, 0, and 4 h hypothalamic and cortical NE were elevated ($p<.01$), whereas cortical DA was reduced ($p<.01$). This may reflect compromised neural systems that facilitate coping which consequently may increase the vulnerability of PS offspring to the deleterious effects of stress.

Supported by NIMH grant MH-43986.

116.6

TYROSINE PROTECTS HUMANS FROM THE ADVERSE EFFECTS OF ACUTE EXPOSURE TO HYPOXIA AND COLD. H. R. Lieberman, L. E. Banderet* and B. Shukitt-Hale*. U.S. Army Research Institute of Environmental Medicine, Natick, MA 01760.

In rodents, administration of tyrosine, a food constituent and precursor of the catecholamines, ameliorates some of the behavioral and neurochemical deficits caused by exposure to acute stressors. In an initial study, tyrosine (100 mg/kg, p.o.) protected humans from some adverse behavioral effects of exposure (4.5 h) to a combination of hypoxia (4200 m and 4700 m) and cold (15° C) (Banderet and Lieberman, Brain Res. Bull., 22:759-762, 1989). To extend these findings we conducted a dose-response study using similar environmental conditions (4700 m and 17° C) and increased the exposure to 7 h. Using a double-blind, placebo-controlled crossover design we administered 85 and 170 mg/kg of tyrosine p.o. to 21 male volunteers. Cognition, symptoms and affect were assessed using: Bakan vigilance task, visual pattern recognition, Environmental Symptom Questionnaire (ESQ) and Profile of Mood States (POMS). Among volunteers with the greatest response to the stressors, both doses of tyrosine significantly ($p<.05$) reduced the adverse behavioral effects of exposure to the stressors. Vigilance, pattern recognition, distress (ESQ), alertness (ESQ), depression (POMS) and confusion (POMS), were less impaired when tyrosine was given.

116.7

RELATIONSHIP BETWEEN URINARY CATECHOLAMINE LEVELS AND SEVERITY OF POST-TRAUMATIC STRESS DISORDER

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We have previously found elevations in urinary norepinephrine (NE) and epinephrine (E) concentrations in combat veterans with PTSD compared to other psychiatric groups. In the present study we set out to replicate these pilot results in a larger sample of veterans with PTSD (n=18) and to explore the relationship between catecholamine levels and severity of PTSD symptoms. The mean urinary NE concentration was significantly higher in the PTSD group compared with normal male controls (51.9 ± 38.4, PTSD; 31.6 ± 9.4, controls). No significant group differences were observed in mean E or dopamine (DA) levels. Correlational analysis showed that both NE and DA, but not E, were significantly related to scores on the Combat Exposure Scale and to overall PTSD symptoms as measured by the Impact of Events Scale (IES). In particular, NE was related to scores on the 'avoidance' subscale of the IES, while DA was related to scores on the 'intrusive' subscale. In contrast, none of the three catecholamine levels were found to correlate with depression of psychotic symptoms. The results support the idea of an enhanced sympathetic nervous system activation in PTSD, and suggest that increased sympathetic arousal may be closely linked to severity of PTSD symptoms.

116.8

PHYSICAL STRESS, EPINEPHRINE, DEXTROSE, & INSULIN INCREASE BOUND SIALIC ACID OF MOUSE BRAIN. L. Cherian and W. R. Klemm. Dept. Veterinary Anatomy, Texas A&M University, College Station, TX 77843

Our previous research showed that ethanol can decrease total brain sialic acid (SA). Because ethanol can act as a physiological stressor, some of ethanol effect might be due to stressor action. In this present study we tested the hypothesis that stress could decrease brain SA by comparing brain SA levels in non-handled/non-injected mice with those given saline and various physical stressors and drugs.

Total whole-brain SA in male outbred mice decreased, compared to non-injected mice, at 3 and 4 hours after IP saline. This lagged behind an earlier increase in free SA. Physical stressors, such as tail and foot shock, *increased* total brain SA 4 hrs later. To explore mechanisms, we tested several stress-related drugs. In one study, total brain SA increased about 32%, compared to saline-injected controls, in mice sacrificed 4 hours after epinephrine (0.1 mg/kg). Prednisolone (8 mg/kg) and morphine (25 mg/kg) did not seem to change brain SA levels from the decreases seen in their respective saline-injected control groups. The epinephrine effect may be due to increased availability of glucose substrate for SA synthesis. Dextrose, given IP at 2 gm/kg, produced a similar increase in total bound brain SA 4 hrs. later. Insulin (10 units/kg) also caused a similar increase. Although insulin decreases blood glucose, glucose is more accessible for SA synthesis because of enhanced transport into cells. We discuss the possibility that brain SA may be a component of the classical pituitary-adrenal stress response. Supported partly by grant AA 06920, NIAAA.

116.9

HEMODYNAMIC AND HUMORAL RESPONSES TO ACUTE EMOTIONAL STRESS. M.H. Zink III* and R.H. Alper, The University of Kansas Medical Center, Kansas City, KS. 66103

The role of stress in the pathogenesis of hypertension has long been postulated. Several models are used to examine the cardiovascular and neuroendocrine responses to chronic and acute stressors. These experiments were designed to study concurrently hemodynamic and hormonal effects of emotional stress in conscious, unrestrained male Sprague-Dawley rats. After acclimation in an operant conditioning chamber, rats were exposed to a warning tone for 1 min followed by a 3 sec, 2 mV inescapable footshock (*Stress*) or no footshock (*Control*). Mean arterial pressure (MAP), heart rate (HR), renal blood flow (RBF), and renal vascular resistance (RVR) were continuously monitored. Plasma renin activity (PRA) was determined 5, 15 and 30 min after the tone. Exposure to tone only produced a mild increase in MAP and HR which returned to baseline within 1 min after cessation of the tone. The *Stressed* rats displayed elevated MAP and HR for 5 min after shock with a return to baseline by 15 min. PRA and RBF were not altered in either group. In *Control* rats tested 3 times on one day at 1 hr intervals, and then a fourth time on the following day, the increase in MAP observed during the tone was attenuated with each exposure; tachycardia was present during the tone in all four trials. In *Stressed* rats, the increase in MAP was greater with each of the four exposures to the tone. Tachycardia was observed only upon the initial exposure to the tone in the *Stressed* rats; the increase in HR was suppressed in subsequent trials. Stress did not increase PRA. In a limited number of rats RBF was not altered but RVR increased 40% due to the increase in MAP. Thus it appears that inescapable footshock produces a conditioned increase in MAP, decrease in HR and little or no effect on RBF or PRA.

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116.10

INDOLE-MEDIATED ADAPTATION: DOES MELATONIN MEDIATE RESISTANCE TO STRESS IN HUMANS? K.G. Walton,[1] G.M. Brown,[3] N. Pugh,*[1] C. MacLean*[1] and P. Gelderloos.*[2] [1]Neurochemistry Lab, Dept. of Chemistry, and [2]Dept. of Psychology, Maharishi International University, Fairfield, IA, USA 52556 and [3]Dept. of Biomedical Sciences, McMaster University, 1200 Main St. West, Hamilton, ON, Canada L8N 3Z5.

Studies in animals suggest the increase of melatonin in response to stressors is important in physiological adaptation. Some authors claim that, in addition to a timekeeping function, melatonin has a tranquilizing or "anti-stress" role. Since excretion of the metabolite 6-sulfatoxymelatonin (6SM) is a gauge of melatonin turnover, we investigated the relationship between 6SM excretion and stress in college students. Urinary 6SM was tested by radioimmunoassay. Levels of stress were indicated by scores on Profile of Mood States and Spielberger's State-Trait Anxiety Inventory as well as by status as practitioner (TM, $n = 22$) or non-practitioner (NonTM, $n = 32$) of the Transcendental Meditation and TM-Sidhi program, a program known to reduce stress. Use of drugs (alcohol, tobacco and caffeine) was monitored by questionnaire and was minimal in the entire TM group but high in some of the NonTM group. Excretion rates of 6SM were higher in the NonTM group and also were affected by drug use and by gender. For example, daytime 6SM for NonTM men was 566 ± 448 ng/h ($n = 8$) while for TM men it was 164 ± 107 ($n = 11$; $t = 2.9$, $p = .01$). In women, nighttime levels differed most; e.g. NonTM drug-user women (799 ± 399, $n = 14$) were higher than the TM women (498 ± 235, $n = 11$; $t = 2.21$, $p < .04$). Psychological tests supported the presumption that drug users were high-stress, drug-free NonTM subjects were intermediate-stress and TM subjects low-stress. Anxiety and mood disturbances were highly correlated with 6SM excretion, but were often opposite for men and women. Positive correlations between 6SM and 5-HIAA also were found, suggesting the functions of serotonin and melatonin are linked. We conclude that high stress and high melatonin turnover are associated, with details dependent upon gender. This relationship could reflect gender-specific roles of melatonin in resisting or eliminating stress.

116.11

EFFECTS OF MILD STRESS ON SEROTONIN TURNOVER AND OPEN-FIELD BEHAVIOR IN STREPTOZOTOCIN-DIABETIC RATS. LL Bellush & SG Reid. Dept. of Psychology, Ohio University, Athens, OH 45701.

Chronically-hyperglycemic diabetic (D) rats have reduced turnover of 5-HT in several brain regions. Increased 5-HT turnover was recently implicated in restraint stress-induced effects on food intake and open field activity. In the present investigation, we noted increased 5-HT turnover in 3 brain regions (frontal cortex, amygdala and brain stem) of D and nondiabetic (ND) rats after 1/2 hr restraint stress. D also had significantly reduced turnover relative to ND, as well as smaller increments in turnover in response to restraint. Both restraint and diabetes were associated with enlarged adrenal glands and reduced adrenal epinephrine concentration, although plasma corticosterone elevations after restraint were similar in D and ND. In a second study, we evaluated the functional relevance of these changes by measuring open field activity in D and ND 24 hrs after 2 hr restraint in round plexiglass tubes. Restraint had no effect on the behavior of ND in the open field, but led to significant reductions in both general activity and exploratory behavior in D rats.

ISCHEMIA II

117.1

THE EFFECT OF CGS-19755 ON LOCAL CEREBRAL pH (LCpH) AND CEREBRAL BLOOD FLOW (CBF) IN MIDDLE CEREBRAL ARTERY (MCA) AND IPSILATERAL COMMON CAROTID ARTERY (CCA) OCCLUDED RATS.
S.Takizawa, M.Hogan*, A.Hakim.* Brain Imaging Center, Montreal Neurological Institute, Montreal, Canada, H3A 2B4.

Systemic administration of CGS-19755, a potent competitive NMDA receptor antagonist, has been reported to result in better clinical outcome and smaller infarct volumes. However, we observed that even in ischemia, CGS-19755 crossed the blood-brain barrier poorly. This suggested that the observed in-vitro binding of the drug may not be functionally relevant. We report here the effect of intravenous infusion of CGS-19755 (10mg/kg bolus + 5mg/kg/hr for 4 hours) on LCpH and CBF in Sprague-Dawley rats with MCA + CCA occlusion compared to ischemic controls without treatment and others receiving CGS-19755 carrier. LCpH and CBF were determinied simultaneously using ^{14}C-dimethyloxazolidinedione and ^{14}C-iodoantipyrine by a double-label autoradiographic technique. Our data are as follows:

Structure on Occluded Side	Control Group (n=4) CBF ml/hg/min	pH	CGS-19755 (n=4) CBF ml/hg/min	pH
Caudate-putamen	6.2 ± 1.5	6.67 ± 0.06	46.6 ± 26.4	6.98 ±0.10
Hippocampus	74.2 ± 7.3	7.16 ± 0.02	130.8 ± 25.3	7.11 ± 0.03
Parietal cortex	11.7 ± 8.1	6.86 ± 0.04	19.4 ± 8.8	6.98 ± 0.10
Sensorimotor cortex	27.2 ± 8.2	6.93 ± 0.06	71.5 ±13.3	7.13 ±0.02

Values are mean ± SEM

These preliminary data suggest that the neuroprotective effect of CGS-19755 in ischemia may be related to vasodilator properties and ability to correct post-ischemic cerebral acidosis.

117.2

EXTRACELLULAR GLUTAMATE IN GERBIL BRAIN FOLLOWING REPEATED ISCHEMIA EVALUATED BY IN VIVO MICRODIALYSIS. N. Saito*, C. Chang*, K. Kawai*, T. S. Nowak, Jr., M. Spatz and I. Klatzo. Lab. of Neuropathol. and Neuroanatom. Sci., NINDS, NIH, Bethesda, MD 20892.

Repeated brief ischemic insults result in cumulative brain injury, particularly evident as severe delayed edema at 24 h recirculation accompanied by widespread serum protein extravasation and morphological damage. A progressive reduction in tissue oxygen tension is observed during the intervals between repeated insults suggesting that persistent hypoxia may play a role in the cumulative effect, but specific functional derangements have not yet been described. In the present study the time course of changes in extracellular glutamate was evaluated by cortical microdialysis in halothane anesthetized gerbils subjected to a series of three 5 min bilateral carotid artery occlusions at 1 h intervals. Preischemic and sham occluded animals showed stable baseline levels of 0.5-2 pmol/µl dialysate with the enzymatic cycling assay employed. A single 5 min occlusion resulted in modest glutamate release, rarely achieving more than 3-4 pmol/µl over baseline, which resolved in 30-60 min. Repeated occlusions generally resulted in several-fold further increments in glutamate release even when levels had returned to baseline between occlusions, often achieving concentrations of 10-20 pmol/µl in the dialysate, comparable to that seen after a longer 15 min single occlusion. Mechanisms responsible for this potentiation remain to be identified. Nevertheless, these results identify progressive increases in glutamate release as a specific component of the pathophysiology of repeated ischemic insults which may further contribute to their cumulative impact.

117.3

GLUTAMATE PLAYS A MINOR ROLE IN THE MEMBRANE EVENTS LEADING TO NEURONAL DEATH IN A PHARMACOLOGICAL MODEL OF ISCHEMIA *IN VITRO*. EMMA R. WOOD, and PETER B. REINER, Depts. of Psychology and Psychiatry, University of British Columbia, Vancouver, B.C., Canada.

A prominent hypothesis concerning the mechanism of ischemic damage is that of glutamate neurotoxicity. We examined the effects of glutamate antagonists in a pharmacological model of cerebral ischemia *in vitro*. 400 μm rat hippocampal slices were maintained in standard ACSF, and intracellular recordings were obtained from pyramidal neurons in the CA1 subfield. Pharmacological ischemia was induced by the addition of 10 mM iodoacetic acid and 1 mM sodium cyanide to the bathing medium. This treatment mimics ischemia *in vivo* in that iodoacteic acid prevents the glycolytic production of ATP by blocking the enzyme 3-phosphoglyceraldehyde dehydrogenase, and cyanide blocks oxidative phosphorylation by inhibiting the mitochondrial enzyme cytochrome a_3. Thus both the hypoglycemia and the hypoxia that occur during ischemia *in vivo* are reproduced. This treatment causes a characteristic hyperpolarisation, loss of membrane resistance, and depolarisation, eventually leading to loss of membrane function. A combination of 200 μM APV, 10 μM CNQX and 1 mM kynurenic acid, (which together block NMDA, AMPA and kainate receptors) was applied to the bathing medium for 10 minutes prior to, and during pharmacological ischemia. Experimental and control slices were always obtained from the same rat. Glutamate antagonists increased both the duration and the magnitude of the hyperpolarisation associated with pharmacological ischemia, and delayed the cell depolarisation significantly. These effects however, were not *enormous*, and cell death occurred rapidly in every case, implying that while glutamate may contribute to the pathophysiology of ischemia, it is only one of a number of mediators of cell death.

117.4

GLUTAMATE-INDUCED NEUROTOXICITY AND THE CYTOPROTECTIVE PROPERTIES OF IFENPRODIL, A NOVEL NMDA ANTAGONIST. D. Graham* and S.Z. Langer. Synthélabo Recherche (L.E.R.S.), 58 rue de la Glacière, 75013 Paris, France.

In animal models of focal cerebral ischaemia ifenprodil has been shown to exhibit cytoprotective activity (Gotti et al., J. Pharmacol. Exp. Ther. 247, 1211, 1989). Interestingly, recent data indicate that ifenprodil labels a polyamine-sensitive site associated with the NMDA receptor complex (Schoemaker et al., Eur. J. Pharmacol., 176, 249, 1990). We have therefore studied the effect of ifenprodil in a neurotoxicity cell culture model. Dispersed cocultures of neurones and glia were prepared from fetal mice cerebral cortices at 15-17 d gestation and maintained in a humidified CO_2 atmosphere at 37°C for 15-19 d. Neurotoxicity was induced by acute L-glutamate exposure and cell-death assessed by measurement of the amount of the cytosolic enzyme, lactate dehydrogenase (LDH), released into the bathing medium after 20 h. L-Glutamate exposure produced a concentration-dependent increase in released LDH activity. This effect of L-glutamate was potentiated in the presence of glycine (10 µM). L-Glutamate (500 µM)-induced neurotoxicity was inhibited in the presence of the NMDA channel blocker MK801 and ifenprodil with IC_{50} values of 30 nM and 200 nM, respectively. These results support the view that the neuroprotective properties of ifenprodil are exerted through an interaction at the polyamine-sensitive site on the NMDA receptor complex.

117.5

COMBINED NICARDIPINE AND MK-801 TREATMENT REDUCES CEREBRAL ISCHEMIC DAMAGE. D. CORBETT AND K. E. HEWITT *. Basic Medical Sciences, Faculty of Medicine, Memorial University, St. John's, NF, CANADA, A1B 3V6.

Excessive calcium influx and prolonged activation of the N-methyl-D-aspartate(NMDA) receptor may contribute to cerebral ischemic damage. The present study examined the anti-ischemic effects of the calcium channel antagonist, nicardipine and the NMDA antagonist, MK-801.

Forty gerbils were subjected to a 5 ' bilateral occlusion of the carotid arteries under halothane anesthesia. The MK-801 group received a 5.0 mg/kg (i.p.) injection 15 ' after occlusion. The nicardipine group was given a 0.5 mg/kg i.p. injection 15' after occlusion plus a 1.0 mg/kg/day infusion of the drug via an osmotic mini- pump. The NMK group received the above MK-801 and nicardipine drug treatments. A control group was treated identically to the NMK group except saline was used in place of MK-801 and nicardipine. Cell counts of CA1 revealed a protective effect of MK-801 and nicardipine given only in combination ($F(3, 27) = 3.641$, $P < .05$, Scheffe test). Combined drug therapy may be a useful approach in treatment of ischemic brain injury. Supported by MRC.

117.6

THERAPEUTIC EFFICACY OF LY-233053, A COMPETITIVE GLUTAMATE ANTAGONIST, IN EXPERIMENTAL CNS ISCHEMIA. K.P. Madden, W.M. Clark*, A. Kochhar, and J.A. Zivin. Department of Neurosciences, University of California, San Diego.

Glutamate antagonists offer a promising new avenue of therapy for CNS ischemia. Non-competitive antagonists have provided neuroprotection in a variety of models of neuronal injury. However, these agents cause marked sedation, which confounds tests of functional outcome following CNS ischemia and limits potential clinical usefulness. We tested the clinical effect and therapeutic efficacy of LY-233053, a new competitive glutamate antagonist, in a well-established rabbit model of spinal cord ischemia. Reversible ischemic insult was induced with temporary compressive occlusion of the infrarenal aorta. Duration of ischemia was varied to provide a range of durations in each experimental group. The P_{50} represents the duration that resulted in permanent paraplegia in 50% of animals. Five minutes after onset of ischemia, animals received IV bolus of either 50 or 100 mg/kg of LY-233053. Fifteen animals receiving 50 mg/kg displayed ataxia without apparent sedation and had a P_{50} of 31.1 ± 1.9 minutes. Fifteen animals receiving 100 mg/kg displayed modest sedation and had a P_{50} of 39.4 ± 2.7 minutes. These values were significantly longer than the P_{50} of 25.2 ± 1.5 minutes for 14 control animals (t= 2.42 and 4.62, respectively). We conclude that competitive glutamate antagonists may provide significant neuroprotection in CNS ischemia without major sedative effects.

117.7

DEXTROMETHORPHAN PREVENTS HYPOXIA-INDUCED LOSS OF HIPPOCAMPAL FUNCTION. R. J. Radek and W. J. Giardina. Neuroscience Research Division, Abbott Laboratories, Abbott Park, Il 60064

Dextromethorphan (DEX), an opioid class antitussive, has neuroprotective effects in animal models of brain ischemia and hypoxic cortical cell cultures. The purpose of this study was to determine whether DEX prevents hypoxia-induced loss of synaptic function in an in vitro hippocampal slice preparation. The population spike (PS) was recorded from from the CA1 pyramidal cell layer of 400µ thick guinea pig-derived hippocampal slices. The PS was irreversibly blocked in slices that were superfused with $N_2(95\%)/CO_2(5\%)$ gassed ACSF for 30 minutes at 37° C. The PS recovered in 9 of 10 slices that were superfused with DEX at 100µM during the exposure to hypoxia. The mean PS amplitude in these 9 slices was 42% of pre-hypoxic amplitude. The PS returned in 1 of 5 slices superfused with 10µM DEX. The PS returned in 4 of 6 slices superfused with 100µM DL-2-amino-5-phosphonovaleric acid (AP-5), an NMDA receptor antagonist. The mean PS amplitude of these 4 slices was 51% of pre-hypoxia amplitude. These results show that DEX, like the NMDA receptor antagonist AP-5, has neuroprotective properties in the hypoxia sensitive hippocampus.

117.8

FUNCTIONAL NEUROPROTECTION WITH MK801: REDUCED RADIAL MAZE IMPAIRMENT IN GERBIL ISCHEMIA MODEL. C.A. Boast and S.A. Maurer*. Wyeth-Ayerst Research, Princeton, NJ 08543-8000.

Forebrain ischemia results in selective CA1 hippocampal damage and disrupts radial maze performance (Volpe, et al, Stroke,15:558,1984). The noncompetitive NMDA antagonist, MK801, provides protection from ischemic brain damage (Foster, et al, Neurosci. Lett., 76:307, 1987). We assessed whether MK801 would reduce a radial maze impairment in ischemic gerbils. Female Mongolian gerbils were trained on an eight arm radial maze (four arms baited) until the criterion of 75% correct on both reference and working memory was met for three consecutive sessions. Gerbils were then subjected to 10 min of bilateral carotid occlusion under halothane anesthesia with and without MK801 (10 mg/kg, i.p. just prior to occlusion). Testing resumed 24 hr later and continued for 4 days. Ischemia significantly impaired radial maze performance, affecting both reference and working memory. MK801 significantly reduced hippocampal damage and prevented the ischemia-induced radial maze impairments. MK801 treatment alone transiently impaired radial maze performance 24 hr after dosing. These findings are consistent with previous data indicating that ischemia disrupts radial maze performance and that MK801 reduces ischemic brain damage. The morphological neuroprotection provided by MK801 is now shown to be functional in that radial maze performance was unimpaired in ischemic gerbils treated with the drug.

117.9

THE ACTIVITY OF BMY 14802 IN MODELS OF NEUROPROTECTION: A COMPARISON WITH SABELUZOLE AND IFENPRODIL. S. L. Moon, J. A. Stanley*, R. C. Lamy*, M.N. Duquette*, and J.M. Libera*. CNS Biology, Bristol-Myers Squibb Co., Wallingford, CT 06492.

BMY 14802 (Bristol-Myers Squibb), a phenyl-akyl-piperidine, is effective in animal models used to evaluate neuroprotective compounds. In an anoxia test, adult Sprague-Dawley rats were exposed to an atmosphere of 100% nitrogen for one min. Without any protection, virtually all of the vehicle-dosed animals (>91.7%) died. A 50 mg/kg dose of BMY 14802 given 30 min. prior to nitrogen exposure protected 63% of the animals. BMY 14802 at 75 mg/kg antagonized NMDA-induced convulsions in Swiss Webster mice, shifting the CD_{50} from 113 to 133 mg/kg NMDA. Also, when assessed by means of a rating scale to denote the severity of cell loss, BMY 14802 at 25 mg/kg one hour pre- and post-surgery significantly attenuated hippocampal neuronal damage induced by a 15 min. bilateral carotid occlusion in the Mongolian gerbil.

BMY 14802 was compared to two putative neuroprotective phenyl-akyl-piperazines that also bind to sigma sites. Sabeluzole (Janssen) at 5 mg/kg protected 100% in the anoxia test and shifted the CD_{50} for NMDA to 179 mg/kg. However, under the dosing regimen used, it was not active in the gerbil carotid occlusion model at 2.5 and 5 mg/kg. Ifenprodil (Synthelabo) showed a maximal protection of 17% against anoxia at 80 mg/kg. It did not alter NMDA-induced convulsions.

In addition to showing activity across all three models of neuroprotection, BMY 14802 did not produce PCP-like effects. Unlike MK-801, BMY 14802 did not produce PCP-like catalepsy in the pigeon, nor did it substitute for PCP in pigeons trained to discriminate PCP.

117.10

PROTECTION AGAINST ISCHEMIC NEURONAL DEGENERATION *IN VITRO* OR *IN VIVO* BY HONGHUA, AN ANTI-EXCITOTOXIC CHINESE HERBAL MEDICINE. H. Y. Bai, H. Xiao*, M.T. Price, J.W. Olney. Washington University School of Medicine, St. Louis, MO. 63110.

CNS ischemia causes glutamate (Glu) and aspartate to accumulate extracellularly and trigger neuronal degeneration by excitotoxic interaction with N-methyl-D-aspartate (NMDA), kainic acid (KA) and/or quisqualate (Quis) receptors. We have shown in the *in vitro* isolated chick embryo retina that simulated ischemia (glucose/oxygen deprivation) causes a Glu-like pattern of neuronal degeneration which is not adequately prevented by anti-excitotoxic agents that block only NMDA or only non-NMDA receptors, but is completely prevented by agents that block both NMDA and non-NMDA receptors. Similarly, we found that a combination of NMDA and non-NMDA antagonists provided optimal protection against ischemic damage induced in the *in vivo* adult rat retina by a dye-photothrombosis approach. Here we used the same retinal ischemia models to test the neuroprotective properties of Honghua, a Chinese herbal medicine derived from safflower. In the isolated chick embryo retina, an extract of Honghua protected against the neurotoxic actions of NMDA, KA, Quis or Glu and against ischemic neuronal degeneration. When injected into the vitreous of the adult rat eye immediately before induction of photothrombosis, Honghua conferred excellent protection against ischemic degeneration of retinal neurons. We conclude that Honghua contains one or more anti-excitotoxic agents that, either alone or in combination, block both NMDA and non-NMDA receptors and, therefore, effectively protect against ischemic neuronal degeneration. Supported by EY 08089 and RSA MH-38894 (JWO).

117.11

FELBAMATE REDUCES NEONATAL HYPOXIC-ISCHEMIC BRAIN DAMAGE IN THE RAT. C. G. Wasterlain, P. H. Schwartz, L. M. Thompson* and H. H. Hattori. Epil. Res. Labs., VAMC, Sepulveda, CA 91343 and Brain Res. Inst., UCLA.

Felbamate, a dicarbamate anti-convulsant, has marked *in vitro* neuroprotective properties against hypoxic damage in the hippocampal slice (Wallis *et al.*, *Neurology* 40 (Suppl. 1):193, 1990). We tested its effect on *in vivo* hypoxic-ischemic brain damage in a neonatal rat model. Ninety-four seven-day old Wistar (Simonsen) rat pups were subjected to bilateral carotid ligation under methoxyflurane anesthesia and, after four hours of recovery, were exposed for 1 hour to 6.5% O_2/93.5% N_2. Temperature was carefully controlled before, during, and after hypoxic exposure. Sixty minutes before hypoxic exposure 37 rats received, i.p., felbamate, 300 mg/kg in DMSO (1 mL/kg), 37 vehicle controls received DMSO (1 mL/kg), and 20 received 0.9% NaCl (1 mL/kg). Animals were perfusion-fixed 3 days later and serial brain sections were stained with hematoxylin and eosin. The volume of neocortical infarction (above the rhinal fissure) was calculated from 6 standardized coronal sections and the percent infarcted cortex was determined. Saline controls had infarcts occupying 70 ± 10 % of the neocortex while vehicle controls had 65 ± 5 % cortical infarction. The felbamate treated animals had 29 ± 6 % cortical infarction, a value significantly lower than both control groups ($p < 0.01$). These data suggest that felbamate may have neuroprotective properties *in vivo* and deserves evaluation in cerebral ischemia and status epilepticus.
This work was supported by the Research Service of the Veterans Administration and by Carter-Wallace, Inc.

117.12

DEPLETION OF ATP LEVELS IN THE HIPPOCAMPAL SLICE DOES NOT PREDICT SYNAPTIC FAILURE DURING HYPOXIA. R.A.Wallis, H Hattori, K.Panizzon, E. Csiszar * and C.G. Wasterlain. Neuronal Recovery Laboratory, VAMC, Sepulveda, CA 91343 and Dept. of Neurology and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

ATP depletion during hypoxia has been thought to be responsible for synaptic transmission failure. To investigate this question, we examined ATP levels in relationship to synaptic activity in the CA1 area of hippocampal slices with dentate and CA4 regions trimmed. Using artificial cerebral spinal fluid containing 4 mM glucose, 1.3 mM magnesium, and 2.4 mM calcium, we exposed slices to 15 min. of hypoxia. ATP levels showed a progressive decline with baseline, 5, 10 and 15 min. of hypoxia yielding ATP values of 15.6±0.6 (SE), 13.0±1.0, 10.7±1.0 and 8.3±0.5 nmole/mg protein respectively. Phosphocreatine levels likewise showed decline throughout the hypoxic exposure with values of 29.4±1.2, 21.8±3.0, 12.9±1.8, 7.9±1.5, at baseline and 5,10 and 15 min. of hypoxia. Synaptic transmission, however, as measured by the mean percent amplitude of the original evoked potential, did not show a progressive decline but rather first declined at 5 min to 2%, then recovered to 36% at 10 min and then disappeared at 15 min. These data show that the initial fall of the evoked potential during hypoxia occurs while energy reserves are still sufficient to sustain major membrane pumps. Initial synaptic failure is unlikely to reflect profound energy failure, since evoked response amplitude later increase when ATP levels continue to decline.

This work was supported by the Research Service of the VA and Grant NS-13515 from NINDS.

117.13

A POST-HYPOXIC MODEL OF MYOCLONUS IN THE RAT. P. H. Schwartz, L. M. Thompson* and C. G. Wasterlain. Epil. Res. Labs., VAMC, Sepulveda, CA 91343 and Brain Res. Inst., UCLA.

Myoclonus is a common consequence of episodes of cerebral hypoxia-ischemia arising from such causes as cardiac arrest. To date, although several chemically-induced or genetic models of myoclonus have been described, no model of post-hypoxic myoclonus has been available for study. Here we describe a model of post-hypoxic myoclonus induced in the rat by reversible cardiac arrest with long-term recovery. Cardiac arrest was induced by intracardiac KCl in temperature-controlled, ketamine anesthetized, paralyzed, and ventilated adult male Sprague-Dawley rats. After ten minutes of zero blood pressure, the rats were restored to spontaneous circulation by cardiopulmonary resuscitation. For at least six days, they showed marked hindlimb extensor hypertonus and the EEG was abnormal and was characterized by a predominance of slow wave activity interspersed with sharp waves. Most animals displayed spontaneous myoclonus involving either the whole body or the face, action myoclonus, involving the whole body, or startle myoclonus (acoustic), involving the whole body which, with repeated stimulation, could give rise to running seizures. Histopathological examination of the brains from these animals revealed selective neuronal necrosis in layers 3-5 of lateral neocortex, the CA_1 pyramidal cell layer and the septohippocampal nucleus. In addition, there was a selective loss of Purkinje cells near the dural surface of the cerebellum, and neuronal necrosis in the medial superior olive as well as a loss of larger cells throughout the rostro-caudal extent of the medullary reticular formation. This model of post-hypoxic myoclonus appears promising for studies of the pathophysiologic mechanisms of myoclonus as well as its pharmacological treatment. A video of the myoclonic animals will be presented.

117.14

BARBITURATES ENHANCE CORTICAL NEURONAL INJURY UNDER CONDITIONS OF SUBSTRATE DEPRIVATION. J.H. Weiss[1], R.G. Giffard[2] and D.W. Choi[1]. Depts. of Neurology[1] and Anesthesiology[2], Stanford Univ. Med. Sch., Stanford, CA 94305.

Barbiturates have been extensively studied as potential neuroprotective agents in ischemia. We found that high concentrations of barbiturates can attenuate excitatory amino acid-induced neurotoxicity in murine cortical cell cultures (Neurology 38:214, 1988). In the current studies, we examined the effect of barbiturates on glucose deprivation-induced neuronal injury in the same system. Cultures exposed to 0 glucose for 6 - 10 hr developed substantial neuronal injury by the next day, apparent either by light microscopy or by the efflux of lactate dehydrogenase to the bathing medium. Addition of 300 - 500 µM secobarbital to the media was not protective; in contrast, neuronal injury was markedly increased. Even in cultures deprived of glucose for only 4 hr, an insult which produced little injury in control cultures, secobarbital addition increased neuronal damage. Similar potentiation of injury was found with thiopental or methohexital, or when barbiturates were added to cultures deprived simultaneously of both oxygen and glucose. Enhancement of neuronal injury under ischemic conditions might be a factor limiting the neuroprotective value of barbiturates in vivo.

117.15

CALPAIN INHIBITORS IMPROVE THE RECOVERY OF SYNAPTIC TRANSMISSION AFTER HYPOXIA IN HIPPOCAMPAL SLICES.
A. Arai, M. Kessler, K. Lee and G. Lynch. CNLM, University of California, Irvine, CA 92717 and Department of Anatomy, Thomas Jefferson Medical School, Philadelphia, PA 19107.

Recently, it was reported that transient ischemic episodes that produce delayed pathology in hippocampus cause an immediate increase in the degradation of spectrin, a primary component of the membrane cytoskeleton (Brain Res, 1989, 492:366). The breakdown products observed were identical to those resulting from activation of a calcium sensitive protease (calpain) suggesting that this enzyme is triggered by even relatively brief periods of hypoxia. In this study, we tested calpain inhibitors for their effects on hypoxia-induced synaptic dysfunction in hippocampal slices.

Synaptic transmission in hippocampal slices continuously declined after substituting N_2 for O_2 and was lost by 4-5 min; the fiber volley disappeared after 5-6 min of hypoxia. Recovery from hypoxia was complete if reoxygenation was begun before the loss of the fiber volley but became progressively more impaired past this point. Treatment with the calpain inhibitors leupeptin (1 mM) and calpain inhibitor I (200 μM) significantly improved the recovery of the evoked response under conditions in which control slices did not show any recovery, i.e. the drug treated slices recovered 50-80% of their initial EPSPs. Calpain inhibitors did not delay the disappearance of the fiber volley and thus presumably do not affect the processes involved in energy and ion gradient dissipation. The data suggest, instead, that a brief activation of calpain may be sufficient to cause an immediate impairment of neuronal function. Thus, calpain may be involved in both acute and delayed neuronal damage when activated during hypoxia or ischemia episodes (supported by NIA AG00538).

117.16

INHIBITION OF PROTEOLYSIS PROTECTS HIPPOCAMPAL NEURONS FROM ISCHEMIA.
K.S. Lee, S. Frank*, P. Vanderklish*, A. Arai and G. Lynch. Dept. of Anatomy Thomas Jefferson Univ., Philadelphia, PA 19107 and CNLM, Univ. of Calif., Irvine, CA 92717.

Transient forebrain ischemia has recently been shown to elicit a rapid and sustained proteolysis of the cytoskeletal protein, spectrin (Seubert et al., '89, Brain Res. 492: 366). Spectrin is a preferred substrate for the calcium-activated protease, calpain; and, the specific pattern of spectrin proteolysis by calpain is identical to that seen following ischemia. The current studies examined whether this proteolytic response to transient ischemia can be inhibited by *in vivo* treatment with a protease inhibitor and whether protease inhibition affects the process of neuronal death.

Adult gerbils were administered saline or the protease inhibitor, leupeptin via a cannula implanted in the right lateral ventricle. Compounds were delivered over a three day period by an Alzet osmotic pump attached to the cannula. Animals were then subjected to a ten minute period of bilateral occlusion of the carotid arteries. Post-ischemic survival periods were 30 minutes for the spectrin assay experiments and 14 days for the histological analysis of cell death. Leupeptin treatment resulted in a substantial reduction in ischemia-induced spectrin proteolysis. The post-ischemic increase in spectrin breakdown products was inhibited by approximately 75% in leupeptin treated animals. Histological studies showed that leupeptin had a pronounced neuroprotective effect on the post-ischemic loss of selectively vulnerable CA1 pyramidal neurons. Saline-treated animals exhibited a 72% loss of CA1 neurons while leupeptin-treated animals showed only 15% cell loss.

Taken together, these observations indicate that neuronal proteolysis (presumably mediated by calpain) is a critical step in post-ischemic neuronal pathology. Moreover, suppression of this proteolytic response represents a promising avenue for limiting cell death following ischemia and perhaps other neurodegenerative responses. Research supported by: NIH grant NS24782-01A2 to K.L and NIA grant AG-00538 to G.L)

117.17

FOCAL CEREBRAL ISCHEMIC INFARCTION AND BRAIN EDEMA ARE REDUCED IN TRANSGENIC MICE OVEREXPRESSING HUMAN SUPEROXIDE DISMUTASE.
H. Kinouchi,* S. Imaizumi,* E. Carlson,* C. Epstein* and P. H. Chan. CNS Injury & Edema Research Center, Depts. of Neurology & Neurosurgery, Pediatrics and Biochemistry/Biophysics, Univ. of Calif., San Francisco, CA 94143, U.S.A.

Oxygen radicals have been implicated in the pathogenesis of brain edema and cell death in cerebral ischemia. We have demonstrated that liposome-entrapped SOD reduced the infarct size significantly in focal cerebral ischemia in rats. Transgenic mice overexpressing human CuZn-SOD (SOD-1) enzymatic activity (1.6-6.0 fold increase) in whole brain have been successfully established (Epstein *et al.*, PNAS 84, 8044, 1987). We now compare the infarct size (IS; infarct area/hemisphere area) and ipsilateral hemisphere enlargement (IHE; ipsilateral hemisphere/opposite hemisphere) at various stereotactic coronal planes in focal cerebral ischemia produced by a combination of middle cerebral artery occlusion and bilateral common carotid artery occlusion (Chen *et al.*, *Stroke* **17**, 738, 1986) in transgenic mice and normal diploid mice littermates. Experiments were done blindly and physiological parameters were monitored throughout the studies. This model provides a reproducible infarct in ipsilateral cortex, as measured by 2,3,5,-triphenyltetrazolium chloride (TTC) stain for mitochondrial dehydrogenase activity at 24 hrs postischemia. The infarct size and the ipsilateral hemisphere enlargement (an indication of brain edema) of transgenic mice were significantly smaller than that of normal mice (coronal level from anterior 3 mm; IS: 15.3% vs 23.7%, IHE: 105% vs 114%). These data suggest that superoxide radicals play an important role in the pathogenesis of focal ischemic brain injury, because the overexpression of human SOD-1 enzymatic activities attenuates the infarct size and brain edema. (Supported by NIH grants NS-14543, NS-25372, HD-17001, and AG-08938.)

117.18

ANTIOXIDANT LEVELS INFLUENCE ASTROCYTE SURVIVAL IN HYPEROXIA.
S.F. Chen, V.S. Woolworth,* and P. H. Chan. CNS Injury & Edema Research Center, Dept. of Neurology, University of California, San Francisco, CA 94143, U.S.A.

A model for astrocyte exposure to hyperoxia has been developed using primary cultures of cerebral cortical astrocytes from neonatal rats. Exposure of these cultures to 95% O_2/5% CO_2 in a modular incubator chamber resulted in cell death between 48 and 72 hours, as indicated by LDH release, morphology changes, and trypan blue nonexclusion. This model has enabled us to examine the effects of alterations in endogenous antioxidant levels on the outcome of hyperoxic treatment. Astrocyte intracellular glutathione (GSH) levels and survival times were measured in cells treated with 50 μM buthionine sulfoximine (BSO) or 35 mM 2-oxothiazolidine-4-carboxylate (OTC). BSO dropped GSH levels to less than 10% of normal and shortened survival times. OTC only slightly elevated GSH levels but significantly lengthened survival times. Treatment of astrocytes with 1 mM diethyldithiocarbamate (DDC) decreased levels of superoxide dismutase and GSH-peroxidase to 70% of normal and also decreased survival times under hyperoxic conditions. None of these compounds affected cell survival under normoxic conditions. Glucose utilization, as measured by the decrease in glucose levels in the medium, was more rapid under hyperoxic than normoxic conditions. OTC treatment slows the glucose utilization by astrocytes under hyperoxic conditions. These results imply that GSH is a critical metabolite in cytoprotection against the damage induced by hyperoxia and confirms that cell death is oxygen radical mediated. (Supported by NIH grants NS-14543 and NS-25372.)

ISCHEMIA III

118.1

HISTOLOGY AND SOMATOSENSORY EVOKED POTENTIALS FOLLOWING BASILAR ARTERY OCCLUSION IN THE RAT.
JC Wojak, V DeCrescito, W Young. Dept. of Neurosurgery, NYU Med. Ctr., New York, NY 10016.

We surgically exposed and occluded the basilar artery in 25 rats. Basilar artery occlusion at any single point between the foramen magnum and the circle of Willis did not produce histologically detectable infarcts in the brain at 12-24 hours. Two point occlusions of the basilar artery produced variable infarcts between the occlusion sites but no ischemic lesions elsewhere. After either single or double point occlusions, the proximal basilar artery refilled within 2-3 minutes. When the basilar artery was occluded above and below the origins of the anterior inferior cerebellar arteries, the arteries between the occlusion points initially collapsed but refilled within 2-3 minutes. In two rats, bilateral carotid occlusion combined with basilar artery occlusion rostral or caudal to the anterior inferior cerebellar arteries did not completely eliminate proximal basilar flow. Basilar artery occlusions invariably suppressed somatosensory evoked potentials by >50%. Regardless of whether a brainstem infarct developed, somatosensory evoked potentials recovered above baseline levels by 4 hours in 7 of 17 rats and returned to baseline levels by 24 hours in every rat tested. We conclude that the occluded basilar artery receives extensive retrograde collateral flow and that somatosensory evoked potentials are exquisitely sensitive to basilar artery occlusion but are insensitive to whether brainstem infarcts develop.

118.2

TRIGEMINAL SEPs IN A REPERFUSABLE MCA OCCLUSION MODEL IN RATS.
Takeo Shimizu[1], Eric H. Chudler[2] and Michael A. Moskowitz[1]. Stroke Research Laboratory[1] and Laboratory of Pain Research[2], Dept. of Neurosurgery, Massachusetts General Hospital, Boston, MA 02114.

We investigated the sequential changes of trigeminal somatosensory evoked potentials (SEPs) recorded over the somatosensory cortex and the consequential histochemical changes occurring after focal cerebral ischemia using transvascular occlusion of the middle cerebral artery (MCA) in rats. Adult Wistar rats (270-340 gm) were anesthetized with chloral hydrate (400 mg/kg, i.p.). Ischemia of the cerebral cortex was induced by the advancement of a thread cylinder through a coaxial catheter into the anterior cerebral artery (A_1). SEPs generated from the primary somatosensory cortex (P1) and thalamocortical radiation (IIIb) usually disappeared within 5 min after obstruction of the MCA. The amplitudes of P1 and IIIb returned to control values when ischemia lasted 15 min. Recovery of P1 and IIIb were delayed after 30 min of ischemia. After 60 min of ischemia, IIIb and P1 did not recover significantly. Histochemical evidence of an infarction involving the somatosensory cortex always coincided with cases that did not show recovery of P1. These data illustrate the correlation between the persistent abolishment of the cortical SEP and irreversible brain damage after ischemia and reperfusion.

118.3

OUABAIN INDUCES SPREADING DEPRESSION-LIKE DEPOLARIZATION. M. Balestrino°, S. Mazzari°°, A. Leon°°
°Department of Neurology, University of Genoa and
°°Fidia Research Laboratory, Abano Terme PD, Italy

In rat hippocampal slices addition of ouabain (100 uM) to the perfusion medium caused the peculiar, sudden, 20-40 mV depolarization that is usually observed in spreading depression (SD). Usually tissue voltage slowly (1-3 min) recovered to baseline despite continuing ouabain perfusion. This did reflect cell repolarization (thus suggesting that the Na-K pump was not yet completely blocked): in fact, in some slices a second identical SD-like cycle occurred minutes later. A slower, profound and sustained depolarization followed, presumably reflecting progressive and complete block of the pump. Since SD-like events occurred well before this complete block, they appeared to be triggered by some intermediate factor. Nevertheless, failure of the Na-K pump (as induced by ouabain) is obviously a key factor in the chain of events that leads to SD-like depolarization. This further suggests that drugs that activate the Na-K ATPase (e.g. GM1 ganglioside: J. Neurochem. 37:350, 1981) may prevent or delay SD-like phenomena (e.g. anoxic depolarization: Neurosci. Abs. 12:1401, 1986).

118.4

ANOXIC-INDUCED CHANGES IN K^+_o AND Ca^{2+}_o IN NEONATAL RABBIT MEDULLA. M.E. Morris and T. Trippenbach*. Department of Pharmacology, University of Ottawa, Ottawa, Canada K1H 8M5.

Changes in K^+_o and Ca^{2+}_o were measured in the brainstem of newborn rabbits (1-14 days old; 53-260 g; urethane anesthesia; controlled ventilation/100% O_2) during brief anoxia (1-3 min 100% N_2). Ion-selective microelectrodes recorded ion and tissue potentials at depths 350-700 μm (1-1.4 mm lateral to obex). N_2 evoked reversible K^+ ↑s (with small phase I and larger phase II) of ≤2.3 mM (>2.5 mM resting level), duration 30-90 s; and ≤5 mV tissue depolarization of similar time course. Brief K^+ ↑s (<5 s, ≤12 mM) were frequently superimposed. K^+ plateau changes for Day 1-4 pups were consistently larger (0.7-2.3 mM) than those for Day 6-14 pups (≤0.7 mM) and times to onset and peak delayed. Ca^{2+}_o ↓s were more variable, small (0.1-1.25 mM below 1.7 mM baseline) and brief (10-60 s). They were often associated with potential bursts and the time of early K^+ change. K^+ ↑s were generally less than those expected to depress synaptic transmission. The higher levels during the first post-natal week may be due to the absence of inhibitory synapses and/or the presence of voltage-independent NMDA responses. It is suggested that the burst activity and Ca^{2+} changes indicate a Ca^{2+} influx which may activate g_K and contribute to the sequence of excitation, gasping and apnea which forms the immature breathing response to hypoxia.

(Supported by The Hospital for Sick Children Foundation).

118.5

ANOXIC DEPOLARIZATION OF NEOCORTICAL PYRAMIDAL NEURONS IN VITRO. A.S. Rosen and M.E. Morris. Department of Pharmacology, University of Ottawa, Ottawa, Canada K1H 8M5.

Brief (≤5 min) anoxic exposure of rat neocortical slices elicited depolarizing responses (AD) of 2.5-5 mV in 18/20 layer II-III neurons. Input resistance (R_N) was generally unchanged (but a 15-20% reduction observed in 2/18 neurons) and PSPs were depressed. AD, recorded at resting potentials between -55 and -100 mV, was reversed during reoxygenation, which also elicited postanoxic hyperpolarization (PAH) -- which has been attributed to Na^+-K^+ pump reactivation (Fujiwara et al. 1987, J. Physiol. 384: 131). An early pump failure as cause of AD has not yet been ruled out. During anoxia K^+ sensitive electrodes record an increase in $[K^+]_o$ of 1 mM. Moreover, at normal pO_2, ouabain 5 μM also elicits a depolarizing response. Of further interest is the finding that AD can be almost entirely blocked in slices superfused for 25-30 min with kynurenic acid 100 μM; this suggests that a release of excitatory amino acids may be partly responsible for the effect.

Since with anoxia hippocampal pyramidal neurons undergo hyperpolarization and a decrease in R_N (a Ca^{++} induced K^+ efflux proposed as the most likely mechanism (Leblond & Krnjevic 1989, J. Neurophysiol. 62: 1), it is concluded that different mechanisms appear to operate during anoxic responses of neocortical versus hippocampal pyramidal neurons.

(Supported by The Medical Research Council of Canada).

118.6

EFFECT OF ANOXIA ON INTRACELLULAR AND EXTRACELLULAR K^+ IN HYPOGLOSSAL NEURONS STUDIED IN-VITRO. C. Jiang, G.G. Haddad and S. Agulian. Department of Pediatrics, Section of Respiratory Medicine, Yale University School of Medicine, New Haven, CT 06510.

Previous reports have shown that extracellular K^+ (Ko) increases in brain slices during O2 deprivation. The source of Ko and the mechanisms by which K^+ leaks from cells are not well understood. To address these issues, we studied changes in Ko and intracellular K^+ (Ki) of hypoglossal (XII) neurons during 3-5 min anoxia in brain slices of adult rats using ion sensitive microelectrodes. Intracellular recordings with double barrelled microelectrodes showed that Ki decreased during anoxia in all XII neurons studied (n=20). The magnitude of drop was 44.5 ± 17.3 mM, (mean ± SD, n=6). To study the mechanisms by which K^+ leaks from XII neurons, we used several pharmacological blockers. K^+ leakage was reduced by 40-50% for each of TEA (20-50 mM), CsCl (4mM) and tolbutamide (1-3mM), an ATP-sensitive K^+ channel blocker. In contrast 4-AP, Apamine, CoCl2 and TTX had little or no effect. Measurements of the extracellular space showed that its shrinkage with anoxia was less than 5%. These results strongly suggest that during anoxia, 1) neurons lose Ki and this could contribute to the increase of Ko; 2) Ki leakage is achieved through specific K^+ channels, possibly related to ATP depletion but not to Ca^{++} activation; and 3) K^+ leakage does not depend on the increased neuronal activity observed in XII neurons.

118.7

ION CHANNEL INVOLVEMENT IN HYPOXIA-INDUCED SPREADING DEPRESSION IN HIPPOCAMPAL SLICES. P.G. Aitken, J. Jing*, J. Young*, G.G. Somjen, Dept. Cell Biology and Div. Neurosurgery, Duke Medical Center, Durham, NC 27710.

When subjected to severe hypoxia *in vitro*, hippocampal tissue exhibits cellular depolarization and ionic redistribution very similar, if not identical, to classical spreading depression (SD). Because hypoxic SD is a causative factor in neuronal injury and death, we are interested in the specific ion channels involved. We previously demonstrated that the K^+ channel blocker tetraethylammonium (TEA) decreases the latency and amplitude of hypoxic SD in the CA1 region of hippocampal slices (SNA, 1988, p. 185). We now report the effects of the K^+ channel blocker 4-aminopyridine (4-AP) and the Na^+ channel blocker tetrodotoxin (TTX). Rat hippocampal slices were kept at 35°C in an interface chamber. Two hypoxic periods were given, one in control artificial CSF and one in ACSF containing 1μM TTX or 50μM 4-AP. The hypoxic periods lasted just long enough to induce SD, and were 1 hour apart. The latency and amplitude of SD were monitored in st. pyramidale of CA1. 4-AP caused a significant reduction in the latency of SD, to 85% of control. TTX had the opposite effect, causing an increase in SD latency to 180% of control. Neither 4-AP nor TTX affected the amplitude of the DC voltage shift or the shape of the SD waveform. We interpret the latency differences as due to changes in neural excitability; 4-AP increases, while TTX decreses, excitability. That TTX and 4-AP do not decrease SD amplitude argues that neither the voltage-gated Na^+ channels nor the delayed rectifier or "A" K^+ channels are major contributors to hypoxic SD. Since TEA has been shown to decrease SD amplitude, Ca^{2+}-activated K^+ channels (blocked by TEA but not by 4-AP) may be important ion carriers during hypoxic SD. Since TEA does not completely block hypoxic SD, other channels must be involved as well. [Supported by NIH grants 17771, 18670, 06233]

118.8

POTASSIUM-INDUCED PROLONGED DEPOLARIZATION IN HIPPOCAMPUS *IN SITU* AND *IN VITRO*: LIMITS OF DELAYED RECOVERY. O. Herreras*, J. Jing*, G.G. Somjen. Div. Physiology, Duke Univ. Med. Ctr., Durham, NC 27710

We have reported that spreading depression (SD) of prolonged duration induced in hippocampal tissue slices by high K^+ was followed by depression of synaptic transmission for 1-3 hours (Kawasaki et al, Brain Res. 457: 322-329, 1988). We now tested prolonged SD in brain of rats anesthetized with urethane. SD was induced by microdialysis of artificial CSF with K^+ replacing Na^+, and/or by superfusion of the surgically exposed hippocampus. Evoked potentials, DC potential and $[K^+]_o$ were recorded from CA1. 30 min or longer depolarization induced by 90 min high K^+ dialysis was followed by hyperexcitability, then depression, then full recovery of antidromic and partial recovery of orthodromic transmission in 3-5 hours. 60 min or longer irrigation combined with dialysis of high K^+ caused complete loss of evoked responses; shorter similar treatment was followed by slow partial or complete recovery. EPSPs recovered more than orthodromic population spikes. We then repeated the experiments on hippocampus *in vitro*, with longer post-exposure observation. Some slices that were intially depressed, recovered slowly over 3-6 hours. We conclude: (1) hippocampal tissue normally perfused by blood tolerates severe depolarization of longer duration than the same tissue *in vitro*. (2) Depression of transmission for 1-3 hours is not necessarily "irreversible". (3) Antidromic conduction and EPSPs can recover to control level before the ability to fire orthodromic spikes is regained.

(Supported by the NIH and the Spanish Min. Education)

118.9

CALCIUM/CALMODULIN-DEPENDENT PROTEIN KINASE II ISOLATED FROM CONTROL AND ISCHEMIC GERBILS. S.B. Churn, W.C. Taft, R.E. Blair and R.J. DeLorenzo. Departments of Neurology and Pharmacology, Medical College of Virginia, Richmond VA 23298.

Calcium/calmodulin-dependent protein kinase type II (CaM kinase II) activity has been shown to be especially sensitive to the effects of transient ischemia (Brain Res. 447:159,1988). However, it is not known whether the decrease in activity observed after ischemia is due to proteolytic digestion of CaM kinase II or some postranslational modification of the enzyme. To examine this question, CaM kinase II was purified, in parallel, from control and ischemic animals. Gerbils were given a 5 min ischemic insult by means of bilateral carotid occlusion and allowed to recover for 24 hrs. Whole forebrain homogenates were made and CaM kinase II purified by previously published methods (J. Biol. Chem. 258:12632, 1983). CaM kinase II enzyme levels were quantitated by a biotinylated-calmodulin binding assay in every step throughout purification. Total protein, calmodulin binding and percent recovery were not significantly different between control and ischemic fractions. However, ischemic CaM kinase II activity remained down relative to control activity in all fractions obtained. Kinetic studies, including exogenous substrate, ATP and calmodulin affinities, phosphorylation rate, and tests for the presence of inhibitors showed no significant differences between control and ischemic CaM kinase II. Ischemic CaM kinase II activity could not be restored by phosphatase treatment. The results support the conclusion that ischemia induces a post-translational modification of CaM kinase II.

118.10

CALCIUM UPTAKE AND PROTEIN SYNTHESIS AFTER GLOBAL ISCHEMIA OF RAT BRAIN. G. Mies*, N. Saito*, G. Nagashima*, K. Kawai*, T.S. Nowak, Jr., G.A. Dienel and I. Klatzo. LNNS, NINDS, NIH, Bethesda, MD 20892.

Postischemic selective vulnerability of the central nervous system not only develops in the hippocampal formation or in the striatum but also in other brain regions such as thalamus, substantia nigra and inferior colliculus. It was therefore of interest to investigate the regional relationship between characteristic features observed during the progression of delayed neuronal injury and characteristic metabolic changes during postischemic recovery possibly associated with the maturation phenomenon. For this purpose, a double tracer technique was established which employs [45Ca]chloride for the detection of jeopardized brain regions and [3H]leucine for the measurement of regional cerebral protein synthesis. Cerebral ischemia was studied in a 10 min cardiac arrest model in rats induced by the compression of intrathoracic large vessels, followed by survival intervals up to one month. During the early postischemic recovery period (6h to 1d), an inhibited cerebral protein synthesis but no 45Ca uptake was observed in brain regions which, at later postischemic stages (2h to 4d), developed morphological criteria of injured neurons and increased 45Ca uptake. At 4 d to 1mo postischemia, depressed protein synthesis was generally associated with 45Ca uptake in areas in which neurons were at risk or irreversibly damaged. Persistent deficits in regional protein synthesis, consequently, appear to predict areas of subsequent 45Ca accumulation in which neuron damage may evolve at later postischemic stages. In contrast, the reticular nucleus of thalamus showed intense 45Ca uptake with apparently normal amino acid incorporation at 4d postischemic recirculation. Morphological alterations of these GABAergic neurons were slight or absent. The thalamus therefore is an area in which the contributions of specific pathophysiology in individual cell types to these different indexes of ischemic injury may be productively investigated.

118.11

HIGH Ca++ LOADING OUTSIDE PRIMARY AND PERI-INFARCT ISCHEMIC AREAS: TERTIARY INJURY PROCESS IN STROKE. S.E. Karpiak, A. Ortiz, C.G. Wakade, N. Hernandez, M. Durkin*, A.I. Barkai*, & S.P. Mahadik. NY State Psych. Inst., & Columbia U. (Physicians & Surgeons), NY,NY.

Ischemia is induced by middle cerebral artery (MCAo) & ipsilateral common carotid artery (CCAo) occlusion with a 1hr temporary contralateral CCAo. The ischemic damage is restricted to cortex. No tissue pathology occurs in subcortical tissues. Maximal Ca++ increases (primary & peri-infarct areas) occur 72hrs after ischemia. To study the anatomical distribution of these increases, 72hrs after ischemia, rats were injected (i.p.) with 100uCi [45]Ca++. After 5hrs rats were sacrificed & 20micron frozen coronal sections were taken throughout. Sections were exposed to x-ray film for 14 days. Autoradiographic analyses parallel our prior finding of Ca++ increases in primary & peri-infarct areas. *Large [45]Ca++ increases were also seen in the ipsilateral thalamic nuclei, tracts & internal capsule.* No tissue pathology has been observed in these areas. The long-term sequelae of this increased [45]Ca++ in areas not directly affected by the ischemic insult may be significant. High levels of Ca++ in tertiary areas may reflect injury processes heretofore not included in stroke pathology. Such tertiary damage may be the basis for psychopathologies associated with stroke (e.g. depression & cognitive disorders), and the primary locus for therapeutic interventions. These data provide a basis for studies of *other* injury processes associated with a singular focal neuronal insult. Supported in part by grants from NINDCS (NS-2525856) and FIDIA Research Foundation.

118.12

HYPOTHERMIA DURING HYPERGLYCEMIC ANOXIA PREVENTS DIFFUSE BRAIN INJURY. K.R. Wagner, M. Kleinholz*, G.M. de Courten-Myers*, R.E. Myers. Research Serv., VAMC; Depts. Neurol. & Pathol., UC Coll. Med., Cincinnati, OH 45220.

Hyperglycemia during anoxic/ischemic insults exacerbates brain injury while hypothermia is markedly brain protective. Presently, we examined whether mild (33°C) or moderate (28-29°C) hypothermia would prevent the development of brain edema and diffuse injury that occurs following similar hyperglycemic anoxic exposures at normal body temperature (38-39°C).

Hyperglycemic cats (serum glucose = 25-50 mM), anesthetized with pentobarbital (35 mg/kg), were made anoxic by respiration with 100% nitrogen. These exposures (range = 8 min in normothermic to 20 min in moderately hypothermic cats) produced markedly elevated lactic acid concentrations in cerebral cortex (22-28 µmoles/g). While postanoxic hyperglycemic normothermic cats developed neurologic signs (focal and tonic-clonic seizures), brain edema, blood-brain barrier damage (Evans blue penetration) and usually died within 24 hours, both groups of hyperglycemic hypothermic cats survived and showed no or only minimal damage to brain.

In conclusion, hypothermia is remarkably brain protective even during hyperglycemic anoxia in which markedly elevated lactic acid accumulations occur in brain. Hypothermia modifies the biochemical mechanisms through which severe tissue lactic acidosis during anoxic/ischemic exposures injures the brain.

118.13

REGIONALLY-SELECTIVE EFFECTS OF BRAIN TEMPERATURE ON ISCHEMIC DAMAGE. K.H. Neill*, B.J. Crain and J.V. Nadler. Depts. Pharmacology, Pathology and Neurobiology, Duke Univ. Med. Ctr., Durham, NC 27710.

Transient forebrain ischemia preferentially destroys neurons in striatum, neocortex and hippocampal formation. We tested the dependence of this neuronal degeneration in the gerbil on brain temperature. A thermistor probe implanted in the striatum monitored brain temperature before, during and after a 5-min bilateral carotid occlusion. During the occlusion, rectal temperature was maintained at 36.5 °C and the brain was maintained at various temperatures between 29 and 37 °C. All animals developed a mild postischemic hyperthermia (0.6 ± 0.1 °C) for several hours. Ischemic damage to different neuronal populations varied with temperature. CA3 hippocampal pyramidal cells were protected from damage by the smallest reduction in brain temperature, followed by striatal neurons, cortical pyramidal cells and CA1 hippocampal pyramidal cells. The experimental anti-ischemic drug MK-801 consistently reduced the mean postischemic brain temperature by about 2 °C and caused the brain temperature to oscillate between hyperthermic and hypothermic. Under these conditions MK-801 abolished striatal damage, but only modestly and inconsistently protected CA1 pyramidal cells. MK-801 did not affect brain temperature in sham-operated gerbils.

The neuroprotective effect of hypothermia seems to depend on how far above threshold the ischemic insult is for the neuron in question. The inconsistent neuroprotective effects of MK-801 on CA1 pyramidal cells cannot be explained by differences in the degree or duration of postischemic hypothermia. (Supported by NIH grant NS 06233.)

118.14

PROTEIN KINASE C BINDING TO CELL MEMBRANES IS STIMULATED BY CEREBRAL ISCHEMIA AND INHIBITED BY HYPOTHERMIA. M. Cardell*, F. Boris-Möller*, H. Bing-Ren* and T. Wieloch. Laboratory for Experimental Brain Research, Lund University, 221 85 Lund, Sweden.

Cerebral ischemia is accompanied by a rapid decrease in energy phosphates, that triggers membrane depolarization leading to a massive increase in intracellular calcium ion concentration, transmitter release and elevation of brain tissue diglycerides and free arachidonate. Using polyclonal antibodies directed against PKC(α), (β_{II}), (γ), we investigated the changes in the distribution of protein kinase C in the cytosolic and particulate fractions of brain homogenates at 15 sec, 1, 2, 3, 5, 10, and 15 min after onset of cardiac arrest ischemia in the rat. Three to ten min after the onset of ischemia the PKC levels increase in the particulate fraction, concomitantly with a decease in the cytosolic fraction, suggesting a firm binding of PKC to cell membranes during ischemia. At 15 min of ischemia PKC levels decreased in both particulate and cytosolic fractions. The ischemia-induced changes in the subcellular distribution of PKC(γ) was marked, while PKC(βII) was less affected, and PKC(α) only marginally affected.

Hypothermia protects neurons against ischemic damage. We found that hypothermia (27 °C) prevents the ischemia-induced increase in binding of PKC to cell membranes, and the decrease in the PKC levels.

We propose that PKC is translocated to cell membranes during ischemia due to elevation of intracellular calcium ion concentrations and/or modification the membrane lipid properties. The cerebro-protective effect of hypothermia may be due to its prevention of PKC translocation-activation.

118.15

DIAZEPAM INDUCED HYPOTHERMIA AND NEUROPROTECTION IN GERBIL FOREBRAIN ISCHEMIA. M.A. Kapin*, R.A. Myers*, V.L. Brazee*, G.W. Dent. Bristol-Myers Squibb Co., Wallingford, CT 06492 In the gerbil, moderate hypothermia results in an attenuation of neuronal injury associated with forebrain ischemia. Benzodiazepines, such as diazepam, have been previously shown to be neuroprotective in mild forebrain ischemia of the gerbil and to be hypothermic producing in the rat. We have previously demonstrated in the gerbil that a marked correlation exists (r=.91) between decreasing colonic temperature and attenuation of neuronal injury. Our goal in this study was to assess in a model of severe gerbil forebrain ischemia whether diazepam could induce a marked hypothermia and at these doses attenuate hippocampal pyramidal cell death. Following methoxyflurane anesthesia, male gerbils (55-80gm) underwent 15 min bilateral carotid occlusion and 96 hrs of reperfusion. At the end of the reperfusion period, the brains were histologically examined for pyramidal cell injury. Colonic temperatures (°C) for vehicle (V), and diazepam at 1 or 10 mg/kg,i.p. (V/1/10) was pre-dose =38.0±.1/37.9±.3/38.3±.1; post-dose at 30 min = 37.2±.2/36.7±.2/33.6±.08 & 90 min 36.8±.2/36.7±.2/33.4±.3. The percent protection of hippocampal pyramidal cell injury by diazepam was 9% (n.s.) and 65% (sig.p<.001) for 1 and 10 mg/kg, respectively. Our data support the contention that diazepam-mediated hypothermia may in part account for the protective actions of this drug.

118.16

Hippocampal Damage following Ischemia: Temperature Maintainence Blocks Protective Effects of the Antiglucocorticoid Metapyrone. J.K.Morse and J.N. Davis, V.A. and Duke University Medical Centers, Durham, NC 27710

Hypothermia has been shown to reduce ischemia-induced hippocampal CA1 damage. Metapyrone, an anti-glucocorticoid, has also been shown to lower temperature and to reduce hippocampal damage following transient forebrain ischemia in the gerbil. We hypothesized that metapyrone prevented CA1 cell death by lowering brain temperature. To test this theory we subjected gerbils to 5 minutes of bilateral carotid occlusion. One group (n=8) received 3 injections of 100 mg/Kg metapyrone and the other group (n=8) received 3 injections of saline. Both experimental groups underwent surgery and sacrifice in a heated room (30°C). During the 72 hours, from the time of carotid reperfusion until sacrifice, the gerbils were kept in a heated chamber, on one of 4 levels. The mean temperature on the top shelf was 32.9°C (± 0.3 SEM); the second shelf 31.9°C (± 0.3); the third shelf 30.6°C (± 0.4); the bottom shelf 29.6°C (± 0.4). There was no difference in the severity of hippocampal damage between experimental groups. However, damage in both metapyrone and control groups was more severe in those animals kept at the higher temperatures. These results suggest that the protective effect of metapyrone on hippocampal cell death may have been mediated by a reduction in brain temperature. (Sponsored by NS06233/NS07274)

118.17

DURATION, TEMPERATURE EFFECTS IN FOUR-VESSEL OCCLUSION ISCHEMIA ON CA1-CA3 DAMAGE IN RATS. P. Bialobok*, G.J. Thomas, J.M. Ordy, T.M. Wengenack, and W.P. Dunlap*. Fisons Pharmaceuticals, Univ. of Rochester, Rochester, NY 14623, and Tulane Univ., New Orleans, LA 70118.

Patients with global cerebral ischemia (IS) in stroke and cardiac failure have memory loss and hippocampal CA1-CA3 damage. Studies with the rat 4-VO model of IS have shown memory loss and CA1-CA3 damage. Differences in CA1 and CA3 vulnerability have been associated with variations in duration of IS and temperature during IS and reperfusion (RP). The aims of this study were to compare the effects of 15 and 30 min. of IS on CA1-CA3 vulnerability at: 1) ambient temp., 2) 37.5°C. body temp. during IS, 3) 37.5°C. during RP, and 4) 37.5°C. during IS and RP. Thirty min. of IS resulted in significantly greater CA1 than CA3 damage, compared to 15 min. of IS. Fifteen min. of IS produced mainly CA1 damage. Temperature interaction effects on CA1-CA3 damage depended upon duration of IS. Valid assessment of neuroprotective drug effects on CA1-CA3 damage requires designs that include comparisons with CA1-CA3 damage attributable to specific durations of IS and temperature during IS and RP.

118.18

DELAYED HYPOTHERMIA REDUCES INFARCT VOLUME FOLLOWING TRANSIENT FOCAL ISCHEMIA IN RAT BRAIN. D.J.Moyer and F.A.Welsh. Div. of Neurosurgery, Univ. of Pennsylvania, Philadelphia, PA 19104.

The protective effect of hypothermia on ischemic injury has been well documented. In the present study, the effect of delayed hypothermia on cortical infarct volume was investigated in a model of transient focal ischemia in rat brain. Focal ischemia was produced by permanent occlusion of the middle cerebral artery combined with transient (1 hr) occlusion of both carotid arteries. Immediately after occlusion, cerebral temperature was lowered from 37.5°C to 32°C over the course of 20 min, maintained at this level for the duration of the 1-hr insult, and returned to 37.5°C during reperfusion. Cerebral temperature was maintained at 37.5°C during ischemia and reperfusion in the normothermic group. After recovery for 24 hr, the brain was sectioned, stained, infarct volume determined by planimetry. Delayed hypothermia significantly reduced infarct volume from 93 ±15 mm^3 (normothermic, N=4) to 13 ±7 mm^3 (hypothermic, N=4, p<0.001). These results demonstrate that hypothermia, even when administered after the onset of ischemia, protects the brain against ischemic injury.

118.19

REGIONAL VULNERABILITY TO HYPOXIA-ISCHEMIA DURING DEVELOPMENT OF IMMATURE RAT BRAIN. K.S.Blumenfeld and F.A.Welsh. Div. of Neurosurgery, Univ. of Pennsylvania, Philadelphia, PA 19104.

To investigate the effect of development on regional vulnerability to hypoxia-ischemia in the brain of immature rat, litters of rat pups aged 7, 15, and 23 days were subjected to unilateral hypoxia-ischemia, followed by recovery for one week. Unilateral hypoxia-ischemia was produced by permanently ligating one carotid artery and placing the pups in an atmosphere containing 8% oxygen for 90 min. After recovery for 1 week, the brains were sectioned, stained, and examined for histopathologic alteration. In the 7-day group, histologic injury was not detected in neocortex, striatum, hippocampus, or thalamus. In the 15-day group, histologic injury was present in neocortex and striatum in the majority of animals, and was occasionally evident in hippocampus and thalamus. In the 23-day group, all four regions suffered severe injury in the majority of animals. Thus, the regional extent and overall severity of ischemic injury was markedly increased in the older groups. However, it is not yet clear whether the rank order of regional vulnerability changes during this stage of brain development.

118.20

EFFECTS OF INCOMPLETE GLOBAL CEREBRAL ISCHEMIA ON CORTICAL EEG SPECTRAL ESTIMATES IN THE UNANESTHETIZED RAT. J. E. Moreton, E. Echevarria, L. Robles and F.C. Tortella. U. of Maryland, Sch. of Pharmacy, Baltimore, M.D. 21201 and Neuropharm. Br., Div. of Neuropsychiat., Walter Reed Army Inst. of Res., Washington, D.C. 20307.

Male S.D. rats were prepared with cerebrocortical EEG electrodes, i.v. jugular and i.a. tail cannulae, bilateral vertebral artery occlusion and common carotid artery (CCA) snares. 24 h later, CCAs were occluded for either 15 or 20 min and recovery evaluated for 24 or 72 h. EEG and physiological parameters were monitored. Four-vessel occlusion resulted in behavioral coma and an associated isoelectric EEG. Despite no convulsive behavior or ictal activity, during recovery the EEG resembled interictal epileptiform activity superimposed on low-voltage slow waves. During the initial 6 h of reperfusion, the frequency of occurrence and amplitude of these EEG complexes increased. By 24 and 72 h recovery they were absent, or much less frequent and low amplitude slow-wave EEG activity predominated. Computer-derived spectral shifts in peak frequency(PF), mean frequency(MF), total power(TP), complexity(COM), mobility(MOB) and edge frequency(EDF) from 0-20 Hz correlated with the direct EEG changes. For example, baseline vs 10 min reperfusion values, respectively, were: PF 5.08/1.5; MF 5.74/2.56; TP 68.8/7.5; MOB 6.9/3.0; COM 9.4/5.0; EDF 15.5/6.7. Although the EEG tended to normalize during recovery decrements persisted at 24 and 72 hr. These data indicate potential value of preclinical EEG spectral analysis in cerebral ischemia research.

119.1

Diazepam Decreases Cerebral Glucose Uptake During Status Epilepticus in Amygdala Kindled Rats: Reversal by Ro15-1788. B.E. Jones and G.G. Buterbaugh, Univ. of Maryland Sch. of Pharmacy, Dept. Pharmacology and Toxicology, Baltimore, MD 21201

Status Epilepticus was evoked in amygdala kindled animals by pilocarpine (20mg/kg; i.p.) and amygdala stimulation. After ten min of SE, rats exhibit a bilaterally symmetric cerebral glucose uptake (CGU) except for the lower midbrain and brainstem regions. Diazepam (Dz; 6mg/kg; i.v.) 15 min after SE onset produced a profound loss of muscle tone, diminished CGU in the hippocampus, striatum and frontoparietal cortices but did not alter the EEG seizure. Ro15-1788 (Ro; 50mg/kg; i.v.) administered 5 min after diazepam completely reversed the behavioral effect of Dz; CGU in these rats was comparable to untreated rats. Ro (25mg/kg; i.v.) partially reversed the behavioral effect of Dz; CGU in these rats was also comparable to untreated rats. These results suggest that the effect of Dz to markedly reduce CGU during SE without attenuating the electrographic component of the seizure involves the GABA/Benzodiazepine receptor complex.

119.2

CHANGES IN INHIBITORY PROCESSES FOLLOWING RECURRENT SEIZURES INDUCED BY SYSTEMIC ADMINISTRATION OF KAINIC ACID. N.W. Milgram, T. Yearwood*, M. Khurgel, and R. Racine. Dept. Psychol., U. Toronto, Toronto, Ontario.

Paired pulse inhibition was monitored prior to, immediately following, and over a one month period after a single subcutaneous injection of kainic acid in rats chronically prepared with stimulation electrodes in the angular bundle and recording electrodes in the dentate gyrus. There was a progressive loss of inhibition in the period immediately following KA which predicted the development of seizures. This effect lasted for a maximum of 24 hours. Over subsequent testing inhibition recovered to levels significantly greater than baseline. Increased inhibition persisted for the duration of the experiment. The long-term increase was restricted to the early phase of inhibition. The late-phase of inhibition (analyzed at interpulse intervals between 200 and 300 msec) showed a transient decrease followed by recovery to baseline levels within 7 days. These findings are inconsistent with previous reports of a long-lasting decrease in inhibition produced by recurrent seizures.
(Sloviter, R.S., Science, 235: 73, 1987).

119.3

ACTIVATION OF SUPERIOR COLLICULUS, PRE-TECTUM AND ZONA INCERTA IN THE LATE STAGES OF STATUS EPILEPTICUS—AN ANTI-ICTAL MECHANISM? A. Handforth & D. M. Treiman*, VAMC Wadsworth and Dept. of Neurology, UCLA, Los Angeles, California 90024.

The later stages of status epilepticus (SE) are characterized electrographically by a transition from fast spiking (FS) to periodic epileptiform discharges (PEDs), and behaviorally by a devolution from frank to subtle convulsions. To investigate the underlying mechanisms of SE devolution, we studied the late stages of SE in the lithium-pilocarpine model with the ^{14}C-2-deoxyglucose (2DG) method. SE was induced in LiCl-pretreated rats with pilocarpine, 20 mg/kg i.p., then 2DG, 30 uCi, given as i.v. bolus at early FS, late FS, late FS with pauses (FS+P), early PEDs with jerks, late PEDs with jerks, or late PEDs with twitches (n=6 per group). Controls received both drugs but did not enter SE. 30 min after 2DG the brain was removed and sections applied to x-ray film. The resulting autoradiographs revealed in the first two FS stages intense generalized forebrain activity. The superior colliculus (SC), pretectum (PT) and zona incerta (ZI) remained low in activity. At the FS+P stage, however, which preceded PEDs, these areas displayed sharply increased activity, which continued to the latest stages of SE. It is proposed that the late activation of the SC-PT-ZI complex may be causally related to the devolution of SE.

119.4

SEIZURES INCREASE ACETYLCHOLINE AND CHOLINE IN RAT BRAIN. X. Gu and R.S. Jope, Dept. of Psychiatry, U. of Alabama , Birmingham, AL 35294.

Seizures induced by three convulsants, each causing status epilepticus, produced differential effects on acetylcholine (ACh) in rat brain. Status epilepticus induced by coadministration of lithium and pilocarpine caused increases in ACh in the cerebral cortex and hippocampus. Status epilepticus induced by a high dose of pilocarpine depleted ACh after 1 hr, followed by an increase. Status epilepticus induced by kainate increased ACh, but the magnitude of the increases were lower and the latencies were longer in comparison with lithium/pilocarpine, both of which may be reflective of the time course and characteristics of the electrical seizure activity. In contrast, ACh in the striatum was either unchanged or decreased by convulsant treatments. The finding that ACh increases in two models of status epilepticus in the cortex and hippocampus is in direct contrast with many in vitro reports in which excessive stimulation causes depletion of ACh.

The concentration of choline (Ch) increased during seizures in all three regions and with all three seizure models. This is likely to be due to activation of phospholipase C and/or D activity causing cleavage of Ch-containing lipids.

The excessive ACh, as well as the Ch, present during status epilepticus induced by Li and pilocarpine was responsive to pharmacological manipulation. Atropine tended to decrease ACh and increase Ch, similar to its effects in controls. The NMDA antagonist, MK-801, reduced the excessive concentrations of both ACh and Ch. Inhibition of Ch uptake by hemicholinium-3 administered icv reduced ACh in controls and when given to rats during status epilepticus.

These results demonstrate that the rat brain concentrations of ACh and Ch can increase during status epilepticus. The accumulated ACh was not in a static, inactive compartment, but was actively turning-over and was responsive to drug treatments. Excessive concentrations of ACh and/or Ch may play a role in seizure maintenance and in the neuronal damage and lethality associated with status epilepticus.

119.5

EFFECTS OF HYPERGLYCEMIA ON SEIZURE INDUCED BRAIN DAMAGE IN THE RAT, W. Andrew Kofke, M.D.*, M.A. Barmada, M.D., Thomas Rudy, Ph.D., University of Pittsburgh Department of Anesthesiology/CCM and Pathology (Neuropathology), Pittsburgh, PA 15261

Objectives. We tested the hypothesis that hyperglycemia exacerbates seizure-induced damage to the substantia nigra pars reticulata (SNPR).

Methods. Rats were anesthetized with halothane, intubated, and mechanically ventilated for surgery. Halothane was stopped for 1h. Three treatment groups (N=20 each) were subdivided by duration of SZ and recovery period, as follows (n=5 each subgroup): Controls (Ctl); 40% mannitol (Man) 40% Glc. Infusions were started 1h PreSZ. Flurothyl SZ were induced for 45 or 75 m. Rats underwent perfusion fixation end SZ or after 2h. At end SZ thiopental 15 mg/kg IV was given. Brains were assessed for eosinophilic neurons (EN) in neocortical areas and substantia nigra with severity of damage graded from 0-5 (0=no damage). SNPR was also graded (0-5) for degree of vacuolation (vac). Ridit analysis was used to test statistical significance.

Results. Glc was 59-107 mg% in ctl and Man groups and was 409 and 452 mg% in Glc groups. Glc administration decreased the SNPR grade (Glc vac = 2.8 vs control = 4.0, Glc EN = 3.3 vs control = 4.0; $P < .05$). No effect was evident for Man infusion or acute groups.

Conclusions. Hyperglycemia can be protective vs SZ-induced SNPR damage.

119.6

RENAL DYSFUNCTION FOLLOWING REPETITIVE SEIZURES IN RATS. B.L. Peronne*, R.S. Hodson*, R.C. Vari*, and N.R. Kreisman. Dept. of Physiology, Tulane Univ. Sch. Med., New Orleans, LA 70112

To determine whether acute renal failure occurs in status epilepticus, seizures were induced periodically in anesthetized, paralyzed rats by i.v. injection of pentylenetetrazol. Measurements were made of arterial blood pressure (BP), hematocrit (Hct), glomerular filtration rate (GFR), renal plasma flow (RPF), renal blood flow (RBF), urine protein concentration, urinary Na^+ and K^+ excretion, and urine flow ($\dot{V}$) both prior to and after 40 min of seizures. Following seizures, $\dot{V}$ increased from 11.3 to 29.3 μl/min, and Na^+ excretion increased from 0.74 to 1.98 μeq/min. RBF decreased from 8.6 to 4.1 ml/min, RBF decreased from 15.6 to 4.5 ml/min, and GFR decreased from 2.6 to 1.1 ml/min. No significant differences were found in BP, urinary K^+ excretion, or urine protein levels. The marked diuresis and large increase in Na^+ excretion, coupled with decreased RBF and GFR, suggests that seizures can produce acute renal failure probably mediated by prerenal vasoconstriction and tubular damage. (Supported by the American Heart Association National Center and La. affiliate).

119.7

PERSISTENT ALTERATIONS OF DENTATE GYRUS INHIBITION FOLLOWING KAINIC ACID-INDUCED STATUS EPILEPTICUS IN MATURE, BUT NOT IMMATURE, RATS. K. Haas, E.F. Sperber, S.L. Moshé and P.K. Stanton. Albert Einstein Coll. of Med., Bronx, NY 10461.

In adult rats, kindling and kainic acid-induced seizures produce sprouting of recurrent collaterals in the dentate supragranular layer and a persistant enhancement of inhibition of dentate granule cells. These changes accompany enhanced seizure susceptibility. In contrast, kainic acid-induced seizures in immature rats do not lead to long term increases in seizure susceptibility. Hence, we determined whether kainic acid-induced status epilepticus in immature (14-15 day old) rats is associated with persistent alterations in dentate gyrus synaptic transmission in hippocampal slices.

Thirty days after status epilepticus, extracellular recording were made in the granule cell body layer of hippocampal slices (400 μm) from seized rats and age-matched controls. Relative levels of inhibition and excitation were assessed by paired-pulse orthodromic stimulation of the perforant path. In controls, afferent stimulation produced a triphasic response consisting of an initial fast inhibition (0-20 msec), excitation (20-200 msec), and a late inhibitory phase (200-6000 msec). The level of granule cell excitability is measured as the change in amplitude of a test pulse delivered at varying intervals after a conditioning stimulus. We measured recurrent, feedback innervation by antidromic-orthodromic paired-pulse activation, and feed-forward inhibition by preceding the test pulse with a weak, epsp-generating orthodromic stimulus.

Adults exhibited enhancement of the late inhibitory component of paired-pulse responses four weeks after status was induced. In contrast, slices from 14-15 day old pups showed no significant changes in paired-pulse physiology four weeks post-status. These data suggest that the immature brain, though more seizure-prone than the adult, may be less susceptible to seizure-induced persistent alterations in neuronal excitability.

119.8

SYNAPTIC REORGANIZATION FOLLOWING STATUS EPILEPTICUS IS AGE-DEPENDENT. E.F. Sperber, K. Haas, P.K. Stanton & S.L. Moshé. Depts. of Neurology & Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461

Lesions of hippocampal afferents induce synaptic reorganization of hippocampal mossy fibers and redistribution of synaptic terminals in the supragranular layer of the dentate fascia. A similar pattern of synaptic sprouting has been observed following seizures in adult rats, suggesting the presence of a seizure induced lesion.

In previous studies, we reported that kainic acid induced seizures produce greater amounts of cell loss in the hippocampal CA3 region of adult rats as compared to pups. However, if undetected cell loss is present in the pup, we would expect synaptic sprouting to occur. To test this hypothesis, we exposed adult rats and 14-15 day old pups to kainic acid induced status epilepticus. Thirty days later, we determined whether sprouting ocurred by using the Timm stain.

Consistent with previous studies of adult rats, the present results indicate that kainic acid induced status epilepticus produced dense Timm staining in the supragranular layer of the hippocampal fascia dentate. In contrast, in rat pups, status epilepticus did not produce any aberrant Timm staining. This suggests that status epilepticus does not induce hippocampal mossy fiber reorganization early in life. These age related differences may be the result of the delayed maturation of the granular cell layer which is still developing and proliferating at 2-4 weeks of age.

119.9

PATTERNS OF 2-DEOXYGLUCOSE UPTAKE AND FOS IMMUNOREACTIVITY ASSOCIATED WITH LIMBIC STATUS EPILEPTICUS IN THE RAT. L.E. White and J.L. Price. Dept. Anat. & Neurobiol., Washington Univ., St. Louis, MO 63110.

Models of nonconvulsive and convulsive limbic status epilepticus were developed in the adult rat by 40 min of continuous electrical stimulation of either the basolateral amygdala, the anterior piriform cortex, or the olfactory bulb. Nonconvulsive status epilepticus is characterized by persistent low frequency (1-2 Hz), sharp wave discharges observed in depth EEG recordings during incessant exploratory behaviors. The associated 2-deoxyglucose (2-DG) uptake is concentrated in the basolateral amygdala and its efferent targets. Three olfactory areas, the anterior olfactory nucleus, the ventral tenia tecta, and the dorsal endopiriform nucleus, are also activated, but not the piriform cortex. Convulsive status epilepticus includes all of these same features, with the addition of emergent convulsive episodes manifested as discrete segments of high frequency (10-20 Hz), sharp wave activity and concurrent clonic behaviors. The accompanying pattern of 2-DG uptake consists of the nonconvulsive pattern, with the addition of several limbic and nonlimbic areas, especially the piriform and entorhinal cortices and the substantia nigra. In general, structures that exhibit high 2-DG uptake also contained many somata that were immunoreactive for the FOS protein, with the exceptions of the substantia nigra and the medial thalamus.

These findings demonstrate that common seizure circuits may be activated by stimulating different forebrain sites. Furthermore, they suggest that activation of the basolateral amygdala underlies nonconvulsive status epilepticus, while activation of other limbic and nonlimbic structures, particularly the piriform cortex and the substantia nigra, is required for the expression of convulsive status epilepticus. Supported by NIH DC00093 and NS07057.

119.10

FOREBRAIN DAMAGE INDUCED BY THE ACH-ESTERASE INHIBITOR SOMAN IS BLOCKED BY MK-801 EVEN WHEN GIVEN AFTER CONVULSION-ONSET. S. Sparenborg, L.H.Brennecke*, D.J. Braitman*. U.S. Army Medical Research Institute of Chemical Defense, APG, Maryland 21010

Diazepam or the NMDA antagonist MK-801 was given to guinea pigs during soman-induced status epilepticus (SE) to determine the time course of inhibitory and excitatory amino acid (EAA) involvement in the development of neuronal necrosis resulting from soman poisoning. Subjects were pretreated with pyridostigmine, challenged with soman ($2xLD_{50}$), and given atropine and pralidoxime chloride as therapy. MK-801 (0.3 or 1 mg/kg) or diazepam (1 or 3 mg/kg) was given at 5 or 120 min after the appearance of electrographic spikes. In other groups, MK-801 (0.3 mg/kg) or diazepam (1 mg/kg) was given at 50 min after spike appearance. The brains of all subjects that survived for 48 hours after soman challenge were prepared (with H & E stain) for light microsopic examination of the amygdala, hippocampus, thalamus, and the parietal and pyriform cortices.

SE and motor convulsions developed in each subject within five minutes of spike appearance. Neuronal necrosis, most severe in the amygdala and least severe in the thalamus, was observed in animals not given MK-801 or diazepam. MK-801 completely protected against necrosis and convulsions when given at the 5- and 50-min delays. Severe necrosis was found in the amygdala, and mild to moderate necrosis was found in the thalamus, hippocampus, pyriform and parietal cortices of the MK-801 120-min delay groups and in all groups treated with diazepam.

Previously, we had found that diazepam (2 mg/kg) given prior to soman completely prevented neuronal necrosis. In this experiment, however, when the administration of diazepam was delayed until only 5 min after the onset of electrical seizures, a substantial level of necrosis developed. It appears that the efficacy of diazepam was decreased when given after a critical period early in the development of SE, but the efficacy of MK-801 continued until 120 minutes after seizure onset. The relative superiority of MK-801 may be attributed to its ability to act directly on the EAA system, which has been shown to be critically involved in the induction of brain damage by several neurological insults.

ALZHEIMER'S DISEASE: BIOCHEMISTRY AND CLINICAL STUDIES

120.1

ELEVATION OF UNSATURATED FATTY ACIDS IN BRAIN MEMBRANE PHOSPHOLIPIDS IN ALZHEIMER'S DISEASE. T. Nakada and I. L. Kwee. Neurochem Res Lab, VA Med Ctr, Martinez, CA 94553 and Dept of Neurology, Univ of California, Davis, CA 95616.

Fatty acid constituents were analyzed in cerebral cortex of pathologically proven Alzheimer' brains and aged matched controls using conventional thin layer chromatography and gas liquid chromatography. Alzheimer's brains were consistently found to have higher mono- (16:1, 18:1) and poly-unsaturated (18:2, 18:3) fatty acids compared to age matched controls ($p < 0.01$). The elevation in essential fatty acids indicated that mechanisms other than diet are likely to be the cause of the observed elevation in unsaturated fatty acids in Alzheimer's cortex. The study strongly suggests that abnormalities in fatty acid metabolism and resultant membrane phospholipid abnormalities may play a role in the pathogenesis of Alzheimer's disease.

120.2

TROPHIC EFFECT OF BRAIN HOMOGENATE AND CEREBROSPINAL FLUID ON NEURONAL CELLS IN ALZHEIMER'S DISEASE

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GRC,NIA,Baltimore,MD.21224,NIDR,NIH,Bethesda,MD.

In order to determine if there is a toxic or a tangle promoting factor in Alzheimer's disease(AD), we tested the effect of brain extract, cerebro spinal fluid (CSF) and serum from both AD patients and control individuals, on neuronal cell branching, process formation and adhesion. Pheochromocytoma (PC-12) cells and neuroblastoma-glioma NG108-15 cells were cultured in serum free defined media containing various concentrations of brain homogenates, CSF or serum from patients with AD and control subjects on substrates of plastic and laminin. We found the brain homogenates from both AD patients and control individuals enhanced neurite outgrowth in PC-12 and NG-108 cells in the presence of laminin. However only AD brain homogenate stimulated an increase in branching per neurite process in PC12 cells after approximately 4 hours of culture on laminin substrate.

The observed neurotrophic effect of Alzheimer's brain tissue extract on neuronal culture may be explained by the presence of a neurotrophic factor giving rise to abnormal sprouting and tangle promoting activity in Alzheimer's disease. Such a factor might be produced in response to neuronal loss or as a part of Alzheimer's pathology, characterization of such a factor may be useful in understanding the pathophysiology of the disease.

120.3

BASIC FIBROBLAST GROWTH FACTOR (FGF) IN ALZHEIMER'S DISEASE. A. Baird*, A.M. Gonzalez*, E.D. Bird#, R. Chorsky*, J. Alvarez*, R.J. Corona*, and E.G. Stopa#. The Whittier Inst., La Jolla, CA, McLean Hosp., Belmont, MA and #Dept. of Path., SUNY Health Science Center, Syracuse, NY

Although nerve growth factor (NGF) has been considered for therapeutic use in the treatment of Alzheimer's disease (AD) (*Neurobiol. of Aging* 10:205-207, 1989), basic (FGF) has been shown to be equally as effective in supporting the growth of basal forebrain cholinergic neurons (*Nature* 332:306-361, 1988). In this study, we examined basic FGF in control and AD brains in an effort to characterize a possible role for the growth factor in the pathogenesis of AD. Immunocytochemical localization with a specific polyclonal antibody (773), revealed that basic FGF is widely distributed in astrocytes and neurons throughout the normal prefrontal cortex and hippocampus (n=5). In AD (n=4), immunoreactive FGF was substantially increased in these regions and could be localized in neuritic plaques and in association with neuronal neurofibrillary tangles. *In situ* hybridization for FGF mRNA suggested an increased expression of the FGF gene within the hippocampus of Alzheimer patients. Western blotting established the presence of basic FGF in all samples studied and quantitative RIAs performed on fresh frozen samples of cerebral cortex confirmed that the concentration of FGF was approximately 2-fold greater in Alzheimer patients. These observations support the hypothesis that basic FGF may have neurotrophic activity in the human brain and suggest that it is increased during the "injury" response of AD. By virtue of basic FGF's affinity for heparan sulfate-like proteoglycans, and the association of these GAGs with amyloid plaques, our data also suggest that the increased FGF observed in AD may not necessarily reflect the bioavailability of the growth factor to surrounding neurons. RVHPGSS00002, AG00295, DK-18811.

120.4

PEPTIDE PROFILE VARIATIONS RESULTING FROM THE ADDITION OF KAINIC ACID TO FETAL RAT HIPPOCAMPAL CELL CULTURES. M.L. Zettel, J.R. Slemmon & P.D. Coleman. Dept. Neurobiology and Anatomy & Dept. Biochemistry, Univ. of Rochester, Rochester,N.Y. 14642

For about a century it has been known that nervous system injury leads to glial cell reaction. More recent evidence from studies in which cell death is limited to a neuronal population suggests that this glial reaction is a response to neuron death (Streit & Kreutzberg, 1988). We propose that this glial response may be mediated by altered release of signal peptides by dying or dead neurons. We have found candidate peptides whose release into the microenvironment is altered following manipulations designed to produce neuron death.

Kainic acid (KA) is a neuroexcitatory agent which has been shown to induce neuron death. We added four concentrations (.0, .1mM, .5mM, 1.0mM) of KA in DME to hippocampal and cerebellar microexplant cultures (Moonen, et al., 1990) derived from fetal day 18 rats as well as to media alone. Reverse phase HPLC showed that KA added to media alone abolishes one large peptide peak. Chromatograms obtained from defined media of explants in which there had been presumptive neuron death show peptide peaks which consistently differ in amplitude from control cultures. Identical experiments performed using cerebellar cultures from the same fetal rats do not show most of these changes. The peptides of some of these peaks are being isolated and added to glial cell culture preparations to examine the effects of the peptides on glial proliferation. Peptides of interest are also being sequenced. Supported by NIH grants: AG01121, AG03644, AG09016.

120.5

NA/CA EXCHANGE ACTIVITY IN CEREBRAL PLASMA MEMBRANE VESICLES IS INCREASED IN ALZHEIMER'S DISEASE. (AD) R.A. Colvin,* J.W. Bennett,* S.L. Colvin,* and G.D. Miner. Pharmacology Dept., ORU School of Medicine and The Alzhiemer's Foundation, Tulsa, OK, 74137

Cerebral plasma membrane vesicles (PMV) were purified by sucrose density gradient centrifugation from frozen postmortem hippocampal/temporal cortex tissue slices derived from age matched brains of normal, AD and Non-Alzheimer dementia (NAD) origin. Na/Ca exchange of PMV increased rapidly at first and then maintained a steady plateau for up to 5 min. When the Ca ionophore A23187 (10μM) was added after 4 min, Ca content was immediately reduced by 90% Ruthenium red (10μM) had no effect on Ca content. Na/Ca exchange was increased in AD brains as evidenced by both an increase in the initial rise in Ca content and in elevated values of peak plateau Ca content. The K_m and V_{max} for Na/Ca exchange was obtained from Lineweaver-Burk analysis of the effect of increasing concentrations of Ca on Ca content after 30 sec. The values obtained for the K_m (μM) and V_{max} (nmol/mg/30 sec) were (respectively): normal (n=6) 57.9±28.1 and 2.23±.29; AD (n=6) 71.2±29.3 and 3.34±.794; NAD (n=4) 66.1±3.3 and 1.88±.75 (mean±S.D.). The V_{max} for Na/Ca exchange in AD brain tissues was significantly ($p<.05$) elevated when compared with normal or NAD brains. The results suggest that Na/Ca exchange activity is probably increased in AD brain in regions suffering the greatest degeneration.

120.6

ALTERED PROTEIN TYROSINE PHOSPHORYLATION IN ALZHEIMER DISEASE. I.P. Shapiro, E. Masliah, T. Saitoh. Department of Neurosciences, School of Medicine (M-024), University of California, San Diego, La Jolla, CA 92093.

Since protein tyrosine kinases (PTKs) and their substrates are important in signal transduction and cellular regulation in the brain, alterations in protein tyrosine phosphorylation might be involved in Alzheimer disease (AD) pathology. The activity of PTKs was determined in extracts from AD autopsy brains and age and post-mortem time matched unaffected control brains using the PTK synthetic peptide substrate, poly(Glu_4Tyr_1). The specific activity of PTKs in the particulate fraction was decreased two-fold ($p< 0.005$) in AD relative to controls. Cytosolic PTK activity was not significantly altered. Quantitative immunohistochemistry and morphometry of frontal cortex sections with anti-phosphotyrosine (anti-PTyr) antibody indicated increased anti-PTyr staining in neurons, although the number of anti-PTyr positive neurons per mm^2 decreased. Also, increased anti-PTyr staining was observed in hippocampal neurons. Analysis of Western blots of proteins cytosol and particulate fractions revealed increases in cytosolic anti-PTyr immunoreactive polypeptides with molecular mass of 60 and 55 kDa. These results suggest that altered PTK activity and protein tyrosine phosphorylation are involved in AD pathology.

120.7

ISOLATION OF A NOVEL 66 kD PHF PEPTIDE FROM ALZHEIMER'S BRAIN. F.P. Zemlan, G.D. Vogelsang, E.F. Schwab and G.E. Dean. Department of Psychiatry, Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0559.

Electrophoretic purification and solubilization of PHF (Vogelsang et al., J. Neurochem. 54 1990 148) yields two groups of PHF peptides, a broad band of PHF peptides immunoreactive with the pAb PAM raised against an enriched PHF preparation and a group of 55-66 kD PHF peptide fragments not immunoreactive with PAM. These two groups of PHF fragments were independently isolated based on their differential affinity for nitrocellulose. The 55-66 kD peptide fraction with low nitrocellulose affinity demonstrated a ladder of 55-66 kD proteins on silver stained SDS-PAGE and no immunoreactivity with either PAM or the MAP-tau mAb Tau-1 on Western blots. This fraction was employed for pAb production in mice. Following immunoblot pAb screening against purified PHF; a pAb M1 was identified which labeled a primary 66 kD PHF peptide fragment on Western blots. Immunocytochemical studies demonstrated that M1 pAb selectively labeled neurofibrillary tangles in formalin fixed AD brain with no labeling observed in age matched normal brain. The present data suggest that a novel 66 kD PHF peptide fragment has been isolated which appears unrelated to MAP-tau. Further characterization studies are ongoing to determine whether this 66 kD PHF peptide fragment represents a novel protein present in low abundance in normal brain.

120.8

COMPARISON OF A SANDWICH ENZYME IMMUNOASSAY FOR ALZHEIMER'S DISEASE ASSOCIATED PROTEIN (ADAP) IN HUMAN BRAIN AND THE PRESENCE OF A68 BY WESTERN BLOT. *Hossein A. Ghanbari and Barney E. Miller.* ABBOTT LABORATORIES, DEPT 9MA/AP20-3, ABBOTT PARK, IL 60064

We have developed a sandwich enzyme immunoassay, ALZ-EIA (Brain) for measuring Alzheimer's Disease Associated proteins (ADAP) in human brain tissue. Although both of the antibodies used in this assay (PR1 and ALZ-50) cross-react with normal brain components (including tau proteins), the combination appears to minimize cross-reactivity and the assay has demonstrated a high level of specificity for Alzheimer's Disease (AD) cases. A-68 is one of the the proteins detected in the human AD brain as indicated by Western blot using ALZ50. This protein is absent in non-AD brains. We have compared ADAP reactivity by ALZ-EIA (Brain) and presence of A68 by Western blot using a total of 61 brain specimens. The brain specimens included: AD(n=21), AD/Down's (n=2), Normal (n=14), Parkinson's Disease (n=7), Huntington's (n=2), Korsakov (n=2), Wernicke (n=1) and Motor Neuron Disease (n=2). The non-AD cases (n=28) had no detectable ADAP by ALZ-EIA (Brain), and showed no A-68 band by Western blot using ALZ-50. The AD cases (n=23, including the two AD/Down's), all were positive for ADAP by the ALZ-EIA (Brain), but only 21 of 23 had visible A-68 band by Western blot. ALZ-EIA (Brain) compared well with the presence of A-68 bands by Western blot and demonstrated very high specificity and sensitivity.

120.9

DISTRIBUTION OF ALZHEIMER'S DISEASE ASSOCIATED PROTEIN (ADAP) IN VARIOUS REGIONS OF HUMAN BRAIN. B.E. Miller[1], W.O Whetsell, Jr.[2], H. Haigler[1], and H.A. Ghanbari[1]. Dept. 9MA, AP20, Abbott Laboratories, Abbott Park, IL 60064[1] and Neuropathology, Dept. of Pathology, Vanderbilt University, Nashville, TN 37232-2561[2].

The distribution of an Alzheimer's Disease associated protein complex (ADAP) was studied by enzyme immunoassay (see Ghanbari, *et al.*, JAMA, June, 1990, for details of the assay) in post-mortem brain tissue from a group of five Alzheimer'sDisease (AD) patients and a group of five non-Alzheimer's Disease (NAD) patients. In each of these ten brains, eighteen different brain regions were examined including frontal, temporal, parietal and occipital cortex, hippocampus, subiculum, amygdala, caudate nucleus, putamen, globus pallidus, thalamus, hypothalamus, subthalamus, basal nucleus of Meynert, substantia nigra, locus ceruleus, medulla, and cerebellum. ADAP is a protein complex of total molecular weight of more than 200 kDaltons which produces three major bands in SDS-PAGE Western blot using ALZ50 antibody. This enzyme immunoassay (ALZ-EIA) has been configured to minimize cross-reactivity with normal brain components by using the antibodies ALZ50 and PR1, a polyclonal antibody against ADAP (ibid.) In all five NAD brains (including one case of multi-infarct dementia without Alzheimer's), no ADAP was detected by ALZ-EIA. ADAP was consistently detected in all five AD brains with highest levels being found in regions associated with cognition and memory.

120.10

THE DISORGANIZATION OF VISUAL COGNITIVE FUNTION IN PATIENTS WITH ALZHEIMER'S DISEASE(AD)-COMPARATIVE STUDY OF AD WITH DEVELOPING CHILDREN-. M.Fujii*., S.Murakami**., J.Miyazawa*., R.Fukatsu*., N.Nakano*., Y.Aizawa*., T.Saito*., N.Takahata*., T.Fukuda***., M.Yamada***. *Dept. of Neuropsychiatry, Sapporo Medical College., **Yamanoue Hospital, Sapporo,060. ***NHK Science and Technical Lab., Tokyo, 156.

There are some evidences suggesting disorganization of visual cognitive function plays an important role in occurance of neuropsychological disturbances, which are observed frequently in patients with AD. Our previous studies showed that a vision analyzer is useful in investigating the visual information processing and that subclinical findings are recognizable similar to unilateral neglect syndrome (UNS) and Balint's syndrome (BS) even in early stage of AD. On the other hand, it is well known that children complete the visual cognitive function during their development. In this study, we compare the visual information processing of AD with that of chilren. We examined 7 patients with AD (range 56-63) and 30 controls (range 6-81 including 6 children below 9), using a vision analyzer (TKK939). Results obtained are (i) in children below 9, each gazing time became shorter than the aged controls and gazing points were seen more on the copied figure than the model, and multisaccades were seen on the model, but BS (e.g. a tendency to be localized gazing points out of two figures) was not seen; (ii) in the elderly children and the other controls, the gazing points distributed both on the model and the copied figure equally; (iii) in the early stage of AD, each gazing time became shorter like children below 9, but in the moderate stage of AD, each gazing time became much shorter and few gazing points were seen on the model. Our results demonstrate some similalities and differences between visual information processing of AD, disorganization of visual cognitive function and that of children below 9 years old, undeveloped visual cognitive function.

120.11

CLASSICAL CONDITIONING OF THE EYEBLINK RESPONSE IN ALZHEIMER'S DISEASE AND MULTI-INFARCT DEMENTIA. R. G. Finkbiner, D. J. Libon, D. K. Sasse, J. M. Coffin, K. E. DeMott, & D. S. Woodruff-Pak. Dept. of Psychology, Temple Univ. and Philadelphia Geriatric Center, Philadelphia, PA 19141.

We reported striking differences in conditioning between patients diagnosed as probable Alzheimer's disease (AD) and non-demented elderly. For this study, it was predicted that AD patients with impaired cholinergic input to hippocampus would all show poor conditioning. MID patients would show poor conditioning only if the vascular lesions affected relevant subcortical areas. Thus, mean MID scores should be higher than AD scores. We tested patients who met NINCDS-ADRDA criteria for the diagnosis of probable AD (N=17; age=84.9) or MID (N=8; age=83.3). They were age-matched to non-demented elderly (N=16; age=83.4). Scores on the Blessed Memory-Information-Concentration test for AD, MID, and non-demented elderly were 17, 14, and 3, respectively. Subjects' hearing was tested, and they received 90 trials of 500 msec, 80 dB tone CS paired with 100 msec, 5 psi corneal airpuff US in the delay paradigm (400 msec CS-US interval). Percent CRs (corrected for alpha responses) for AD patients were 12.1, for MID patients were 23.6. and for non-demented elderly were 30.7. Eyeblink conditioning differentiates among demented patients as predicted. (Supported by an Alzheimer's Association/NJAHCF Research Grant).

120.12

HIPPOCAMPAL GLUCOSE METABOLISM IN ALZHEIMER'S DISEASE. W.J. Jagust, J.L. Eberling, M.G. Baker*, T.E. Nordahl*, P.E. Valk*, B.R. Reed*, T.F. Budinger*. Donner Laboratory, Lawrence Berkeley Laboratory, University of California, Berkeley, CA 94720.

Measurement of glucose metabolism in mesial temporal lobe structures in Alzheimer's disease (AD) has been limited by the spatial resolution of existing tomographs. We utilized a high resolution (2.6 mm in-plane) PET tomograph and ^{18}F-fluorodeoxyglucose to study glucose metabolism in the temporal lobes in 7 patients with very mild to moderate AD and 6 age-matched controls. Beginning 30 min following injection of the tracer, 3 tomographic levels were scanned -20^0 to the canthomeatal line contiguously through the temporal lobes; a fourth scan was obtained at a level including the basal ganglia. Previously determined rate constants were used with an operational equation to calculate regional cerebral metabolic rates for glucose (rCMRglc), which were then averaged over three levels for temporal lobe structures.

AD patients showed lower rCMRglc than controls in all regions studied, with significant differences ($p < .005$) in all temporal lobe regions and in visual cortex. No significant differences were seen between the groups in frontal lobes or subcortical regions. The pattern of metabolism was similar, with the lowest metabolic rates seen in anterior temporal neocortical and mesial temporal lobes in both groups (AD patients averaged 26±8 μmol/100g/min in anterior temporal and controls 42±3 μmol/100g/min). There was no overlap between AD patients and controls in anterior temporal and mesial temporal regions. Thus, while AD involves a generalized decline in glucose metabolism which is most severe in temporal lobes, the anterior and mesial temporal lobe regions differentiate AD patients from controls best.

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SYMPOSIUM. THE OLIVO-CEREBELLAR SYSTEM: ITS POSSIBLE ROLE IN LEARNING. J.A. Harvey, Med. Col. of Pennsylvania (Chairperson); R.R. Llinás New York Univ.; J.P. WELSH, Univ. of Iowa; C.H. YEO, Univ. Col. London, England; R.G. BAKER, New York Univ; S.G. LISBERGER, Univ. of California.

The purpose of this symposium is to review and critically evaluate recent data that have advanced our knowledge concerning the participation of the olivo-cerebellar system in the acquisition of motor skills. Cerebellar involvement in learning has been examined primarily within two motor systems: the vestibulo-ocular reflex and the classically conditioned nictitating membrane response. Sites of plasticity for these examples of motor learning have been suggested to involve various loci within the olivo-cerebellar system. However, a growing body of data suggests that many of these conclusions about the cerebellum as a site of learning and memory have been premature and need to be modified. While the olivo-cerebellar system is clearly important for learning, there is mounting evidence that the actual site or sites of plasticity may be in other parts of the brain. This symposium will explore the recent data and the issues involved, and provide some suggestions for resolving the controversies in this field.

125

SYMPOSIUM. DIRECT ION CHANNEL GATING BY INTRACELLULAR IONS AND MOLECULES. P.E. Hockberger, Northwestern Univ. Medical School (Chairperson); P. Gardner, Stanford Univ. School of Medicine; G. Matthews, SUNY - Stony Brook; L.D. Partridge, Univ. of New Mexico; S.Dryer, Florida State Univ.; P.R. Stanfield*, Univ. of Leicester.

The list of intracellular molecules and ions that can affect membrane ionic conductances has been growing in recent years. This symposium will focus on examples of ion channels that are gated by intracellular ligands and supported by single-channel studies using isolated membrane patches. Dr. Gardner will describe a voltage-insensitive calcium channel that is opened by intracellular IP3 elevation in T-lymphocytes. Regulation of this channel is coupled to the T3-Ti antigen-major histocompatibility receptor complex, and activation of the complex results in clonal proliferation through a calcium-dependent mechanism. Dr. Matthews will describe a sodium current found in vertebrate photoreceptors, olfactory receptors, and invertebrate neurons that is regulated by the level of intracellular cGMP (or cAMP) which in turn is controlled by the level of phosphodiesterase activity. Dr. Partridge will describe a Ca^{2+}-activated, non-specific cation channel that is important in excitation-secretion coupling and is involved in shaping neuronal firing patterns. Dr. Dryer will describe how elevation of intracellular Na^+ opens a novel potassium channel in both vertebrate and invertebrate neurons that also contributes to shaping neuronal firing patterns. Dr. Stanfield will describe how intracellular ATP is responsible for regulating a novel potassium channel in nerve and muscle cells. Under conditions of energy depletion this channel would open, thereby preventing maintained excitation and possibly protecting the cell from extreme fatigue and/or excitotoxicity.

126.1

PROCESSING OF THE LHRH PRECURSOR IN THE GT1 CELL LINES DERIVED FROM TRANSGENIC MICE. **W.C. Wetsel, P.L. Mellon*, R.I. Weiner, and A. Negro-Vilar*. Lab. of Mol. & Integrat. Neurosci., NIH/NIEHS, Res. Tri. Pk., NC 27709; Reg. Biol. Lab., Salk Institute, La Jolla, CA 92037; Reprod. Endo. Center, Univ. of Cal., San Francisco, CA 94143.**

Several cell lines (GT1-1, GT1-3 and GT1-7) were developed by genetically targeted tumorigenesis in transgenic mice (Mellon et al., Neuron, in press). These cells biosynthesize and secrete immunologically recognizable LHRH. We have characterized this material using size-exclusion chromatography. LHRH-like immunoreactivity (IR) was measured by A772 antisera, while MC-2 antisera were used to measure pro-LHRH- and GAP-like IR. When extracts or media from GT1-1, GT1-3 and GT1-7 cells were separated according to molecular weight (MW), multiple peaks of immunoreactivity were detected. Cell extracts contained GAP-like materials at approximately 14000-16000, 8200 and 6500 molecular weight (MW). The LHRH antisera bound materials at approximately 8200 and 1200 MW. By comparison, media contained GAP-like IR at approximately 6500 MW, while LHRH-like material was bound at approximately 1200 MW. When the 1200 MW material was added to an anterior pituitary cell culture, secretion of LH was stimulated in a dose-dependent manner. These data indicate that processing of the pro-LHRH precursor in the GT1 cells is similar to that found in the rat. The development of the GT1 cells is important because they allow the molecular mechanisms which underlie the biosynthesis, processing and secretion of pro-LHRH peptides to be investigated. **[Supported by the NIEHS Intramural Program (WCW and ANV) and Grants HD 20377 (PLM) and HD 08924 (RIW)].**

126.2

DYNAMICS OF GnRH SECRETION FROM PERIFUSED GT1-1 CELLS. G. Martinez de la Escalera*, A. L. H. Choi* and R. I. Weiner. Reprod. Endocrinol. Ctr., Univ. of Ca., San Francisco, CA. 94143.

GnRH cell lines were developed in transgenic mice by genetically targeted tumorigenesis. The cells synthesize and release GnRH, express neuronal but not glial markers and have a neuronal phenotype. We have examined the dynamics of GnRH release in perifusion. GT1-1 cells were cultured on coverslips for three days, placed in a Sykes-Moore chamber and perifused (0.3 ml/min) with Locke's medium containing bacitracin. Samples obtained every 2 min were radioimmunoassayed with the Nett R1245 GnRH-specific antiserum. Basal secretion from GT1-1 cells varied between 5 and 10 pg/min. Over a 2 h collection period occasional pulses were observed with amplitudes 1.5 to 6 fold over basal. Removal of Ca^{2+} inhibited spontaneous pulses of GnRH release. Depolarization for 2 min with 56m*M* K^+ caused a 50 fold increase in GnRH release. Maximal levels were reached within 2 min and returned to normal within 10 min. The time course of the response to 15m*M* K^+ was similar but only approximately 10% the amplitude. The removal of Ca^{2+} blocked the response to K^+. Increases in cAMP levels with 2.5m*M* 8-Br-cAMP or 10u*M* forskolin, induced a 3 or 10 fold sustained increase in GnRH, respectively. Basal secretion during relatively short term studies is characterized by the release of pulses of GnRH which are absent following removal of Ca^{2+}. The release of GnRH from GT1-1 cells is stimulated by depolarization with K^+ via a Ca^{2+} dependent mechanism and by increasing intracellular cAMP. Supported by NIH Grant HD08924(R.W.) and the Rockefeller Foundation (G.M.E.)

126.3

INHIBIN/ACTIVIN SUBUNITS ARE CO-STORED WITH GONADOTROPINS IN SECRETORY GRANULES OF THE RAT ANTERIOR PITUITARY GLAND. V.J. Roberts, C.A. Peto*, W.W. Vale and P.E. Sawchenko, The Salk Institute, La Jolla, CA 92037.

The gonads are the major source of circulating inhibin (α/β_A or α/β_B) and activin (β/β). These protein dimers regulate FSH release from the anterior pituitary. Recently, we reported that the gonadotropes are also sources of inhibin and activin subunits (α and β_B) and mRNAs (Endocrinology 124:552, 1989). Here, we determined the extent to which the α- and β_B-subunits might be colocalized with gonadotropins in secretory granules. Dual postembedding immunogold staining methods were used on pituitary from adult male rats, which were cryofixed, molecular distillation dried, and resin-embedded (LifeCell Corp., The Woodlands, TX). Sections were stained on one side with antiserum against either the α- or β_B-subunit and on the other with antiserum against FSH or LH. Secondary antisera were labeled with 5 nm or 15 nm colloidal gold. The inhibin-α subunit colocalized with FSH in 32% of all positively stained granules and with LH in 36% of the granules. The β_B-subunit was found with FSH in 28% of the granules and with LH in 25% of the granules. Approximately 50% of the granules contained only FSH or LH and 10% to 30% were positive only for the inhibin subunits. At least 30% of the granules that are immunopositive for the inhibin proteins show positive signals for both subunits. These results suggest that inhibin and activin may be packaged, and released, either alone or along with FSH and LH.

126.4

REPETITIVE IV INJECTIONS OF L-GLUTAMIC ACID, IN CONTRAST TO THOSE OF NMDA, FAIL TO SUSTAIN INTERMITTENT HYPOTHALAMIC GnRH RELEASE IN THE PREPUBERTAL RHESUS MONKEY. R. Medhamurthy*, V.L. Gay* and T.M. Plant. Dept. of Physiology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261.

In prepubertal male rhesus monkeys, repetitive iv injections of an NMDA receptor agonist (NMDA) elicit sustained trains of hypothalamic GnRH release as reflected by the LH response of the pituitary gland. The purpose of the present experiment was to determine whether similar stimulation with the putative endogenous ligand, L-glutamic acid (Glu), is also able to elicit intermittent hypothalamic GnRH release. An earlier study indicated that a single iv bolus of Glu at 150 mg/kg BW induced a robust GnRH discharge from the hypothalamus of prepubertal monkeys (Medhamurthy, et al., Ann. Meeting Am. Fed. Clin. Res., 1990). Four prepubertal males primed with exogenous GnRH to heighten the responsivity of the pituitary to this releasing hormone, received an iv injection of Glu (150 mg/kg BW) every 3 h for 9 h. The mean (± SE) peak concentration of the LH discharge in response to the 1st, 2nd and 3rd injection of Glu was 68.5, 44.9 and 34.1 ng/ml, respectively. In a separate experiment (n=3), in which the iv intermittent Glu treatment was extended to 24 h, the LH releasing ability of Glu was abolished by the 9th injection. However, administration of NMDA (2-5 mg/kg BW) at the end of 9 or 24 h of intermittent Glu treatment resulted in a robust LH discharge, and the ability of repetitive NMDA administration to sustain GnRH release in these particular animals was confirmed during a 12 h study. One possible explanation for the finding that repetitive injections of Glu fail to sustain GnRH release, without concomitant loss of sensitivity to NMDA stimulation, is that Glu induced GnRH release may be mediated primarily by non-NMDA receptors that are unable to respond to intermittent stimulation with a periodicity of 3 h. Experiments are underway to resolve whether prolonged activation of non-NMDA receptors, such a kainate or quisqualate receptors, also leads to a progressive loss in the ability to release GnRH.

126.5

DISTRIBUTION AND NEUROENDOCRINE ROLE OF KAINATE AND OTHER EXCITATORY AMINO ACID RECEPTORS IN MONKEY HYPOTHALAMUS. A.J. Billy, R. Medhamurthy*, C. Shaw and T.M. Plant. Dept. of Physiology, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261 and Depts. of Physiology, Zoology and Ophthalmology, Univ. of British Columbia, Vancouver, Canada, V6T 1 W3.

The finding that repetitive iv injections of NMDA elicit a sustained train of GnRH discharges from the hypothalamus of the prepubertal monkey prompted the present study to determine the distribution of Glu receptors throughout the primate hypothalamus. Serial, frozen, coronal sections (20μ) of the hypothalamus from a juvenile and adult male rhesus monkey were thaw mounted onto subbed glass slides for subsequent autoradiographic analysis of Glu receptor subtypes, using H^3 labelled KA, MK-801 and CNQX as KA, NMDA, and Q receptor ligands, respectively. Binding sites labelled with H^3 KA, MK-801 or CNQX were found throughout the mediobasal hypothalamus (MBH). A striking feature of binding in this region of the primate brain was an extremely high density of KA receptors in the region of the arcuate nucleus and median eminence: this contrasted with a relatively unremarkable and diffuse pattern of NMDA and Q receptors in MBH. In addition, KA and Q receptors were heavily labelled in the SON, and KA receptor was detected in the lamina terminalis, the tuberal portion of the SON, and the PVN. To study the role of non-NMDA receptors in the regulation of hypothalamic GnRH release, the effects of iv KA and Q receptor agonists on GnRH release were examined in juvenile male monkeys utilizing LH release from a bioassay for the decapeptide. Injections of KA (0.5 mg/kg BW) elicited distinct discharges of LH. Q injections (0.5-2.5 mg/kg BW), however, produced a less robust and inconsistent response. Prior treatment with AP5, an NMDA receptor antagonist, failed to block KA induced LH release. Studies are in progress to confirm that KA exerts its action on LH release at a suprapituitary level. These findings suggest that KA receptors, most probably localized in the region of the arcuate nucleus and median eminence, play a role in regulating GnRH release from the primate hypothalamus.

126.6

EFFECTS OF N-METHYL-D,L-ASPARTATE (NMDA) ON THE REPRODUCTIVE AXES OF HAMSTERS IN LONG VS. SHORT-DAY PHOTOPERIODS. J.M. Meredith, F.W. Turek, J.E. Levine. Dept. Neurobiol/Physiol, Northwestern Univ., Evanston, IL 60208

Hamsters exposed to short-day photoperiods (LD 6:18) develop a reversible inhibition of the reproductive axis which includes decreased LH pulse frequency, decreased FSH and testosterone (T) levels and testicular regression. This study examines the role of hypothalamic LHRH neurons in the short-day inhibition of the reproductive axis. Golden hamsters were housed for 10 wks under LD 14:10 and maintained for another 10 wks under either LD 14:10 or LD 6:18. Animals were bled every 10 minutes from 1300-1800 h and given 4 hourly injections of the LHRH secretagogue, NMDA (10 mg/kg). Long-day animals responded to successive NMDA administrations with mean LH pulse amplitudes of 2.65±.09, 1.22±.50, 1.22±.43, and 1.15±.42 ng/ml respectively. Short-day animals responded to NMDA with mean LH pulse amplitudes of 1.61±.31, 2.89±.34, 3.08±.59 and 2.65±.32 ng/ml. Baseline T levels and paired testes weights were significantly greater in long-day animals. Robust LH responses in short-day animals suggest that photoperiodic inhibition of the hamster reproductive axis is not due to a reduction in the secretory capacity of LHRH neurons, viz. a reduction in size of the releasable LHRH pool. Instead, inhibition of the reproductive axis by short-day photoperiods may occur at or above the level of the LHRH pulse generator. Supported by NIH grants RO1-HD20677, PO1-HD21921, and KO4-HD00879.

126.7

N-METHYL-D,L-ASPARTIC ACID (NMDA)-INDUCED LH RELEASE IN HYPOGONADAL FEMALE MICE WITH FETAL PREOPTIC AREA GRAFTS (HPG/POA). Y. Saitoh, A.J. Silverman, and M.J. Gibson.Div. Endocrinology, Mount Sinai Sch. of Med., New York,NY 10029

In order to clarify the mechanism of NMDA-induced LH release, HPG/POA were challenged by venous injection of NMDA, and the results were compared with those of normal female mice. Most HPG/POA showed persistent estrus and ovarian and uterine development by 60 days after successful grafts; a few started cycling after pregnancies by reflex ovulation. One group of normal and HPG/POA mice were ovariectomised (OVEX); a second group were OVEX and estrogen primed (OVEX/E2), and a third group included intact cycling normal and cycling HPG/POA that were tested in the estrous state. After blood sampling via intrajugular catheters, HPG/POA were perfused for light and/or electron microscopic immunohistochemical studies. There were significant increases in plasma LH at +10 min after NMDA challenge in normal OVEX (baseline: 0.58±0.20; +10 min: 1.85±0.41 ng/ml) and OVEX/E2 (baseline: 0.33±0.02; +10 min: 0.80±0.21ng/ml). HPG/POA OVEX/E2 mice (n=7), which had 4-12 GnRH cells and GnRH axonal outgrowth, responded to NMDA with increased plasma LH (baseline: 0.36±0.03; +10 min: 1.03±0.24 ng/ml). HPG/POA OVEX/E2 mice (n=5) with sparse GnRH fiber outgrowth did not respond to NMDA. Neither normal estrous nor cycling HPG/POA estrous mice responded to NMDA. These results suggest that NMDA stimulation of GnRH cells is estrogen-sensitive and may be mediated by interneurons.

126.8

PHOTOPERIODIC MODULATION OF *N*-METHYL-D-ASPARTATE (NMDA) RECEPTOR BINDING IN THE SYRIAN HAMSTER. H.F.Urbanski and M.Pierce*. Division of Neuroscience, Oregon Regional Primate Research Center, Beaverton, OR 97006.

The involvement of excitatory amino acid receptors in the photoperiodic control of seasonal breeding is suggested by the finding that administration of NMDA to Syrian hamsters can block the inhibitory influence of short days on the reproductive axis. To investigate the effect of photoperiod on the binding properties of NMDA receptors adult male hamsters were either maintained under long days (LD) or induced to revert to a juvenile condition by exposure to short days (SD). The procedure of Price *et al.*, (Eur. J. Pharmacol. 158:279, 1988) was then used for the *in vivo* labeling of NMDA receptors with [^{3}H]MK-801, a specific noncompetitive antagonist. Under both photoperiods, the specific binding of MK-801 to NMDA receptors was high in the cerebral cortex (ca. 50 dpm/mg wet tissue), significantly lower ($P<0.05$) in the medial basal hypothalamus (ca. 10 dpm/mg w.t.), and undetectable in the pituitary gland. In contrast, the specific binding of MK-801 in the pre-optic area (POA) was significantly ($P<0.05$) higher in the sexually active (LD) than in the sexually quiescent (SD) animals (31 *vs.* 7 dpm/mg w.t., respectively). Since this brain region is particularly rich in neurons that secrete luteinizing hormone-releasing hormone, photoperiodic modulation of POA NMDA receptor binding might play a pivotal role in the neuroendocrine control of seasonal breeding.

Supported by NIH grants HD-24312 and RR-00163.

126.9

EFFECTS OF PHOTOPERIOD AND MELATONIN ON IN VITRO HYPOTHALAMIC GONADOTROPIN RELEASING HORMONE (GnRH) RELEASE IN DJUNGARIAN HAMSTERS. AE Jetton, NB Schwartz and FW Turek. Dept. of Neurobiology & Physiology, Northwestern University, Evanston, IL 60208.

Following photoperiodically induced gonadal regression, circulating levels of the gonadotropins luteinizing hormone (LH) and follicle stimulating hormone (FSH) are low in Djungarian hamsters (Phodopus sungorus). The goal of this study was to investigate hypothalamic changes which may be involved in the photoperiodic response. Young male Djungarian hamsters were maintained on 6L:18D (short days) or 16L:8D (long days) for 4-6 wks. Following decapitation the hypothalami were removed, and the testes were weighed to confirm photoperiodic response. Hypothalami were placed 3 per chamber into a dynamic perfusion system and perfused for 4-5 hr. with supplemented Medium 199 with or without 10^{-6}M melatonin. Three, 20 min. stimulations of 50 mM K^+ were administered during each perfusion. Medium was collected in 5 min. fractions and assayed for GnRH by RIA using the EL-14 antibody. GnRH release in response to each stimulation was analyzed by ANOVA. There was no significant difference in GnRH release from short day hypothalami compared to long day hypothalami. In the presence of melatonin, however, long day tissue had a significantly lower GnRH response to K^+ stimulation than short day tissue ($p<0.05$). We conclude that there is little or no difference in readily releasable GnRH during photoperiodically induced inhibition of the neuroendocrine-gonadal axis. Following photic inhibition of reproductive activity, melatonin appears to have a reduced acute effect in blocking stimulation of GnRH release. Supported by P01-HD21921 and HD-09885.

126.10

DOPAMINE (DA) FIBERS ORIGINATING IN THE ANTERO VENTRAL PERIVENTRICULAR NUCLEUS (AVPN) INNERVATE THE RAT MEDIAL PREOPTIC AREA (MPO) GAD AND LHRH NEURONS. C. Leranth[1,2] and F. Naftolin.[1] Dept. of Obstetrics and Gynecology[1] and Section of Neuroanatomy[2], Yale Univ. Sch. of Med. New Haven, CT. 06510

Interconnections between the GABA-, DA-, noradrenaline-, and LHRH-systems may play an important role in the episodic release of luteinizing hormone. We previously demonstrated GABAergic innervation of LHRH cells, and that while both MPO GABAergic and LHRH neurons were synaptic targets of tyrosine hydroxylase (TH) immunoreactive axon terminals, following transection of the ascending noradrenergic bundles only the GABA cells contacted degenerated TH positive noradrenaline-containing boutons, indicating DA connections on LHRH neurons. Experimental: since DA input to the MPO LHRH and GABAergic neurons may derive from the rostral periventricular DA cell group, including the sexually dimorphic AVPN (Simerly et al. '85), 30 minutes after i.p. desipramine HCL (25mg/Kg) injection to protect noradrenaline fibers, 1mg 6-OH DA (1mg in 1µl saline with 0.2% vitamin C) was stereotaxically injected into the AVPN of adult female rats (B.W. 260g). To increase density of immunostaining, 24 hrs later animals were colchicine treated (intracerebroventricular injection of 80µg colchicine in 20µl saline). One day later they had transcardial perfusion of fixative. Consecutive MPO vibratome sections were immunostained for GAD, LHRH, or TH. EM analysis of GAD and LHRH stained material revealed degenerated, autophagous cytolysosome-containing boutons establishing synaptic contacts with both GAD and LHRH neurons. Conclusion: since degeneration was observed in TH immunoreactive axon terminals, DA innervation of the MPO's GAD and LHRH neurons is suggested with DA fibers originating in the sexually dimorphic AVPN that has been implicated in the positive feedback control of gonadotrophin release (Simerly et al. '85). Supported by NIH grant HD23830.

126.11

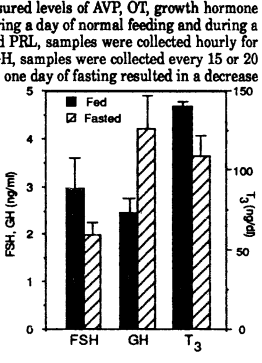
THE STIMULATORY EFFECT OF METHOXAMINE ON *IN VIVO* LHRH RELEASE IS MEDIATED BY PROSTAGLANDIN E_2 (PGE_2) IN OVARIECTOMIZED UNPRIMED MONKEYS. M. Gearing and E. Terasawa. Neuroscience Training Prog. and Wisconsin Reg. Primate Res. Ctr., Univ. of Wisc., Madison, WI 53715.

Recently, we have reported that the α_1-agonist methoxamine (MTX) stimulated pulsatile LHRH release in ovariectomized rhesus monkeys in the absence of estrogen (*Endocrinology* 123:1808,1988). In the present study, the mechanism by which MTX stimulates LHRH release is investigated; specifically, the role of PGE_2 is examined. *In vivo* LHRH release in the stalk-median eminence (S-ME) was measured by push-pull perfusion in conscious, ovariectomized monkeys, and perfusate samples were collected on ice. MTX (10^{-5} M) or PGE_2 (10^{-7} M) was infused into the S-ME through the push cannula for 10 min at 90-min intervals. LHRH and PGE_2 in aliquots of the same perfusate samples were measured by RIA. Infusion of MTX significantly stimulated LHRH release (1.2±0.2 pg/ml before vs. 3.7±0.9 pg/ml after MTX; n=12; $P<0.01$) and PGE_2 release (76.1±8.5 pg/ml before vs. 112.1±18.7 pg/ml after MTX; $P<0.05$). Furthermore, infusion of PGE_2 through the push cannula significantly stimulated LHRH release (1.5±0.5 pg/ml before vs. 2.8±1.1 pg/ml after PGE_2; n=23; $P<0.05$). Infusion of the vehicle had no effect on LHRH or PGE_2 release. These results suggest that the stimulatory effect of MTX on LHRH release is mediated by PGE_2, since MTX stimulates not only LHRH but also PGE_2 release, and since PGE_2 itself stimulates LHRH release. Moreover, the stimulatory effects of MTX and PGE_2 can be observed in the absence of estrogen in the rhesus monkey, unlike in rodents. Therefore, PGE_2 may be an important endogenous mediator of α_1-adrenergic input stimulating pulsatile LHRH release. Our results also demonstrate the usefulness of the push-pull perfusion technique for studies of cellular mechanisms in neuroendocrine research. (NIH HD15433)

126.12

CHANGES IN HYPOTHALAMIC-PITUITARY FUNCTION DURING BRIEF PERIODS OF FASTING IN RHESUS MONKEYS (*Macaca mulatta*) D.L. Helmreich, L. G. Mattern*, and J.L. Cameron. Depts. of Behavioral Neuroscience, Physiology and Psychiatry and the Center for Neuroscience, Univ. of Pittsburgh, Pittsburgh PA 15260.

States of chronic undernutrition suppress the activity of hypothalamic-pituitary-gonadal (HPG) axis, frequently leading to complete suppression of reproductive function. We have previously shown that in adult male rhesus monkeys there is already a decrease in the central drive to the reproductive axis, as indicated by a slowing of pulsatile LH secretion, after only one day of fasting. This suggests that the signal(s) that eventually lead to complete inactivity of the HPG axis are already present after one day of fasting. To determine if the activity of other hypothalamic-pituitary systems is modified by brief periods of fasting, we measured levels of AVP, OT, growth hormone (GH), prolactin (PRL), FSH, T3 and cortisol during a day of normal feeding and during a day of fasting. For analysis of AVP, OT, T3 and PRL, samples were collected hourly for 24 hr. For analysis of cortisol, LH, FSH, and GH, samples were collected every 15 or 20 min for 24 hr (n = 3-6). As previously reported, one day of fasting resulted in a decrease of LH pulse frequency from 8.33 ± 1.2 to 4.33 ± 0.33 pulses/24 hr. Additionally, there was a decrease in mean FSH and T3 levels and an increase in mean GH levels, as illustrated in the figure. Circulating cortisol levels were slightly elevated in the evening of the fasted day. We found no changes the levels of OT, AVP or PRL. These results indicate that one day of fasting causes a significant metabolic stress that results in changes in hypothalamic-pituitary drive to a number of systems that regulate energy balance. These findings support the hypothesis that "metabolic changes" may provide the signal(s) that lead to the suppression of reproductive function in undernourished states.



127.1

THE ROLE OF COGNITIVE FLEXIBILITY IN FRONTAL LOBE MEMORY DISORDERS. L.M. GRATTAN AND P.J. ESLINGER, Depts. of Neurology, Univ. of Maryland (Baltimore, MD, 21201) and Penn State (Hershey, PA, 17033) Colleges of Medicine.

The memory disorders associated with frontal lobe damage in humans appear to be different from those after limbic system damage (particularly in the medial temporal region and diencephalon). In this study we examined the relationship between memory and several frontal lobe mediated cognitive functions, which have been anecdotally implicated in the organizational and encoding aspects of memory. Forty subjects with CT or MR verified lesions to the frontal lobes or connected subcortical and posterior cerebral structures (including limbic system lesions) were extensively studied with standardized measures of cognitive flexibility and Rey's verbal learning and memory paradigm. Results indicated significant associations between certain aspects of cognitive flexibility and memory in the frontal lobe sample, but not in the posterior cerebral or subcortical lesion groups. We conclude that learning and memory performance after frontal lobe lesion may be related to select aspects of cognitive flexibility, including the abilities to initiate, generate, and adopt alternative ways of managing information. (Supported by grant P50 NS26985, Boston University Memory Disorders Research Center).

127.2

THE ABILITY OF PATIENTS WITH FRONTAL- OR TEMPORAL-LOBE EXCISIONS TO ENCODE AND RECALL VISUAL DISTANCE AND LOCATION. (G. Leonard and B. Milner). Montreal Neurol. Inst., McGill Univ., Montreal, Canada H3A 2B4.

Patients with large right frontal-lobe lesions are impaired in their ability to encode and/or reproduce large movements of the arm (with vision excluded), and also in recalling the end position of their arm movements (Leonard, 1987). The present experiments examined the ability of 60 patients with focal cerebral excisions and 13 normal control subjects to judge visual distance and location, to determine if the above deficits are specific to kinesthesis. Subjects viewed a dot moving across a screen and immediately, or after a 15 or 30 s delay (with or without a distracting task) reproduced the distance through which the dot had moved (Exp. 1), or pinpointed its final location (Exp. 2).

All groups performed normally on the location task, but patients with large right frontal-lobe lesions were impaired on the distance task. It is concluded that the right frontal lobe not only contributes to the encoding and recalling of kinesthetic information but also to recalling distance information presented visually.

127.3

A DISSOCIATION BETWEEN VERBAL AND SPATIAL MEMORY FOLLOWING UNILATERAL TEMPORAL LOBECTOMY. A. Chiba, R.P. Kesner, F. Matsuo, P.M. Heilbrun. Depts. of Psychology, Neurology, and Neurosurgery, Univ. of Utah, Salt Lake City, Utah 84112.

Computerized memory tests were administered to assess verbal and spatial memory abilities of patients following unilateral temporal lobe resection for refractory complex partial epilepsy of temporal lobe origin (CPET). The extent of tissue resected for each patient in the testing population typically included 3-5 cm of the anterior temporal lobe, 1-4 cm of the hippocampus, and surrounding mesio-temporal structures unilaterally. Matched preoperative control subjects with CPET were also tested. Patients were tested on a series of four tests of episodic memory emphasizing explicit information for the recognition of item and order information for words and spatial location. Postoperative test results revealed a dissociation between each patients memory for verbal information as compared to their memory for spatial information. Each patient showed a statistically significant persistent memory deficit for either verbal or spatial information relative to that of preoperative control subjects. These data suggest the existence of interhemispheric parallel memory processes for spatial and verbal information. Neither hemisphere appears to be capable of the sole support of memory function. These data also correspond well with animal data collected on analogous tasks of item and order memory for spatial location. Since hippocampal lesioned rats show a significant deficit on such tests, it can be concluded that mesio-temporal structures play an important role in memory for spatial location across species.

127.4

DIFFERENT PROFILES OF HUMAN AMNESIA AND CEREBRAL DAMAGE ASSOCIATED WITH ENCEPHALITIS. **P.J. Eslinger, M.P. Alexander*, L.S. Cermak*, L.M. Grattan, Memory Disorders Res. Ctr., Penn State (Hershey, PA), Boston Univ. (Boston, MA), Univ of MD (Baltimore, MD) Colleges of Medicine.**

Several remarkable cases of human amnesia after herpes simplex encephalitis have been described. While study of individual cases continues to advance our understanding of the neural basis of memory, extant data also reveal differences among these patients. To examine this issue, we studied a group of 4 encephalitics who developed amnesia. Neuropsychological, cognitive and neuroimaging studies revealed distinct differences among patients with variation in both memory disorder and cerebral damage. Limbic system structures were most significantly affected. Bilaterality of lesion and its neocortical extension were different in each case and from the encephalitic patient Boswell (Damasio et al. 1985). In correlated fashion, the degree of anterograde and retrograde amnesia was unique to each case. In conclusion while encephalitis consistently produced amnesia and limbic system damage, the extent of memory loss and cerebral damage varied significantly. **(Supported by grant P50 NS26985)**

127.5

DATA BASED (EPISODIC) MEMORY FOR MOTOR RESPONSES IN HYPOXIC PATIENTS. R.O. Hopkins and R.P. Kesner. Dept. of Psych., University of Utah, Salt Lake City, UT 84112.

Patients with hypoxic or minor brain injury, and normal age matched controls were tested for memory impairments. The Denman memory scale was given to all subjects as a baseline memory assessment. The subtests for digits and paired associates show a decreased performance for the hypoxic subjects, but not for minor brain injury or control groups. All subjects were then tested for item and order recognition memory for lists of six words, pictures, abstract pictures, spatial locations, and motor responses.

Results indicated that compared to age matched controls and minor brain injury control groups, hypoxic brain injured individuals displayed on item and order recognition tasks, deficits for words, pictures, abstract pictures, and spatial locations with a slight recency effect for words, pictures, and abstract pictures, and a larger recency effect for spatial locations. For motor response item and order recognition, no major deficits compared to controls were obtained.

In conclusion, it appears that subjects with hypoxic brain injuries in the face of severe memory deficits for words, pictures, abstract pictures and spatial locations display residual capacity for remembering motor responses, using episodic, working, declarative, or data-based memory.

127.6

A COMPUTATIONAL STUDY INDICATES CROSS-MODAL ASSOCIATION ARISES NATURALLY IN NEOCORTEX VIA FEEDBACK PROJECTIONS Paul Rhodes, Univ. Of California, San Diego, CA

Anatomical studies (Pandya and coworkers) have revealed that unimodal association cortex may be viewed as a cascade of reciprocally interconnected regions. Further, the unimodal association areas project into polymodal association areas which themselves are similarly organized. The forward-going projections tend to terminate in a columnar fashion in layer IV, and the reciprocal recurrent projections tend to terminate in layer I. To explore the flow of neural activity that might be found in this structure, I have done simulations which include 2 regions of unimodal association cortex and a polymodal region to which they convergently project, and which reciprocally projects back. Each region is comprised of a 10x10 grid of cortical modules. Each module possesses layer III and V pyramidals, a layer I Cajal-Retzius (horizontal) cell, and a layer III basket. Each is modelled as a 2-compartment neuron with active calcium, sodium, and potassium conductances and fairly realistic firing properties. The neurons are laterally interconnected within regions, vertically interconnected within columns, and reciprocally interconnected between regions as described above. Synapses between pyramidals are assumed modifiable by a covariance version of the Hebb rule. Baskets project to pyramidal somata The model includes 1200 model neurons and 50,000 modifiable synapses. The simulated activity suggests a mechanism for cross-modal associative memory. After bimodal sensory input during a plastic period, a fragment of the original activity is then input to one modality. This input projects to the bimodal region, driving firing there which results in pattern completion for the full associated bimodal activity pattern. Recurrent projections to the unimodal regions then produce activity in each that reconstructs their original activity during the plastic period. Attention is called to the vital role of the horizontal fiber system of layer I, which provides the network of large-scale lateral connections giving rise to pattern completion. Note that layer I is primarily activated by the feedback projections. We hypothesize that both pattern completion and cross-modal associative memory are particularly dependent upon feedback projections.

127.7

THE NEURAL REGIONALIZATION OF KNOWLEDGE ACCESS DEVICES A. R. Damasio, H. Damasio, D. Tranel and J.P. Brandt, Department of Neurology, University of Iowa College of Medicine, Iowa City, Iowa.

We performed experiments involving visual recognition and naming of numerous entities of varied categories in 40 subjects with damage to different temporal association cortices. Results indicate that the linkage mechanisms that permit access of concepts given the names (or vice-versa), are neurally segregated and subcompartmentalized. Different lesion probes correlate with different recognition and naming profiles, e.g., damage to left temporal pole disrupts access to proper names of unique entities but not recognition of those entities, nor access to common names; damage to inferior occipito-temporal cortex disrupts recognition of certain classes of natural entities but leaves intact recognition of other natural kinds and man-made kinds. The results suggest a parcellation of the systems that support neural inscriptions of entities sharing certain characteristics, (e.g., visually ambiguous natural kinds versus manipulable, nonambiguous man-made kinds), and a regionalization of the neural devices that permit access to the reference lexicon, e.g., the devices that access proper nouns is clearly separate from those that access common nouns, which are, in turn, subregionalized relative to different kinds.

127.8

MEMORY FOR SPATIAL LOCATION AND OBJECT MEMORY ARE EQUIVALENTLY IMPAIRED IN HUMAN AMNESIA. C. B. Cave[1], J. Janowsky[2], and L. R. Squire[1], VA Med. Ctr., and Dept. of Psychiatry, UCSD, La Jolla, CA 92093[1]; Univ. of Oregon, Eugene, OR 97403[2].

One hypothesis about the function of the mammalian hippocampus emphasizes its special role in spatial cognition. We tested this idea by asking whether amnesic patients with damage to the hippocampal formation, or other amnesic patients, are especially poor at spatial memory tasks. Sixteen amnesic patients were tested, seven with alcoholic Korsakoff's syndrome, eight with confirmed (N=5) or suspected (N=3) damage to the hippocampal formation, and one with a bilateral thalamic infarction. Patients and control subjects first studied each of 16 toy items randomly arrayed on a 60 cm^2 surface (Smith & Milner, *Neuropsychologia, 19*:781-793, 1981). After a delay of 5 min for amnesic patients and 5 min to 5 wks for different groups of control subjects, object recall and recognition were tested. Finally, subjects were given a new surface and were asked to place the toys where they had previously appeared. For the amnesic patients, memory for the objects (both recall and recognition) was equivalent to that of the control subjects who were tested after 3-5 wks, and poorer than control subjects who were tested after 5 min or 1-2 wks. If amnesic patients are disproportionally impaired in their memory for spatial location, then their spatial memory for the toys should be more impaired than their object memory, relative to the control subjects. However, spatial location memory of the amnesic patients was equivalent to the control subjects who were tested after 1-2 wks and was therefore, if anything, better than their object memory. A second condition showed that the object memory tests were not being influenced by spatial strategies. The results were the same when the amnesic patients were considered as a single group and when patients with diencephalic or hippocampal damage were considered separately. The results show that in human amnesia, even in patients with direct damage to the hippocampal formation, spatial memory is no more affected than memory for objects.

127.9

IMPLICIT MEMORY: NO EVIDENCE FOR RAPID ACQUISITION OF NEW ASSOCIATIONS IN AMNESIC PATIENTS OR NORMAL SUBJECTS. G. Musen and L. R. Squire VA Med Ctr & UCSD Psychiatry Dept., La Jolla, CA 92093. Amnesic patients perform normally on many nondeclarative (implicit) memory tasks such as skill learning, priming, and classical conditioning. For example, amnesic patients exhibit normal facilitation of reading speed after a single reading of new text. It is unclear whether this phenomenon, or any other intact memory ability in amnesia, depends on the rapid acquisition of new associations. We attempted to replicate the only study known to us to suggest that memory-impaired patients can rapidly acquire new associations (Moscovitch et al., 1986 JEP:G. 115:331-347). Nine amnesic patients and 12 control subjects studied a list of 30 word pairs (5 sec/pair). Then, as quickly as possible, they read three different lists: 10 previously studied word pairs, 10 new word pairs and 10 recombined word pairs consisting of new pairings of previously studied word pairs. As expected, both amnesic patients and normal subjects read the old pairs more quickly than the new pairs (amnesic patients: 14.5 sec vs. 16.5 sec; normal subjects: 16.4 sec vs. 17.8 sec). If new associations are acquired during the study phase, then recombined pairs should later be read more slowly than the old pairs. However, we found that amnesic patients and normal subjects read old and recombined pairs equally rapidly (amnesic patients: 14.5 sec vs. 15.0 sec; normal subjects: 16.4 sec vs. 16.8 sec). We also replicated this finding in a second study of normal subjects. Finally, in two experiments, we found that normal subjects did acquire new associations, i.e., they read recombined word pairs more slowly than old pairs, when they were given 10-20 repetitions of the study pairs. Implicit memory may not be adapted for the rapid acquisition of new associations.

127.10

EXTENSIVE RETROGRADE AMNESIA IN TWO SEVERELY AMNESIC PATIENTS ON TESTS OF FAMILIARITY AND NAME COMPLETION ABILITY. F. Haist[1], L.R. Squire[1], and A.R. Damasio[2], [1]VA Med. Ctr. and UCSD Dept. of Psychiatry, La Jolla, CA 92161, and [2]Dept. of Neurology, Univ. of Iowa, Iowa City, IA 52242.

In a single-case study, Warrington and McCarthy suggested that retrograde amnesia is not observed when remote memory tests for previously acquired facts are re-designed as semantic memory tests, e.g., when they assess simple familiarity or name completion ability rather than associative memory (Brain & Cog. 7:184-200,1988). This result raises the possibility that retrograde amnesia can be mitigated by simple changes in test procedures. However, an impairment may have been difficult to detect due to the high (ceiling) level performance of their patient. We administered remote memory tests like those used by Warrington and McCarthy to 8 control subjects and 2 amnesic patients (Boswell and WI), both of whom have severe and extensive retrograde amnesia as measured by standard recognition tests. In the Familiarity task, subjects attempted to select the famous name when it was presented together with two nonfamous distractor names. In the Name Completion test, subjects attempted to complete a famous name, given the first name and part of the surname (e.g., Marilyn Mon___). The names for both tests were taken from a standard Famous Faces test and spanned the time period 1940 to 1985. Control subjects scored better than 96% correct in all 5 decades on the Familiarity test and better than 80% for all decades on the Completion test. On both tests, the 2 patients scored outside the range of control subjects for every decade and were more than 2 standard deviations below the control mean. In summary, the amnesic patients had severely impaired remote memory, even when the tests were designed to assess simple familiarity or name completion ability. The findings suggest that retrograde amnesia reflects impairment of a single (declarative) memory system. Thus, there seems little basis for fractionating retrograde amnesia into semantic and cognitively-mediated components.

127.11

IMPAIRED HABIT LEARNING FOLLOWING CEREBELLAR HEMORRHAGE: A SINGLE-CASE STUDY. J. Fiez, S.E. Petersen, & M.E. Raichle. Dept. Neurol. & Neurol. Surg., McDonnell Ctr. for Higher Brain Function, Mallinkrodt Inst. of Rad., Wash. Univ. Sch. of Med., St. Louis, MO 63110.

Previously, using positron-emission tomography (PET), increased blood flow to a right posterior cerebellar region was found when subjects were asked to "generate" verbs for presented nouns. A similar change was not found when subjects merely repeated aloud the presented nouns.

In an attempt to account for this activation, we have studied a 49-year old male lawyer (M.M.) who suffered a stroke affecting a large portion of his right posterior cerebellum. Neither MRI nor CT scans revealed any other neurological abnormalities. M.M. has returned to full-time practice and reports no significant motor or cognitive deficits.

However, M.M. was impaired on the generate task in two ways. First, in initial testing, 60% of his responses were errors (compared to 2% in normals). Second, M.M.'s median reaction time decreased by only 8% across trials (compared to a 27% decrease in normals). Consistent with M.M.'s failure to learn the generate task is his performance on concurrent discrimination tasks -- tasks used previously to dissociate habit formation from another type of long-term (declarative) memory in monkeys (Phillips, et. al., Exp. Brain Res., 27:99-107). While normals reach criterion on word and pattern discriminations after 4-6 trials, M.M. failed to do so after even 14 trials. On the Tower of Toronto puzzle, a task amnesics learn normally, M.M. scored only a 2, far below the normal 13-16 (Saint-Cyr, et. al., Brain 111:941-959).

In contrast, M.M. scored normally on tests of word fluency, short-term and declarative memory, language, and frontal function. These dissociations lead us to conclude that M.M.'s deficits are attributable to a specific impairment in habit learning resulting from damage to the right cerebellum.

127.12

VISUALIZATION OF THE MODULAR AND LAMINAR ORGANIZATION OF THE HUMAN CEREBRAL CORTEX *IN VIVO* WITH MAGNETIC RESONANCE. **H. Damasio, R.O. Kuljis, W.T.C. Yuh, G.W. Van Hoesen, J.C. Ehrhardt and A.R. Damasio**. Departments of Neurology, Radiology and Anatomy, The University of Iowa College of Medicine, Iowa City, Iowa 52242-1053.

Several neurologic and psychiatric illnesses result in a disruption of the internal structure of the cerebral cortex, which can only be visualized *postmortem* by histological examination. Current neuroimaging methods lack sufficient resolution to visualize cortical organization at the microscopic level, imposing limitations on diagnostic studies *in vivo*. Here we report that a modified protocol for magnetic resonance imaging at 1.5 Tesla may permit the visualization of some features of the internal organization of the cerebral cortex *in vivo*. The protocol included revisions to pulse sequences, field of view, number of repetitions, and sectioning plane in 14 living human subjects and two fixed brains. The latter were prepared for histological analysis of the cell (Nissl) and myelin (Gallyas) architecture after scanning. In all cases, a periodic pattern of up to 1mm thick low-signal slabs was present in the entorhinal cortex. The slabs were perpendicular to the pial surface, separated by relatively high-signal septa, arranged in a pattern similar to the modules defined histologically by the clusters of neurons in layer II, and traversed at mid-cortical thickness by a low-signal lamina that corresponds to the *lamina dissecans*. Our observations suggest that the modular and laminar architecture of the cerebral cortex can be visualized *in vivo* by magnetic resonance imaging.

Supported by NINDS grants PO1 NS19632 and 14944.

128.1

ACUTE NEUROTOXICITY IN CULTURED RAT CEREBELLAR GRANULE CELLS: ENDOGENOUS GLUTAMATE AND THE EFFECTS OF ACIDIC AMINO ACIDS, pH AND Zn^{++}. M. Schramm and S. Eimerl*, Dept. Biological Chemistry, Hebrew University, Jerusalem 91904, Israel.

During 14 days in culture cells accumulate 40 mM glutamate; about x 4000 the final medium concentration, and become extremely sensitive to addition of this amino acid. Release from the endogenous store might contribute to the toxicity evoked by addition of 20 μM glutamate. 5 min exposure killed 70% of the cells within 1 hr. Of 6 other acidic amino acids tested cysteine sulfinate was most active. Even with cystein sulfinate, 500 μM were required to produce the high toxicity of 20μM glutamate. The pH dependence of acute toxicity corresponded to the function of the NMDA receptor channel (Tang et. al., Soc. Nerosci. Abstr. 15: 326 (1989). Glutamate, 20μM had a negligible effect at pH7.0 but produced maximal toxicity at pH 8.0.Zn^{++}, 2μM inhibited 66% of the toxicity of 20μM glutamate, but the metal ion, by itself, killed, 40% of the cells at 20μM. It is suggested that endogenous glutamate, pH and low Zn^{++} concentrations, play an important role in acute toxicity in this system.

128.2

NMDA ANTAGONISTS PREVENT KAINATE TOXICITY IN RAT RETINAL GANGLION CELLS IN VITRO. Nikolaus J. Sucher, Elias Aizenman† and Stuart A. Lipton, Dept. of Neurology, Children's Hospital & Harvard Medical School, Boston, MA 02115. (†Present address: Univ. of Pittsburgh).

Under defined culture conditions, exogenous glutamate (Glu), *N*-methyl-D-aspartate (NMDA), or an endogenous Glu-related toxin are lethal to rat retinal ganglion cells. These detrimental effects are NMDA receptor-mediated since specific NMDA antagonists can prevent cellular injury. In the presence of the endogenous Glu-like toxin, 125 μM kainate (KA) further increases the proportion of retinal ganglion cells that die, but the toxicity (due to both KA and the endogenous toxin) is totally prevented by 200 μM APV, a specific NMDA receptor antagonist. The fraction of toxicity attributed directly to KA can be blocked by the relatively specific non-NMDA antagonist, CNQX (10 μM), but under these conditions a proportion of neurons still die due to the endogenous Glu-like toxin. In patch-clamp recordings, the response to 125 μM KA was totally unaffected by 200 μM APV, even under conditions of $[Mg^{2+}]_o = 0$ in which nonspecific KA-activation of NMDA receptors would be expected to be maximal were it to occur in this preparation. These findings suggest that *(i)* KA-induced retinal ganglion cell death is mediated via NMDA receptors, and *(ii)* KA may possibly lead to the net efflux of additional endogenous Glu-related toxin that acts at the NMDA receptor under these *in vitro* conditions.

128.3

Degeneration of Neurons in Organotypic Hippocampal Culture is Delayed Over 24 Hours After Brief Exposure to Glutamate Agonists J. J. Vornov, R. C. Tasker* and J. T. Coyle Depts. Neurology, Anesthesiology, Neuroscience and Psychiatry, The Johns Hopkins School of Medicine, Baltimore, MD 21205

Glutamate release appears to be responsible for neuronal injury in some animal models of ischemia, with degeneration often delayed by hours or days. We have observed such a delayed time course of morphological degeneration of neurons in organotypic hippocampal cultures after exposure to glutamate agonists. Cultures were prepared using the roller tube method of Gahwiler. After 14 days *in vitro*, cultures were exposed for 30 minutes to glutamate agonists at 37°C and allowed to survive in fresh growth media for 24 hours or 5 days. There was no LDH released during exposure to glutamate (10mM), NMDA (100μM) or kainate (100μM). However, media from all cultures contained LDH activity 24 hours later, indicating significant injury. Despite the large amounts of LDH released, thionin staining showed many morphologically intact neurons at 24 hours. Neuronal degeneration, denoted by pyknotic neuronal nuclei was most apparent after NMDA exposure. Kainate exposure caused less morphological damage, though shrunken nuclei with surrounding halos were seen (as observed 24 hours after striatal kainate injection *in vivo*). The effects of glutamate were the least evident. After 5 days of recovery, neuronal degeneration was widespread in all treated cultures, but cultures exposed to glutamate still contained neurons, that while not normal in appearance, were not pyknotic. These results suggest that injury in organotypic hippocampal culture, as *in vivo*, is a prolonged process.

128.4

MUSCIMOL ATTENUATES SLOW EXCITATORY AMINO ACID-INDUCED INJURY OF CULTURED CORTICAL NEURONS. H. Monyer[1], D.M. Hartley[2], H. Ehsani[2], P.H. Seeburg[1] and D.W. Choi[2]. [1] Cntr. for Molecular Biology, Heidelberg, FRG; [2]Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305.

Mixed cortical cell cultures containing both neurons and glia were exposed to 25 - 30 μM kainate for 24 hr in a defined medium. At the end of this exposure, widespread submaximal neuronal degeneration was evident by phase-contrast microscopy or efflux of lactate dehydrogenase to the bathing medium; this injury could be attenuated by 10 μM CNQX. Addition of 10 μM to 1 mM concentrations of the $GABA_A$ agonist, muscimol, partially attenuated kainate-induced neuronal damage, with IC_{50} about 100 μM; maximal injury reduction produced by 300 μM - 1mM muscimol generally ranged between 40 - 70%. High concentrations of muscimol also produced partial protection against the slow excitotoxic neuronal death induced by 24 hr exposure to 10 μM AMPA or 15 μM NMDA. However, 1 mM muscimol had little or no protective effect against the excitotoxic damage induced by 5 min exposure to 500 μM NMDA.

These observations are consistent with the hypothesis that neuronal depolarization, perhaps leading to activation of voltage-gated calcium channels, may be an important factor in the slow excitotoxic injury of cortical neurons induced either by non-NMDA agonists, or by low concentrations of NMDA agonists.

128.5

THE PROTEIN KINASE C AGONIST 7-OCTYLINDOLACTAM V ATTENUATES SLOW EXCITATORY AMINO ACID-INDUCED NEUROTOXICITY IN CORTICAL CELL CULTURES. D.M. Hartley and D.W. Choi. Dept. of Neurology and Neurosciences Program, Stanford Univ. Med. Sch., Stanford, CA 94305.

Protein kinase C (PKC) has been suggested to play an important role in glutamate receptor-mediated neuronal death (Favaron et al., PNAS 85: 7351, 1988). We attempted to modify slow excitatory amino acid-induced neuronal injury in mixed cortical cell cultures, using the new synthetic indolactam derivative, 7-octylindolactam V (7-OIL). Cultures exposed to 30 - 35 μM kainate for 24 hr developed widespread submaximal neuronal degeneration. Addition of 10 μM 7-OIL, either 24 hr before kainate exposure or upon initiation of kainate exposure, reduced neuronal damage by 30 - 90 %; lower concentrations of 7-OIL were ineffective. 10 μM 7-OIL also partially attenuated the neuronal injury induced by 24 hr exposure to 10 μM AMPA or 15 μM NMDA.

Further study will be required to elucidate the mechanism of this protective action of 7-OIL. Patch clamp studies showed no acute attenuation in kainate-induced whole cell currents (voltage-clamped at -60 mV and +30 mV) with application of 10 μM 7-OIL. Arguing against a simple interaction with neuronal PKC is the high concentrations required for neuronal protection; furthermore, (-) and (+) enantiomers were equivalent in reducing kainate-induced injury.

128.6

NORDIHYDROGUAIARETIC ACID (NDGA) ATTENUATES SLOW EXCITATORY AMINO ACID-INDUCED NEURONAL DEGENERATION IN CORTICAL CULTURES. K. ROSE, V.M.G. BRUNO, R. OLIKER*, and D.W. CHOI. Dept. of Neurology, Stanford Univ. Med. Sch., Stanford, CA 94305

Recent evidence suggests that free radical formation may be responsible for a portion of the neuronal injury resulting from excess exposure to excitatory amino acids (Monyer et al., Soc. Neurosci. Abstr. 15: 479, 1989; Murphy et al., Neuron 2: 1547, 1989). An important mechanism of glutamate receptor-induced free radical formation may be augmentation of arachidonic acid metabolism. These experiments examined the ability of the lipoxygenase inhibitor NDGA to attenuate excitatory amino acid neurotoxicity in murine cortical cell cultures.

30 μM NDGA did not protect cortical neurons against the fast excitotoxic injury induced by 5 min exposure to either 100 μM NMDA or 150 μM glutamate; higher concentrations of NDGA were toxic. However, 3 - 30 μM NDGA attenuated the slow excitotoxic injury induced by 24 hr exposure to 10 μM AMPA, 30 μM kainate, 20 μM NMDA, or 600 μM quinolinate; at 30 μM, neuronal injury in all these paradigms was reduced by about half. 3 - 30 μM NDGA also protected cultures against the neuronal injury induced by glucose deprivation for 12 hr.

NDGA may be a useful alternative to receptor antagonists for attenuating slow excitotoxic injury.

128.7

NMDA-INDUCED CALCIUM TRANSIENTS IN CALBINDIN-CONTAINING STRIATAL NEURONS IN PRIMARY CULTURE. S. Weiss, D. Hochman and B.A. MacVicar. Neuroscience Research Group, University of Calgary, Calgary, AB, Canada T2N 4N1.

It has been suggested that the selective presence of calcium-binding proteins, e.g. calbindin-D_{28K} (CaBP), in neurons may confer resistance to neurodegeneration mediated by excitatory amino acids. In striatal neurons in primary culture, 15-20% of the cells contained CaBP-like immunoreactivity as revealed by indirect immunocytochemistry. We examined the actions of excitatory amino acid agonists on intracellular calcium ($[Ca^{2+}]_i$) in CaBP-immunoreactive (IR) striatal neurons by combining imaging of $[Ca^{2+}]_i$ using FURA-2 with post-hoc immunocytochemistry. Increases in $[Ca^{2+}]_i$ were observed in CaBP-IR neurons in response to NMDA (100μM), kainate (100μM) or elevated K^+ (50mM). With a single administration of NMDA (100μM), neither the peak increase in $[Ca^{2+}]_i$ nor the rate or extent of recovery in CaBP-IR cells were significantly different from those that did not contain CaBP. In preliminary experiments with repeated agonist application, increases in $[Ca^{2+}]_i$ in neurons that did not contain CaBP were more prolonged than those in CaBP-IR neurons. These findings support the hypothesis that the presence of calcium binding proteins may serve to protect neurons from sustained increases in $[Ca^{2+}]_i$.

Supported by the Medical Research Council of Canada.

128.8

GANGLIOSIDES NORMALIZE DISTORTED SINGLE CELL $[Ca^{++}]_i$ DYNAMICS AFTER TOXIC DOSES OF GLUTAMATE IN CEREBELLAR GRANULE CELLS. G. A. de Erausquin*, G. Brooker*, H. Manev, A. Guidotti and E. Costa. Fidia-Georgetown Institute for the Neurosciences and Dept. of Biochemistry and Molecular Biology, Georgetown University Medical Center, Washington, D.C. 20007.

Glutamate induced delayed neurotoxicity after abusive and paroxismal activation of its receptors has been proposed to depend upon a sustained increase in intracellular free $[Ca^{++}]_i$. To elucidate the temporal and causal relationship between glutamate induced changes in $[Ca^{++}]_i$ and neuronal death, we simultaneously studied the dynamics of $[Ca^{++}]_i$ changes in single neurons with fura-2 and the cell viability by imaging the nuclear penetration of propidium iodide. The main difference between toxic (50 uM) and non-toxic (5 uM) doses of glutamate is the lack of rectification in $[Ca^{++}]_i$ 20 min after glutamate is removed. This protracted rise in $[Ca^{++}]_i$ in a single given cell is correlated with ($r=0.87$, $p<0.01$, Spearman's test), and consequently predictive of, the time of appearance of neuronal death, as measured by propidium iodide fluorescence. In addition, glutamate receptor antagonists (MK-801 or CPP) reduce the acute increase of $[Ca^{++}]_i$ induced by glutamate but fail to revert the protracted increase of $[Ca^{++}]_i$, elicited by toxic doses of glutamate. In contrast, ganglioside GM_1 and the semisynthetic analogs LIGA-4 and LIGA-20 (JPET, 1990, 252, 419) failed to change the immediate rise of $[Ca^{++}]_i$ elicited by glutamate but prevented the protracted increase in $[Ca^{++}]_i$ after toxic doses of glutamate. Voltage dependent Ca^{++} channel blockers (nifepedine, etc.) did not change the initial or protracted responses to glutamate. Supported by NIH Grants HL 28940 and NS 28130.

128.9

INTRACELLULAR CALCIUM CONCENTRATIONS DURING COMBINED 'CHEMICAL HYPOXIA' AND EXCITOXIC NEURONAL INJURY J.M. Dubinsky and S.M. Rothman Dept. of Anatomy and Neurobiology and Dept. of Pediatrics, Washington University School of Medicine, St. Louis, Mo. 63110.

Since ischemic and hypoxic neuronal damage can be attenuated by NMDA antagonists, it is assumed that the initial metabolic insult causes a release of endogenous glutamate that itself is neurotoxic. To test the hypothesis that hypoxic damage might be additive to glutamate neurotoxicity, we have examined neuronal survival and rises in intracellular calcium after exposure to glutamate and/or cyanide, as a model of 'chemical hypoxia'. Experiments were performed on cultures of dissociated postnatal hippocampal neurons after 2 weeks in vitro. Intracellular Ca_i levels were measured in fura-2-AM loaded cells with a photometer based system. Dose response curves for rises in Ca_i show that glutamate maximally increased Ca_i 250-300nM with a K_d of 30μM, while 3-10mM NaCN produced Ca_i levels approaching 1 μM. 300μM NaCN, the minimal dose to produce a response, shifted the glutamate dose-response curve left and raised the maximal Ca_i level in an additive fashion. The rise in Ca_i produced by either glutamate or NaCN was greatly reduced by coapplication with MK801 or 7-chlorokynurenic acid. Simultaneous application of glutamate and NaCN produced a rise in Ca_i which was reduced by MK801 plus CNQX. Glutamate killed 80% of neurons after a 5 min exposure while 3 mM NaCN was not toxic for 30 min. We suspect that in NaCN, a small increase in Ca_i probably triggered by glutamate accelerates release from intracellular stores, producing a rise in Ca_i far above that seen with glutamate alone. Thus, an extremely large increase in Ca_i is not toxic when the Ca is liberated from internal sites. Supported by NS19988 and Monsanto.

128.10

ACTIVATION OF A TRANSLATIONAL DEATH PROGRAM IN CNS NEURONS BY EXCITATORY AMINO ACIDS. S.D. Skaper, A. Morandi*, L. Facci, D. Milani* and A. Leon. Fidia Research Laboratories, Abano Terme 35031, Italy.

Excitatory amino acids (EAAs), especially glutamate, appear to play a key role in the delayed neuronal death of CNS insults like hypoxia, hypoglycemia or cerebral ischemia. To explore the molecular mechanisms underlying EAA induced cellular injury, postnatal rat cerebellar granule neuron cultures were used which, when exposed to 100 µM glutamate (15 min, 24°C) in Mg^{2+}-free medium, undergo a massive and delayed degeneration during the next 24 hr. Prior treatment of the cultures with inhibitors of protein synthesis (1 ug/ml cycloheximide, CHX; 2 µg/ml puromycin, PUR) for 18-24 hr eliminated 80-90% of the subsequent neuronal loss induced by glutamate. Actinomycin D (5 µg/ml, AMD), a blocker of RNA synthesis, reduced this neuronal loss by only 50-60%. A pretreatment time of 6 hr was less effective in limiting glutamate neurotoxicity. Similar neuroprotective effects of CHX, PUR and AMD were seen against acute exposure to kainate. EAA receptor-operated Ca^{2+} influx was not affected by these inhibitors. 2-D PAGE analysis revealed a time-dependent increase by glutamate of several proteins (mainly 54 and 61 KD); gangliosides, which are also neuroprotective, appear to prevent this latter response. Translationally-linked death programs may thus participate in dysfunction of EAA transmission systems.

128.11

AIDS VIRUS COAT PROTEIN SENSITIZES NEURONS TO NMDA RECEPTOR-MEDIATED TOXICITY. Stuart A. Lipton, Peter K. Kaiser, Nikolaus J. Sucher, Evan B. Dreyer, and Jeffrey T. Offermann. Dept. of Neurology, Children's Hospital & Progr. in Neurosci., Harvard Medical Sch., Boston, MA 02115.

Recently, we have shown *in vitro* that picomolar doses of HIV coat protein gp120 produce a dramatic increase in $[Ca^{2+}]_i$ in rodent retinal ganglion cell and hippocampal neurons. Further, in retinal ganglion cells this rise in $[Ca^{2+}]_i$ is associated with neurotoxicity that can be prevented with calcium channel antagonists (Dreyer et al., *Science* 1990;248:364). In other experiments, it was demonstrated that these cultures contain an endogenous glutamate-related toxin that activates NMDA receptors to produce retinal ganglion cell death; however, this form of neurotoxicity is evident only in elevated Ca^{2+}/low Mg^{2+} medium (Levy & Lipton, *Neurology*, in press). In the present experiments, performed under normal ionic conditions ($[Ca^{2+}]_o$ = 1.8 mM; $[Mg^{2+}]_o$ = 0.8 mM), we found that the toxic effect of 20 pM recombinant gp120 on retinal ganglion cells was attenuated by the NMDA antagonists APV (100 µM) or MK-801 (12 µM) but was unaffected by CNQX (5-10 µM) (n = 9 experiments). When glutamate-pyruvate transaminase was added to the culture medium to break down endogenous glutamate, 20 pM gp120 no longer killed retinal ganglion cells. Taken together, these results suggest that low (20 pM) doses of gp120 may enhance NMDA receptor mediated toxicity in this preparation.

128.12

INCREASED RATIO OF QUINOLINATE:KYNURENATE AND INCREASED CEREBRAL CORTEX AND LUNG INDOLEAMINE-2,3-DIOXYGENASE ACTIVITY IN BOTH SRV-D AND SIV-INFECTED MACAQUES M.P. Heyes, K. Saito*, M. Gravell*, E.K. Jordan*, A. Lackner*, M. Smith* and S.P. Markey* NIMH and NINDS, Bethesda, MD 20892 and California Primate Center, Davis, CA 95616.

Increased quinolinate (QUIN) and kynurenate (KYNA) in CSF occur in patients with AIDS and have been implicated in pathogenesis of the AIDS dementia complex. Sustained increases in CSF and blood QUIN occurred in macaques infected with either simian retrovirus type-D (SRV-D, D/1/California serotype) or simian immunodeficiency virus (SIV). Increases in CSF KYNA also occurred, but to a lesser degree than the increases in CSF QUIN. Highest CSF QUIN concentrations were observed in macaques with neurologic signs and encephalitis (>5,000 nM vs 25 nM in control macaques). The activity of indoleamine-2,3-dioxygenase (IDO), the first enzyme of the kynurenine pathway from L-tryptophan (L-TRP) to QUIN and KYNA, was increased in both cerebral cortex and lungs of viremic macques. Hepatic tryptophan-2,3-dioxygenase was unaltered. Highest IDO activity was found in SIV-infected macaques with encephalitis. Increased lung and cerebral cortex IDO activity likely accelerate the formation of kynurenine pathway metabolites in both systemic and central tissues. However, it remains to be determined which kynurenine pathway metabolites, produced either intracerebrally or systemically or in both tissues, provide substrate for the synthesis of QUIN and KYNA in CSF. Further studies are required to determine whether the depletion of L-TRP and consequential production of QUIN, KYNA have any detrimental neurologic consequences in infection.

129.1

MAGNETIC RESONANCE IMAGING OF THE RHESUS MONKEY BRAIN: FUNCTIONAL IMAGING OF CEREBRAL PERFUSION USING DYSPROSIUM-DTPA-BMA. R.C. Saunders, J.A. Frank*, T. Aigner, D. Doudet, S. Rocklage*, & S. Quay*, NIMH and NIH, Bethesda, MD 20892 and Salutar Inc., Sunnyvale, CA 94086.

We used rapid magnetic resonance (MR) scanning in combination with a paramagnetic contrast agent, dysprosium-DTPA-BMA (DYS), to differentiate cerebral perfusion in monkey visual cortex during stimulated and non-stimulated conditions. Monkeys were prepared with either a unilateral optic tract section/forebrain commissurotomy or with a complete commissurotomy of the corpus callosum, anterior commissure, and optic chiasm. Thus, in each animal, one hemisphere was blind and the other was not, so that each animal served as its own control. Animals were scanned with visual stimulation and in complete darkness. MR imaging was performed in a GE Signa 1.5 T scanner with a 5" receive-only surface coil. Sixty sequential dynamic GRASS images through striate cortex were collected with TE=11 ms, TR=20 ms, flip angle=10°, FOV=16 cm, NEX=0.75, with 128 views (1.92 s/image) after a bolus injection of DYS. Signal intensity of the DYS was determined in several regions in both visual and nonvisual cortical areas. In contrast to control monkeys, in which no differences were observed between hemispheres under either stimulated or non-stimulated conditions, comparison of the visual cortex in the normal and blind hemispheres of the operated animals revealed a mean signal intensity difference of 9%. These results demonstrate that modest changes in cerebral perfusion resulting from physiological stimulation can be quantified by DYS-enhanced MR imaging.

129.2

CEREBRAL BLOOD FLOW IN THE VISUAL SYSTEM OF THE RHESUS MONKEY BY PET. D.J. Doudet, R.C. Saunders, J. Frank and R.N. Nakamura. Clinical Brain Imaging/LCM, NIMH, Clinical Brain Disorders Branch, NIMH, Dept. of Radiology, NIH, Bethesda MD 20892

Regional Cerebral Blood Flow (rCBF) using ^{15}O labelled water for PET (Positron Emission Tomography) was used to study the effects on brain activity of deafferentation of the visual system. PET scans were collected in normal rhesus monkeys and animals in which one hemisphere was blinded by unilateral optic tract section together with forebrain commissurotomy, at rest, in a darkened room, and during simple visual stimulation. rCBF was studied in several neocortical areas and in the striatum.

No left/right differences were found in the normals under both conditions. A significant decrease in rCBF existed in the occipital, preoccipital and parietal areas of the deafferented hemisphere compared to rCBF in the intact side. rCBF in striatal and non visual cortical areas in both hemispheres of the lesioned monkeys were not significantly different from rCBF in normal rhesus. Visual stimulation induced increased rCBF (8-12%) at the level of the intact primary occipital cortex only. Magnetic Resonance Imaging scans did not present evidence of pathology in the cortical and striatal areas studied

PET can be used to evidence functional abnormalities in an integrated system after deafferentation of one element of this system.

129.3

THE INFLUENCE OF AMPHETAMINE TREATMENT ON SOMATOSENSORY FUNCTION IN THE NORMAL AND INFARCTED RAT BRAIN. W.D. Dietrich, O.F. Alonso, R. Busto and M.D. Ginsberg. Cerebral Vascular Disease Research Center, Univ. Miami School Medicine, Miami, FL 33101.

The consequences of d-amphetamine (d-AMP) on the metabolic responsiveness of the cerebral cortex to physiological activation were studied in normal and barrel field (BF) infarcted rats. Treated rats received 4 mg/kg d-AMP 1 hour prior to unilateral vibrissae stimulation and 2-deoxyglucose study. In nontreated rats, activation led to significiant increases in glucose metabolism (lCMRglu) restricted to the cortical BF. In d-AMP treated rats, stimulation-induced increased lCMRglu was enhanced in layer 4 of the BF compared to nontreated rats (120 vs. 81 umols/100g/min). In addition, ipsilateral and contralateral cortical regions outside the BF demonstrated metabolic activation under d-AMP. A 84% increase in lCMRglu above control was seen in cortical areas anterior to the BF. Stimulation-induced increased lCMRglu was severely depressed in nontreated rats which had undergone left BF infarction two weeks previously. In contrast, bilateral increases in lCMRglu were observed within multiple cortical regions of treated infarcted rats. A 50% increase in lCMRglu was detected in areas bordering the BF infarct. Thus, in the normal and infarcted rat, d-AMP promotes alternate circuit activation - a pharmacological property which may be advantageous for post-injury recovery.

129.4

SEVERE HYPERTENSION AND SYMPATHETIC STIMULATION: LOCAL HETEROGENEOUS CHANGES IN CEREBRAL BLOOD FLOW. U.I. Tuor. Neonatology, Hospital for Sick Children and University of Toronto, M5G 1X8, Toronto, Canada.

This study mapped the local influence of sympathetic stimulation on the cerebral circulation during extreme hypertension. Local cerebral blood flow was measured in 11 anesthetized rats with 14C-iodoantipyrine autoradiography after 10 minutes of severe hypertension (MABP approx.190 mmHg, angiotensin II,IV). Animals also underwent: (i) unilateral section of the sympathetic nerve trunk (denervated) or (ii) electrical stimulation of the superior cervical ganglion + contralateral denervation (stimulated).

In both groups, severe hypertension resulted in heterogeneous areas of marked hyperemia within the cortex, thalamus, and cerebellum. In the denervated group, local blood flow during hypertension was similar within innervated and denervated hemispheres. In the stimulated group, most foci of hyperemia had similar levels of flow in the stimulated and denervated hemispheres. In contrast, within some regions with homogeneous perfusion, blood flow was reduced 15-25% ipsilateral to the stimulated side (eg. caudate nucleus and globus pallidus). Thus, sympathetic stimulation during severe hypertension limits elevations in global cerebral blood flow by a diffuse but selective increase in cerebrovascular resistance.

(Supported by the Heart and Stroke Foundation of Ontario).

129.5

DECREASED LOCAL CORTICAL BLOOD FLOW ELICITED BY ELECTRICAL STIMULATION OF THE DORSAL RAPHE NUCLEUS IN RAT. *M.D. Underwood, M.J. Bakalian*, V. Arango and J.J. Mann.* Labs. of Neuropharmacology, University of Pittsburgh, Pittsburgh PA 15213.

Electrical stimulation of the dorsal raphe nucleus (DRN) elicits a widespread reduction in cerebral blood flow (CBF) as shown by autoradiography (Bonvento et al., JCBFM 9:251-5, 1989). Using a laser-Doppler flowmeter, we sought to determine the time-course and magnitude of change in local cortical blood flow (CrtBF) following electrical stimulation of DRN.

Rats were anesthetized (chloralose: 30-40 mg/kg), paralyzed and artificially ventilated. Arterial pressure (AP), heart rate (HR) and blood gases were continuously monitored and controlled. The effect of stimulus frequency (1-200 Hz) and intensity (10-100 μA) on AP and HR was examined. CrtBF was measured using a laser-Doppler flowmeter with the probe placed extradurally over parietal sensorimotor cortex. Stimulation sites were histologically verified.

Stimulation of DRN (8 sec train; 0.5 msec; n = 6) elicited stimulus-locked frequency and intensity dependent increases in AP, HR and CrtBF. Maximal elevation in AP (34 ± 4 mmHg) was observed at 100 μA and 200 Hz. In contrast, continuous stimulation of DRN (200 Hz; 1 sec on/1 sec off; 0-70 μA slowly increased then sustained 10 min) elicited a slow onset (approximately 1 min) decrease in CrtBF ($p < 0.05$) with no change in AP. The greatest decrease in CrtBF was observed 4 min after stimulus onset (88.7% ± 2.5% of baseline), CrtBF thereafter gradually increased (to 93% of baseline) and was sustained, remaining below pre-stimulation levels ($p < 0.05$).

Acute activation of the DRN, perhaps via serotonergic pathways, elicits prominent effects on the systemic circulation, while continuous stimulation reveals reduced cerebrocortical blood flow. Partially supported by P50 MH46745.

129.6

BLOOD FLOW:METABOLISM COUPLING IN EFFERENT SITES OF ELECTRICALLY STIMULATED AREA POSTREMA

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Neurosurgical Research Unit, Departments of Surgery & Physiology, Queen's University, Kingston, Canada K7L 3N6

Neurons of the brainstem circumventricular organ, area postrema (AP), innervate specific nuclei in the medulla oblongata and pons. Some of this innervation may be destined for the microvasculature to control blood flow (F) and capillary function. We tested the hypothesis that electrical stimulation of AP would couple F precisely to the increases in glucose metabolism (GM) found previously in medullary and pontine structures (*Am. J. Physiol.* 258:R788, 1990). A microelectrode was positioned stereotaxically to stimulate the dorsocentral AP of anesthetized, ventilated albino rats (ES, 150 μA, 15 pps, 1 ms). F and GM were determined in stimulated and sham-prepared rats by autoradiographic analysis of iodo[^{14}C]antipyrine and [^{14}C]deoxyglucose images, respectively. ES caused a 13% decrease in arterial blood pressure. There was little change in F or GM during ES in a medullary structure not receiving prominent AP innervation, the spinal trigeminal nucleus. In nucleus tractus solitarius, nucleus ambiguus, locus coeruleus, and lateral parabrachial nuclei, however, ES increased GM by 28-62%, decreased cerebrovascular resistance (CVR) by 41-46%, and increased F by 47-62% (changes in F:GM ratio of -9 to 27%). In the C1 adrenergic cell group, ES decreased CVR by 58% and increased F by 108% (increase in F:GM ratio of 68%). The results are evidence for active dilatation of resistance vessels in AP-innervated structures during ES and indicate a particularly strong influence of AP projections on capillary perfusion in the medullary C1 cell group.

Supported by the Medical Research Council of Canada

129.7

STIMULATION OF THE FASTIGIAL NUCLEUS PRODUCES A FOCAL ELEVATION IN CEREBROCORTICAL INTERSTITIAL K^+. C. Iadecola[1] & R.P. Kraig[2]. Departments of Neurology, Univ of Minnesota, Minneapolis, MN 55455[1] & Univ. of Chicago[2]

Electrical stimulation of the fastigial nucleus (FN) increases cerebral blood flow (CBF), an action which in cerebral cortex is mediated by local neurons. These neurons might influence local microvessels by the interstitial release of vasoactive ions, such as K^+. We studied if FN stimulation produces interstitial K^+ (K^+_o) accumulation in cortex and, if so, whether changes are focal or diffuse. Rats were anesthetized (halothane 1-5%), paralyzed and artificially ventilated. FN was stimulated through electrodes stereotaxically implanted. K^+_o was measured in parietal cortex by ion-sensitive microelectrodes. FN stimulation (5-10V, 50Hz, 8 sec) elicited sustained K^+_o increases ranging from 0.1 to 2.9 mM (n=47). Increases were stimulus-locked, stimulus frequency and intensity dependent (n=7) and were not evoked from other cerebellar sites (n=5). K^+_o elevations were consistently greater 600-900μ below the pial surface (0.5-2.9 mM; n=9). Smaller or no changes (0.0-0.7 mM) occurred at depths of 250-500μ (n=15) and 1000-2000μ (n=23). Thus the rise in cortical K^+_o evoked from the FN is highly focal and may reflect activation of a subset of afferent fibers and/or intrinsic neurons. Such restricted K^+_o change is unlikely to mediate the widespread increase in cortical CBF evoked from the FN. Rather, if a focal K^+_o rise participates in the vasodilation, a mechanism resulting in a wide dispersal of K^+, possibly within syncytial glia, must be involved.

129.8

CEREBRAL VASODILATATION ELICITED BY ELECTRICAL STIMULATION OF THE CENTROMEDIAN-PARAFASICULAR THALAMUS IS MEDIATED IN PART BY NICOTINIC CHOLINERIC NEURONS S. Mraovitch, P.J. Goadsby[1] and J. Seylaz* Laboratoire de Physiologie et Physiopathologie Cérébrovasculaire, Universite Paris VII, Paris France & [1]The National Hospitals for Nervous Diseases, Maida Vale, London England.

The centromedian-parafasicular complex of the intralaminar thalamus (CM-Pf) is a collection of neurons that have been implicated in cortical electrical activity, nociception and more recently in cerebrovascular regulation since the CM-Pf can increase cerebral blood flow in the absence of an associated rise in cerebral metabolism. Using laser Doppler flowmetry (LDF) the pharmacology of the cerebral vasodilator response to CM-Pf stimulation has been studied. Adult Wistar rats were anesthetised with halothane and chloralose (40mg/kg), paralysed and artificially ventilated. Cerebral cortical perfusion (CBF_{LDF}) was measured continuously with a BPM 403a (TSI) LDF monitor. The CM-Pf was electrically stimulated with varying frequencies (1-500/s) and intensities (50-250uA). Stimulation produced a frequency and intensity dependent increase in CBF_{LDF} up to a maximum of 114%. At 150uA/200/s the response was tested after intravenous injection of both atropine (300ug/kg; no effect) and mecamylamine (4mg/kg; response attenuated by 80%). We conclude that the marked reduction of the CM-Pf elicited cerebral vasodilator response by mecamylamine implicates a cholinergic nicotinic mechanism.

129.9

LOCALIZATION OF NICOTINE-INDUCED INCREASES IN CORTICAL CEREBRAL BLOOD FLOW (CBF). D.G. Linville and S.P. Arneric, Department of Pharmacology, Southern IL University School of Medicine, Springfield, IL 62702 and Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064-3500

Electrical stimulation of the basal forebrain (BF) elicits increases in parietal cortical CBF (+150%) as measured by laser-doppler flowmetry (LDF), and this increase is selectively attenuated by the nicotinic antagonist, mecamylamine (Neurosci. Abstr. 13:288.12, 1987). We sought to determine: 1) Does nicotine receptor activation in the BF and/or cortex influence cortical CBF? 2) Does the BF-elicited increase in cortical CBF require intrinsic cortical neurons? Sprague-Dawley rats (3-6 months) were anesthetized (chloralose), paralyzed, artificially ventilated and arterial blood gases controlled. Phosphate buffered saline or L-nicotine (0.0, 0.125, 0.25 & 0.5 nmol/100nl) were microinjected into the cortex (immediately beneath the LDF probe) or BF. BF-injected nicotine increased cortical CBF by +39, +116, +163, +127%, respectively (N=4-5, $p<0.05$), whereas no detectable increases were obtained with cortical microinjections (N=3). Corticovascular responsivity was confirmed at each injection site with glutamate, 10 nmol/100nl. Secondly, 5-7 days following selective unilateral destruction of intrinsic cortical neurons with ibotenic acid, BF-elicited increases in cortical CBF were determined within the lesion and compared to the homologous sham-injected side. No significant differences were observed when lesions were restricted to superficial layers of cortex. However, when lesions disrupted all cortical layers, marked attenuation of responses was observed. CONCLUSIONS: 1) Nicotinic receptor activation in BF, but not cortex, enhances cortical CBF. 2) BF-stimulated cortical CBF responses appear to require synaptic mediation through intrinsic cortical neurons, which is similar to the increases in cortical CBF elicited from the fastigial nucleus of the cerebellum (Iadecola, et al., Am. J. Physiol. 252:1082-91, 1987). (Supported by the American Health Assistance Foundation for Alzheimer Disease Research)

129.10

UNILATERAL ABLATION OF NUCLEUS BASALIS MAGNOCELLULARIS IN RAT INCREASES FUNCTIONAL INTERACTIONS BETWEEN DENERVATED NEOCORTEX AND REMAINING CHOLINERGIC FOREBRAIN NUCLEI. T.T. Soncrant, Y. Lamour, D.M. Larson*, H.W. Holloway*, B. Horwitz, S.I. Rapoport. Lab. Neurosciences, National Institute on Aging, NIH, 10/6C103, Bethesda, MD 20892.

Unilateral destruction of the nucleus basalis magnocellularis (nbm) in rats reduces cholinergic inputs to the ipsilateral frontoparietal neocortex and disrupts behavior. Regional cerebral metabolic rates for glucose (rCMRglc) of denervated cortex initially are reduced, but normalize by 2 wk. To examine brain functional interactions, correlational analysis of rCMRglc was performed on 2 groups of 16 young rats at 2 wk after stereotaxic ablation of the right nbm with ibotenate or sham surgery. rCMRglc was measured in 117 brain regions of awake rats with the [14C]deoxyglucose method. For each region pair, a partial correlation coefficient (to control for effect of mean CMRglc) was calculated for rCMRglc across animals. Most correlations between cholinergic nuclei (medial septum and diagonal band) and right (66/72, mean increase 0.44) but not left (39/72) frontoparietal cortical regions were larger ($P < 0.001$) in lesioned rats, as were those between most frontoparietal region pairs (516/630, $P < 0.001$). These results suggest that functional interactions within frontoparietal neocortex, and between denervated cortex and remaining cholinergic nuclei, are altered at 2 wk after unilateral nbm ablation.

129.11

NITRIC OXIDE (NO): ITS ROLE IN MEDIATING INCREASES IN CORTICAL CEREBRAL BLOOD FLOW (CBF) ELICITED BY ELECTRICAL STIMULATION OF THE BASAL FOREBRAIN (BF). S.P. Arnerić, D.G. Linville, J.F. Kerwin, Jr., and F. Murad. Neuroscience Research, Abbott Laboratories, Abbott Park, IL 60064-3500, and Dept. of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62702.

NO is an EDRF (endothelium-derived relaxing factor) released from endothelial cells in response to transmitters such as acetylcholine (ACh), and may mediate multiple messenger functions in macrophages and brain tissue. It diffuses into smooth muscle where it causes activation of guanylate cyclase and relaxation. Recent evidence suggests that neurons arising from the BF participate in a regionally selective regulation of cortical CBF (Arneric, Excerpta Int. Cong. Series 869: 381-384, 1989). The BF-elicited CBF response appears, in large part, to be cholinergically mediated since physostigmine potentiates (Lacombe et al., Brain Res. 491:1-14, 1989), and nicotinic cholinergic antagonists attenuate this response (Arneric, 1989). This study sought to determine: Is the release of NO an important physiologic link required to mediate increases in cortical CBF governed by the BF? To do so, an inhibitor of NO synthetase (i.e. N^G-nitro-L-arginine, LNNA) was infused i.v. and changes in resting and BF-elicited increases in cortical CBF were assessed using laser-doppler flowmetry (LDF). Sprague-Dawley rats (3-5 months) were anesthetized (urethane), paralyzed, artificially ventilated and arterial blood gases controlled. Flow probes were stereotaxically positioned on the dural surface (2.5 mm rostral & 2.6 mm lateral to Bregma). Unilateral electrical stimulation (100 uA; 10 sec.) of BF at 5, 10, 25 and 50 Hz resulted in graded, stimulus-locked increases in CBF (up to 271% above resting control). Infusion of LNNA (60 mg/kg) maximally attenuated the BF-elicited increases from +271 % to +165 % at 25 Hz (i.e. a -44 ± 10 % reduction in the ability to increase CBF, $p< 0.05$, N=4); an effect that appeared frequency-dependent. Resting CBF was nonsignificantly reduced (-13 ± 7%). Although arterial blood pressure was elevated 15-25 mmHg, it was maintained within the autoregulated range. These data suggest: 1) NO formation has an important role in mediating regulation of cortical CBF by BF neurons; 2) The discharge freqency of BF neurons may influence the contribution of NO-formation to increase CBF; and 3) In contrast to effects on systemic circulation, the tonic production of NO in the cerebral circulation is small under these experimental conditions. Supported by ABBOTT LABORATORIES & AMERICAN HEALTH ASSISTANCE FOUNDATION FOR ALZHEIMER'S DISEASE.

129.12

AGE-DEPENDENT CEREBRAL METABOLIC EFFECTS OF UNILATERAL LESION OF NUCLEUS BASALIS MAGNOCELLULARIS IN RATS. F. De Micheli, H.W. Holloway*, D.M. Larson*, S.I. Rapoport, T.T. Soncrant. Laboratory of Neurosciences, National Institute on Aging, NIH, 10/6C103, Bethesda, MD 20892.

Unilateral ablation of the nucleus basalis magnocellularis (nbm) in rat causes degeneration of cholinergic projections to the ipsilateral frontoparietal neocortex and, in young rats, reduces regional cerebral metabolic rates for glucose (rCMRglc) in denervated cortex at 3 days. To evaluate the age-dependent cerebral metabolic effects of unilateral nbm destruction, rCMRglc was determined, using the [14C]deoxyglucose method, in 48 brain regions of 3- and 24-month old Fischer-344 rats at 3 days after stereotaxic injection of ibotenate into the right nbm. Lesion placement was confirmed in adjacent Nissl-stained sections. Immunohistochemical acetylcholinesterase staining of coronal tissue slices was markedly reduced in frontoparietal cortex ipsilateral to nbm lesion in all brains. rCMRglc was reduced in frontoparietal regions ipsilateral to nbm ablation (compared to the contralateral side) to a greater extent ($P < 0.05$) in 3 month old (-25%) than in 24 month old (-8%) rats. These results suggest that the tonic activity of cortically-projecting cholinergic neurons is reduced in aged rats, or that cerebral metabolic changes after nbm lesion follow different time courses in young and old rats.

130.1

ORGANIZATION OF RHESUS MONKEY PRIMARY VISUAL CORTEX AS REVEALED BY MAP_2 ANTIBODY STAINING. A. Peters. Dept. of Anatomy, Boston Univ. Sch. of Med., Boston, MA 02118.

Area 17 has been analyzed using an antibody to MAP_2 (Kosik et al., Proc. Natl. Acad. Sci., 81:7941, 1984) which can be used to label the cell bodies and dendrites of neurons. It is found that the cortex contains vertically oriented modules of pyramidal cells. The modules average 30 μm in diameter and are centered around the layer V pyramids. The apical dendrites of these cells ascend through layer IV in narrow clusters or bundles. When these clusters pass through layer II/III the apical dendrites of pyramids in those layers are added to them. The apical dendrites of layer VI pyramids also form bundles, but these appear to be disposed randomly. MAP_2 staining also shows the presence of groups of pyramidal cells in layer IVA. The cell bodies of the neurons in these groups are in cones, from the tops of which emerge bundles of apical dendrites. There are about 120 cones of layer IVA pyramids beneath 1 mm^2 of cortical surface, and they seem to occupy the spaces within the horizontally oriented honeycomb pattern that is revealed in layer IVA by cytochrome oxidase reactions. Consequently, it is likely that the parvocellular input from lateral geniculate body to layer IVA terminates in relation to these cones of pyramidal neurons. Supported by NIH grant NS 07016.

130.2

DENDRITIC ARBORIZATIONS OF VISUAL CORTEX NEURONS AND THEIR RELATION TO THE CYTOCHROME OXIDASE (CO) RICH BLOBS IN MONKEY STRIATE CORTEX. R. Malach Weizmann Inst. Rehovot, Israel 76 100.

The CO dense blob system is intriguing in being dispersed throughout striate cortex while maintaining clear connectional and physiological compartmentalization. Is this organization caused by the developmental superposition of two segregated systems, or does it subserve enhanced cross-talk at blob margins? These alternatives imply different levels of dendritic segregation of blob and interblob neurons. To investigate this issue CO and Golgi staining were combined within the same sections of monkey striate cortex.

Dendritic arbors were either traced relative to CO borders using a computer aided microscope or scanned in 3-D using confocal, laser scanning, microscope. Neurons with cell bodies located at the centers of blobs and interblob regions had dendrites which were mostly confined to the same compartment. However, in the population of neurons located near blob boundaries there were many instances in which dendrites of interblob neurons appeared to penetrate deep into the blobs and vice versa. Quantitative analysis is being conducted to test for more subtle relationships between CO topography and dendritic architecture. Supported by BSF 88-0275 and BRF 527/89.

130.3

CYTOCHROME-OXIDASE ACTIVITY IN CAT VISUAL CORTEX: IS IT PERIODIC?. K.M. Murphy, R.C. Van Sluyters & D.G. Jones*. Dept of Psychology, McGill University, Montreal PQ H3A 1B1, School of Optometry, University of California, Berkeley CA 94720. Computer Science, Stanford University, Stanford CA

The inputs from the two eyes to visual cortex are largely segregated in layer IV, forming the anatomical correlate of physiologically identified ocular dominance. In the cat visual cortex, examination of the 2D anatomical ocular dominance pattern in unfolded and flattened sections reveals an irregularly branching and beaded arrangement[1]. Previously, we analyzed the complete tangential arrangement of ocular dominance in normal and monocularly deprived cats[2]. The total number of ocular dominance beads in area 17 was similar for all animals studied, furthermore, the center-to-center spacing of the beads was not perturbed by monocular deprivation. These results indicate that there is some modularity to the arrangement of inputs to visual cortex. In primates the arrangement of ocular dominance has been related to the array of patches of high cytochrome oxidase activity in layers II-III. However, in the cat examinations of cytochrome oxidase staining of radial sections have failed to demonstrate any periodicity, making it unclear as to whether there is an intrinsic cortical periodicity that may relate to the pattern of geniculocortical inputs.

Since the complete pattern of cortical ocular dominance in the cat is most easily recognized in tangential sections from unfolded and flattened cortex we have used this same approach to examine cytochrome oxidase staining in area 17 of the cat. In the tangential plane cytochrome oxidase staining of area 17 reveals marked periodicity in the supragranular layers. This indicates that a periodic pattern of cytochrome oxidase staining is not unique to the primate visual cortex.

1. Anderson, Olavarria, Van Sluyters (1988) J. Neurosci., 8, 2183-2200.
2. Murphy, Van Sluyters, Jones (1989) Soc. for Neurosci. Abstracts, 795.

130.4

***IN VIVO* THREE DIMENSIONAL OPTICAL IMAGING OF FUNCTIONAL ARCHITECTURE IN PRIMATE VISUAL CORTEX.** *D. Malonek, D. Shoham, E. Ratzlaff* & A. Grinvald.* Lab. of Neurobiology, The Rockefeller Univ., New York, NY 10021, IBM Research Division & The Weizmann Institute of Science.

High resolution optical imaging of cortical intrinsic signals has facilitated the visualization of the two-dimensional functional architecture in the living brain. However a central feature of cortical organization is its functional segregation into layers; distinguishing different layers requires discriminating signals originating from different depths. Our previous experiments provided 100-150 µm resolution in two dimensions, but the depth resolution was not examined systematically.

Here we report that the depth resolution of previous measurements was limited by the optics used. The resulting images represented a weighted average of the signals originating from the pial surface to a depth of 600-2000 microns (depending on the illuminating wavelength). To study the functional architecture in three dimensions we have designed a new optical system which offers computer controlled optical sectioning of cortical maps *in vivo*. A tandem lens arrangement provided the CCD camera with a depth of field resolution of 45 µm (nominal). Images of the macaque primary visual cortex were taken at several depths (using 630nm illumination), interlacing different visual stimuli and different depths in a single experiment. Vascular artifacts seen with previous measurements virtually disappeared when focusing 300µm below the cortical surface. To show that optical sectioning was feasible we imaged the blobs, the orientation and the ocular dominance columns at different depths and obtained very similar maps of all these three functional features between depths of 100 and 600 µm. However, as expected from electrophysiological studies, both the blobs and the orientation columns were not detected at depths of 700-1000 µm, corresponding to layer 4C, while the ocular dominance columns were still visible at those depths. These results suggest that further improvements in the three dimensional resolution should be feasible using deconvolution of serial images and/or confocal microscopy.

130.5

NOVEL ASPECTS OF THE FUNCTIONAL ARCHITECTURE OF AREA 18 IN CAT VISUAL CORTEX. T. Bonhoeffer and A. Grinvald. Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021; IBM Research Division & The Weizmann Institute of Science, Rehovot, Israel.

In cat visual cortex, it has been shown that cells with similar ocular dominance or orientation tuning are grouped together, forming columns which run from the pia to the white matter. *In vivo* optical imaging of intrinsic signals was used to investigate the functional organization of area 18. We measured neuronal activity by recording the changes in light reflectance with a high resolution CCD camera. This technique allowed us to obtain multiple maps of the functional architecture of the same cortical area as a function of various parameters of the visual stimulus. In our maps of orientation preference we observed that orientation was most often organized radially rather than in parallel slabs; orientations from 0 to 180° were arranged in a pinwheel-like fashion around singularities which we termed "orientation centers". We found on the average 1.2 orientation centers / mm^2. Currently we are investigating the receptive field properties of single cells which are located in these optically-imaged singularities.

More recently, direction selectivity has been proposed to be a parameter according to which neurons are clustered in the visual cortex. Direction selectivity maps were also evaluated. In seven hemispheres we found that the cortical areas responding best to gratings moving in one direction exactly coincided with the areas showing the strongest response to the same stimulus moving in the opposite direction. Given the resolution of optical imaging we conclude that cells in area 18 do not form "directional" cortical columns. If cells with similar direction selectivity are clustered at all, then the size of these clusters is below 100 µm (the resolution of our method) or cells with opposite direction selectivity are grouped on top of each other in one column but at different cortical depth.

130.6

COLUMNS BEYOND V1 AND V2 IN MACAQUE VISUAL CORTEX: A DOUBLE-LABEL DEOXYGLUCOSE STUDY. R.B.H. Tootell and R.T. Born. Dept. Neurobiology, Harvard Med. Sch., Boston, MA 02115.

Several lines of evidence suggest that functional columns exist in cortical visual areas beyond V1 and V2, but they have never been directly labeled, nor analyzed, with functional anatomical techniques. To do this, we developed a double-label (using ^{14}C and ^{3}H-DG) deoxyglucose (2-L-DG) protocol which shows, for the first time, cleanly separable regions of high and low brain activity in the original (^{14}C- vs. ^{3}H-sensitive) films, without subtracting one image from the other. We used this technique in conjunction with specifically chosen stimulus pairs to test whether cells were systematically grouped into columns on the basis of preferred orientation, color, binocular disparity and other stimulus parameters. We examined all of visual cortex in flattened sections from 11 macaque monkeys.

We found many types of columns, especially in areas MT, VP, V3/V3A, V4, and two discrete, previously-undescribed areas which we call V8 (dorsal to MT) and VM (on the ventromedial bank). Drifting square wave gratings, at orientations/directions 90^{o} apart, were used to produce clearly interdigitated orientation/direction columns in areas V1, V2, MT, and what appears to be VP. The same stimulus produced columns of cells which were not discriminable by orientation/direction changes in areas V3/V3A and V4. Area MT also has a columnar patch/interpatch architecture, in which cells highly responsive to texture are segregated from those more responsive to contours (Tootell and Born, ARVO 1990). Spatially-diffuse variations in color consistently produce numerous columns of active cells in areas V8 and VM, and scattered columns in VP, V3A and V4.

We conclude that the 2-L-DG approach has clear value for mapping unknown columnar systems present in visual cortex, and for doing preliminary (2-condition) functional analysis of them. Supported by EY0798 and NIMH 14275.

130.7

FUNCTIONAL ARCHITECTURE OF COLOR AND DISPARITY IN VISUAL AREA 2 OF MACAQUE MONKEY. *D.Y. Ts'o, C.D. Gilbert, T.N. Wiesel.* Laboratory of Neurobiology, The Rockefeller University, NY, NY 10021.

Guided by functional maps of primate area 18 (V2) obtained from *in vivo* optical imaging, we have used single unit electrophysiology and tracer injections to study the organization and subcompartmentalization of functional properties within the cytochrome oxidase-rich "thick and thin stripes". Within the thick stripes, a high proportion of cells showed sharp disparity tuning. The disparity tuned regions showed separate clusters of tuned excitatory, near and far cells. On a finer scale, sites along a given electrode penetration represented disparities that showed regular shifts in position in depth relative to the fixation plane. The clusters of the different disparity tuned cell classes lay within regions of relatively uniform orientation preference, whereas penetrations in non-disparity tuned cortex often encountered cells with rapid shifts in orientation preference.

The thin stripes of V2 contained color selective cells. Color oriented cells were found primarily at the border of the thin stripe, between the unoriented color cells and neighboring regions of non-color selective oriented cells. In some cases, color selective regions bordered disparity selective regions, and here cells exhibiting both color and disparity selectivity were found.

Retrograde labeling after injections of multiple tracers within disparity and color selective regions of V2 revealed long range horizontal connections between stripes of the same functional type. A further segregation of connections was seen in the distribution of cells labeled after injections at sites containing near cells versus those containing far cells. Taken together, our studies reveal a functional sub-structure within each stripe of area V2. (Supported by grants EY07968, EY05253, and the Whitaker and Rita Allen Foundations).

130.8

DISTRIBUTION OF NEURONAL RESPONSE PROPERTIES IN MACAQUE V2. J.B. Levitt, D.C. Kiper, and J.A. Movshon. Department of Psychology and Center for Neural Science, New York University, NY, NY, 10003.

Visual area V2 in the macaque contains alternate thick and thin stripes of cytochrome oxidase (CO) rich tissue separated by CO-sparse interstripes. It has been reported that the specificity of the anatomical connections of these regions is matched by the physiological properties of neurons within them. We have quantitatively examined the response properties of neurons in V2 and related their properties to the local CO architecture. We recorded the activity of single units representing the central 5 deg in all laminae and CO division of V2 in anesthetized, paralyzed macaque monkeys. After mapping receptive fields with colored and black-and-white bars, slits, or spots, we measured the responses of V2 neurons to drifting sinusoidal gratings that varied in orientation, spatial frequency, drift rate, and contrast.

While there was a tendency for neurons with certain response selectivities to be segregated (for example, directional cells in thick stripes and color-selective cells in thin and interstripes), the striking feature of V2 was its uniformity. Unoriented, end-stopped, color-selective, and direction-selective cells could be found in all CO divisions; the majority of cells in all CO compartments of V2 were oriented, nondirectional, and not color-selective. Furthermore, cells in the different CO divisions differed little in their spatial, temporal, or contrast sensitivity. Nor did directional cells differ from color-selective cells in their sensitivity or responsiveness. We did note a difference in the laminar distribution of certain cell types. We found direction-selective cells predominantly in layer 4 and the bottom of layer 3, and color cells outside of 4 in the supra- and infragranular layers. We also identified a group of neurons, all within the central 2 deg, having simple receptive fields similar to those seen in V1. These were found mainly in layer 4, had low spontaneous firing rates, and were tuned to lower spatial frequencies than were complex cells. These simple cells were found in all CO regions. Our results suggest that the physiological organization of V2 is substantially more homogeneous than has been suggested by others.

Supported by NIMH grant MH09692 and NIH grants EY02017 and EY05864.

130.9

NEURONS WITH END-STOPPED RECEPTIVE FIELDS DETECT OCCLUSION CUES. E. Peterhans and R. von der Heydt*, Dept. of Neurology, University Hospital, 8091 Zürich, Switzerland.

Figures like the Kanizsa triangle mimic situations of spatial occlusion that induce illusory contours in perception. Based on previous experiments we have proposed a neural mechanism that produces such contours by integrating the signals of end-stopped cells. Under this aspect we have now studied end-stopped cells in areas V1 and V2 of the alert monkey.

We found that cells that responded to short lines or edges nearly always responded also to the ends of long lines or to corners. Line-ends and corners often evoked similar, sometimes even stronger responses than the short stimuli. Exclusive responses to corners were also found. Half of the cells (32/61) had asymmetric receptive fields: they preferred the line-ends or corners to cover one end-zone, but not the other. Moreover, they also responded to line-ends and corners of illusory-contour figures where these features indicated occlusion. The responses were reduced or abolished when a thin line was added to a corner so as to contradict the assumption of occlusion, or when a T-junction was changed into a cross.

We conclude that end-stopped cells, and especially those with asymmetric fields, may serve the detection of occlusion cues and may provide input to the proposed contour mechanism.

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130.10

THE NEURAL BASIS OF STEREOSCOPIC DEPTH DISCRIMINATION: PHASE CODING BY SIMPLE CELLS IN THE VISUAL CORTEX. G.C. DeAngelis*, I. Ohzawa, and R.D. Freeman, Bioengineering and Neurobiology Groups, School of Optometry, Univ. of California, Berkeley, California 94720.

Neural processing of stereoscopic depth information is thought to be initiated in the visual cortex, where binocular neurons can encode retinal image disparity. Traditionally, it has been assumed that the neural basis for depth discrimination involves disparity selective neurons whose preferred disparity is determined by the horizontal offset of identical receptive fields in the two eyes. This notion has been supported by the claim (Maske et al. 1984) that receptive field profiles of binocular simple cells are similar for the two eyes.

We have proposed an alternative mechanism (Freeman and Ohzawa, 1990) in which binocular simple cells encode disparity by having different receptive field profiles, which are in retinal correspondence, in the two eyes. To test this hypothesis in anesthetized and paralyzed cats, detailed two-dimensional receptive field profiles are constructed for both eyes using a reverse correlation technique (Jones & Palmer, 1987). An optimization method is then used to fit a Gabor function to each receptive field profile. The spatial phase parameter of the best-fitting Gabor function describes the relative positions and strengths of the receptive field subregions, and provides a quantitative measure for comparison of left and right eye profiles.

In contrast to the results of Maske et al., we find that the left and right eye receptive field profiles of simple cells often differ substantially. The difference in spatial phase between the two receptive field profiles ranges from 0 to 180 degrees. Moreover, the data indicate that cells with preferred orientations near horizontal tend to have similar receptive fields (small phase differences) in the two eyes, whereas cells tuned to orientations near vertical exhibit a wide variety of phase differences between the left and right eye receptive field profiles.

These results provide support for a neural mechanism of stereoscopic depth discrimination in which disparity is encoded among simple cells in terms of phase at multiple size or spatial frequency scales. (EY01175)

130.11

RECEPTIVE FIELD LENGTHS IN CAT STRIATE CORTEX CAN INCREASE WITH DECREASING STIMULUS CONTRAST. B. Jagadeesh and D. Ferster. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

We have found that the lengths of cortical receptive fields measured with low-contrast stimuli can be far longer than those measured with high-contrast stimuli in the same cells. Length summation curves were made using drifting sine-wave gratings with contrasts of 1% to 64%, and lengths of 0.25° to 24.0°. As previously shown within the classical receptive field, length can compensate for contrast: At low contrasts, longer stimuli are required to evoke the same response evoked by shorter, high-contrast stimuli (Schumer & Movshon, Vision. Res. 24:565). This compensation between length and contrast, can extend beyond the length of the receptive field measured at high contrasts. In other words, the optimal length of the receptive field can increase. For example, cells that showed complete summation at only 2° with high-contrast stimuli, could show length summation to 11° at low contrasts. In end-inhibited cells, summation at low contrasts could extend into the region in which the response had been decreasing at high contrasts.

One possible origin for the increased summation lengths observed at low contrasts may be input from layer 6 cells with long receptive fields, which provide an excitatory projection to layer 4 (Ferster & Lindström, J. Physiol. 367:233). In end-inhibited cells, the mechanism may be more complex, depending on the initial difference in length thresholds of both excitatory and inhibitory mechanisms. We speculate that the increase in summation lengths of individual cells at low contrasts might improve the detection of low-contrast stimuli, but only at the expense of spatial discrimination.

130.12

PROCESSING OF FORM IN MONKEY VISUAL AREA V1. K. Purpura and J. Victor. Cornell Univ. Med. College and The Rockefeller Univ., New York, N.Y. 10021.

Physiological and anatomical evidence indicate that visual information processing is divided among submodalities in both the striate (V1) and extrastriate cortex. The analysis of visual form must, however, require some unification across these modalities and may not be the function of any one cortical area. We present evidence for the analysis of form in V1 of the monkey.

Visual stimuli consisted of achromatic and chromatic isodipole textures produced on a CRT with specialized computer-driven hardware. Isodipole textures have identical spatial frequency spectra but differ in particular local features. Interchange between textures will produce a differential response only in mechanisms sensitive to the form elements; spatial filtering mechanisms will respond similarly to all such textures. The stimuli were presented to anesthetized and paralyzed cynomolgus monkeys. Visual evoked potential recordings (VEPs) were made with electrodes placed on the V1 pia and, in separate experiments, local field potentials and multiunit activity (MUA) were simultaneously monitored with tungsten-in-glass electrodes inserted into the cortex. Both recording arrangements revealed differential responses to the interchange of isodipole textures. The epicortical VEPs were striking in their similarity to human scalp VEPs elicited by isodipole textures. The differential MUAs were characterized by a drop in the population firing rate during the appearance of a structured texture in a structured/random interchange. These responses indicate that analysis of local form is present in V1.

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131.1

STIMULATING PERIPHERAL 5-HT$_1$-LIKE SEROTONERGIC RECEPTORS INCREASES WATER INTAKE BY A VAGALLY-DEPENDENT MECHANISM IN RATS. K. J. Simansky and K. Eberle-Wang, Dept. of Pharmacology, Med. Coll. of Pennsylvania, Philadelphia, PA 19129.

This study analyzed the mechanisms for the dipsogenic action of subcutaneously administered serotonin (5-HT) in rats given free access to dry food and water. 5-HT increased water intake during the 2-h test period in a dose-related manner with an approximate ED_{70} of 10 µmol/kg, SC. Drinking produced by this dose of 5-HT was antagonized by the 5-HT$_1$/5-HT$_2$ antagonists, metergoline, methysergide and 1-(1-naphthyl)-piperazine (1-NP), but not by ketanserin, mianserin, ritanserin or xylamidine. Thus, 5-HT$_1$ receptors other than the 5-HT$_{1C}$ subtype probably mediated the dipsogenic action of 5-HT. The 5-HT analog, 5-carboxamidotryptamine (5-CT), a 5-HT$_1$ agonist, also increased water intake but the peripheral 5-HT$_2$ agonist, α-methyl-5-HT, did not. The antagonist profile for blocking drinking produced by 5-CT was identical to that for 5-HT. The 5-HT$_{1A/1B}$ and beta-adrenergic antagonist, propranolol, stereo-selectively decreased drinking caused by either 5-HT or 5-CT. This effect was probably due to beta-blockade as atenolol also prevented drinking by 5-HT or 5-CT. Total abdominal vagotomy (VGX) prevented drinking caused by 5-HT and 5-CT and the 5-HT$_3$ antagonist, ICS 205-930 neither restored 5-HT-induced drinking in VGX rats nor blocked it in controls. These data suggest that stimulating peripheral 5-HT$_1$-like receptors produces drinking by a vagally-dependent mechanism in rats. Supported by MH 41987 (KJS).

131.2

ANORECTIC ACTION OF PERIPHERAL SEROTONIN (5-HT) AFTER ABDOMINAL VAGOTOMY IN RATS. K. Eberle-Wang and K.J. Simansky, Dept. of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA, 19129.

Previous work by Fletcher and Burton (1985) demonstrated an increased responsiveness to 5-HT anorexia after vagotomy (VGX) in rats. We reexamined this issue by determining dose-response curves for 5HT-anorexia in male VGX rats under various testing conditions. Nondeprived VGX rats given a 30-min test meal of sweetened mash during the dark period displayed comparable percentage reductions in mash intake following 5-HT (.25-4.0 µmol/kg, i.p.) when compared to laparotomized (LAP) controls. A separate group of food-deprived VGX/LAP rats was tested during the light period. 5-HT (2.0, 4.0, 8.0 µmol/kg, i.p.) produced anorexia in both groups; in contrast, CCK-8 (4.5 and 8.0 nmol/kg, i.p.) reduced food intake only in the LAP rats. In the same animals, 5-HT (4.0-16.0 µmol/kg, s.c.) decreased mash intake in both groups; once again, VGX did not enhance this action. Immunocytochemistry for α-neurofilament protein was used as a novel verification procedure for vagotomy. These results demonstrate that total VGX does not reliably produce supersensitivity to the anorectic action of peripheral 5-HT. Together with similar evidence using liquid diets (Simansky, in preparation), our data establish that 5-HT reduces food intake via an extravagal mechanism. Moreover, these data suggest that separate peripheral neural substrates mediate anorexia after 5-HT and CCK-8. Supported by MH 41987 (KJS).

131.3

STIMULATION OF FEEDING BY CNS METERGOLINE VARIES WITH INJECTION SITE. D.V. Coscina, J.N. Nobrega and D. Feifel, Sect. of Biopsychology, Clarke Inst. of Psychiatry, and Dept. of Psychology, Univ. Toronto., Toronto, Ont. Canada M5T 1R8.

Past research has shown that systemic administration of the serotonin antagonist, metergoline (MET), elicits feeding in satiated rats as well as blocks the anorectic action of the serotonergic agonist, d-fenfluramine, in both hungry rats and humans. What remains unclear is whether MET produces these effects by acting in the periphery (e.g., gastrointestinal tract) or in the brain. To begin addressing this question, ad libitum fed adult male rats were implanted unilaterally with CNS cannulae terminating in either the lateral cerebroventricle (LCV) or the paraventricular nucleus of the hypothalamus (PVN), a site at which MET has been shown to block food intake stimulated by local injections of norepinephrine (NE). One hr feeding tests after LCV injection of 0, 50, 100 or 150 nanomoles MET produced reliable enhancements of feeding at the two highest doses. To probe the specificity of this finding, separate 90 min tests of water intake, spontaneous activity, oxygen (O_2) intake and carbon dioxide (CO_2) output were run in the absence of food following 100 nanomoles MET vs. vehicle. The only difference observed was a small (+ 6.9%) but reliable increase in CO_2 output. Contrasting to this, PVN injections of 0, 5, 10, 20, 40 or 60 nanomoles MET produced no reliable changes in one hr feeding. Separate tests confirmed that NE injected into the PVN produced reliable enhancement of feeding, thereby eliminating the likelihood of negative findings because of improper cannula placements. These results indicate that the stimulation of feeding by systemic MET may be mediated partially or wholly through actions on the CNS, but the PVN is an unlikely site responsible for this effect.

131.4

SEROTONIN PARTICIPATES IN SUPPRESSION OF FOOD INTAKE BY EXOGENOUS CCK BUT NOT BOMBESIN. R.C. Ritter, L.A. Brenner and A. Zetino*. Dept. of VCAPP, Washington State University, Pullman, WA 99164-6520.

Stallone et al. (1987) observed that suppression of food intake by CCK is attenuated by metergoline, a relatively nonselective serotonin (5HT) receptor antagonist, suggesting that 5HT-sensitive neurons participate in CCK satiety. To further assess the involvement of 5HT in suppression of food intake by CCK, we examined the effect of several 5HT receptor antagonists on suppression of real feeding by exogenous CCK or bombesin. We also assessed the effects of 5HT antagonists on CCK-induced suppression of sham feeding. Four 5HT receptor antagonists exhibiting some selectivity for 5HT 1, 2 or 3 receptors were examined: metergoline, mianserin, ketanserin and MDL72222. Both metergoline and mianserin attenuate suppression of real feeding and sham feeding by intraperitoneal (IP) CCK. Neither drug affected suppression of real feeding by IP bombesin. Neither ketanserin nor MDL72222 altered suppression of feeding by CCK or bombesin. Metergoline alone increased intake during real feeding and produced an even greater increase during sham feeding. No increases in intake were observed with the other receptor antagonists by themselves. Our results suggest that 5HT$_1$ receptors participate in suppression of food intake by CCK but not bombesin. While the effect of mianserin appears somewhat selective for attenuation of CCK's effects on feeding, metergoline directly stimulated feeding, especially in the absence of GI feedback signals. Therefore, the actions of mianserin and metergoline may represent interference with more than one neural system. Supported by NIH grant NS20561.

131.5

5-HT$_{1A}$ AND 5-HT$_{1B}$ RECEPTOR BINDING SITES IN DISCRETE HYPOTHALAMIC NUCLEI: RELATION TO CIRCADIAN RHYTHM AND GENDER. S.F. Leibowitz and M. Jhanwar-Uniyal. The Rockefeller University, New York, N.Y. 10021, U.S.A.

Hypothalamic serotonin (5-HT) has a suppressive effect on feeding behavior, which is found to be strongest at the onset of the dark (active) cycle and more potent in female as compared to male rats. In this study, 5-HT receptor binding sites, of 1A and 1B subtypes, were biochemically examined in 8 discrete hypothalamic areas, across the light/dark (12:12 hr) cycle and also in female versus male rats.

Hypothalamic nuclei [paraventricular (PVN), ventromedial (VMH), medial preoptic (POM), dorsomedial (DMH), supraoptic (SON), suprachiasmatic (SCH), arcuate-median eminence (ARC-ME) and lateral perifornical hypothalamus (PLH)] were micropunched and assayed. Employing the radioligand binding technique, we measured the binding of the 5-HT receptor agonist [3H]8-OH-DPAT (2 nM) to 5-HT1A receptors (in presence or absence of 10 µM 5-HT). For 5-HT$_{1B}$ receptors, [3H]5-HT (2 nM) was incubated with 8-OH-DPAT (100 nM) and mesulergine (100 nM), in presence or absence 10 µM 5-HT.

The results revealed a significant light/dark rhythm of 5-HT$_{1A}$ receptor binding sites in 5 of the 8 hypothalamic nuclei. In the PVN and SCH, a single peak of 5-HT binding to 1B sites was detected in the middle of the light cycle; this peak was followed by a sharp decline in 5-HT binding at dark onset and a subsequent rise starting after midnight. A different temporal pattern was observed in the ARC-ME and PLH; these two nuclei showed greatest binding in the middle of the dark cycle and lowest binding at dark onset. In the VMH, a small but significant rise in 5-HT$_{1A}$ receptor sites occurred at dark onset. Analysis of 5-HT receptor binding in female as compared to male rats demonstrated that 5-HT$_{1A}$ receptor sites in females were significantly higher in the PVN, ARC-ME, and PLH, while lower in the VMH. Concentration of 5-HT$_{1A}$ receptor sites in females was greater in the ARC-ME, PLH and SON but lower in the POM. These results indicate circadian and gender differences in 5-HT receptors consistent with pharmacological findings.

131.6

BRAIN D-2 RECEPTOR BLOCKADE REDUCES SUCROSE REWARD. L.H. Schneider, J.D. Davis, E. Rauhofer*, J. Gibbs and G.P. Smith. Dept. Psychiatry, NY Hospital-Cornell Medical Center, White Plains, NY 10605 and Dept. Psychology, University of Illinois, Chicago, IL 60680

PURPOSE: To determine and compare the efficacy of the selective D-2 receptor antagonist sultopride administered centrally (icv) or peripherally (ip) to inhibit sucrose sham feeding in the rat.

METHODS: 8 male S-D rats were prepared with chronic gastric and lateral ventricular cannulas. They sham fed a 20% sucrose solution for 30-min tests after 4.75h pellet deprivation and pretreatment at -1h with vehicle-only (saline; 10 ul icv and 0.8 ml ip) or with sultopride (gift of SESIIF, France).

RESULTS: Data are Mean (SE) ml/30 min sham intake of 20% sucrose.

Lateral Ventricle

Dose	0	0.1	0.15	0.2	0.4
	29.4	26.5	20.0*	12.7*	11.8*
	(1.3)	(2.3)	(2.9)	(3.1)	(2.8)

Intraperitoneal

Dose	0	4.0	10.0
	28.9	29.6	3.0*
	(1.3)	(2.8)	(1.3)

Dose is mg sultopride/rat. *significant inhibition from vehicle icv and ip.

CONCLUSIONS: Since sultopride is about 30 times more potent when infused centrally than injected peripherally, central D-2 receptors are critical to the orosensory positive reinforcing effect of sucrose on sham feeding. [Supported by NIH (NINDS) R29 NS24781 (LHS); NIMH RSA MH00149 (GPS).]

131.7

TRANSIENT DECLINES IN BLOOD GLUCOSE PRECEDE MEAL INITIATION IN EXPERIMENTALLY INDUCED DIABETES IN RATS. F.J. Smith, L.A. Campfield and D. Howell*. Neurobiology and Obesity Research, Hoffmann-La Roche Inc. Nutley, NJ 07110

The objective of this study was to determine if transient declines in blood glucose (BG) similar to those previously observed prior to meal initiation in lean and obese rats preceded feeding in experimentally diabetic rats. Female Wistar rats were chronically implanted with cardiac cannulas. Following recovery from surgery, a single subcutaneous injection of streptozotocin (40 mg/kg BW) was administered to induce hyperglycemia. Two to seven days following streptozotocin induced diabetes, continuous monitoring of BG was performed at the light/dark transition of the 12/12 light cycle. All meals (n=8) were preceded by a fall in BG of -9.7 ± 1.9% below baseline at 17.4 ± 3.2 min. The total duration of the transient decline in BG was 34 ± 5 min and the meal occurred at 19 ± 4 min following the beginning of the decline. The parameters of these transient declines were similar to those previously reported for non-diabetic rats. Mean baseline BG was 294 ± 17 and the nadir was 265 ± 13 mg/dl. These data provide additional support for the relationship between transient declines in blood glucose and meal initiation and further emphasize the role of relative changes in systemic blood glucose rather than absolute glucose concentrations. These findings directly address one of major challenges to the glucostatic theory of Mayer (1955) and extend the pattern of blood glucose dynamics as a signal for meal initiation to the hyperglycemic, hyperphagic rat.

131.8

INCREASING THE SATURATION OF A FATTY ACID INCREASES ITS SATIATING POTENCY IN RATS. D.R. Lewis, J.M. Philopena*, and D. Greenberg. New York Hospital-Cornell Medical Center, White Plains, NY 10605.

Polyunsaturated fatty acids are essential in mammalian nutrition. In order to evaluate whether the degree of saturation of a fatty acid is important in determining satiating potency, we tested the effects on feeding of duodenal infusions of monounsaturated oleic acid or diunsaturated linoleic acid in sham feeding rats.

Rats (n=14) were fitted with gastric cannulas and duodenal catheters. Rats were given equivolumetric duodenal infusions of several concentrations (total caloric loads from 0.625 kcal to 13 kcal) of either oleic or linoleic acid and of 0.15M NaCl vehicle. Linoleic acid was significantly more potent in reducing sham fed intakes for all concentrations tested. The percent inhibition from intake during vehicle infusion for representative concentrations are:

	Oleic Acid	Linoleic Acid
Concentration:	6.25 kcal	1.25 kcal
Percent Inhibition:	55 ± 6	88 ± 4

Since the potency for inhibition of intake is at least four times greater for linoleic than for oleic acid these results suggest that degree of saturation is a critical stimulus for fat-induced satiety.

Supported by: The Weight Watchers Foundation, and NIH DK38757(DG).

131.9

NEURONS REQUIRED FOR LIPOPRIVIC FEEDING ARE NOT LIMITED TO A SINGLE VAGAL BRANCH. S. Ritter, B.W. Hutton* and T.T. Dinh*. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

Our previous work indicates that feeding responses stimulated by decreased glucose and fatty acid utilization (glucoprivation and lipoprivation, respectively) rely on different and distinct neural pathways. Lesion of vagal sensory neurons with capsaicin and total subdiaphragmatic vagotomy both abolish lipoprivic, but not glucoprivic, feeding. Thus, lipoprivic feeding requires intact vagal sensory neurons innervating the abdominal viscera. In the present work, we lesioned specific vagal branches in order to trace the pathway for lipoprivic feeding in more detail. After recovery from surgery, rats were adapted to a medium fat (40% of calories) diet for at least two weeks. Subsequently, glucoprivic and lipoprivic feeding were measured in 6 hr tests beginning immediately after drug injection. Fatty acid oxidation was blocked with mercaptoacetate (400 or 600 μmole/kg, i.p.) and glucose utilization with 2-deoxy-D-glucose (100 or 200 mg/kg, s.c.). None of the lesions blocked glucoprivic feeding. In contrast, lipoprivic feeding was abolished by transection of the gastric branches, but not transection of any other single vagal branch or total liver denervation. However, if all vagal branches were transected, leaving only the gastrics intact, the response was also impaired. Thus, sensory neurons required for lipoprivic feeding are not exclusively located in one vagal branch and may be distributed widely in the abdomen.

Supported by grant PHS #DK 40498.

131.10

CAROTID AND SYSTEMIC NUTRIENT INFUSIONS REDUCE FOOD INTAKE. E.K. Walls*, P.H. Reinhardt*, A.E. Willing* and H.S. Koopmans. Med. Physiology, Univ. of Calgary, Calgary, Canada T2N 4N1.

Both the glucostatic and aminostatic theories propose that food intake is regulated by cells in the brain that sense the levels of circulating nutrients and/or their intracellular utilization. To determine whether glucose and amino acid sensitive cells in the brain or in the periphery are more important in the control of food intake, two levels of dextrose (5 and 10 kcal/day) and 10 kcal/day of amino acids were each infused for 24 hr periods into rats with chronic catheters in the common carotid artery (C) or the superior vena cava (VC). Daily food intake was unchanged by 5 kcal dextrose. However, intake decreased from a 3 day baseline by 6.2 ± 2.3 kcal for the C rats and by 6.4 ± 1.3 kcal for the VC rats during the 10 kcal infusion ($p < 0.01$). Infusing 10 kcal of amino acids produced compensatory reductions in food intake of 12.1 ± 3.0 kcal and 10.8 ± 2.2 in the C and VC rats. The similar decreases in food intake for C and VC rats clearly show that nutrients fail to produce greater effects when infused directly into the brain. Instead, these findings suggest that the effect of infused nutrients on daily food intake is predominantly a function of nutrient-specific sensory receptor activation in the periphery.

131.11

COMPARISON OF BRAINSTEM METABOLIC ACTIVITY ASSOCIATED WITH SHAM AND NORMAL FEEDING. C.B. Phifer. Louisiana Scholars' College, Northwestern State Univ., Natchitoches, LA 71497.

Gastric distension is the only gastrointestinal stimulus for termination of ingestive behavior in very young rat pups. Thus the sham-feeding infant rat offers an excellent model for investigation of neural activity associated with the presence or absence of gastric distension. Chronic gastric fistulas were installed in six-day-old rat pups; pyloric nooses were also installed to prevent any leakage of stomach contents into the intestines. Pups were then allowed to ingest a milk diet with either open or closed fistulas during a 1 hr [14-C] 2-deoxyglucose incorporation period (30 uCi/100g, sc). Autoradiographic images corresponding to several levels in the brainstem were averaged for sham-feeding pups and pups feeding normally. Differences in activity between treatment groups were revealed by subtracting one average image from another.

Preliminary analyses of difference images revealed that sham-feeding animals had decreased metabolic activity relative to animals feeding normally in the nucleus tractus solitarius, particularly in medial regions at the level of area postrema. Further results will be discussed in relation to earlier findings on metabolic changes associated with gastric distension alone. (Supported by NICHD Grant HD-17457.)

132.1

EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR (NGFrec) AND TYROSINE HYDROXYLASE (TH) mRNAs IN THE IMMATURE RAT OVARY. G.A. Dissen, G. Nilaver, D.F. Hill,* and S.R. Ojeda. OR Reg. Prim. Res. Ctr., Beaverton, OR 97006, and OR Hlth. Sci. Univ., Portland, OR 97201.

It has recently been shown that NGF is produced in the ovary and that development of the sympathetic innervation of the gland is NGF-dependent. In the present study, NGFrec mRNA was detected in the immature ovary by RNA blot hybridization and the NGFrec protein was detected by ^{125}I-NGF cross-linking/immunoprecipitation/PAGE. NGFrec mRNA levels, readily detectable in juvenile rats, became maximally elevated on the day of first proestrus and declined markedly, after ovulation, suggesting that NGFrec synthesis is tightly coupled to the development of preovulatory follicles. No such fluctuations in NGFrec protein were observed, presumably because of active retrograde transport of the receptor by the ovarian nerves. Denervation of the gland reduced NGFrec protein levels during the first 6 days after the transection, but did not change NGFrec mRNA values. This suggests that the extrinsic nerves bear a significant fraction of the ovarian NGFrec and that receptor synthesis is not limited to the innervating neurons, but also occurs in ovarian cells. That some of these NGFrec bearing cells may be catecholamine-producing, is suggested by the hybridization of a rat TH cRNA to multiple ovarian TH mRNA-like transcripts and by the surprising finding that after denervation TH immunoreactivity, normally present in nerve fibers, becomes expressed in granulosa cells of a subpopulation of small to medium size follicles. The results suggest that the immature ovary is influenced by a dual catecholaminergic control provided by the extrinsic sympathetic nerves and ovarian cells able to synthesize catecholamines. While the relative contribution of the latter remains to be established it is likely that the function of both is under NGF influence. (Supported by NIH Grants HD24870 and RR00163).

132.2

EFFECT OF DECORTICATION AND TROPHIC FACTOR TREATMENT ON THE ChAT IMMUNOREACTIVE FIBER NETWORK OF THE ADULT RAT CORTEX. L. Garofalo, A. Ribeiro-da-Silva and A.C. Cuello. Dept. Pharmacology & Therapeutics, McGill Univ., Montréal Qué., Canada H3G-1Y6.

Unilaterally decorticated rats treated with both NGF and the monosialoganglioside GM1 show large increases, over control values, of cholinergic markers in the remaining cortex adjacent to the lesion site (Cuello at al., PNAS, 86:2056, 1989). In order to determine the nature of these biochemical increases we have measured using an image analysis system changes in cortical ChAT immunoreactive fibers and varicosities. Decorticated rats received i.c.v. either : vehicle (artificial CSF + 0.1% BSA) or NGF (12µg/day) and GM1 (1.5mg/day) via minipump for 7 days beginning immediately post-lesion. A decrease in the number of ChAT positive processes, detected at the light microscopic level, was observed thirty days post-lesion in the remaining cortex adjacent to the lesion site of vehicle treated rats. In contrast, cortical ChAT immunoreactive processes quantified from decorticated rats treated with both NGF and GM1 were moderately increased over control values. At the ultrastructural level, quantification of ChAT immunolabelled varicosity area showed that those observed in trophic factor treated lesioned rats were significantly greater than those seen in control or vehicle treated decorticated animals. These results together with previous data suggest that NGF and GM1 modulate neuroplasticity in the adult mammalian CNS and may favor functional cortical cholinergic recovery following injury. Supported by MRC (Canada) and the Canadian Center of Excellence Network for Neural Repair and Functional Recovery. L.G. receives a studentship from FRSQ (Québec).

132.3

TROPHIC ACTIONS OF RECOMBINANT HUMAN NERVE GROWTH FACTOR ON CULTURED RAT EMBRYONIC CNS CELLS. L.E. Burton[1], B. Knusel[2], F. Hefti[2], F.M. Longo[3], W.C. Mobley[3], V.E. Koliatsos[4], and D.L. Price[4]. [1]Genentech, Inc., S. San Francisco, CA 94080; [2]Andrus Gerontology Cent., USC Los Angeles, Los Angeles, CA 90089; [3]Dept. Neurology, UCSF, San Francisco, CA 94100; [4]Dept. Pathology, Johns Hopkins Univ., Baltimore, MD 21205.

Recombinant human nerve growth factor (rhNGF) (>95% pure by SDS-PAGE) was tested for actions on fetal rat brain neurons in culture including, in particular, the cholinergic neurons of the basal forebrain. rhNGF was more potent in increasing choline acetyltransferase (ChAT) activity in septal cultures than NGF purified from mouse salivary glands (mNGF). ED50s were 4.9 pM for rhNGF and 13.8 pM for mNGF. The maximal ChAT activity response was achieved at approx. 35 pM with both NGFs and their efficacies were not significantly different. The two NGFs were not additive in effect. Identical to the results with mNGF, rhNGF strongly enhanced the intensity of ChAT immunostaining in septal cultures. Neither rhNGF nor mNGF affected the appearance of the cultures under phase contrast illumination. Survival of cells at very low plating density on polyornithine/laminin coated culture dishes were not affected by either factor. Protein content and the uptake of GABA were also unaffected. At concentrations of up to 10 ug/ml, rhNGF did not significantly increase uptake of dopamine into cultures of ventral mesencephalon. We conclude that rhNGF produces potent and selective actions on cholinergic neurons of the basal forebrain as previously shown for mNGF.

132.4

RECOMBINANT HUMAN NERVE GROWTH FACTOR PREVENTS RETROGRADE DEGENERATION OF AXOTOMIZED BASAL FOREBRAIN CHOLINERGIC NEURONS. V.E. Koliatsos, M.D. Applegate, R.E. Clatterbuck, L.E. Burton, W.C. Mobley, F. Hefti and D.L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The present investigation tested *in vivo* the efficacy of a recombinant human nerve growth factor (rhNGF) assessed *in vitro* in an accompanying study. In rats and monkeys, axons of cholinergic neurons of the basal forebrain magnocellular complex were transected in the fimbria-fornix; animals survived for two weeks. One group received intraventricular mouse NGF (mNGF), a second group was given rhNGF, and a control group received vehicle. Animals in the latter group showed a significant reduction in the number and size of choline acetyltransferase-immunoreactive cell bodies within the medial septal nucleus ipsilateral to the lesion. Treatment with either mNGF or rhNGF completely prevented these abnormalities in size and transmitter phenotype of basal forebrain neurons. Our results indicate that intraspecies differences in NGF do not influence the responsiveness of neurons to this trophic factor and that a potent rhNGF is now available for use in other settings characterized by degeneration of basal forebrain cholinergic neurons.

132.5

PREVENTION OF CHOLINERGIC NEURON DEGENERATION IN THE RAT AND PRIMATE BRAIN WITH RECOMBINANT HUMAN NERVE GROWTH FACTOR. M. H. Tuszynski[1], D. G. Amaral[1,3], H.-S. U*[2], J. Barnett*[4], H. Chen*[4], and F. H. Gage[1]. Dept. Neurosciences[1] and Neurosurg.[2], Univ. California-San Diego, La Jolla, CA. 92093, Salk Inst. Biol. Stud.[3], La Jolla, CA. 92037, Syntex Research[4], Palo Alto, CA.

Previous studies have shown that infusions of the 2.5S form of mouse ß-NGF prevent the retrograde degeneration of both rat and primate adult basal forebrain cholinergic neurons after axotomy. In the current set of experiments, we assayed the ability of recombinant **human** NGF (rhNGF) to prevent retrograde cholinergic neuron degeneration in both rats and primates.

The coding sequence of human ßNGF was inserted into a baculovirus expression vector and used to infect insect cells. *In vitro*, the cells secreted microgram quantities of ßNGF which was biologically active on PC12 cells and SY5Y human neuroblastoma cells. Equivalent doses of rhNGF and mouse NGF (mNGF) were then tested in two *in vivo* models. Adult Sprague-Dawley rats underwent unilateral fimbria fornix transections and continuous infusions of either 1) artificial CSF (controls), 2) 50 ug/ml mNGF, 3) 100 ug/ml mNGF, 4) 50 ug/ml rhNGF, or 5) 100 ug/ml rhNGF. After 2 week survival periods, immunocytological markers for ChAT in the medial septum showed persistent ChAT labelling in 43±3% of control animals, 97±6% of low-dose and 101±3% of high-dose mNGF-treated animals, and 79±8% of low-dose and 93±5% of high dose rhNGF-treated animals.

Similarly, primates underwent unilateral fornix lesions and continuous infusions of either artificial CSF (controls), 180 ug/ml mNGF, or 180 ug/ml rhNGF. Persistent medial septal ChAT labelling 4 weeks later was found in 45% of control animals, 80% of mNGF treated animals, and 86% of rhNGF treated animals. Thus, rhNGF is biologically active *in vitro* and *in vivo*, and prevents the retrograde degeneration of cholinergic neurons in both rats and primates.

132.6

RECEPTOR-MEDIATED TRANSPORT OF RECOMBINANT HUMAN NGF FROM OLFACTORY BULB TO FOREBRAIN CHOLINERGIC NUCLEI. C. A. Altar and C. Bakhit Developmental Biology, Genentech Inc. South San Francisco, CA 94080.

Receptors for nerve growth factor (NGF) are present in the olfactory bulb and the nuclei of its cholinergic afferents. The degeneration of the bulb and its cholinergic afferents in Alzheimer's disease warranted a study of the transport of biologically active [^{125}I] labeled recombinant human NGF (rhNGF) following its injection into the olfactory bulb.

[^{125}I]rhNGF (103 nM; 330,000 cpm, 1.5 ul) was injected into the left olfactory bulb of 6 male Sprague-Dawley rats. Rats were glutaraldehyde-paraformaldehyde perfused 18 h later. Autoradiography revealed [^{125}I] label in the ipsilateral horizontal and vertical limbs of the diagonal band (DB) and in no other brain region except within the injected olfactory bulb. The transport of label to the diagonal band was blocked by a coinjection with 17 uM of unlabeled rhNGF. Cerebellar injections of [^{125}I]rhNGF (652,000 cpm, 2 ul), with or without 19.5 uM unlabeled rhNGF, did not label the diagonal band, nor the lateral vestibular or red nuclei, from which originate the primary cholinergic afferents to cerebellum.

The receptor-dependent transport of rhNGF from olfactory bulb to forebrain cholinergic nuclei and the ability of exogenous rhNGF to increase *in vivo* calbindin-28K content in the olfactory bulb (Iacopino et al, this meeting) suggests that the bulb and its innervation from the DB may be responsive to endogenous NGF or exogenously administered rhNGF.

132.7

REGULATION OF NGF EXPRESSION DURING DEVELOPMENT AND IN HIPPOCAMPAL CULTURES. W.J. Friedman and H. Persson. Lab. of Molecular Neurobiology, Karolinska Institute, Stockholm, Sweden

NGF is a well-characterized neurotrophic factor which is synthesized in the hippocampus and cortex and supports the function of basal forebrain cholinergic neurons. We have examined expression of NGF and NGF receptor mRNA during development using *in situ* hybridization. We have further studied regulation of NGF mRNA expression *in vitro* using a dissociated culture system of embryonic rat hippocampus.

We previously demonstrated that agents which mediate inflammatory responses, such as interleukin-1 (IL-1) and prostaglandin E_2 (PGE_2), enhance levels of NGF mRNA. Immunosuppressing agents such as dexamethasone suppress NGF expression. We have further examined the role of tumor necrosis factor ($TNF\alpha$), which synergizes with IL-1 in many peripheral systems, on NGF regulation. TNF enhances NGF mRNA expression in the hippocampal cultures and may have a synergistic effect with IL-1.

Further work has investigated possible second messenger pathways involved in mediating the Il-1 induction of NGF expression. Pretreatment of hippocampal cultures with phorbol ester to exhaust the protein kinase C pathway did not inhibit the ability of IL-1 to induce NGF mRNA, suggesting that either this pathway is not involved, or is not the only pathway necessary to regulate NGF expression. Studies are in progress to examine the possible involvement of cAMP and other second messenger pathways in regulation of NGF mRNA expression.

132.8

EFFECTS OF PERIPHERAL INJURY ON THE DISTRIBUTION OF NERVE GROWTH FACTOR RECEPTOR (NGFR) AND NGFR mRNA IN THE SPINAL CORD AND BRAIN. C.E. Hulsebosch, W.T. Stamps, T.E. Kruger, and B.A. Urschel. Dept. of Anat. and Neurosci., Marine Biomed. Inst., Univ. Texas Med. Br., Galveston, TX 77550.

NGFR has been postulated to be involved with neurite guidance and regeneration by providing a "pathway" of NGF which is associated with the low affinity, fast disassociating NGFR on Schwann cells. Since central projections of somatosensory fibers are known to sprout under certain conditions, it is of interest to determine if the distribution of NGFR and mRNA for NGFR correlate with the occurrence of sprouting. The present paradigm employs a forelimb amputation paradigm in which minimal somatosensory sprouting occurs. Five neonates were anesthetized by hypothermia at birth, either right or left forelimbs were amputated and the animals sacrificed 7 days later. The NGFR distribution was determined using the monoclonal 192 and standard immunohistochemical techniques. In situ hybridization was used to determine the presence of the mRNA for NGF using a synthetic oligonucleotide probe corresponding to the membrane-spanning domain of the rat NGFR sequence as determined by Radeke, et. al. The NGFR distribution was particularly sparse in laminae II (inner zone) both ipsi- and contralateral to the amputation. The forelimb region of the motorcortex contralateral to the amputated side demonstrated heavy labeling of neuron like cells. We hypothesize that the presence of NGFR is involved with successful regeneration of somatosensory components of the spinal cord and in the absence of restoration, surviving neurons in the motor pathway upregulate NGFR mRNA.

132.9

QUANTITATION OF NGF RECEPTOR mRNA REGULATION IN DISSOCIATED RAT BASAL FOREBRAIN CULTURES. R. C. Elliott, B. Lu, I. B. Black, and C. F. Dreyfus. Cornell U. Med. Coll., N.Y., NY, UMDNJ/Robt. Wood Johnson Med. Sch., Piscataway, NJ.

Although recent study is characterizing the regulation of nerve growth factor receptors (NGF-R), quantitative characterization of NGF-R mRNA in defined populations in rigorously controlled culture systems remains unexplored. We have used a sensitive nuclease protection assay to monitor steady-state levels of NGF-R mRNA in dissociated cultures of embryonic day 17 rat basal forebrain (BF) in serum-free, fully defined medium to reduce support cell number. This approach allows precise quantitation of alteration in NGF-R mRNA levels in a well characterized system. In initial experiments we examined effects of NGF on expression of its own receptor mRNA. Cultures were maintained for 1, 4, or 7 days in the presence or absence of the factor. In control cultures, NGF-R message expression per total mRNA increased 2-fold over 7 days. Exogeneous NGF increased NGF-R message over controls as early as day 4, and elevated receptor mRNA 5-fold by day 7. These data suggest that NGF is capable of selectively elevating receptor message with respect to total cellular mRNA. Furthermore, the effects of NGF appear to be exerted locally, since the increases occurred in dissociates enriched for BF neurons. We are currently investigating mechanisms underlying NGF-R mRNA regulation by NGF, and characterizing potential regulatory effects of other growth and trophic factors. (Support: NINDS, NICHD, and McKnight Fdn.)

132.10

MECHANISM OF NGF mRNA REGULATION BY INTERLEUKIN-1 AND BASIC FIBROBLAST GROWTH FACTOR IN RAT ASTROCYTES. X. VIGE and B.C. WISE. FGIN, Georgetown University, Washington, D.C. 20007.

Neonatal rat cortical astrocytes in primary culture synthesize and secrete nerve growth factor (NGF). Interleukin-1 (IL-1) and basic fibroblast growth factor (bFGF) treatment of astrocytes increased NGF mRNA content by about 2-fold. The concentrations of IL-1 and bFGF causing half-maximal stimulation were 4 and 31 pM, respectively. The increase in NGF mRNA elicited by IL-1 and bFGF was maximal at 3 hours of incubation. In the presence of IL-1, this increase persisted for 72 hrs, whereas in the presence of bFGF, the increase was of shorter duration, and after 24 hrs of incubation the NGF mRNA content was below control levels. The combined treatment of astrocytes with maximally effective doses of IL-1 and bFGF produced an additive increase in NGF mRNA content, which suggests that different mechanisms are operative. Astrocyte treatment with cycloheximide increased (about 4.5-fold) NGF mRNA content and this content failed to increase further by IL-1 or bFGF treatment. Experiments using actinomycin D indicated that IL-1 increased by 40% the half-life and reduced by 30% the rate of degradation of the NGF mRNA. bFGF treatment failed to change these parameters. Thus, in astrocytes, IL-1 increases NGF mRNA content, at least in part, by stabilizing mRNA, whereas bFGF does not affect mRNA stability, but may act at the level of NGF gene transcription.

132.11

NGF REGULATION OF CHOLINE-ACETYLTRANSFERASE (ChAT) mRNA EXPRESSION IN RAT CNS. L. Cavicchioli, R. Dal Toso, M. Fusco, G. Vantini, T. Flanigan[1*], G. Dickson[1*], F. Walsh[1] and A. Leon. Fidia Research Laboratories, 35031 Abano Terme, Italy and [1] Dept. Exp. Pathol., Guy's Hospital, London SE1 9RT, UK

Choline-acetyltransferase (ChAT) is a specific cholinergic marker and both enzymatic activity and immunoreactivity have been widely used to monitor the effects of NGF on CNS cholinergic neurons. However, almost no information is available on possible mechanisms of NGF-induced ChAT regulation at transcriptional, translational and post-translational levels. To this end, we have developed a PCR procedure allowing for detection and relative quantification of ChAT mRNA in descrete CNS areas (Cavicchioli et al., Neurosci. Abst., 29.9, 1989). Here we report that: i) detection of ChAT mRNA occurs only in CNS areas featuring cholinergic cell bodies; ii) the amount of ChAT mRNA in the septum is subject to ontogenic development reaching adult levels between postnatal day 14 and 25; iii) levels of ChAT mRNA are increased following i.c.v. NGF administration in the septum of both young and adult rats. These results indicate that NGF effects on ChAT activity are, at least in part associated with increased levels of ChAT mRNA. As such, this parameter is now being utilized in defining mechanisms underlying ganglioside GM_1 induced potentiation of NGF effects in vivo.

132.12

DEVELOPMENTAL EXPRESSION OF NERVE GROWTH FACTOR (NGF) RECEPTOR mRNA IN THE RAT BASAL FOREBRAIN: INFLUENCE OF NGF ADMINISTRATION. W.C. Mobley, T. Barrall,* B.J. Gwag, and J.E. Springer. Department of Neurology, University of California at San Francisco, San Francisco, CA 94143, and Dept. of Neurology, Center for Neurological Research, Hahnemann University, Philadelphia, PA 19102-1192.

The distribution and actions of nerve growth factor (NGF) in the central nervous system (CNS) support its role as a neurotrophic molecule for developing and adult basal forebrain cholinergic neurons. CNS infusions of NGF during development will increase the activity of choline acetyltransferase, an enzyme responsible for acetylcholine production. Cholinergic axotomy in adult animals will result in neuronal loss and/or dysfunction, which can be ameliorated with exogenous NGF application. While the mechanism(s) of action of this neurotrophic molecule is unclear, recent studies indicate that NGF may regulate the expression of NGF receptors (NGFR). Following release from target structures (i.e. hippocampus and neocortex) NGF binds to specific receptors located on cholinergic neurons and is transported to the cell body. We used in situ hybridization histochemistry to analyze the developmental expression of NGFR mRNA in the basal forebrain at postnatal days (PD) 1, 4, 8, 11, 21, and adult. In addition, NGFR mRNA expression was determined in PD4 and PD8 animals following intraventricular infusions of NGF. Radiolabeled sense or antisense NGFR mRNA probes were transcribed from a 3.4kb cDNA and hybridized to 16 μm thick brain sections. Basal forebrain cells expressing NGFR mRNA were first detected at PD4 with a striking increase in mRNA levels per cell observed at PD8 and PD11. In addition, NGFR mRNA levels at these two developmental time periods were greater than levels detected in adult animals. Animals infused with NGF at PD3 and analyzed at PD4 showed a dramatic increase in NGFR mRNA expression. In addition, a similar increase in NGFR mRNA was observed in animals infused every other day starting at PD3 and analyzed at PD8. We suggest that basal forebrain cholinergic neurons are sensitive to the presence of NGF throughout development and into adult. In addition, this study demonstrates that NGF can influence the expression of NGFR mRNA and thus the cells ability to respond to NGF at very early developmental periods. Supported by NIH grant AG-08969.

133.1

EVIDENCE FOR GLYCINE-LIKE IMMUNOREACTIVE INPUT ONTO RETROGRADELY LABELED SYMPATHETIC PREGANGLIONIC NEURONS IN THE RAT AND PIGEON. J. Cabot, A. Bushnell* and V. Alessi, Dept. of Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

Iontophoresis data collected in vivo and in vitro in the cat and rat thoracic spinal cord suggest that glycine may be an inhibitory neurotransmitter released from synapses directly contacting sympathetic preganglionic neurons (SPN's). This study provides light and electron microscopial evidence showing the presence of glycine-like immunoreactive(GLY-IR) processes within the SPN neuropil.

Male rats and pigeons were sacrificed 72 hrs after HRP injections into the superior cervical ganglion or paravertebral ganglion 14, respectively. Following intracardial perfusion, thoracic spinal cord was vibratome sectioned in the longitudinal plane and DAB reacted for HRP. For light microscopy, postembedding protocols using ABC immunohistochemistry were followed. For electron microscopy, preembedding immunohistochemical procedures were used.

Light microscopically, GLY-IR processes and punctate swellings are found in all the SPN subnuclei within rat thoracic spinal cord. Some GLY-IR profiles closely appose HRP-labeled SPN somata and proximal dendrites. GLY-IR cell bodies are observed among intermediolateral cell column (IML) neurons, within medial lamina V and throughout lamina VII. In the pigeon, the SPN neuropil contains a dense network of GLY-IR processes. GLY-IR profiles closely appose and appear to encircle HRP-labeled SPN's in both subnuclei. GLY-IR interneurons are found in the principal SPN cell column and throughout lamina VII.

To date, the ultrastructure of GLY-IR processes has only been examined within the rat IML. GLY-IR terminals have been observed making contact with HRP-labeled SPN somas and proximal dendrites. They form symmetrical contacts and contain spherical vesicles. GLY-IR dendrites and axons are also present in the IML. (Supported by HL24103; JBC is an Established Investigator of AHA.)

133.2

RELATIONSHIPS BETWEEN ACTIVITY OF PREGANGLIONIC SYMPATHETIC NERVES: PHASE AND COHERENCE. B. Kocsis, S.M. Barman and G.L. Gebber. Dept. of Pharmacol., Michigan State Univ., E. Lansing, MI 48824.

We (Kocsis *et al.*, Soc. Neurosci. Abstr., Vol. 15, Part 1, p. 335, 1989) previously described the frequency-domain relationships between the activity of different cat postganglionic sympathetic nerves. Coherence analysis revealed that the discharges of the inferior cardiac and renal nerves are correlated between 0.2 and 12 Hz. Phase spectral analysis showed that the interval between activity in these nerves is either constant between 0.2 and 12 Hz or frequency-dependent. The current study was designed to test whether the constant and frequency-dependent interval patterns represent different states of the central system responsible for basal sympathetic nerve activity. For this purpose, we studied the relationships between the discharges of preganglionic rather than postganglionic nerves. Recordings were made from the second thoracic preganglionic white ramus (T2WR) and splanchnic preganglionic nerve (Spl.N.) in baroreceptor-denervated cats anesthetized with chloralose. We found that 1) the peak coherence value and coherent frequency band relating T2WR and Spl.N. discharges were 0.77±0.05 and 0.2 to 12 Hz, respectively, 2) the interval between T2WR and Spl.N. discharges was either constant or frequency-dependent under normocapnic conditions and 3) the constant interval pattern was switched to the frequency-dependent pattern when end-tidal CO_2 was raised to hypercapnic levels. These results parallel those obtained in our earlier experiments with postganglionic nerve recordings. We conclude that the constant and frequency-dependent interval patterns of relationship between the discharges of different sympathetic nerves arise in the central nervous system. (Supported by NIH grants HL13187 and HL33266.)

133.3

RELATIONSHIPS BETWEEN ACTIVITY IN POSTGANGLIONIC SYMPATHETIC NERVE PAIRS IN UNANESTHETIZED DECEREBRATE (UD) CATS. S. M. Barman and G. L. Gebber. Dept. of Pharmacol., Michigan State Univ., E. Lansing, MI 48824.

The coherence function and phase spectrum were used to evaluate the relationships between inferior cardiac nerve (ICN) and renal nerve (RN) activity in eight UD and baroreceptor-denervated cats. Midbrain transection at stereotaxic plane A3 was completed under short-lasting ketamine hydrochloride (20 mg/kg, im) anesthesia. Decerebration was indicated by rigidity in all four limbs and the failure to alter the EEG in response to pinching. Analyses were made between 4 and 8 hr after decerebration. Mean arterial pressure was 126±10 mm Hg. As was the case in barbiturate-or chloralose-anesthetized cats, most of the activity in these sympathetic nerves in UD cats was between 2 and 6 Hz. However, there were important differences among these three groups of animals. First, the peak coherence value (0.48±0.07) relating ICN and RN activity in the 2- to 6-Hz band was significantly lower in UD cats than in barbiturate-(0.63±0.02; n=24) or chloralose-anesthetized (0.74±0.03; n=16) cats. Second, the coherence values and phase differences were considerably more labile in UD cats than in anesthetized cats. In some UD cats the coherence of ICN and RN activity disappeared spontaneously without an apparent change in the general condition of the animal or the shape of the autospectra of nerve activity. Third, mild hypercapnia (5% end-tidal CO2) often was accompanied by a loss of coherence in the 2- to 6-Hz band in UD but not anesthetized cats. Fourth, bimodal autospectra and coherence functions were seen only in UD cats (3 of 8 experiments). ICN and RN activity cohered between 2-6 Hz and 7-11 Hz in these experiments. We conclude that coupling between the components of the central system responsible for the 2- to 6-Hz rhythm in sympathetic nerve activity is weaker and more sensitive to change in UD than in anesthetized cats. (Supported by HL-13187 and HL-33266).

133.4

MECHANISMS ACCOUNTING FOR RAPID RHYTHMS IN SYMPATHETIC NERVE DISCHARGE (SND). G.L. Gebber, S.M. Barman and B. Kocsis. Dept. of Pharmacol., Michigan State Univ., E. Lansing, MI 48824.

The cardiac-related rhythm recorded from sympathetic nerves in baroreceptor-innervated cats and its free-running 2- to 6-Hz counterpart in baroreceptor-denervated cats might reflect 1) the oscillatory activity of a population of single central neurons or 2) the output of a central network of neurons whose discharges are probabilistically rather than strictly related to the phases of the population rhythm. These models were tested by studying the frequency- and time-domain relationships between the discharges of single medullary neurons and inferior cardiac postganglionic SND in diallylbarbiturate-urethane anesthetized cats. Our findings are as follows. First, although statistically significant, the coherence values relating SND to the activity of neurons in the medullary lateral tegmental field, rostral ventrolateral medulla and raphe were closer to zero than to unity. Second, whereas most of the power in the autospectra of SND was contained between 2 and 6 Hz, that in the autospectra of medullary unit activity was more evenly distributed over a much wider frequency band. Third, the interspike intervals of medullary unit activity were much more variable than the intervals between the cardiac-related bursts of SND in baroreceptor-innervated cats. Thus, the rapid rhythms in SND are not strongly reflected in the spike trains of individual medullary neurons believed to comprise the network responsible for the population rhythms. Rather, it appears that the rapid sympathetic nerve rhythms are emergent properties of a network composed of "non-oscillatory" brain stem neurons. Our data further indicate that each cycle of the population rhythm is generated by a different subset of the network. Regarding this point, individual medullary neurons with sympathetic nerve-related activity did not fire in every cycle of SND. (Supported by NIH grants HL13187 and HL33266.)

133.5

CHEMICAL LESION OF ROSTRAL VENTROLATERAL (RVLM) OR VENTROMEDIAL (RVMM) MEDULLA PREVENTS NEUROGENIC HYPERTENSION. K.J. Varner, E.C. Vasquez and M.J. Brody. Dept. of Pharmacology & Cardiovascular Ctr., Univ. of Iowa, Iowa City, IA 52242.

Integrity of neurons in RVLM and/or RVMM is required for the maintenance of arterial pressure, sympathetic activity and baroreceptor and cardiopulmonary reflex function in anesthetized rats. The purpose of this study was to determine whether chemical destruction of neurons in RVLM or RVMM prevents the increases in mean arterial pressure (MAP) and heart rate (HR) produced by sinoaortic deafferentation (SAD). Male Sprague-Dawley rats were anesthetized with pentobarbital and mechanically ventilated. RVLM or RVMM was lesioned bilaterally by microinjecting N-methyl-D-aspartic acid (NMDA) (200 nl, 30 nMoles) through one barrel of a 3 barrel pipette. Block of pressor responses to NMDA (20-40 ng, 50-100 nl) confirmed the effectiveness of the lesion. SAD significantly increased MAP and HR in sham lesion rats. Lesion of RVLM or RVMM prevented the increases in MAP or HR following SAD. Resting MAP and HR were not significantly reduced by either lesion but MAP was lowered by ganglionic blockade. From these studies we conclude that cells in both RVLM and RVMM are involved in the hypertensive response produced by acute SAD, perhaps by mechanisms unrelated to maintenance of arterial pressure.

133.6

THE NON-NMDA SUBTYPE OF EXCITATORY AMINO ACID RECEPTOR PLAYS THE MAJOR ROLE IN CONTROL OF CARDIOVASCULAR FUNCTION BY THE SUBRETROFACIAL NUCLEUS IN CATS. T.P. Abrahams and R.A. Gillis*, Dept. of Pharmacology, Georgetown University, Washington, D.C. 20007.

Recent studies (Am. J. Phsiol. 256:R722, 1989) have reported that microinjection of kynurenic acid (KYN; 12.5-25.0nmol), the non-selective Excitatory Amino Acid (EAA) antagonist, into the rostral ventrolateral medulla of the cat decreases arterial blood pressure (BP) and inferior cardiac sympathetic nerve discharge. The purpose of our study was to confirm this finding and determine the subtypes of EAA receptor(s) responsible for mediating this effect. This was done by microinjecting various EAA antagonists bilaterally into the subretrofacial nucleus (SRFN) of chloralose anesthetized animals while monitoring BP and heart rate (HR). KYN (12.5nmol; N=5) produced a decrease in mean BP (-31±9mmHg, P<0.05) with no significant change in HR. To determine the subtype of EAA receptor responsible for eliciting tonic sympathetic outflow from the SRFN, specific antagonists of NMDA and non-NMDA EAA receptors were tested. The NMDA receptor antagonist 3-((RS)-Carboxypiperazin-4-yl-)-propyl-1-phosphonic acid (CPP; 2.25nmol; n=3) microinjected into the SRFN produced a small but significant decrease in BP (-13±1mmHg; p<0.05). This effect of CPP on BP was significantly lower than that seen with KYN. Two antagonists of the non-NMDA subtype of EAA receptor,6-Cyano-7-nitroquinoxaline-2,3-dione (CNQX; 2.25nmol; n=4) and g-D-Glutamylaminomethyl sulphonic acid (GAMS; 2.5nmol; n=4), were microinjected into the SRFN. Both of these drugs produced decreases in BP (-29±4 and -23±3mmHg, respectively; p<0.05) similar to that observed with KYN. No significant changes in HR were noted with CPP, CNQX or GAMS. These data indicate that a non-NMDA EAA receptor plays the major role in control of cardiovascular function by the SFRN. Supported by USPHS grant 1P01 NS28130.

133.7

INTERACTION BETWEEN A5 AND BARORECEPTOR AFFERENT INPUTS IN THE NUCLEUS OF THE SOLITARY TRACT. M. B. Hug and J. Ciriello, Dept. of Physiology, Univ. of Western Ontario, London, Canada, N6A 5C1.

The A5 noradrenergic cell group has been shown to alter cardiovascular reflexes. In this study, to investigate if A5 neurons could alter these reflexes by influencing the activity of nucleus of the solitary tract (NTS) neurons, single unit extracellular recordings were made from NTS units during A5 stimulation in alpha-chloralose anesthetized rats. Of 139 spontaneously active NTS units tested, 25 were excited and 4 were inhibited during A5 stimulation. Nine of these 25 units were also excited orthodromically by stimulation of the aortic depressor nerve (ADN). When a conditioning stimulus was applied to the A5 region and the ADN was stimulated at several delay intervals ranging from 0 ms to 300 ms, the ADN response of 6 units was decreased at intervals of 0 to 80 ms. An additional 4 units in NTS were antidromically activated by A5 stimulation. These antidromic units also responded orthodromically with an excitation (n=2) or inhibition (n=2) to A5 stimulation and 2 were further excited by ADN stimulation. These data have demonstrated that A5 and ADN inputs converge on NTS neurons, that A5 inputs to NTS decrease the response of NTS neurons to ADN inputs and that A5 and NTS are reciprocally connected. These data suggest that A5 neurons alter cardiovascular reflexes by inhibiting the activity of NTS neurons to cardiovascular afferent input.
(Supported by MRC of Canada).

133.8

FOREBRAIN PROJECTIONS OF THE VENTROLATERAL MEDULLA. S.Roder and J. Ciriello, Dept. of Physiology, Univ. of Western Ontario, London, Canada, N6A 5C1.

The present study was done to provide a detailed mapping of forebrain structures innervated by ascending fibers from catecholaminergic neurons in the ventrolateral medulla (VLM). Fibers and terminals in the forebrain of the rat from neurons in the caudal (c) VLM, the rostral (r) VLM and the intermediate (int) VLM, were identified using the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L) combined with either tyrosine hydroxylase (TH) or phenylethanolamine-N-methyltransferase (PNMT) immunohistochemistry. PHA-L labelled cells within the injection sites overlapped the catecholaminergic cell groups as indicated by PHA-L and TH double labelled cells. The heaviest projection of PHA-L labelling in the forebrain originated from the intVLM, followed by cVLM and then by rVLM. Labelling from intVLM was observed in medial (MS) and lateral (LS) septum, median preoptic nucleus (MnPO), bed nucleus of the stria terminalis (BST), lateral (LPO) and medial (MPO) preoptic area, paraventricular nucleus of the hypothalamus (PVH), supraoptic nucleus (SON), paraventricular thalamic nucleus (PVA), lateral hypothalamus (LH), substantia inominata (SI), zona incerta, central amygdaloid nucleus, arcuate nucleus (Arc), ventromedial (VMH) and dorsomedial (DM) hypothalamus, posterior hypothalamus and posterior thalamus. PHA-L labelling from a cVLM injection was observed in MnPO, LS, BST, PVH, SON and PVA, whereas PHA-L labelling from a rVLM injection was observed in MnPO, BST, MPO, LPO, LS, MS, PVH, SON, PVA, LH, Arc, DM, VMH and SI. These data demonstrate that the projections from VLM neurons have a wide distribution in the forebrain and may alter the activity of structures previously implicated in the control of the circulation. (Supported by MRC).

133.9

IDENTIFICATION OF ENKEPHALIN-CONTAINING NEURONS IN THE CENTRAL NUCLEUS OF THE AMYGDALA PROJECTING DIRECTLY TO THE NUCLEUS AMBIGUUS. M.M. Caverson and E.T. Kiriakopoulos, Departments of Anatomy and Physiology, University of Western Ontario, London, Canada N6A 5C1.

The central nucleus of the amygdala (Ce) has been shown to influence the vagal outflow to the heart. However, relatively little is known about the neurochemical specificity of amygdala neurons innervating medullary regions containing preganglionic vagal cardiomotor neurons (VCN). Therefore, the present study was done to investigate the putative neurotransmitter content of amygdala neurons projecting directly to regions of the nucleus Ambiguus (AMB) shown to contain VCN. Iontophoretic injections of the retrograde tracer Fluorogold were made into the region of AMB in adult male Sprague-Dawley rats. After survival periods of up to 24 days, the animals were treated with colchicine, and subsequently perfused transcardially. Transverse sections of the forebrain and brain stem were cut and processed using a streptavidin immunofluorescence technique for the presence of enkephalin (ENK), CRH, CGRP, neurotensin or substance P in Fluorogold retrogradely labelled neurons. Injection sites were centred in AMB and included the compact, semicompact and ventral external formation of the nucleus at levels primarily rostral to obex. Within the amygdala, a dense cluster of Fluorogold retrogradely labelled neurons was observed throughout the rostro-caudal extent of the Ce, bilaterally. Of the substances examined only ENK was observed in a small number of Fluorogold retrogradely labelled neurons in the medial aspect of the Ce. These data indicate that for the five neuroactive substances investigated in this study there exists a neurochemical specificity in amygdala neurons projecting directly to AMB. The role of these medullary projecting ENK-containing amygdala neurons in the vagal control of the heart has yet to be determined. (Supported by the Medical Research Council of Canada).

133.10

EVIDENCE FOR A NOVEL NEUROPHYSIN II HYPOTHALAMO-MEDULLARY PATHWAY. E.T. Kiriakopoulos, M.M. Caverson and J. Ciriello, Depts. of Anatomy and Physiology, Univ. of Western Ontario, London, Canada N6A 5C1

We have previously shown that neurons in paraventricular nucleus that project directly to regions of nucleus ambiguus (AMB) contain neurophysin II (NII) or oxytocin (OXY). This study was done in Sprague Dawley rats to identify the location of NII and OXY fiber pathways that innervate AMB. Frozen transverse sections of the brainstem were cut and adjacent sections were processed for either NII or OXY immunoreactivity using the streptavidin immunofluorescence technique. The distribution of NII or OXY immunoreactive fibers was mapped on projection drawings of the brainstem. NII fibers were consistently more dense than OXY, throughout the entire brainstem. The most notable difference in their distribution was observed in the dorsal medulla. A discrete collection of NII fibers was seen running caudally along the dorsal medullary surface. This pattern of labelling was not evident for OXY fibers. The dorsal bundle of NII fibers appeared to course through the ventrolateral aspects of the central grey and continue caudally to innervate the parabrachial nuclei, locus coereleus, vestibular nuclei, nucleus of the solitary tract (NTS) and spinal trigeminal nucleus. An additional pathway coursing along the ventral surface appeared to contribute to the innervation of the A5 region, AMB, ventral and medial reticular formation and raphé nuclei. OXY fiber labelling in the dorsal medulla was observed in the regions of NTS, dorsal motor nucleus of the vagus and AMB and appeared to originate from a pathway coursing along the ventral medullary surface. These results provide evidence for the existence of a specific NII-containing pathway innervating dorsal medullary structures. (Supported by the MRC).

133.11

CHARACTERISTIC DYNAMICS OF CARDIAC R-R INTERVAL VARIATION IN OBSTRUCTIVE APNEA, NOCTURNAL MYOCLONUS, AND CONGESTIVE HEART FAILURE SYNDROMES DURING SLEEP. R.B. Trelease*, A. Garfinkel and R.M. Harper. Department of Anatomy & Cell Biology, Department of Kinesiology and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Variation in cardiac R-R intervals (RRIs) characteristically occurs in the fundamental respiratory frequency (respiratory sinus arrhythmia) and vasomotor oscillation frequency (1-3/minute) ranges, with sleep-waking states selectively enhancing or attenuating the expression of these components of variation. We previously reported that additional large oscillations in RRIs occur during sleep in human subjects in association with nocturnal myoclonus and hypoxemic sleep apneas. Conventional procedures for assessing cardiac interval variation such as spectral analysis and other linear statistics mask dynamic aspects of interval change because such procedures average characteristics over time. We employed interval "phase plane diagrams" that plot each RRI against the subsequent RRI for entire nights of sleep-waking data in 6 normal subjects, 6 patients with obstructive sleep apnea, and 6 patients with congestive heart failure. Such plots provide a type of nonlinear "autocorrelation" indicating the range of subsequent RRI variation for each RRI. Normal subjects demonstrated RRI plots with points scattering less in the lower left quadrant and greater variability at long RRIs. Patients with sleep apnea syndrome showed patterns with few short RRIs and a larger range of long RRI variation relative to normals. Patients with congestive heart failure showed a reduced range of RRIs with an absence of variation in long RRIs. These results suggest that normal and pathophysiologic characteristics may be differentiated on the basis of the nonlinear dynamics of RRI variation during nocturnal sleep studies.

134.1

SIMULTANEOUS EFFECTS OF MULTIPLE RECEPTOR POPULATIONS ON A COMMON SECOND MESSENGER SYSTEM. G.A. Palcos and J.J. Mann, Labs. of Neuropharmacology, University of Pittsburgh, Pittsburgh, PA. 15213

The interaction of multiple receptor populations on a common second messenger system in neurons is a critical aspect of nervous system function and may be involved in human neuropsychiatric disorders. We therefore studied the interactions of the 5-HT_2, alpha$_2$-adrenergic and prostaglandin (PGI_2) receptors on phosphoinositide (PI) turnover in human platelets. Epinephrine (EPI) and serotonin stimulated PI hydrolysis in a dose-dependent manner. Yohimbine (20 μM) completely blocked the PI response to EPI indicating this effect was mediated by alpha$_2$-adrenergic receptors. The 5-HT_2 and 5-HT_{1C} receptors are both coupled to PI turnover. Competition experiments indicated that in the platelet the response to serotonin was mediated by the 5-HT_2 receptor. A weak synergistic response was generated by the combination of 5-HT and EPI. EPI (0.1 mM) and serotonin (0.2 mM) increased PI turnover by 47 ± 8 (S.D.)% and 55 ± 8%, respectively, but these two agonists together elevated PI hydrolysis by 129 ± 9%. The maximal response to EPI (54 ± 2%) and 5-HT (66 ± 5%) was less than the maximal response to the combination. Prostacyclin at 1μg/ml reduced PI turnover induced by 0.1 mM EPI or 0.2 mM 5-HT by 44 ± 2% and 77 ± 3% respectively. In contrast, prostacyclin reduced PI hydrolysis stimulated by a combination of EPI (0.1 mM) and 5-HT (0.2 mM) by only 16 ± 13%. These studies indicate: 1) The pool of phospholipases appears to exceed the maximal capacity of the individual alpha$_2$-adrenergic and 5-HT_2 receptor populations to activate this second messenger system. 2) Some potentiation occurs when two receptor populations are both activated. 3) Inhibition of PI turnover by prostacyclin can be partly reversed by activation of a combination of these receptor populations. Future studies should examine other neuron and cell systems to assess the generalizability of these findings. This work was partly supported by MH40695.

134.2

(+) 3PPP SELECTIVELY DIFFERENTIATES EFFECTS OF SIGMA LIGANDS IN VIVO: EVIDENCE FOR SUBTYPES. S. Iyengar, S.J. Mick, V. Dilworth, P.C. Contreras, N. M. Gray, J.M. Farah, T.S. Rao and P. L. Wood. CNSDR, G. D. Searle, St. Louis, MO 63198.

The effects of sigma ligands, (+) 3PPP, (-) butaclamol, (+) SKF 10,047 and (+) pentazocine were evaluated in vivo on striatal dopamine (DA) turnover, ACTH and prolactin (PRL) release and cerebellar cGMP release. (+)SKF 10,047 and (+) pentazocine increased both PRL and DA turnover. (+) 3PPP and (-) butaclamol decreased PRL release and did not affect DA turnover. All 4 compounds increased ACTH release; however, only the increases caused by (+) SKF 10,047 and (+) pentazocine were reversed by pretreatment with CPP, a NMDA antagonist, while those caused by the other compounds were not. (+) 3PPP and (-) butaclamol potentiated NMDA receptor mediated cGMP increase while the benzomorphans did not. In addition to identifying a functional interaction of sigma receptors with DA receptors as well as NMDA receptors, these studies further divide the observed effects into two groups characterized by benzomorphan compounds and non-benzomorphan compounds, suggesting the existence of sigma receptor subtypes in vivo. The effects of sigma ligands ifenprodil, BMY 14802, WY 47384 and SC 48960 will be discussed in light of these data.

134.3

PET INVESTIGATIONS OF DOPAMINERGIC AND CHOLINERGIC INTERACTIONS IN THE PRIMATE BRAIN. S.L. Dewey, J.D. Brodie, J.S. Fowler, R.R. MacGregor, D.J. Schlyer, P.T. King, D.L. Alexoff, N.D. Volkow, C.-Y. Shiue, A.P. Wolf, and B. Bendriem. Dept. of Chemistry, Brookhaven National Lab, Upton, NY 11973. Dept. of Psychiatry, NYU Medical Center, NY NY 10016.

Central nervous system (CNS) function requires proper neurochemical balances between neurotransmitter systems. Neurotransmitter interactions provide the fundamental basis for homeostatic balance and for appropriate responses by the CNS to changes in the internal and external environment. Interactions between the dopaminergic D_2 and the muscarinic-cholinergic receptor systems in the primate brain (*Papio anubis*) were examined using positron emission tomography (PET) combined with [^{18}F]-N-methylspiroperidol (NMSP). Pretreatment with benztropine (Cogentin), a long lasting anticholinergic drug, bilaterally reduced, by an average of 13.0 % (n=4), the incorporation of radioactivity in the corpus striatum but did not alter that observed in the cerebellum or the rate of metabolism of [^{18}F]-NMSP in plasma. This reduction exceeded the normal variation (1.6 %) in tracer incorporation in repeated studies without any intervention in the same animals. This study demonstrates the usefulness of PET for investigating neurotransmitter interactions in vivo and provides insight into the consequences of multiple pharmacologic administration. Supported by USDOE, OHER and NS-15638 from NIH.

134.4

RELATIONSHIP OF GABAERGIC TO CHOLINERGIC NEURONS WITHIN THE LATERODORSAL AND PEDUNCULOPONTINE TEGMENTAL NUCLEI. B.E. Jones. Montreal Neurological Institute, McGill University, Montreal, Quebec, Canada, H3A 2B4.

In assessing the potential contribution of noncholinergic neurons to the activity and role of neurons in the laterodorsal and pedunculopontine tegmental (LDT and PPT) nuclei, the identification, distribution and frequency of neurons synthesizing the inhibitory neurotransmitter GABA were studied. Immunohistochemical staining using the peroxidase-antiperoxidase (PAP) technique was utilized for choline acetyltransferase (ChAT) and glutamic acid decarboxylase (GAD) in the rat brain. ChAT and GAD were stained singly in adjacent sections or doubly in a sequential procedure in the same sections using as respective chromogens: diaminobenzidine (DAB, producing a homogenous brown staining) and benzidine dihydrochloride (BDHC, producing a granular blue staining). The two enzymes were found in separate and different cells. GAD-immunoreactive cells were smaller than ChAT-immunoreactive cells and were either more numerous (in the LDT) or equally as numerous (in the PPT) than the ChAT-immunoreactive neurons in these nuclei. These results suggest that GABAergic neurons in the LDT/PPT may modulate the activity of the intrinsic cholinergic neurons and/or contribute to the efferent projections to the rostral or caudal target structures of these nuclei, as is currently being investigated.

Supported by the MRC of Canada.

134.5

ANATOMIC EVIDENCE FOR GABA-ERGIC AFFERENTS TO THE RAT LOCUS COERULEUS IN THE DORSAL MEDIAL MEDULLA: A IMMUNOCYTOCHEMICAL AND RETROGRADE TRANSPORT STUDY. *Vincent A. Pieribone[1], Michael T. Shipley[2], Matthew Ennis[2] and Gary Aston-Jones[3], [1]Dept. Bio. New York Univ., NY, NY, [2]Dept. Anat. Cell Bio., Univ Cinn. Med. Col., Cinncinati., OH and [3]Dept. Mental Health Sci., Hahnemann Univ., Philadelphia PA.*

Recent physiologic studies have indicated that the rat locus coeruleus (LC) recieves a potent GABA-ergic input from the dorsal medulla (Ennis and Aston-Jones, 1989). Anatomic studies have shown that the medial suprafascicular nucleus prepositus hypoglossi (PrH) innervates the locus coeruleus. The present study uses anatomic methods to investigate this pathway.

Immunocytochemistry for GABA or glutamic acid decarboxylase revealed that neurons of the LC recieve a very dense network of GABA-ergic terminal boutons (Shipley *et. al.* Neurosci. Abs. 1988). There were no GABA-ergic somata found within the LC, however colchicine pretreated was not used.

Only with colchicine pretreatment (75 μg i.c.v. or 5 μg within the medulla) were large numbers of GABA-ergic neurons found in the medulla. With such treatments, a collection of large (20-30μm) GABA-ergic neurons were found clustered beneath the IVth ventricle above the medial longitudinal fasciculus (mlf) and extending alongside the mlf ventrolaterally. These neurons extended rostrally from the hypoglossal nucleus to the level of the VIIth nucleus and were distinct in size (larger) and location (more medial) to GABA-ergic neurons found in the lateral PrH and nucleus of the solitary tract. Injections (150 nl) made into the LC of WGA-apoHRP/colloidal gold particles resulted in retrogradely labeled neurons in the suprafascicular PrH. When these sections were then processed for GABA immunocytochemistry numerous doubly labeled neurons were identified. Over 60 percent of LC afferent neurons in the dorsal medulla contain GABA immunoreactivity following colchicine pretreatment identifing this as a major inhibitory afferent to the LC. This work upported by PHS Grant NS24698.

134.6

NEUROPEPTIDE Y (NPY) REVERSIBLY ABOLISHES ACTIONS OF GLUTAMATE IN NUCLEUS TRACTUS SOLITARII

L. Grundemar, C. Wahlestedt, R. Håkanson*, and D.J. Reis. Dept. Pharmacol., Univ. of Lund, Sweden and Div. of Neurobiol., Cornell Univ. Med. Coll. NY, NY 10021 USA.

NPY and its receptors are found in the nucleus tractus solitarii (NTS). Here baroreceptor afferents terminate to initiate reflex hypotension and bradycardia presumably by release of l-glutamate (GLU). We sought to determine whether NPY might modify the actions of GLU upon arterial pressure (AP) and heart rate (HR) in the NTS. Rats were anesthetized (α-chloralose and urethane), paralyzed and ventilated. NPY microinjected into NTS unilaterally elicited a dose-dependent fall in AP and HR (max at 90 pmol of -42.1 ± 6.1 mmHg and HR -65 ± 13.7 bpm; n=8). GLU (0.7 nmol) injected into NTS also evoked transient falls in AP and HR (-64.6 ± 4.06 mmHg and -95.0 ± 24.0 beats per minute; n=8). Injection of NPY (90 pmol), but not vehicle or galanin, unilaterally into NTS reversibly abolished, for hours, the hypotensive actions of GLU; the contralateral NTS still responded normally to GLU. The effect was not anesthetic, however, since, unlike 2% lidocaine, NPY did not block the hypotension elicited by electrical stimulation at the injection site. NPY injected bilaterally in NTS inhibited, by over 70%, the baroreceptor HR response in response to phenylephrine (10 μg/kg i.v.; n=5). We conclude that in NTS, NPY reversibly inhibits the cardiovascular responses elicited by local application of GLU and inhibits baroreflex responses.

134.7

NEOSTRIATAL SUBSTANCE P AND SOMATOSTATIN NEURONS RECEIVE A DIRECT NIGRAL DOPAMINERGIC INPUT: AN ULTRASTRUCTURAL DOUBLE LABELING IMMUNOCYTOCHEMICAL STUDY. I. Mendez, K. Elisevich, C.C.G. Naus and B.A. Flumerfelt. Depts. of Anatomy and Clinical Neurological Sciences, University of Western Ontario, London, Canada, N6A 5C1.

Evidence for substance P (SP) and somatostatin (SS) containing neurons in the neostriatum has been provided by numerous studies. A dopaminergic input from the substantia nigra to the neostriatum is also well documented. However, a direct synaptic interaction between the dopaminergic input and the SP and/or SS containing cells has not yet been demonstrated. In this study, tyrosine hydroxylase (TH) and either SP or SS were simultaneously localized using the PAP method and two chromogens with distinct reaction products. TH-immunoreactive terminals were first demonstrated using 3,3' diaminobenzidine tetrahydrochloride (DAB), then SP or SS immunoreactivity was localized in neurons using benzidine dihydrochloride (BDHC). Ultrastructural examination revealed that TH-positive terminals made synaptic contacts on neostriatal SP and SS immunoreactive dendrites. The present study thus provides evidence of a direct dopaminergic synaptic input on neostriatal SP and SS neurons which suggests the anatomical basis for dopaminergic control of striatal SP and SS activity.

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134.8

EFFECTS OF COCAINE ADMINISTRATION ON THE NEUROPEPTIDE CONTENT OF THE BASAL GANGLIA IN THE RODENT. E.L. Lynd, S.N. Haber. Department of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14624.

Cocaine is a widely abused drug that acts by lowering reinforcement thresholds for brain stimulation reward. The cellular mechanism for cocaine's reinforcing properties is its ability to inhibit dopamine reuptake. The basal ganglia consists of several interconnected subcortical nuclei which are thought to subserve both motor and limbic function. Both the nucleus accumbens and the ventral pallidum, limbic related regions of the basal ganglia, have been implicated as sites for cocaine's actions. Studies have demonstrated that dopamine can regulate the expression of several neuropeptides in the basal ganglia. Most work on cocaine has focused on behavioral studies, and changes in dopamine and other transmitters detectable by assays. We examined the effects of repeated cocaine administration on enkephalin, substance P, and tyrosine hydroxylase staining. Furthermore we studied its effect on the regulation of these transmitters.

Subcutaneous injections of cocaine (40mg/kg) were made in adult male rats once a day for four days. One hour after the last injection animals were sacrificed and perfused with paraformaldehyde. Immunocytochemistry revealed changes in staining patterns of transmitters. In situ hybridization techniques were performed using probes to enkephalin, substance P, and tyrosine hydroxylase to examine changes in regulation.

The results indicate that cocaine administration alters the relationship between these neurotransmitter systems and their regulation.

134.9

SEROTONIN INCREASES NEOSTRIATAL SUBSTANCE P AND PREPROTACHYKININ mRNA. **P. D. Walker, L. A. Riley, R. P. Hart and G. M. Jonakait. Dept. of Biological Sciences, Rutgers University., Newark, NJ 07102.**

The trans-synaptic regulation of substance P (SP) in the neostriatum (NS) has been of considerable interest since it was found that loss of activity in the dopaminergic nigrostriatal pathway decreases SP biosynthesis. Previous studies from our laboratory have shown that the depletion of serotonin (5-HT) similarly lowers striatal levels of the mRNA coding for preprotachykinin (PPT), the prohormone precursor of SP (Walker et al., Soc. Neurosci Abstr. 15:581).

The present studies examined the effects of increasing 5-HT neurotransmission on levels of PPT mRNA and SP peptide in the NS. Adult rats received injections of saline or the 5-HT uptake inhibitor zimelidine (10 mg/kg i.p. twice daily for 5 days). Northern blot analysis of individual striata revealed that PPT mRNA levels were increased to 168±10% ($p<0.0005$) of control in the NS suggesting that raising extracellular 5-HT leads to increases in striatal SP biosynthesis. To begin to identify the receptor subtype mediating the action of serotonin, saline or the 5-HT_2 agonist DOI (7.5 mg/kg s.c. for 9 days) were infused via Alzet minipumps into adult rats. In DOI-treated rats ($n=10$), PPT mRNA levels in the NS were increased to 208±26% ($p<0.0005$) of control. Similarly, SP peptide levels in the NS were increased to 128± 5% ($p<0.0005$, $n=10$) of control. These results taken together suggest that 5-HT, acting at a 5-HT2 receptor, helps to regulate SP biosynthesis in the NS. (Supported by MH43365 and AHA-NJ).

134.10

SEROTONIN UPTAKE INHIBITION DECREASES PREPROTACHYKININ mRNA AND SUBSTANCE P IN MEDULLARY RAPHE NEURONS. L.A. Riley, R.P. Hart and G.M. Jonakait, Dept. of Biol. Sci., Rutgers Univ., Newark, NJ 07102.

Substance P (SP) is colocalized with serotonin (5-HT) in neurons of the medullary raphe, but its role and regulation there is not well understood. We have sought to determine whether changes in 5-HT levels affect the biosynthesis of colocalized SP. Recently we showed that depletion of 5-HT (by chronic inhibition of 5-HT biosynthesis) increased levels of the mRNA coding for the prohormone precursor of SP, preprotachykinin (PPT; Walker et al., Mol. Brain Res., in press).

In order to determine whether increases in 5-HT cause the opposite effect and whether changes in terminal SP also occur, rats were treated with the specific 5-HT uptake inhibitor zimelidine. Medullary raphe RNA prepared from individual animals was subjected to Northern blot hybridization using a [^{32}P]-labeled probe for rat PPT mRNA (a gift from James Krause, Washington Univ., St. Louis, MO). Zimelidine treatment lowered PPT mRNA levels 35.4±5.3% ($p<0.001$, $n=20$). The SP content of ventral thoracic spinal cord was determined by radioimmunoassay. Zimelidine treatment also slightly decreased the SP terminal content (16%, $p<0.05$, $n=10$). These results suggest that 5-HT uptake inhibition decreases SP biosynthesis in medullary raphe cells. Supported by MH43365.

CELLULAR AND MOLECULAR STUDIES I

135.1

DETERMINATION OF THE PROSPECTIVE FATE AND POTENCY OF CELLS OF THE EARLY AVIAN EPIBLAST. I.S. Alvarez and G.C. Schoenwolf. University of Utah School of Medicine, Salt Lake City, UT 84132.

We have been generating quail/chick transplantation chimeras and labeling localized regions of chick blastoderms with cell markers to construct prospective fate maps of the early (H&H stages 3,4) avian epiblast. Additionally, we are testing the fates and behaviors of marked epiblast cells placed in atypical locations to ascertain their state of determination. The locations of three major cell populations have been delineated in the prenodal epiblast: prospective surface epithelial cells, prospective L (lateral) cells of the neural plate, and prospective MHP (median hinge point) cells of the neural plate. The fates of these three types of cells is *not* yet determined at stage 4. However, their characteristic rearrangement (migratory) behavior has already become established by the mid-primitive streak stage (stage 3c). For example, when prospective L cells are replaced with prospective MHP cells, the graft cells are able to form both L and MHP cells, as well as mesodermal cells, but they cannot intercalate with prospective L cells. Rather, they extend toward adjacent MHP cells and intercalate with them. This suggests that different populations of epiblast cells each contain unique surface properties that allow them to recognize each other. Additional experiments are under way to define the nature of these properties. Supported by NIH grant no. NS 18112 and a Fulbright Fellowship no. FU89-8797464.

135.2

THE MICROENVIRONMENT OF THE ROSTRAL HALF-SOMITE ENHANCES GROWTH AND REGULATES SEGMENTATION OF SYMPATHETIC GANGLIA. C. Kalcheim and R.S. Goldstein. Dept. Anat. and Embryol., Hebrew Univ., Jerusalem 91010 ISRAEL.

Unilateral creation of a paraxial mesoderm with only rostral somitic (RS) halves, leads to the development of non-segmented dorsal root ganglia (DRG) that are larger and contain more cells than the sum of the contralateral, control DRG. Increased cell number is the result of a specific mitogenic effect on neural crest (NC) cells developing into DRG (Goldstein et al, Proc. Natl. Acad. Sci. USA, 1990, in press). We report here that grafts of RS halves also produce unsegmented sympathetic ganglia (SG) of 380 to 809% greater volume than the sum of the volumes of control SG in E6 and E10 quail-chick chimeras. Therefore, the RS mesoderm exerts a general mitogenic effect on NC cells developing into peripheral ganglia. In the same embryos, the increase in DRG volume on the operated side ranged between 28 and 74%, one-tenth the increase in SG volume. Moreover, on E10, the average volume of the SG is 56% of that of the corresponding DRG on the grafted side, in contrast to only 16% on the control side. These data suggest that: 1) SG precursors may be more sensitive to the mitogenic effect of the RS than DRG precursors, and/or 2) the extended presence of sympathoblasts with mitotic potential is responsible for the 10-fold greater effect on the SG. Funded by the MDA, the Israel Acad. of Sci., and the Isr. Council for R & D.

135.3

A MARKER SYSTEM FOR THE DORSAL EMBRYONIC RETINA.
P. McCaffery*, H. Roth* and U.C. Dräger. Department of Neurobiology, Harvard Medical School, Boston, MA 02115.

In a search for mechanisms underlying retinal polarity formation we generated monoclonal antibodies (mab's) that label strongly the dorsal part of the undifferentiated embryonic retina. These mab's recognize a 44kD protein (p44) that has been named the high-affinity laminin receptor; this term may not, however, describe its function. Immunohistochemically the mab's label a granular cytoplasmic antigen in dorsal retina. In cells grown *in vitro* the granules labeled by the mab's form a subpopulation of the granules labeled by an antiserum to ribosomes. In subcellular fractionations we find all of p44 associated with very large sedimentable complexes, from which it is quantitatively released by divalent cation chelators. We find no evidence for laminin binding to p44. The divalent-cation dependent binding to part of the ribosomal system is consistent with Brawerman's hypothesis that p44 constitutes an initiation factor.

Despite the pronounced dorso-ventral asymmetry seen immunohistochemically, a large range of biochemical tests show an even distribution of p44 throughout the embryonic retina. However, *in situ* digestions with trypsin reveal a higher susceptibility of p44 in dorsal than ventral retina, which points to a more accessible, open conformation of the protein in dorsal retina. As a possible candidate involved in this conformation difference we identified a 53kD protein whose distribution mimics the immunohistochemical labeling pattern by the p44 mab's: it is present only in the dorsal part of the undifferentiated embryonic retina. From these observations we conclude tentatively that the dorso-ventral difference in p44 does not consist in a difference in laminin binding, but possibly in a differential control of the protein translation system. (Supported by EY 01938)

135.4

THE EXPRESSION OF ASTROTACTIN, A NEURON-GLIA ADHESION LIGAND IN MOUSE CEREBELLUM, IS DEVELOPMENTALLY REGULATED T.N. Stitt, C.A. Mason, M. Berger, P. Joyce and M.E. Hatten, Center for Neurobiology and Behavior and Department of Pathology, College of Physicians and Surgeons, Columbia University, New York, NY 10032

In the developing brain, glial guidance provides a primary mechanism for the positioning of young neurons. We have previously described an immune activity, which we named astrotactin, which blocks neuron-glia binding, the formation of neuron-glia contacts, glial organization of neuronal positioning, and glial-guided neuronal migration of early postnatal mouse cerebellar cells *in vitro*. By imunoprecipitation and Western blot analysis, blocking antibodies, purified by absorption against adult brain membrane fractions, recognize a neuronal glycoprotein of apparent molecular weight 100Kd. By Western blot analysis, the level of expression of astrotactin varies with the developmental age of the cerebellum, with low levels of expression in the later embryonic period, high levels in the postnatal period and low levels in the adult.

Immunocytochemical localization reveals that astrotactin is expressed by granule cells, but not by astroglial cells *in vitro*. In addition, astrotactin is expressed by neurons migrating along glial processes *in vitro*. Astrotactin is not expressed by PNS neurons, including PC12 or sympathetic neurons. In tissue sections of postnatal cerebellum, astrotactin is expressed by neurons migrating along Bergmann glia in the molecular layer and by granule neurons in the internal granule cell layer. Cells in the EGL are unstained as are axons of the white matter. A similar pattern is seen in the developing cortex. These experiments suggest that astrotactin is expressed in zones, and at developmental periods, where neuronal migration along glial fibers and glial-mediated neuronal assembly into cell layers is occurring. Supported by NS 15429 (MEH).

135.5

REGULATION OF N-CADHERIN DURING NEURAL DEVELOPMENT: PHOSPHORYLATION, CYTOSKELETAL ASSOCIATION AND mRNA. Laura A. Lagunowich and Gerald B. Grunwald. Department of Anatomy, Thomas Jefferson University, Phila., PA 19107.

We have previously described expression of N-cadherin during retina and brain development including down-regulation of protein expression and posttranslational modifications such as sulfation and phosphorylation. The present studies, directed at elucidating regulatory mechanisms and their possible functional significance, demonstrate that N-cadherin phosphorylation and detergent extractibility change in a coordinated, tissue and age-specific fashion suggesting that cytoskeletal association of N-cadherin may be regulated in part by phosphorylation. During neural development, N-cadherin protein decreases while the degree of phosphorylation increases concomitantly with enhanced apparent cytoskeletal association. In the lens and heart all three parameters remain consistently high at all ages tested. Northern blotting studies indicate that the decrease in N-cadherin protein expression is mediated in part by a reduction in the N-cadherin mRNA level. Supported by NIH grants NRSA-EY06067 to LAL and RO1-EY06658 to GBG.

135.6

REGULATION OF N-CADHERIN DURING NEURAL DEVELOPMENT: A POSSIBLE ROLE FOR EXTRACELLULAR PROTEOLYSIS. Gerald B. Grunwald, Nancy E. Paradies and Eileen F. Roark*, Department of Anatomy and Developmental Biology/Teratology Training Program, Thomas Jefferson University, Phila., PA 19107.

During retinal development N-cadherin expression sharply decreases and a soluble fragment of N-cadherin is released from the cell surface. To relate these observations, we have used an in vitro retinal organ culture system to examine the role of extracellular proteases in regulating N-cadherin expression. The results indicate that 1) N-cadherin turnover is blocked by metalloprotease inhibitors; 2) retinal tissues secrete metalloproteases; 3) the rate of turnover of retinal N-cadherin increases during development; 4) the profile of secreted retinal proteases changes during development; 5) both the soluble fragment of N-cadherin and proteases observed to accumulate during in vitro retinal culture also accumulate in vivo in the vitreous humor. The identity of the secreted proteases and their possible role in directly mediating N-cadherin cleavage are presently being investigated. Supported by NIH grants T32-HD07326 and RO1-EY06658 (GBG.)

135.7

DOES THE MESODERM INDUCE HOX GENE EXPRESSION IN THE ZEBRAFISH NERVOUS SYSTEM? M. Westerfield. Inst. of Neurosci., Univ. of Oregon, Eugene OR 97403.

Axial polarity within the vertebrate embryo may be determined by the spatially restricted expression of transcriptional regulators. Hox genes are expressed in specific regions of the embryonic nervous system and may serve this function. To learn how Hox gene expression is regulated, we injected 1-cell zebrafish embryos with DNA constructs containing the promoter regions of mammalian Hox 1.1 or Hox 3.3 genes fused to sequences coding for β-galactosidase. The constructs were first expressed at late gastrula stages in cells entering the posterior embryonic axis. Later, expressing cells were found in the spinal cord and somites of the trunk. Expression was missing in the trunk segments of *spt-1* mutants that lack muscle. Instead, ectopic expression appeared in both the spinal cord and somites of the tail, the region where mutant mesodermal cells inappropriately migrate. These results suggest that early interactions between the mesoderm and the neurectoderm, which fail to occur in the trunk of *spt-1* mutants, normally induce Hox gene expression in this region.

Supported by the NIH; promoters provided by A. Puschel and E. DeRobertis.

135.8

ALTERNATE NEUROMUSCULAR TARGET SELECTION IN DROSOPHILA LARVAE TRANSFORMED BY HOMEOTIC MUTATIONS. S. Cash* & H. Keshishian. Dept. of Biology, Yale Univ., New Haven, CT 06511.

The innervation of the bodywall musculature of *Drosophila* larvae is highly stereotyped, permitting the identification of individual muscle fibers as well as their specific synaptic contacts. In larvae carrying the homeotic mutation *abx pbx bx^3/Df(3R)P2*, segment T3 of the CNS is transformed toward T2. In contrast, it is the abdominal segments that are transformed in the musculature. Thus, abdominal muscle fibers have a different segmental identity relative to their innervating motoneurons. We have examined this mismatch between CNS and periphery to determine whether motoneuronal ending anatomy is a feature conferred by the neuron or the target. The transformation also results in the loss of specific muscle fibers. We have examined the behavior of motor endings orphaned by the loss of these fibers. Where muscle fiber 5 is missing, an ectopic ending is found on the adjacent muscle fiber 12 in one third of the bodywalls, and at lower frequencies on the adjacent fibers 4, and 21-24. The overall architecture of nerve pathways, however, remains intact. Focal iontophoresis of glutamate shows that active receptors are present on both the native and supernumerary sites. These results show that *Drosophila* synaptic targets can tolerate inappropriate endings, that synapses can be made on membrane domains other than the normal sites, and that ectopic endings may be functional. Where the normal muscle fiber is missing, motoneurons select one of several alternate targets. We are investigating the extent to which ectopic ending morphology recapitulates the normal ending, and are attempting to phenocopy this result by laser ablations of embryonic muscle fibers.

135.9

ENHANCER TRAP MUTAGENESIS OF THE EMBRYONIC NEUROMUSCULAR SYSTEM IN DROSOPHILA MELANOGASTER. E. Harkins, A. Fluet*, E. Farrell*, and H. Keshishian. Dept. of Biology, Yale Univ., New Haven, CT 06511.

The neuromuscular system of *Drosophila* embryos and larvae consists of a set of highly stereotyped bodywall muscles which receive morphologically characteristic motoneuronal inputs. We are currently conducting a search for embryonically expressed molecules which may play a role in establishing the observed neuromuscular stereotypy.

Enhancer trap mutagenesis is a means of single insert mutagenesis that allows the screening of regulatory elements by temporally and spatially regulated expression of a marker gene, *E. coli* lacZ. From approximately 10,000 lines generated in collaboration with Allan Spradling at the Carnegie Institute of Washington, we are looking for staining patterns which reflect the expression of an endogenous gene that may be involved in the specificity of muscle pattern formation, paths taken by motoneuronal growth cones, target recognition, and/or synaptogenesis. In particular, we are looking for patterns with subsets of motoneurons, muscle fibers, muscle precursors, and combinations thereof. We have isolated lines which demonstrate such intriguing patterns, with staining in CNS, muscle, or specific regions of the epidermis where muscle fiber insertion occurs. Selected lines will be used for molecular genetic and functional analyses. Mutant phenotypes, if not obtained by the initial insertion event, will be generated by inducing imprecise excision of the element. Supported by grants from NIH and the March of Dimes.

135.10

EXPRESSION AND CHARACTERIZATION OF NERVOUS SYSTEM ANTIGENS IN GRASSHOPPER EMBRYOS. Ellen M. Carpenter and Michael J. Bastiani, Dept. of Biology, University of Utah, Salt Lake City, UT 84112

Using immune suppression, we have generated six monoclonal antibodies which recognize epitopes expressed in the central nervous system (CNS) of developing grasshopper embryos. Three of these antibodies were selected for further characterization based on their recognition of different elements of the developing CNS. The 10H11 antibody recognizes most neuronal cell bodies and axons in the CNS. Labeling using 10H11 is restricted to the CNS; no peripheral nervous system structures are labeled. 10H11 labeling appears early in development associated with the earliest identifiable neurons and antigen expression is maintained on most or all CNS axons and neuronal cell bodies during early embryonic development. The 10E6 antibody recognizes a small population of CNS neuronal cell bodies and a discrete subset of axons comprising one fascicle running through the longitudinal connective and three fascicles crossing the midline in the anterior and posterior commissures. 10E6 also labels peripheral sensory structures. The 1B5 antibody labels glial cells as evidenced by discontinuous labeling overlying the surfaces of the longitudinal connectives corresponding to the location of identified glial cells. 1B5 labeling also surrounds laterally positioned neuronal cell bodies, but does not appear to label the cell bodies themselves. All three antibodies recognize epitopes expressed on the surfaces of cells as the labeling patterns obtained after labeling live embryos closely match the patterns seen in fixed tissue.

Preliminary biochemical characterization of the antigens using periodate treatment to remove carbohydrates and chloroform-methanol extraction to remove glycolipids suggests that 1B5 may recognize a carbohydrate epitope expressed on a glycoprotein while 10E6 and 10H11 may recognize epitopes expressed on glycolipids. 10H11 labeling was particularly striking following chloroform-methanol extraction as labeling became restricted to a subset of axon fascicles and to localized regions of the axons in these fascicles.

Supported by NIH grants NS25378, NS08404, and the McKnight Foundation.

135.11

FURTHER CHARACTERIZATION AND CLONING OF A SURFACE ANTIGEN EXPRESSED IN A STRIPED PATTERN DURING SEGMENTATION AND LATER SPECIFIC TO THE NERVOUS SYSTEM IN GRASSHOPPER EMBRYOS. Rolf O. Karlstrom and Michael J. Bastiani. Dept. of Biology, University of Utah, Salt Lake City, UT 84112.

Cell-cell interactions play a central role in insect development, guiding such processes as segmentation, cell differentiation, and axonal outgrowth. We are characterizing cell surface molecules which may be involved in intercellular interactions during embryogenesis. The 1C10 MAb recognizes a surface antigen (Ag) specific to the nervous system in 40% grasshopper embryos. Before neurogenesis, just prior to segmentation, the Ag is expressed in a pattern of broad stripes consisting of approximately one third of the cells in a future segment. As segmentation proceeds, labelling becomes restricted to the neurogenic ectoderm, then is further restricted to neuroblasts and their progeny, ganglion mother cells and neurons. Axons express the 1C10 antigen immediately upon axonogenesis. Limb neurons express the 1C10 Ag as they segregate from the limb epithelium. EM observations confirm the 1C10 Ag is expressed on the surfaces of most or all axons, neurons, and some neuroblasts at 40% of embryonic development. After 50% of development axonal expression becomes restricted to a dorsal longitudinal pathway.

A MAb affinity column was used to purify the 40 kD 1C10 antigen and a serum antibody (SAb) was generated against the purified protein. The SAb labels embryos in a pattern identical to that of the MAb and recognizes a 40 kD doublet on an immunoblot of embryonic membrane proteins. This SAb has been used to screen an expression library prepared from 40% embryonic grasshopper mRNA. Three clones have been independently identified which produce epitopes recognized by the SAb. At least one of these clones produces an epitope recognized by both the SAb and MAb. *In situ* hybridization is being used to determine whether one or all of these clones represents the 1C10 gene.

Supported by NS25378, McKnight Foundation, and NSF graduate fellowship to R.K.

135.12

EMBRYONIC DEVELOPMENT OF THE LOBSTER *HOMARUS AMERICANUS*: MORPHOGENESIS AND SEROTONIN APPEARANCE. S. M. Helluy and B. S. Beltz. Bio. Dept., Wellesley College, MA. 02181.

During the development of *Homarus* in the egg, three periods of organogenesis are distinguishable: that of the nauplius, postnauplius and first larval stage (L1). Behavioral and morphological landmarks have been examined and their times of appearance related to Perkins' eye index based on the size of the lateral eyes. This index has been transformed to a percentage scale of embryonic development.

Serotonin immunoreactivity (5-HT IR), studied as a marker of developing nervous structures, is first seen in the protocerebrum at approximately 10% development (E10). By E20, 5-HT IR forms a "U"-shaped path from the medial edges of the optic lobes, along the protocerebral tracts, and in the protocerebral bridge. By E30, 5-HT IR is also clear in the central body, and in the circumesophageal and subesophageal ganglia. At that stage, the olfactory lobes emerge. The deutocerebral giant neurons show staining in their cell bodies and axons projecting to the olfactory lobes by E40. Presumptive sensilla are forming then, inverted in each of the uniramous antennulae, and the axons of the sensory neurons are seen at the base of the antennulae. By E50, all the prominent stained structures present in adults in the optic lobes, brain and nerve cord have appeared, except for the accessory lobes of the deutocerebrum which are labeled as soon as they begin to emerge at about E60. These lobes continue to develop during the organogenesis of the L1 and after hatching. Based upon the late development of the accessory lobes in *Homarus*, and upon neuroanatomical and ontogenetic evidence from different crustacean taxa, we are proposing that delayed hatching and slow maturation may be among the primary factors that enable the growth of a complex deutocerebrum in crustaceans. (Supported by BNS-8718938 and 8958169, and NIH NS-25915)

DRUGS OF ABUSE: AMPHETAMINE AND COCAINE

136.1

HIGH-AFFINITY ACTIVE TRANSPORT OF [^{3}H]-d-AMPHETAMINE INTO RAT STRIATAL SYNAPTOSOMES. R. Zaczek, S. Culp* and E.B. De Souza. NIDA Addiction Research Center, Baltimore, MD 21224.

The incorporation of [^{3}H]-d-amphetamine (^{3}H-dAMPH) into rat brain synaptosomes was examined using physiological buffer at 37°C. Saturation curves of ^{3}H-dAMPH incorporation into striatum revealed a high affinity site (K_i = 73 nM) which was ouabain-sensitive, suggesting the presence of active transport. 6-Hydroxydopamine lesion of the striatum caused approximately 50% reductions in both ^{3}H-dAMPH and [^{3}H]-dopamine transport, suggesting that ^{3}H-dAMPH transport occured into dopamine terminals. Furthermore, there was an absence of high-affinity ^{3}H-dAMPH transport into brain areas such as cerebral cortex, which have a paucity of dopamine terminals. The high-affinity transport of ^{3}H-dAMPH into striatum was inhibited by the monoamines: dopamine (IC_{50} = 257 nM), norepinephrine (IC_{50} = 437 nM) and serotonin (IC_{50} = 7.9 μM). The pharmacological profile of high-affinity ^{3}H-dAMPH transport into striatum also showed sterospecificity: d-amphetamine (IC_{50} = 60 nM) was an 8-fold more potent inhibitor than its l-isomer (IC_{50} = 466 nM). High-affinity transport of ^{3}H-dAMPH was potently inhibited by putative dopamine uptake blockers such as methamphetamine (IC_{50} = 48 nM), methylphenidate (IC_{50} = 53 nM) and cocaine (IC_{50} = 172 nM). Other compounds such as diethylpropion (IC_{50} = 4.2 μM) and phendimetrazine (IC_{50} = 4.9 μM) were weak inhibitors. In summary, ^{3}H-dAMPH is actively transported into dopamine terminals and may lead to release of dopamine through a heteroexchange process. This process may be important in low-dose effects of amphetamines such as increased locomotor activity in rats and stimulant activity in man.

136.2

HETEROGENEITY OF THE METABOLIC RESPONSE OF THE VENTRAL STRIATUM TO PSYCHOSTIMULANT ADMINISTRATION AS ASSESSED BY THE 2-[^{14}C]DEOXYGLUCOSE METHOD. L.J. Porrino and M.L. Garnett. Unit on Brain Imaging, NINDS, Bethesda, MD 20892.

Previous studies have shown that the acute intravenous administration of psychostimulants such as cocaine (COC) and methamphetamine (MA) increases local cerebral metabolic rates in the nucleus accumbens and olfactory tubercle, the major components of the ventral striatum (Porrino et al, 1988; Pontieri et al, 1990). We have conducted a more detailed analysis of the topographical distribution of the metabolic effects in these structures. Rates of glucose utilization ($LCMR_{glc}$) were measured at three anterior-posterior levels of the ventral striatum (AP +2.7, +1.7 and +1.0; Paxinos and Watson) following administration of COC (0, .5, 1, 5 mg/kg, iv) or MA (0, .05, .5, 2.5 mg/kg, iv). At moderate and high doses of COC, $LCMR_{glc}$ was increased in the olfactory tubercle and nucleus accumbens at all levels. However, at low doses $LCMR_{glc}$ was increased only in the most rostral portions of these ventral striatal structures. Low doses of MA produced similar selective increases in $LCMR_{glc}$ in the rostral accumbens, but only at the highest dose level was metabolism altered in the olfactory tubercle. These data demonstrate that the ventral striatum is heterogenous in its metabolic response to psychostimulants with more rostral regions appearing uniquely sensitive to low doses of these drugs. In additions, they provide evidence that the olfactory tubercle and nucleus accumbens are functionally distinct.

136.3

REGULATION OF ACETYLCHOLINE AND γ-AMINO BUTURIC ACID IN NUCLEUS ACCUMBENS BY AMPHETAMINE. N. Lindefors, Y. Hurd, S. Brené*, U. Ungerstedt* and H. Persson. Department of Pharmacology and Department of Medical Chemistry, Karolinska Institute, Stockholm,Sweden.

In situ hybridization and in vivo microdialysis were used to study the effect of amphetamine on expression of choline acetyl transferase (ChAT) and glutamic acid decarboxylase (GAD) mRNA and in vivo release of acetylcholine (ACh) and γ-amino buturic acid (GABA), in rat medial nucleus accumbens. The effect of repeated systemic amphetamine (1.5 mg/kg; twice daily for 7 days) was compared with the effect of saline injections and/or a single amphetamine injection. The level of ChAT mRNA was not affected by amphetamine while GAD mRNA was decreased (28%) by repeated injections. Extracellular ACh showed a two-fold increase after an acute amphetamine injection and a 60% increase after repeated injections. Extracellular GABA was decreased (45%), but only in rats receiving repeated amphetamine injections. Thus systemic amphetamine stimulate ACh release in medial nucleus accumbens without stimulating expression of ChAT mRNA. GABA release is attenuated by repeated amphetamine injections and a concomitant decrease in GAD mRNA expression is observed. ChAT is the key enzyme in ACh synthesis but probably not rate limiting in the formation of ACh, while changes in GAD activity are more closely correlated to changes in the formation of GABA. This may explain why an association is only seen between GABA release and GAD mRNA expression. Evidence are thus provided showing that amphetamine induce changes in neuronal function postsynaptic to limbic dopamine terminals, in medial nucleus accumbens. GABA release is decreased by a slow mechanism that is associated with decreased expression of GAD mRNA, while ACh release is increased by a fast mechanism not associated with any signs of change in ChAT mRNA levels.

136.4

PERSISTENT EFFECTS OF 3,4-METHYLENEDIOXYAMPHETAMINE (MDA) ON DOPAMINE AND SEROTONIN NEURONS IN REAGGREGATE TISSUE CULTURE. L.A. Won, P.C. Hoffmann and A. Heller. Dept. of Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637.

MDA is a synthetic amphetamine analog which is neurotoxic to serotonin (5-HT)-containing, but not dopamine (DA)-containing neurons in adult animals (Ricaurte et al., Science 229, 986-988, 1985). We have examined the effect of MDA on developing murine DA and 5-HT neurons in reaggregate tissue cultures formed from dissociated cells of fetal mesencephalic tegmentum and corpus striatum. Both (+) and (-) isomers of MDA cause reductions in DA and 5-HT (Soc. Neurosci. Abstr. 15, 686, 1989). Moreover, reaggregates treated for 7 days with 10^{-4}M MDA, showed cellular damage to both DA and 5-HT neurons. The present study examined whether DA and 5-HT neurons would recover from MDA treatment in neurotransmitter levels and cellular morphology. Reaggregates were exposed to (+) or (-) MDA at 10^{-4}M between 15 and 22 days. At 22 days, half of the flasks continued in drug-containing media while the other half grew in drug-free media for an additional 6 days. At the end of the 28 day culture period, all of the reaggregates were analyzed for endogenous DA and 5-HT levels using HPLC. DA and 5-HT cells were visualized using tyrosine hydroxylase (TH)- and 5-HT-immunocytochemistry. Light microscopic examination of TH- and 5-HT-immunoreactive cells revealed no extensive cytoplasmic vacuolization in reaggregates allowed to recover, in contrast to the cellular damage seen after 7 days of drug exposure. Reaggregates treated with either (+) or (-) MDA at 10^{-4}M over the 13 day period had significant reductions in DA and 5-HT levels. In the case of (+) MDA, DA levels returned to control values following 6 days of recovery, while 5-HT remained significantly depressed (25%) below control values. For (-) MDA, neither DA (-16%) nor 5-HT (-26%) levels fully recovered following 6 days in drug-free medium as compared to control cultures. Supported by MH42134.

136.5

NEUROTOXIC EFFECTS OF RING-SUBSTITUTED PHENYLETHYLAMINE ANALOGS. M. S. Kleven, G. M. Farfel, W. L. Woolverton, and L. S. Seiden. Department of Pharmacological and Physiological Sciences, Drug Abuse Research Center, The University of Chicago, Chicago, IL 60637

Amphetamine, methylamphetamine and ring-substituted analogs including MDA (3,4-methylenedioxyamphetamine), MDMA (3,4-methylenedioxymethylamphetamine) and MMDA (3-methyoxy-4,5-methylenedioxy-amphetamine) cause long-lasting depletions of brain dopamine (DA) and/or serotonin (5-HT). To determine whether different ring substitutions of the amphetamine moiety affect neurotoxic activity, long-lasting neurochemical effects were examined in rats treated with a 4 day regimen of a variety of amphetamine analogues. Doses were approximately 0, 5, 10, and 20 times the ED50 for suppression of milk intake in rats allowed to drink sweetened condensed milk during 15 min sessions. Rats (n=6-8/group) were administered mescaline (0, 50, 100, or 200 mg/kg), 2,5-dimethoxy-amphetamine (DMA; 0, 50, 100, or 200 mg/kg), 2,5-dimethoxy-4-methyl-amphetamine (DOM; 0, 5, 10, or 20 mg/kg), 2,5-dimethoxy-4-ethyl-amphetamine (DOET; 0, 5, 10, or 20 mg/kg), 4-methoxy-amphetamine (PMA; 0, 12.5, 25, or 50 mg/kg) or 2-methoxy-4,5-methylenedioxyamphetamine (MMDA-2; 0, 25, 50, or 100 mg/kg) s.c. twice daily for 4 days and sacrificed 2 or 8 weeks after the last injection. Monoamines and metabolites were assayed in somatosensory cortex, striatum, hippocampus, or hypothalamus. Only the highest dose of PMA (50 mg/kg) significantly depleted striatal and hypothalamic 5-HT and 5-HIAA, an effect which was not observed 8 weeks after the 4 day regimen. None of the other drugs tested had long-lasting effects on 5-HT or 5-HIAA. Additionally, none of the drugs had long-lasting effects on DA or norepinephrine. The results suggest that hallucinogenic amphetamine analogs (mescaline, DOM, DMA, MMDA-2, and DOET) do not have neurotoxic activity, whereas drugs which also have amphetamine-like stimulant effects (e.g., MDA, MDMA, MMDA, and PMA) are potentially neurotoxic to 5-HT neurons. (Supported by DA-0085)

136.6

AMPHETAMINE EXERTS ITS PSYCHOSTIMULANT EFFECTS VIA A WEAK BASE MECHANISM. David Sulzer, Wei-Xing Shi and Stephen Rayport. Depts. Psychiatry, Anatomy & Cell Biology, and Ctr. Neurobiology & Behavior, Columbia Univ.; Dept. Neuropathology, Psychiatric Inst., New York City 10032.

Several psychostimulants act in the brain by increasing dopamine (DA) levels at mesolimbic synapses (Koob & Bloom, 1988). Amphetamine works primarily at the vesicular level, unlike cocaine which is thought to act by binding to the plasma membrane uptake transporter. However, how amphetamine affects vesicular DA levels has been unclear. Acidification by an ATP-dependent proton pump in synaptic vesicles is required for accumulation of monoamine transmitters. We have found that amphetamine and other lipophilic weak base psychostimulants inhibit storage vesicle acidification (Sulzer & Rayport, 1989); we now show that amphetamine-induced alkalinization is sufficient to explain its action.

We observed the effect of pharmacologically-relevant concentrations of amphetamine in mesolimbic DA cell cultures. We visualized acidic organelles with the weak base vital dye acridine orange under low-light, non-bleaching conditions using a chilled-CCD camera. Areas that contain synaptic vesicles in the cell body perimeter and varicosities were stained, as well as lysosomes distributed primarily in the perinuclear region. 1 μM amphetamine caused alkalinization at both sites; the reduction in staining was dose-dependent up to 100 μM.

To examine the effects of amphetamine on transmitter storage vesicles directly, we used isolated bovine adrenal chromaffin granules. Supporting our findings in cultured DA neurons, similar concentrations of amphetamine inhibited the granule pH gradient. This resulted in a parallel decrease in uptake and increase in release of ^{3}H-serotonin. Amphetamine, several other psychostimulants, native monoamine neurotransmitters and *classic* weak bases such as ammonium all had first-order dose-response curves. The weak base mechanism may also contribute to the action of other psychostimulants including cocaine, phencyclidine and tyramine.

136.7

EXPERIENTIAL AND INDIVIDUAL FACTORS IN THE ACQUISITION OF AMPHETAMINE SELF-ADMINISTRATION. G.Mittleman,J.M.Deminiere*,M.Le Moal,H.Simon,P.V. Piazza. I.N.S.E.R.M. U-259, Universite de Bordeaux II, 33077 Bordeaux, France.

When low drug doses are used, some, but not all, rats develop amphetamine self-administration (SA). Similarly, when food deprived and given intermittent presentations of small amounts of food, some, but not all, rats develop schedule-induced polydipsia (SIP). These individual differences are related to differences in the response to activating or arousing conditions as well as the response to amphetamine (AMPH). These experiments studied the behavior of animals tested in the self-administration and SIP paradigms in order to determine if the previously observed individual differences in behavior were consistent in both conditions. In Experiment I, 29 rats were allowed to acquire AMPH SA (10 ug d-amphetamine sulfate/20 ul saline/injection) during 30 min sessions over 5 days. Rats that acquired SA were designated SA-pos and those that did not were designated as SA-neg. All animals were then given 10 daily SIP tests where a 45 mg food pellet was dispensed every minute of the 30 minute tests. The results indicated that SA-pos rats acquired SIP more rapidly than SA-neg animals. In Experiment II 16 animals were first tested for SIP. Rats that acquired drinking were designated as SIP-pos; those that failed to acquire drinking were classified as SIP-neg. All animals were then tested for AMPH SA using the same procedure as above. It was found that SIP-neg rats acquired SA more rapidly than SIP-pos rats.

136.8

COCAINE AND AMPHETAMINE AFFECT THE LOCOMOTOR BEHAVIOR OF *DROSOPHILA MELANOGASTER*. R.C. Richmond, T. L. Mench*, M. F. Gerteisen*, M. D. Boyer* and P. E. Mitchell*. Department of Biology, Indiana University, Bloomington, IN 47405.

The long-term objective of our work is to develop *Drosophila* as a system for exploring the genetic bases for responses to psychoactive drugs. We tested the hypothesis that cocaine and amphetamine affect the locomotor behavior of *D. melanogaster* larvae and adults. Larvae were allowed to ingest sucrose solutions containing cocaine at concentrations up to 1.0% and their locomotor activity was measured as the length of a track left in a thin suspension of yeast on a petri plate. Cocaine solutions of 0.25% and greater reduced locomotor activity below that of paired controls fed sucrose alone. Adult flies were induced to feed on sucrose solutions containing cocaine and their locomotor activity measured as the distance walked in a glass tube under standard conditions. Cocaine solutions of 0.001% were effective in reducing the locomotor activity of adult flies below that of paired controls. Both larval and adult locomotion were tested after ingesting amphetamine to determine if effects on locomotion were present for a compound which does not act as a local anesthetic. Flies which ingest amphetamine also show a reduction in locomotor behavior although the sexes appear to differ in their response to this drug. These results suggest that cocaine and amphetamine act on the CNS of *Drosophila*. *Drosophila* may be a particularly useful system for identifying specific genes which affect responsiveness to cocaine and other psychoactive drugs.

136.9

CHARACTERIZATION OF SIGMA BINDING SITES IN HUMAN PLACENTA: EFFECT OF PERINATAL COCAINE EXPOSURE. D.D. Flynn, A.A. Vaishnav, Y. Itzhak, L. Sanchez*, and D. C. Mash. Depts. Pharmacology, Neurology and Biochemistry, University of Miami School of Medicine, Miami, FL 33101 and the Deptartment of Obstetrics and Gynecology, University of Florida, Jacksonville, FL. 32209

Recent studies suggest that the psychotropic actions of opiate benzomorphans are mediated by sigma receptors. In addition, putative sigma receptors have been characterized in peripheral tissues and cells including the ovary, spleen and lymphocytes (Wolfe et al., 1988; 1989). We report here the characterization of a high affinity sigma binding site in the human placenta. Placental tissue was obtained from patients admitted for delivery at the University of Florida Medical Center at Jacksonville. Cocaine users were identified both by self-reported drug histories and urine screens for cocaine metabolites. Sigma receptors were labeled in placental membranes with R(+)[3H]-3-3-hydroxy[phenyl]-N-(1-propyl)piperidine ([3H]-PPP). Saturation analysis revealed an apparent single class of sites (Kd = 80 nM; Bmax = 20-30 pmol/gram tissue). Haloperidol, pentazocine, 1,3 di (2-tolyl)guanidine, and (+) SKF 10,437 displaced [3H]-PPP binding in placental membranes. The rank order of potency and stereoselectivity demonstrated in competition assays with [3H]-PPP were identical in human cerebellum and placenta. Progesterone competitively displaced [3H]-PPP binding in the placenta (Ki = 0.2 μM). Cocaine was equipotent at sigma binding sites labeled in placental and cerebellar membranes. The density of sigma binding sites in the placentas taken from mothers who tested positive for urinary cocaine metabolites was significantly reduced compared to control values matched for gestational age. These preliminary findings suggest that peripheral sigma receptors may be regulated by cocaine exposure. DA 06227 and NIH BRSG

136.10

BLOCKADE OF THE CONVULSANT EFFECTS OF COCAINE BY MK-801 IN A DIAZEPAM-INSENSITIVE MOUSE MODEL. J. M. Witkin and F. C. Tortella. NIDA Addiction Research Ctr., Baltimore, MD 21224 and Walter Reed Army Inst. Res., Washington, DC 20307

Experimental focus on both the convulsive and lethal effects of cocaine and the finding that these toxic manifestations can be dissociated (Witkin *et al.*, Life Sci. 44: 1285, 1989) has led to the use of a model of cocaine toxicity using supramaximal seizure-inducing doses of cocaine. In male Swiss Webster mice where cocaine HCl (ip) produces convulsions with an ED 50 of 57 mg/kg and death with an LD 50 of 73 mg/kg, 75 mg/kg of cocaine was used to evaluate the anticonvulsant and life-protectant effects of potential therapeutic entities. After 75 mg/kg cocaine, convulsions occurred in 100% and death in 50% of the mice tested. Diazepam (1 - 10 mg/kg, sc, 30 min prior) protected against lethality without altering cocaine-induced clonic convulsions. However, diazepam produced dose-dependent protection against convulsions induced by 60 mg/kg cocaine. In contrast, the N-methyl D-aspartate (NMDA) antagonist, MK-801 (0.1 - 0.3 mg/kg, sc, 30 min prior), produced dose-dependent protection against convulsions induced by 75 mg/kg cocaine without completely blocking lethality. Cocaine did not produce convulsions in the presence of 0.3 mg/kg MK-801 even when given at about 2 X ED 50. These results suggest a role for NMDA antagonists in the treatment of severe cocaine convulsions.

136.11

A PHARMACOGENETIC EVALUATION OF THE COCAINE-KINDLING PROCESS. R. J. Marley, J. M. Witkin & S. R. Goldberg. NIDA-Addiction Res. Ctr., Box 5180, Baltimore, MD 21224.

Repeated administration of cocaine has been shown to result in the development of increased susceptibility to the convulsant properties of cocaine (i.e. kindling). Cocaine's local anesthetic actions have been implicated in the cocaine-kindling process. Genetic differences in susceptibility to cocaine- and lidocaine-kindled seizures were evaluated in 4 inbred mouse strains and compared with susceptibility to seizures induced by the acute administration of the two drugs. The acute administration of cocaine produced convulsions in mice from all 4 genotypes with the following rank order for susceptibility: C57 > BALB = DBA > SJL. The rank order of the 4 strains for sensitivity to the development of cocaine-kindled seizures was opposite to that observed for initial susceptibility to cocaine-induced seizures. In 3 of the genotypes, however, the cocaine-kindled state did not persist upon further exposure to cocaine. Following a period of increased sensitivity to cocaine-induced seizures, a high degree of tolerance to the convulsant properties of cocaine developed among C57 mice. Partial tolerance was also apparent among BALB and DBA mice. Only among the SJL mice did the development of a kindled state persist upon repeated exposure to cocaine. The rank order of the 4 strains for susceptibility to lidocaine-induced seizures and for the development of susceptibility and/or tolerance to lidocaine-induced seizures were not the same as observed for cocaine, suggesting that while the local anesthetic properties of cocaine may underlie, at least in part, its convulsant and epileptogenic actions, the effects of cocaine on these parameters do not completely parallel those of lidocaine.

136.12

DEVELOPMENTAL CORTICAL ANOMALIES AFTER PRENATAL EXPOSURE TO COCAINE. W.E. Kaufmann. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Experimental and clinical studies have shown that cocaine exposure *in utero* leads to neurobehavioral sequelae, particularly as regards disorders of attention and learning. The biological substrate of these problems is not known but may involve lesions of the cerebral cortex. Therefore, using conventional histological and immunocytochemical techniques, cortical organization was studied in three infants (1-3 months of age) born to cocaine abusers. Cases showed increased numbers of neurons in white matter as compared to controls. In prefrontal and anterior temporal areas, ganglionic and fusiform elements, sometimes in clusters, were located mainly superficially ($p = 0.003$ and $p = 0.002$, respectively). Slight astrocytosis and decreased staining of myelin were seen in the two older subjects. Temporal and occipital regions showed decreased immunoreactivity for nonphosphorylated neurofilaments in layers V and VI , possibly reflecting disturbed cytodifferentiation, whereas findings in white matter suggested abnormal neuronal migration and/or failure of physiological cell death. The involvement of cells in association cortices may play a role in some of the neurobehavioral manifestations reported in cocaine-exposed offspring.

TRANSMITTERS IN INVERTEBRATES I

137.1

FMRFamide-like peptides in *C. elegans*: Developmental expression and cloning and sequencing of the gene. C. Li. Dept. of Biology, Boston University, Boston, MA 02215.

About 25 neurons, many of which have been identified, stain with an antibody against the neuropeptide FMRFamide (see Neurosci. Abst. 12:246). The onset of FMRFamide-like expression in these neurons, as determined immunocytochemically, appears to be developmentally regulated. All neurons arise by the first larval stage in *C. elegans*. The first visible staining occurs in the nerve ring of young L1 animals. Shortly thereafter during L1, the cell bodies of about 8 neurons in the nerve ring region and anterior ventral cord processes become visible. As the animal progresses through the four larval stages and into adulthood, more neurons throughout the animal and processes along the entire length of the ventral, lateral, and dorsal nerve cords become visible. All neurons continue to express the peptide into adulthood, and no neuron is stained in larval animals that is not present in adult animals.

Degenerate oligonucleotides against Phe-Met-Arg-Phe-Gly-Lys(Arg) were used to screen a *C. elegans* cDNA library (from S. Kim). One of the 16 hybridizing clones has been subcloned and sequenced thus far. The deduced amino acid sequence of the translation product reveals 8 potential neuropeptide sequences, each of which is flanked by possible endoproteolytic cleavage sites (1-3 basic residues) and ends with a C-terminal Gly, a potential amide donor. Seven of the peptides terminate with the sequence -Pro-Asn-Phe-Leu-Arg-Phe-Gly . These FLRFamide-like peptides would be recognized by the FMRFamide antiserum, and are likely to account for some, if not all, of the immunoreactivity seen in the animal.

137.2

AF2, A NEMATODE NEUROPEPTIDE. **C. Cowden* and A.O.W. Stretton, Dept. of Zoology, University of Wisconsin-Madison, 53706.**

We are interested in the role of neuropeptides in the control of locomotion in Ascaris suum. Our strategy is to isolate peptides that cross-react with an antiserum which binds to neural antigens in Ascaris heads. The RIA used to monitor the isolation is specific for C-terminal RFamide (Marder et al, 1987).

The isolation of AF1 (KNEFIRFamide) from head extracts of Ascaris suum and its physiological effects have been reported (Cowden, Stretton, and Davis, 1989). A second FMRFamide-like peptide has been isolated using 3 HPLC steps: n-butanol in TFA; acetonitrile in HFBA; and acetonitrile in TFA. The natural peptide has the sequence KHEYLRF. Its mass is 991 daltons which is the same as the mass of the synthetic peptide KHEYLRFamide; its elution times in 3 HPLC systems are the same as those of the synthetic peptide. The synthetic peptide is active at 10^{-6} M in injected worms and in a muscle strip assay. We conclude that AF2 is a bioactive neuropeptide with the chemical structure KHEYLRFamide.

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137.3

LOCALIZATION OF GLUTAMATE-LIKE IMMUNOREACTIVITY IN THE LEECH CENTRAL NERVOUS SYSTEM. P.D. Brodfuehrer and A.H. Cohen. Neurobiology and Behavior, Cornell University, Ithaca, NY, 14853.

In the medicinal leech, Hirudo medicinalis, pressure ejection of L-glutamate onto a single segmental ganglion or subesophageal ganglion can induce swimming activity along the ventral nerve cord. This suggests that glutamate may play a role as an excitatory neurotransmitter in leech central nervous system. Using a monoclonal antibody for glutamate (IMN) we tested whether the leech central nervous system contains glutamate-like immunoreactivity.

In 20 µm cryostat sections of leech ganglia, we found positive staining for glutamate-like immunoreactivity distributed along the ventral nerve cord. However, to date cell bodies have only been stained in the supraesophageal ganglion (SupraEG), subesophageal ganglion (SubEG) and segmental ganglion (SG) 1. No neuronal cell bodies labeled in any other ganglia tested (SG 2, 5, 6, 10, 20, 21 and tail ganglion). Neuronal processes, on the other hand, were labeled reliably in all ganglia examined. The distribution of cell body staining consisted of the following: In both the SupraEG and SG 1 up to 5 cell bodies generally labeled, while in the SubEG a pair of 30 µm cell bodies on the ventral surface of the most posterior division of the SubEG, with anteriorly projecting axons ipsilateral to their cell bodies, consistently stained. Moreover, up to 3 other cell bodies occasionally stained in the SubEG. Throughout both the SupraEG and SubEG neuronal processes routinely labeled, while only a small group of bilaterally symmetrical processes were clearly labeled in the other SG examined. Preabsorption controls blocked all positive staining in our sections. Supported by NIMH grant# MH44809 to A.H.C.

137.4

IDENTIFICATION OF FOUR RFAMIDE PEPTIDES IN LEECH . B.D. Evans, J. Pohl*[1], and R.L. Calabrese. Dept. of Biology, Emory University, Atlanta, GA 30322 [1]Microchemical Facility, Emory University, Atlanta, GA 30322

RFamide peptides are potent modulators of neuromuscular interaction in leech (Norris & Calabrese, 1990). Each ganglion of the CNS of the leech *Hirudo medicinalis* contains neurons which are FMRFamide-like immunoreactive as determined with two different antisera (Kuhlman et al., 1985; Evans & Calabrese, 1989).

Using a four step rpHPLC separation, we have purified four FMRFamide-like immunoreactive peptides from leech CNS. Gradients of acetonitrile/H_2O were used for separation, with either 0.1% TFA or HFBA as counterion. Prior to the third separation, samples were incubated in 1.5% H_2O_2 , which oxidizes methionyl residues, thus changing the retention times of peptides containing methionine. FMRFamide-like immunoreactivity was detected with RIA, using antiserum 671C (Marder et al., 1987). The first peptide we isolated cochromatographed with FMRFamide in all systems tested. Sequence analysis indicated Phe-Met-Arg-Phe. The specificity of the antiserum used in RIA for amidated peptides identifies this peptide as FMRFamide. The second peptide isolated was similarly shown to be FLRFamide. YMRFamide and YLRFamide were also purified and sequenced, but coelution with synthetic peptides remains to be tested.

Our results indicate the presence of at least four RFamide peptides in the central nervous system of the annelid *Hirudo*. At least three of these four endogenous peptides can modulate neuromuscular interactions in leech (Kuhlman et al., 1985; Norris & Calabrese, 1990). The identification of multiple species of RFamide peptide in leech should lead to further investigation into the nature of the biological actions of these neuropeptides.

137.5

REGULATION OF PHARYNGEAL MOTILITY IN THE LEECH *HIRUDO MEDICINALIS*. B. A. O'Gara. Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

The role of serotonin in the feeding behavior of the leech is well established (Lent et al., Amer. Zool. 29:1241, 1989). Application of serotonin to the pharynx or stimulation of the serotonin-containing LL cells causes repetitive pharyngeal peristalsis with a frequency of about 1 Hz (Lent and Dickinson, J. Comp. Physiol. A 154:457, 1984). In addition, biting behavior is dependent on CNS serotonin levels. However, the roles of other neurotransmitters as well as the neuronal circuitry underlying feeding in the leech are largely unknown.

In the present study, FMRFamide immunoreactivity was localized to neural processes of the pharynx associated with the longitudinal muscles as well as the circular muscles of the posterior sphincter, the accessory ganglia associated with the pharynx and jaws, and the cephalic ganglia. Application of FMRFamide to the isolated pharynx induced phasic contractions of 20-35 s duration superimposed upon a dose-dependent increase in maintained tension (threshold 1 nM). The small cardioactive peptide B (SCP_B) also induced phasic contractions (20-35 s duration) and slightly increased maintained tension. However, the threshold for these effects was high (10-100 µM).

Several neurons that caused pharyngeal contractions were discovered which have somata in the first neuromere of the subesophageal ganglion. Each of these neurons has a ventral soma with a contralateral axon that enters the circumesophageal connectives and exits the CNS through the stomatogastric nerve of the supraesophageal ganglion. Stimulation of one identified cell caused quite complex effects, longitudinal contractions when depolarized and pharyngeal peristalsis when the depolarization was terminated. Stimulation of other cells caused longitudinal or circular contractions. Supported by NIH grants NS08263 to B.A.O. & NS21778 to W.O. Friesen.

137.6

ION CHANNELS IN SKELETAL MUSCLE MEMBRANE OF LOBSTER. M.K. Worden, N. Cherbuliez, E.A. Kravitz and R. Rahamimoff. Dept of Neurobiology, Harvard Medical School, Boston, MA 02115 and Dept. of Physiology, Hebrew University Medical School, Jerusalem, Israel.

To examine the cellular and molecular mechanisms responsible for hormonal modulation of muscle activity in the lobster Homarus americanus we began characterizing ion channels in lobster muscle membrane using a modification of the bleb (spherical outpocketing of membrane)-forming technique (Burton, et al, Muscle and Nerve 11:1029 (1988)) and the bleb attached and inside out versions of the patch clamp technique. Data were recorded from the accessory flexor muscle in 50 patches with seal resistance > 2 gigaohms. At least three different single channel activities were observed. The first type had a single channel conductance (γ) between 121-153pS (n=6) in symmetrical KCl (460mM). This channel displays bursting activity, and the closed time distribution can be fitted with at least two exponentials. The mean channel open time (mcot) is 0.36ms at -40mV; the probability of opening has a slight voltage dependence between -50 and +50mV. For the second channel type γ is between 33 and 42pS (n=3) and the mcot is 1.8ms over a voltage range betwewen -100 and +50mV. The third channel type has a γ between 250 and 310pS (n=6), and the mcot is 5.0 ms. These channels may regulate electrical and contractile activity in muscle. (Supported by NIH.)

137.7

ISOLATION OF A NEUROPEPTIDE THAT MEDIATES PROLONGED INHIBITION OF BAG CELL TARGET NEURONS IN APLYSIA. S.M. Rajpara*, J.C. Eliassen*, M.L. Block and E. Mayeri. Department of Physiology, University of California, San Francisco, CA 94143

The neuroendocrine bag cells of the marine mollusk *Aplysia* utilize α-bag cell peptide (αBCP), egg laying hormone (ELH) and 2 other neuropeptides as transmitters to produce several types of effects on abdominal ganglion neurons. αBCP mediates inhibition that lasts the duration of the bag cell burst discharge, approx. 30 min., in about 50% of ganglion neurons. In cells L3 and L6, however, inhibition lasts for more than 2 hr and αBCP application mimics only an early component of the response.

To identify the substance mediating the prolonged component of inhibition we purified material from acid extracts of abdominal ganglia using gel filtration HPLC on TSK 250-125 followed by reverse phase HPLC on C4. Elution peaks were assayed for inhibitory activity on L3 and L6 by arterial perfusion application.

We isolated two inhibitory factors whose effects persist after a wash of seawater applied 5-10 min after initial perfusion. Mass spectroscopy and partial amino-acid sequence analysis of one factor indicates that it is ELH[2-36], i.e., ELH that lacks the first amino acid. This peptide appears to be effective at a higher concentration than the other inhibitory factor, which is presently being characterized. This inhibitory activity is the first to be described for an ELH-related peptide. It remains to be determined whether both factors or just one of them is involved in mediating the prolonged inhibition.

137.8

ULTRASTRUCTURAL LOCALIZATION OF SCP AND BUCCALIN LIKE IMMUNOREACTIVITY IN THE ACCESSORY RADULA CLOSER MUSCLE OF *APLYSIA*. F.S. Vilim[1], I. Kupfermann1, and K.R. Weiss[2]. [1]Cntr. Neurobiol. & Behav., Columbia Univ., NYS Psych Inst., NY, NY, and [2]Dept. Physiol. & Biophys., Fishberg Res. Ctr. in Neurobiol., Mt. Sinai School of Med., NY, NY.

The accessory radula closer muscle (ARC) and its innervation provide a model system for studying the role of neuromodulation and cotransmission. Motorneuron B15, which innervates the ARC, has been shown to synthesize peptides that modulate ARC contractions when applied exogenously. These peptides fall into two classes which are coded for by different genes, the SCPs and the buccalins. The SCPs potentiate and the buccalins depress motorneuron induced ARC contractions. The ultrastructural localization of buccalin and SCP have important implications for understanding the functioning of this system. We have undertaken immunogold double labeling at the EM level using rabbit primary antibodies against buccalin and rat antibodies against SCP. Double labeling using these antibodies at the light microscope (LM) level indicated distinct patterns of labeling that were consistent with known biochemical data. Post embedding EM double labeling using these antibodies and secondary antibodies conjugated to different sized colloidal gold particles was carried out on ARCs and buccal ganglia. Immunostained ARC sections revealed that a large proportion of dense core vesicles (DCVs) were labeled for both SCP and buccalin. Control experiments indicate that these results are not due to some artifact of antibody cross reactivity. Immunostained sections of buccal ganglion revealed processes in the neuropil whose DCVs stained only for buccalin or only for SCP. Omitting one of the primary antibodies or preabsorption of the primary antisera with SCP or buccalin eliminated the labeling of the appropriate size gold particles. Furthermore, switching the gold particle size of the secondary antibodies did not substantially alter co-labeling. These results suggest that SCP and buccalin are localized in the same vesicles.

137.9

DIFFERENTIAL EFFECTS OF BUCCALIN ON PEPTIDERGIC AND CHOLINERGIC COMPONENTS OF B15-INDUCED CONTRACTIONS OF THE ARC MUSCLE. E. C. Cropper[1], S. L. Hooper[3], I. Kupfermann[3], and K. R. Weiss[1,2]. [1]Dept. Physiol./Biophysics, [2]Fishberg Ctr. Neurobio., Mt. Sinai Schl. Med., NY, NY 10029, [3]Ctr. Neurobio./Behav., Columbia Univ., NY, NY 10034.

Evidence suggests that B15-induced contractions of the accessory radula closer (ARC) muscle have a cholinergic component produced by release of ACh, and a peptidergic component produced by release of SCP_A and SCP_B. In addition to the SCPs and ACh neuron B15 contains two peptides, buccalin A and buccalin B, that have an inhibitory rather than an excitatory effect on contractile activity of the ARC. It has been demonstrated that the inhibitory effect of the buccalins is exerted on the cholinergic component of muscle contractions: in this study we sought to determine whether it is also exerted on the peptidergic component.

We bilaterally stimulated B15 neurons while we unilaterally exposed ARC muscles to buccalin at a concentration that has an inhibitory effect on the cholinergic component of muscle contractions (10^{-6} M). After stimulation, cAMP levels in buccalin-exposed and nonbuccalin-exposed muscles were compared. (cAMP levels are a sensitive measure of peptidergic effects of B15 stimulation.) The presence of buccalin had no significant effect on cAMP levels produced by motor neuron stimulation. In other experiments we measured the cAMP stimulation produced by direct application of SCP to the ARC (10^{-7} M) in the presence and absence of buccalin (10^{-6} M). Again buccalin had no effect on cAMP levels.

Thus, in these experiments buccalin had an inhibitory effect on the cholinergic component of ARC muscle contractions but it did not significantly affect the peptidergic component.

137.10

STRUCTURE AND DISTRIBUTION OF MYOMODULIN RELATED NEUROPEPTIDES IN *APLYSIA*. M.W. Miller, F. S. Vilim, E.C. Cropper, A. Alevizos, R. Tenenbaum*, D. Karagogeos, I. Kupfermann, and K.R. Weiss. Ctr. for Neurobiol. & Behav., Coll. P. & S, and NYS Psychiat. Inst., NY, NY 10032; FRCN, Mount Sinai Sch. of Med., 1 Gustave Levy Pl., NY, NY 10029.

The neuropeptide myomodulin, originally purified and sequenced from the accessory radula closer (ARC) muscle of *Aplysia*, has been shown to be present in one of the cholinergic motor neurons (B16) innervating this muscle, where it has been proposed to act as a modulatory cotransmitter (Cropper et al., 1987). The identification of a related peptide, myomodulin B, also present in B16 (Vilim et al., Neurosci. Abstr., 15:665, 1989), suggested that these peptides may belong to a larger neuropeptide family. Four additional bioactive peptides with sequence homology to myomodulin and myomodulin B have been purified from the ARC muscle: GWSMLRLa, GLSMLRLa, GLQMLRLa, and SLDMLRLa. Antibodies raised against the first of these peptides revealed myomodulin-like immunoreactivity in cell bodies and clusters in each of the central ganglia and in fibers in the major connectives. Identified neurons exhibiting immunoreactivity include the buccal motor neuron B16 and the abdominal cholinergic interneuron L10. In the periphery, immunoreactive varicosities are present on tissues associated with the cardiovascular, digestive, and reproductive systems. Richly innervated tissues include the large hermaphroditic duct, the pericardium, and the esophagus. The widespread distribution and multiple forms of the myomodulin related peptides suggest that they are involved in a number of neural circuits where they may exert a diversity of actions.

137.11

RELEASE OF PEPTIDE COTRANSMITTERS FROM AN IDENTIFIED MOTOR NEURON MODULATE NEUROMUSCULAR EFFICACY IN *APLYSIA* M. D. Whim and P.E. Lloyd. Dept. Pharm. Physiol. Sci. & Comm. on Neurobiol. Univ. Chicago, Chicago, IL 60637.

Buccal muscle I5 is innervated by two motor neurons (B15 and B16). In addition to the classical transmitter ACh, B15 also contains the two SCPs. We have previously shown that the SCPs are released from the terminals of B15 in the I5 muscle and that peptide release is dependent on the pattern of stimulation (Whim & Lloyd, PNAS 86: 9034, '89). We examined the possibility that the SCPs released from B15 modulate I5 muscle contractions produced by stimulation of B16. Application of exogenous SCPs to I5 muscles increased the amplitude and relaxation rate of B16-evoked contractions. Stimulation of B15 using paradigms known to cause release of the SCPs resulted in a long-lasting increase in the amplitude and relaxation rate of muscle contractions evoked by B16. This modulation of B16-evoked contraction amplitude and relaxation rate was still observed when the B15 contractions were blocked by a cholinergic antagonist. Stimulation of B15 at frequencies which produce no measurable release of the SCPs did not elicit significant modulation of B16-evoked contractions. The minimum B15 stimulation frequency required to modulate B16-evoked contractions was found to be within the physiological range at which B15 fires during feeding (Cropper et al., PNAS 87: 933, '90). Thus, the mechanism underlying this modulation involves the release of the SCPs from B15 terminals in the muscle. Supported by NS 23569.

137.12

RELEASE OF PEPTIDE COTRANSMITTERS (SCPs) FROM AN IDENTIFIED CHOLINERGIC MOTOR NEURON IN *APLYSIA* IS HIGHLY TEMPERATURE DEPENDENT P.E. Lloyd and M. D. Whim Dept. Pharm. Physiol. Sci. & Comm. on Neurobiol. Univ. Chicago, Chicago, IL 60637.

Cholinergic motor neuron B15 innervates buccal muscle I5 (also termed ARC) and contains and synthesizes the SCPs (A & B). Release of SCPs from terminals of B15 in the I5 muscle has been demonstrated (Whim & Lloyd, PNAS 86: 9034, '89). One of the methods used to demonstrate release was that stimulation of B15 with certain paradigms increased cAMP levels in I5 muscle fibers. Increased cAMP levels were critically dependent on stimulation frequency, burst duration, and interburst interval. At 22°C with 4 s bursts and 3s interburst intervals, the lowest frequency that produced significant increases in cAMP was 15 Hz. With the same pattern of stimulation, the minimum frequency needed to elevate cAMP levels was reduced to 10 Hz at 16°C . The effects of 0.1 µM synthetic SCP_B on cAMP levels in muscle fibers were the same at 16°C and 22°C. Thus, it appears that the SCPs were released at significantly lower stimulation frequencies at 16°C. Consistent with this conclusion, the potentiation of B16-evoked contractions by stimulation of B15 (which appears to be a consequence of release of the SCPs; see Whim & Lloyd, these abstracts) was also more effective at 16°C. Supported by NS 23569.

EPILEPSY: HUMAN STUDIES AND ANIMAL MODELS

138.1

DEVELOPMENT OF KINDLED SEIZURES FOLLOWING ELECTRICAL STIMULATION VIA THE CORNEA. G. Skeen[*1], J. Woodhead[*1], H. Wolf[*1], E. Swinyard[*1], E. Tietz[2], S. White[1] [1]Anticonvulsant Drug Development Program, Dept. of Pharmacol. & Toxicol., Univ. of Utah, S.L.C., UT 84108 and [2]Dept. of Pharmacol., Medical College of Ohio, Toledo, OH. 43699

The observation that anticonvulsants which are effective in human complex partial seizures are also effective against amygdala kindled seizures in laboratory animals has led to acceptance of amygdala kindling as a viable model for complex partial seizures in man. The results of the present report represent an effort to develop a model wherein rats could be effectively kindled to Stage 5 motor seizures (Racine, Electroenceph. Clin. Neurophysiol., 32:281, 1972) by the daily administration of a subconvulsive current (8 ma, 4 sec) delivered through corneal electrodes following topical corneal anesthesia with 1% butacaine sulfate. The developmental curves and the pharmacological profile of this model closely approximate those reported previously for other kindling models. Of the prototype anticonvulsants tested, carbamazepine, valproate and clonazepam were all effective in nontoxic oral doses in preventing the expression of Stage 5 seizures (ED50's: 28.9, 118 and 1.9 mg/kg, respectively). Phenytoin (100 mg/kg) and ethosuximide (1000 mg/kg) were both ineffective following oral administration. In contrast, phenytoin was effective following i.p. administration (ED50: 48.3 mg/kg). These observations, coupled with the demonstration that there is transfer between corneal kindling and amygdala kindling, suggest that the corneal-kindled rat may represent a useful animal model for identifying potentially useful compounds for the treatment of partial seizures secondarily generalized in man. One distinct advantage of this model is that it is useful for the large-scale pharmacological screening of potentially useful anticonvulsant substances. (Supported by NIH Contract NO1-NS-9-2328 from the NINDS)

138.2

KINDLING ENHANCES SENSITIVITY OF CA3 HIPPOCAMPAL PYRAMIDAL CELLS TO NMDA. D. Martin, J.V. Nadler and J.O. McNamara. Depts. Pharmacology, Neurobiology and Medicine (Neurology), Duke Univ. Med. Ctr., Durham, NC 27710.

Several reports suggest that increased NMDA receptor density and/or enhanced NMDA receptor function at least partially explains the hyperexcitability associated with kindling. This idea was investigated with use of a grease-gap preparation for assaying the depolarizing responses of CA3 or CA1 hippocampal pyramidal cells to amino acid excitants.

At both 1 day and 1-2 months after the last evoked seizure, CA3 pyramidal cells from kindled rats were significantly more sensitive to NMDA than pyramidal cells from implanted or unimplanted controls. Kindling did not alter the ability of Mg^{2+} to reduce NMDA potency. In both control groups NMDA potency declined 2.5 to 4-fold over the 1-2 month experimental period, apparently as a result of increasing age. This age-related loss of sensitivity to NMDA was completely prevented by kindling. Thus 1-2 months after the last evoked seizure, NMDA was 4 to 6-fold more potent on CA3 pyramidal cells from kindled rats than on pyramidal cells from implanted or unimplanted control rats. Neither Mg^{2+}, kindling nor aging significantly altered the potency of AMPA or L-glutamate. In addition, kindling did not affect the sensitivity of CA1 pyramidal cells to NMDA or AMPA.

These findings suggest that kindling prevents a loss of NMDA receptor function in CA3 pyramidal cells that normally occurs during early adulthood. Such a change could contribute to maintenance of the kindled state. (Supported by NIH grants NS 17771 and NS 16064.)

138.3

KINDLING-INDUCED DECREASE IN PARTICULATE PKC LEVELS IN THE HIPPOCAMPUS AND MOTOR CORTEX. T. Saitoh, M. Sundsmo*, K. Uéda*, M. Hsu, Z. Horvath, G. Buzsaki, UCSD, School of Medicine, Department of Neurosciences, M-024, La Jolla, CA 92093

PKC has been suggested to play an important role in the modification of synaptic connection. In the LTP model, high frequency stimulation of hippocampal afferents induces translocation of PKC from the cytosol to the membrane. Here we examined the PKC changes in kindled animals, a possible model for epilepsy. Young female rats were implanted with stimulating electrodes in the angular bundle and recording electrodes in the hippocampus. Hippocampal afterdischarges were evoked by high frequency trains once (control) or daily until the rats displayed class 5 seizures on 3 consecutive days (kindling). Despite daily high frequency activation, evoked responses failed to potentiate during the course of kindling. Rats were decapitated 24 hr after the last kindling trial. PKC was determined by Western blotting using polyclonal antibodies raised against specific peptides for isozymes (α), (βI), (βII) and (γ). The single episode of kindling trial did not alter the levels and distribution of any PKC isozymes in hippocampus or in motor cortex. The kindled animals, however, demonstrated a reduced level of particular PKC(α) and -(γ) in hippocampus. In the kindled motor cortex, the level of total PKC isozymes did not change, whereas the fraction associated with particulate fraction was reduced. The results demonstrate the possibily important involvement of PKC in the development of kindling.

138.4

AMYGDALA KINDLING ALTERS HIPPOCAMPAL PROTEIN KINASE ACTIVITY S.J.Chen, M.A.Desai, E.Klann, J.D.Sweatt and P.J.Conn, Division of Neurosciences., Baylor College of Medicine, Houston, TX 77030 and Department of Pharmacology., Emory University, Atlanta, GA 30322.

Kindling is a model of epilepsy and neuronal plasticity in which repeated electrical stimulation of certain CNS structures gradually leads to development of generalized clonic convulsions. To investigate the molecular mechanisms responsible for kindling, we have measured protein kinase activity in several rat brain regions. Rats were kindled in the basolateral nucleus of the amygdala to a criterion of 2-5 stage 5 seizures. Brain tissues from kindled and control animals were dissected 2 hours or 24 hours after the last stage 5 seizure and homogenized in buffer containing 50 mM HEPES, pH 7.4, 10 mM $MgCl_2$, 1 mM EDTA and 1 mM EGTA. Homogenates were added to reaction mixtures containing 5 mM sodium pyrophosphate to nhibit protein phosphatase, 100 μM ^{32}P-ATP and 2 μg of the kinase substrate myosin light chain (MLC). After SDS-PAGE, incorporation of ^{32}P-PO_4 into MLC was quantitated using autoradiography and densitometric scanning. All values were normalized to the values obtained from control, shem operated, animals. Elevated kinase activity was observed in kindled hippocampal CA3 region (167±7% of control, N=3) as well as in the dentate gyrus (162% of control, N=1) when tissue was assayed two hours after the last seizure. In contrast, the kinase activity in CA1 region was found to be depressed (72.2% of control, N=1). No significant difference in protein kinase activity between control and kindled samples was observed in amygdala/pyriform cortex or limbic forebrain. Twenty-four hours after the last seizure, however, a different pattern of altered kinase activity was observed. The kinase activity was found to be elevated in CA1 region (150±4% of control, N=2) and depressed in the dentate gyrus (35±2% of control, N=2). No consistant change in CA3 region was observed. These results indicate kindling is associated with a regionally specific and time-dependent alteration of protein kinase activity.

138.5

SECOND MESSENGERS IN THE HIPPOCAMPUS IN HUMAN TEMPORAL LOBE EPILEPSY. S. Sundaresan, N.C. de Lanerolle, M.L. Brines and D.D. Spencer. Sections of Neurosurgery and Neuroendocrinology, Yale University School of Medicine, New Haven, Connecticut 06510.

The distribution of the second messengers adenylate cyclase and protein kinase C (PKC) in the hippocampus in tumor related (TTLE) and cryptogenic (CTLE) temporal lobe epilepsy was compared. Autoradiographic detection of adenylate cyclase by [3H]-forskolin binding revealed significantly increased levels on a per neuron basis in the molecular layer (ML), CA4, CA3, CA2 and CA1 of CTLE hippocampi compared to TTLE. Autoradiographic detection of PKC by [3H]-phorbol-12,13-dibutyrate binding revealed a similar pattern in that levels on a per neuron basis were increased in CTLE compared to TTLE in all hippocampal fields and in the dentate molecular layer. Most CTLE hippocampi displayed substantial PKC levels within the granule cell bodies. Adenylate cyclase is thought to mediate inhibitory effects in hippocampal cells, whereas PKC is thought to have excitatory effects. Levels of both second messengers were increased by similar percentages in the molecular layer of CTLE, but the overall levels of PKC are comparatively higher than adenylate cyclase in the hippocampus. This, together with the observation that PKC is found in granule cell bodies in CTLE but not TTLE or autopsy controls, suggests that these second messenger mechanisms may result in an overall increased excitation of the granule cells.

138.6

HIPPOCAMPAL DOPAMINE RECEPTOR CHANGES IN HUMAN TEMPORAL LOBE EPILEPSY. N. C. de Lanerolle, J. R. Tompkins* and D.D. Spencer, Section of Neurosurgery, Yale University School of Medicine, New Haven, CT 06510

The distribution of dopamine D2 receptors in hippocampi surgically removed from patients with tumor related temporal lobe epilepsy (TTLE) and cryptogenic temporal lobe epilepsy (CTLE) was compared to that in autopsy specimens obtained from neurologically normal subjects, by autoradiographically localizing the binding of [^{125}I] iodosulpride. The mean density of ligand binding was lower in the granule cell layer, areas CA4, CA1 and the subiculum of autopsy specimens compared to both TTLE and CTLE. The density of binding on the granule cell bodies of CTLE was higher than in TTLE, with a marked increase in receptor density in the inner molecular layer of only CTLE. Ligand binding per cell was greater in almost all areas of CTLE than TTLE. There is also a significant inverse correlation between the density of ligand binding in the granule cell layer and dentate molecular layer with the number of granule cells in CTLE. It is suggested that the upregulation of D2 receptors in the CA fields of CTLE and TTLE may be an effect secondary to seizures, whereas the increase in granular layer and molecular layer of CTLE may be a change contributing to epileptogenesis in CTLE. However, these changes must be taken in conjunction with previously reported (Brain Res., 495: 387-395, 1989) in the chemical neuroanatomy of CTLE hippocampi, in evaluating the epileptogenic mechanisms in this disorder.

138.7

AUTORADIOGRAPHIC QUANTITATION OF CENTRAL AND PERIPHERAL BENZODIAZEPINE RECEPTORS IN HUMAN EPILEPTOGENIC TISSUE. E.W. Johnson, N.C. de Lanerolle, J.H. Kim, S. Sundaresan, D.D. Spencer, and R.B. Innis. Depts. Psychiatry and Neurosurgery, VA Medical Center and Yale University School of Medicine, West Haven, CT 06516.

The distribution and density of benzodiazepine (BZ) binding sites were determined with in vitro receptor autoradiography to hippocampal tissue obtained from patients treated neurosurgically for medication-refractory epilepsy. Tissue was studied from three groups: 1) Patients with complex partial seizures (CPS) (n=6), where pathological examination of the neurosurgical tissue showed mesial temporal sclerosis ("Epilepsy" group). 2) Patients with CPS (n=5), who were found on neurosurgery to have a tumor adjacent to the hippocampus as the cause of their seizures ("Tumor" group). 3) Autopsy samples (n=5) obtained from subjects with no neurologic or neuropathologic disorder ("Autopsy" group). Autoradiography was performed with two radioligands: ^{125}I-Ro16-0154 (selective for the "central" type BZ receptor, thought to be located on neurons); and ^{3}H-PK11195 (selective for the "peripheral" type BZ receptor, thought to be located on glia).

The Epilepsy group showed large and regionally selective losses of ^{125}I-Ro16-0154 binding compared to the Tumor and Autopsy groups, with the latter two not being significantly different. Binding of ^{125}I-Ro16-0154 in various hippocampal regions of the Epilepsy group were: CA1-14%; CA2-42%; CA3-19%; CA4-28%; molecular layer of dentate-61%; and subiculum-100% (expressed as percentage of regional levels in Tumor and Autopsy groups). The decreases of ^{125}I-Ro16-0154 binding closely correlated with neuronal cell losses. The binding of ^{3}H-PK11195 in the Epilepsy group were most increased in those regions which showed decreased ^{125}I-Ro16-0154 binding: CA1-286%; CA2-186%; CA3-174%; CA4-260%; molecular layer of dentate-230%; and subiculum-100% (relative to regional levels in tumor and autopsy groups.

138.8

GRANULE CELL DISPERSION IN HUMAN TEMPORAL LOBE EPILEPSY. C.R. Houser. Dept. of Anatomy and Cell Biology, and Brain Research Institute, UCLA, and VA Medical Center, Los Angeles, CA 90024.

The distribution of dentate granule cells has been studied in control autopsy and temporal lobe epilepsy (TLE) specimens. At mid rostro-caudal levels of the dentate gyrus, the granule cell somata in control specimens were closely approximated and formed a distinct, narrow lamina. In a subpopulation of TLE specimens, granule cell somata were dispersed and extended into the molecular layer to varying extents, creating an irregular border between the granule cell and molecular layers. Granule cell dispersion was observed in specimens with and without obvious granule cell loss. However, there appeared to be a relationship between the extent of granule cell dispersion and the amount of cell loss in the polymorph region of the dentate gyrus. The most common feature in the histories of cases with granule cell dispersion was febrile seizures during the first four years of life. The dispersion of granule cells suggests that there has been some alteration in the migration of these neurons, resulting in an extended distribution of the granule cell somata into the molecular layer. Loss of the precise laminar pattern of the dentate gyrus could affect the functional circuitry of this region. Supported by NIH grant NS21908 and VA Medical Research Funds.

138.9

PHYSIOLOGIC PROPERTIES OF DENTATE GRANULE CELLS WITH DENDRITIC DEFORMITIES IN HUMAN EPILEPTIC HIPPOCAMPAL SLICES. M. Isokawa, T. Babb and J. Engel, Jr. Brain Research Institute and Dept. of Anatomy and Neurology, University of California Los Angeles, CA 90024.

Neuronal excitability and synaptic responses were studied in 23 human epileptic dentate granule cells which showed varying degrees of morphological deformities, previously reported as characteristic of human temporal lobe epilepsy by the Golgi study (Scheibel et al., 1974). Intracellular recording was obtained through Lucifer Yellow or Biocytin filled electrodes to measure resting membrane potentials, membrane resistances and synaptic responses. Dye injection subsequent to each recording demonstrated pathologic cell morphology in agreement with Golgi studies: 1) loss of spines, 2) nodule formation, 3) dendritic swelling, 4) development of "string-of-beads", and 5) restricted dendritic arborization. The first three features were observed in tight correlation, and the "string-of-beads" dendrites were seen as advanced pathology. However, these dendritic spine deformities were not necessarily correlated with the degree of restriction in their dendritic domains. Resting membrane potentials of these neurons were -57.4 mV in average (N=22) and typically-observed synaptic responses were EPSPs by perforant path stimulation. In 2 cases, IPSPs were detected upon depolarizing current injection as hyperpolarization following EPSPs. In one neuron, an extreme case of "string-of-beads" morphology was observed, retaining no normal-appearing dendritic branches or identifiable pattern of cell body. Nevertheless, this neuron was capable of maintaining a membrane potential at - 30 mV at the time of penetration and generating EPSPs. Intracellular current injection hyperpolarized the cell to -78mV or depolarized it to generate a spike, but no burst firing was elicited. Further studies in i) synaptic responses in correlation to the degree of dendritic deformity and ii) membrane properties in correlation to dendritic arborization patterns are in progress. Supported by NIH Grant NS02808.

138.10

INDUCTION OF HEAT SHOCK PROTEINS GENES IN KINDLED BRAINS. M-L Wong, M.A. Smith, S.R.B. Weiss, R.M. Post*, P. W. Gold*. Clinical Neuroendocrinology Branch, National Institute of Mental Health Intramural Research Program, Bethesda, MD 20892.

Heat shock proteins (HSP) have been implicated in a number of neuronal responses to stress. HSP may play a role in cellular repair/protection mechanisms, and they have been suggested to be markers for neural injury (Gonzalez *et al.*, 1989). HSPs gene expression increases locally during tissue injury, ischemia, adenovirus infection, and hypertermia. More recently, HSP have also been implicated in generalized status epilepticus (Lowenstein *et al* 1989). We examined the induction of mRNAs for HSPs 70 (HSP of about 70 kilodaltons) and 84 (HSP of about 84 kilodaltons), in rat hippocampi that were subjected to electrical kindling. Male Sprague-Dawley rats were stereotaxically implanted with electrodes in the amygdala and received subconvulsive electrical stimulation (800 μA, 60 Hz, 1ms for 1 sec.), 1-2/days for 2 weeks. Animals were considered kindled after the development of 3 stage-5 seizures and were sacrificed 2 hours, 24 hours, or 3 weeks after their last seizure. Control animals with amygdala electodes never received electrical stimuli. Brain sections were cut through the dorsal hippocampus sections and *in situ* hybridization was done using ^{35}S-dATP labelled oligodeoxynucleotide probes for HSP70 and HSP84. Section were apposed to films and quantified by densiometry and dipped in NT-2 emulsion (Kodak). Both HSP mRNAs had a transient increase ($p<0.05$) 2 hours after their last seizures. Increased mRNA induction was not present either 24 hours or 3 weeks after their last seizures.

138.11

IMMUNOHISTOCHEMICAL STUDY OF QUINOLINIC ACID CATABOLISM IN EPILEPTIC HUMAN HIPPOCAMPUS. F. Du, W.O. Whetsell[1], B. Abou-Khalil*[1], B. Blumenkopf*[1], E. Okuno and R. Schwarcz. Md. Psych. Res. Ctr., Baltimore, MD. 21228 and [1]Vanderbilt University School of Medicine, Nashville, TN 37232.

Quinolinic acid phosphoribosyltransferase (QPRT), the specific degradative enzyme of the excitotoxin quinolinic acid, was studied immunohistochemically in human epileptic hippocampi surgically resected from 6 patients with temporal lobe epilepsy. As compared with normal hippocampi (J. Comp. Neurol., 295:71, 1990), a clearly disease-related pattern of QPRT-immunoreactivity (QPRT-i) was consistently noted in all hippocampal specimens showing sclerosis (5 out of 6). Thus, QPRT-i, mainly associated with proliferated glial cells, increased substantially in the severely degenerated CA1 region, where QPRT-i is very sparse under normal conditions. In contrast, a pronounced decrease in QPRT-i was observed in the largely degenerated end folium which normally contains the highest density of QPRT-i in the hippocampus. QPRT-positive neurons were preserved in areas of CA1 where most other neurons had degenerated. Preliminary double-labeling studies demonstrated that almost all surviving QPRT-i neurons also exhibited glutamic acid decarboxylase-immunoreactivity, which has been reported to be spared in human epileptic hippocampus. The specific pattern of QPRT-i in the pathological specimens may indicate a role of quinolinic acid in temporal lobe epilepsy. (Supported by USPHS grant NS 16102).

138.12

STIMULATION EVOKED CHANGES IN INTRINSIC OPTICAL SIGNALS IN THE HUMAN BRAIN. B.A.MacVicar, D.Hochman, F.LeBlanc and T.Watson. Neuroscience Research Group, University of Calgary, Calgary, Alberta T2N4N1.

Neuronal activity in several systems has been shown to be associated with changes in the optical properties of nervous tissue. We have asked whether activity in the human neocortex is also associated with changes in intrinsic optical signals. The cortex of patients was exposed during operations to remove seizure foci for the treatment of intractable epilepsy. Stimulation by silver ball electrodes was normally performed during these operations to map key motor and sensory areas. During electrical stimulation, video imaging of the cortex was performed to ascertain whether there were changes in the reflectance of the tissue during stimulus-evoked activity. Digitized images were averaged during stimulation and were subtracted from averages obtained during control periods. During stimulation there was a progressive decrease in tissue reflectance around the stimulating electrodes. This change was repeatable and was graded with changes in stimulus intensity. These results indicate that non-invasive video imaging of changes in intrinsic optical signals could potentially be used to map areas of cortical neuronal activity during neurosurgery.

Supported by the Medical Research Council (Canada).

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS II

139.1

AXONAL PATHFINDING IN THE CORD OF ZEBRAFISH MUTANTS MISSING THE FLOOR PLATE (FP). J.Y. Kuwada & K. Hatta, Dept. Biology, U. of Michigan, Ann Arbor, MI 48109 & Institute of Neurosci., U. of Oregon, Eugene, OR 97403.

In the cord of zebrafish embryos the growth cones of the ascending commissural (Co) and VeLD neurons exhibit cell-specific behaviors in the immediate vicinity of the FP cells. Both neurons initially project growth cones ventrally towards the FP. The Co growth cone crosses the midline and turns rostral and dorsal while in apparent contact with the FP. The VeLD growth cone does not cross the midline, instead it turns caudal along the FP. These observations suggest that the FP may have multiple, cell-specific effects on spinal growth cones in the zebrafish embryo. We have examined this by analysis of cyc-1 mutants in which FP cells fail to develop. We find that Co and VeLD neurons (n=77) all extend to the ventral midline but that approximately 40% then make errors in pathfinding. Two examples of errors are Co axons fail to cross the midline but turn rostral and dorsal, and VeLD axons ascend. These results 1) suggest that the FP is not necessary for extension of growth cones to the ventral midline, and 2) are consistent with the existence of multiple, partially redundant growth cone guidance mechanisms. The removal of one mechanism may degrade the fidelity of cell-specific pathfinding normally insured by multiple and redundant mechanisms of growth cone guidance in the zebrafish cord. (Supported by NIH, MOD, and NSF .)

139.2

FLOOR PLATE (FP) ABLATIONS INDUCE AXONAL PATHFINDING ERRORS BY SPINAL COMMISSURAL CELLS IN THE ZEBRAFISH EMBRYO. R.R. Bernhardt & J.Y.Kuwada, Dept. Biology, Univ. of Michigan, Ann Arbor, MI 48109.

The growth cones of ascending commissural (Co) cells extend ventrally and cross the ventral midline by inserting between a row of FP cells and the basal lamina surrounding the cord. They then extend rostrally and dorsally along a diagonal pathway to reach and ascend in a dorsal tract (DLF). We have tested the role of FP cells for guidance of Co growth cones by laser ablating the FP. Ablations did not affect ventral outgrowth of Co axons indicating that the FP is not necessary for ventral extension. However, 3 kinds of errors were observed at the midline. Axons crossed the midline and ascended in an abnormal ventral pathway for at least 2 segments before drifting dorsally to reach the DLF. Other axons did not cross the midline and either turned rostrally and dorsally or caudally and dorsally to ascend or descend, respectively, in the ipsilateral DLF. Normal Co axons were also observed in experimental segments. Together with control ablations of ventrolateral cells, this argues against mechanical blockage as a cause of errors. The increase in Co abnormal trajectories suggests that the FP cells are one of a set of pathfinding cues at the midline. Removal of one cue leads to errors but does not eliminate the capacity for directed axonal outgrowth. (Supported by Swiss NSF, NIH, MOD, and UM.)

139.3

A MUTATION THAT DELETES THE FLOOR PLATE AND DISTURBS AXONAL PATHFINDING IN ZEBRAFISH. K. Hatta*, R.K. Ho*, C. Walker*, & C. B. Kimmel, Inst. of Neuroscience, Univ. of Oregon, Eugene, OR 97403.

In the early zebrafish embryo, the floor plate (FP) is a single row of immunologically distinctive cells extending through the spinal cord and into the brain. The entire floor of the CNS, including the FP, fails to develop in embryos bearing the "cyclops" mutation *cyc-1(b16)*. The phenotype appears during late gastrulation and is rescued by orthotopic transplantation of wild-type gastrula ectoderm into mutants, suggesting, but not yet establishing, that the mutation acts cell-autonomously to block FP specification.

cyc-1 also causes defasciculation and invasion into the midline of longitudinal axonal pathways that normally neighbor the FP. These pathways include axons derived from identified reticulospinal neurons such as the Mauthner cells. The pathways look normal where they border wild-type FP transplanted into mutants, and ablation of the FP in wild-type embryos by laser-irradiation produces a local phenocopy of the axonal disruption. These findings suggest that the axonal disturbances in *cyc-1* arise because of loss of the FP, that normally provides essential cues for axonal guidance. (Supported by the NIH.)

139.4

EARLY GENERATION OF TRACTS IN THE ZEBRAFISH BRAIN. L.S. Ross, T.J. Parrett* and S.S. Easter, Jr. Dept. of Biology, University of Michigan, Ann Arbor, MI 48109.

The zebrafish brain at 24 hours is characterized by a small number of spatially distinct axon tracts that form a simple scaffold (Wilson et al., *Dev.* 108:121, 1990). We have examined the earliest formation of these axon tracts in the forebrain and midbrain of the embryonic zebrafish using acetylcholinesterase (AChE) expression to label differentiated neurons as well as antibodies to HNK-1 to label axons.

The first AChE-positive cells in the brain appeared at 16 hours as small clusters in the presumptive telencephalon, diencephalon, ventral tegmental area, and hindbrain. At each stage thereafter, more labeled cells were added to these pre-existing populations. At some stages, new clusters of labeled cells appeared elsewhere in the brain These new clusters were in the anlage of the epiphysis at 18 hours, in the region of the posterior commissure, rostral tectum, and pituitary at 24 hours, and in the caudal hypothalamus at 30 hours. The number of labeled cells increased most rapidly in the telencephalon and diencephalon, and cells continued to be added to these two regions after the counts in other regions had reached a plateau.

The first HNK-1 positive cells in the forebrain and midbrain also appeared at 16 hours, as a small (2-3 cells) cluster in the ventral tegmental area. The cells of the ventral tegmental area extended axons caudally toward the hindbrain, pioneering the ventral longitudinal tract. At 18 hours, a second tract was established by the caudal extension of axons in the diencephalon to form the tract of the postoptic commissure. At 20 hours, the supraoptic tract was formed by a large cluster of telencephalic cells.(Supported by NIH EY-00168 and EY-07022).

139.5

Interactions of optic fibers with the pre-existing tracts of the embryonic zebrafish. J. D. Burrill and S. S. Easter, Jr., Dept. of Biology, Univ. of Michigan.

The first retinal ganglion cells of the zebrafish extend an axon at 32-36h, and reach the tectum about 46-48h, after crossing three pre-existing tracts.

The optic axons of fixed embryos were labelled by intraocular injections of DiI, and in some the tract of the posterior commissure (TPC) and the dorsoventral diencephalic tract (DVDT) were also labelled with DiO. Wholemounts were viewed in a fluorescence microscope, and some were photoconverted and viewed as wholemounts or in sections.

The leading growth cones were visualized at 47-49h in 10 doubly labelled preparations, and in 23 with only the optic fibers labelled. The only deviations from the normal optic projection were at intersections with other tracts. In 8, some optic axons grew parallel to TPC fibers. In 2, some failed to leave the tract of the postoptic commissure with the others, and continued caudally. In no cases did the optic axons join the supraoptic tract (SOT), DVDT, or the contralateral optic nerve. 28 hatchlings, 5-7d, were labelled intraocularly. In 6, a single axon deviated from the normal pretectal projection pattern, and turned rostrally along the SOT into the telencephalon.

These observations suggest that optic axons are led astray by other axons, and raise the question of why such deviations are so infrequent.

Supported by R01-EY-00168 and T32-EY-007022.

139.6

APPEARANCE AND DISTRIBUTION OF GFAP IMMUNOREACTIVITY IN THE EMBRYONIC ZEBRAFISH CNS. R.C. Marcus and S.S. Easter, Jr. Department of Biology, Neuroscience Program, University of Michigan, Ann Arbor, MI 48109.

The earliest tracts in the brain appear between 16 and 18h of development and enlarge thereafter (unpublished, and Wilson et al., Development 108:121, 1990). To better characterize the cellular environment which axons traverse, an antibody against goldfish GFAP (Nona et al., Glia 2:189, 1989) was used to stain sections and wholemounts of zebrafish at 12, 14, 15, 16, 18, 21, 24, 27, 30, 33, and 48h.

GFAP immunoreactivity was first seen at 15h. At 16h it was distributed uniformly along the ventro-lateral pial surface throughout the CNS, but the dorsal surface and the ventral midline were GFAP-negative. The presence of label in regions where tracts are formed suggests GFAP-positive endfeet may contribute to the permissiveness of the superficial lamina for axonal growth. This same distribution is maintained at later times, but modified in three ways: 1) long GFAP-positive radial processes intersperse with the axons in the developing tracts, 2) the GFAP-positive superficial label gradually becomes restricted to the tracts, and 3) GFAP-positive tangentially oriented fibers cross the ventral midline in the mid and hindbrains. The persistence of label in the tracts may reflect a role for glial cells in the maintenance of axonal pathways. The relationship of the GFAP-positive cells to the developing tracts was confirmed by showing that the neuronal marker, the HNK-1 antibody, labeled in the same regions as anti-GFAP. Electron microscopic studies are under way to further investigate this relationship. Supported by EY00168 to SSE

139.7

DYNAMICS OF AXONAL GROWTH AND ARBOR FORMATION DURING DEVELOPMENT OF THE RETINOTECTAL PROJECTION IN ZEBRAFISH EMBRYOS. R.J. Kaethner and C.A.O. Stuermer, Friedrich-Miescher-Laboratorium der Max-Planck-Gesellschaft, Tuebingen, FRG.

Individual retinal axons were observed over 3 to 13 hrs during their growth to their target and during the process of terminal arbor formation in the tectum of living zebrafish embryos. Axons were labeled by inserting crystals of DiI or DiO into defined regions of the retina in embryos of 50-60 hrs postfertilization (pF) and viewed with low intensity light and a SIT Camera between 70-120 hrs pF.

During their growth through the optic tract and the tectum the axons are lead by a single growth cone and only occasionally emit transient sidebranches of up to 20μm in length. The average axonal growth velocity is in the optic tract 18μm/hr and 10μm/hr in the tectum. Axons grow directed towards their retinotopic target. Here they cease to elongate and emit sidebranches. Branches extend and retract lamellipodia and filopodia in a rapid sequence. Initially neither one of the branches is stable and primary, secondary and higher order branches are withdrawn and new ones created. With time, one or two branches become stable. The rapid sequence of branch extension and retraction, however, continues on the higher order sidebranches, and is still seen in embryos at 120 hrs pF.

This branch turn-over may last longer and could perhaps be necessary for the process of "shifting" (Easter and Stuermer, 1984). Whether these dynamical changes during arbor formation involve formation and breakage of synapses is currently under investigation.

139.8

MONOCLONAL ANTIBODY E21 RECOGNIZES NEW AND REGENERATING RETINAL AXONS IN GOLDFISH. K.A. Wehner and C.A.O. Stuermer, Friedrich-Miescher-Laboratorium, Max-Planck-Gesellschaft., Tuebingen, FRG.

In adult goldfish growing retinal axons from newly added ganglion cells and axons regenerating after optic nerve section (ONS) express several growth specific cell surface proteins. These were detected by monoclonal antibodies, D3, ϵ587 and Reggie, and are N-CAM 180 (Bastmeyer et al., 1990), a 200 kD (Vielmetter and Stuermer, 1989) and a 50 kD protein (Wehner and Stuermer, 1989).

By immunizing mice with cell surface membranes of regenerating goldfish optic nerves we obtained a new monoclonal antibody, E21, and detected another cell surface associated protein, that is expressed on newly added and re-expressed on regenerating goldfish retinal axons. Like N-CAM 180, the 200 kD and 50 kD proteins, the re-expression of the E21-antigen on regenerating retinal axons declines with time after ONS and is no longer detectable at 10 months after ONS.

The immunoaffinity-purified antigen has an apparent MW of 84 kD, is glycosylated and carries the HNK-1 sugar moiety. Sequence analysis of 15 amino acids at the N-terminus revealed, to this point, no homology with known neuronal proteins.

139.9

SUBPLATE NEURONS "PIONEER" THE OUTPUT PATHWAY OF RAT CORTEX BUT NOT PATHWAYS TO BRAINSTEM OR SPINAL TARGETS.

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Preplate (PP) cells are the first cortical neurons generated and aggregate below the pial surface. The later generated, cortical plate (CP) neurons accumulate in the PP and split it into a marginal zone and subplate (SP). McConnell et al (1989 Science 245:978) find in cats that SP neurons send the first axons through the internal capsule (IC), the path taken by layer 6 axons to thalamus and by layer 5 axons enroute to brainstem and spinal cord. Layer 5 axons exit cortex via the IC and bypass their brainstem targets (e.g. superior colliculus - SC) as they extend through the cerebral peduncle, pyramidal tract (PT) and into the spinal cord; later collaterals form along the primary axons and take distinct paths to the brainstem targets (O'Leary & Terashima 1988 Neuron 1:901; 1989 Soc NS Abs 15:875). Here we address in rats whether SP neurons "pioneer" the subcortical paths of layer 5 axons by using the tracers DiI and RITC. DiI labeled axons are seen in the developing cortex on E13, and first exit cortex and enter the nascent IC on E14. These axons are from rostral cortex, axons from caudal cortex are growing rostrally in the nascent intermediate zone. Backfilling from rostral cortex on E13 and IC on E14 identifies the cells of origin as PP neurons based on position and morphology (also the first CP neurons are born on E14 and the CP is first identified on E15-E16). No afferent axons are labeled from the IC on E14. Labeled axons reach the cerebral peduncle on E17; at this age both SP and CP cells are backfilled with DiI from the IC. To determine if SP axons grow down the PT or to the SC, RITC was injected into them in newborn rats. Layer 5 axons reach PT on E21 and SC at P1.5. SP cells are not labeled from either site. We conclude that in rats SP neurons send the first axons into the IC, but SP axons do not "pioneer" the more distal subcortical path of the primary axons of layer 5 neurons, nor the paths taken to their targets by their later forming collaterals.

139.10

AXON TARGETING POTENTIALS OF LAYER 5 PROJECTION NEURONS ACROSS THE DEVELOPING CORTEX.

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Neurons in layer 5b of the mammalian neocortex are the source of cortical projections to targets in the brainstem and spinal cord. DiI labeling of developing 5b axons shows that each brainstem target is contacted exclusively by collateral branches extended from spinally-directed primary axons. 5b neurons in motor and visual areas develop a similar set of axon branches from which the area-distinct projections in the adult emerge via the selective loss of axon branches and/or segments of the primary axon (O'Leary & Terashima 1988 Neuron 1:901; O'Leary & Terashima 1989 Soc NS Abtsr 15:875). Here we address whether subcortically projecting 5b neurons are equipotential in axon targeting by focusing on branched projections to two brainstem targets, the superior colliculus (SC) and dorsal column nuclei (DCN). We provide evidence that: (1) as a population 5b neurons in all neocortical areas initially form this set of branches, (2) individual 5b neurons are competent to form each of the branches, (3) individual 5b neurons can form both branches. The retrograde dyes Diamidino Yellow and Fast Blue were injected in the SC and DCN of neonatal rats to label neurons that send branches to them, and into the spinal cord (SpCd) to label the primary axon. 5b neurons that send branches to the SC or DCN are distributed across the entire, tangential extent of neocortex. Neurons that send branches to the SC or DCN and/or a primary axon to the SpCd are coextensive over the radial extent of layer 5b. Many 5b neurons are double labeled when one dye is injected into the SC or DCN and the other into SpCd, and when the SC and DCN are injected with different dyes. Our findings suggest that subcortically projecting layer 5b neurons throughout the developing neocortex have similar potentials in axon targeting, and belong to the same neuronal class, even though they may ultimately have distinct projections in the adult. Support: NEI grant EY07025.

139.11

IN VIVO EVIDENCE FOR TARGET CONTROL OF COLLATERAL FORMATION AND DIRECTIONAL AXON GROWTH IN MAMMALIAN BRAIN

A. Missias, L.J. Kutka', B.S. Reinoso and D.D.M. O'Leary, Depts of Neurosurgery and Anatomy & Neurobiology, Washington Univ Sch Med, St Louis, MO 63110.

The corticopontine projection develops by a delayed interstitial budding of collaterals from layer 5 corticospinal axons, rather than by a direct ingrowth of primary axons or by bifurcation of the growth cone. Branches form in the axon tract overlying the basilar pons (BP) and extend directly into it (O'Leary & Terashima 1988 Neuron 1:901). In vitro studies suggest that a diffusible tropic substance released by the BP can elicit the formation and directional growth of collateral branches from layer 5 axons (Heffner, Lumsden & O'Leary '90 Science 247:217; Heffner & O'Leary '90 Soc NS Abs). Here we present in vivo experiments that support this chemotropic mechanism. First, we x-irradiated fetal rats during the generation and migration of BP neurons, resulting in a reduction in the BP and the formation of ectopic islands of BP neurons beneath the path of layer 5 axons. DiI labeling reveals that layer 5 axons form collateral branches directly over the reduced BP, and over the ectopic islands of BP neurons - points where branches normally do not form. No branches form at positions that would normally overlie BP. Thus, the BP, rather than local cues in the axon tract, seems to control the location of cortical axon branching. Second, we prevented cortical axons from invading the right BP. We reasoned that if BP was attracting cortical innervation by a diffusible tropic activity, developing cortical axons on the left side should be lured to the right BP. Indeed, DiI labeling shows that an abnormally large number of layer 5 axons cross the midline at the level of the BP and grow into the denervated side. Finally, we find that late arriving layer 5 axons will grow directly into the BP if early arriving axons are removed. Thus, the growth cone of primary layer 5 axons is receptive to cues that identify the BP. Based on these and our in vitro findings, we conclude that the basilar pons attracts its cortical innervation through the release of a diffusible chemotropic molecule.

139.12

SPATIALLY COMPLEX EXPRESSION OF NEURON-SPECIFIC ANTIGENS DURING PERIPHERAL PATHFINDING.

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In the chick limb the axons of sensory and motor neurons exhibit a series of specific pathway choices as they project into the limb between st 23-30. To study the molecular basis of specific pathfinding, monoclonal antibodies were generated to a membrane preparation of st 27-30 peripheral nerve using the cyclophosphamide immune suppression method to reduce the response to common and/or abundant antigens; and the resultant clones screened on frozen sections. Among the neuron-specific clones, the following exhibited staining patterns consistent with a role in pathfinding. 9C11 recognizes a cell surface epitope expressed on cells in the lateral motor column from motoneuron outgrowth (st 22) through muscle nerve formation (st 28). Although 9C11 uniformly stains axons within major nerve trunks and muscle nerves, it does not stain the intramuscular portion of the axon. 5E10 recognizes an antigen sequentially expressed on different subsets of sensory and motor axons at precise proximo-distal levels, staining axons only from the point at which they diverge to follow specific pathway choices, until they reach their targets; within muscle staining was weak. Thus this antigen appears to be part of the neuron's mechanism to detect and/or respond to specific target-related cues.

Support: NIH NS-19640

139.13

MOTONEURON SPECIFICATION IN THE LUMBOSACRAL SPINAL CORD OF THE CHICK EMBRYO.

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Studies in the chick embryo indicate that lumbosacral (LS) motoneurons are specified with respect to muscle target before sending axons into the hindlimb. How and when does this specification occur? LS motoneurons may be specified as they form a motor column, a mode of specification likely to be dependent on interactions with other spinal cord components. To begin to address this hypothesis I have asked if motoneurons within a piece of ventral neural tube, 1/3-1 segment in length, can project appropriately when placed in a foreign motor column site. At stage 15 the neural tube piece with some underlying notochord was removed from axial levels LS1, 2, or 3 in a quail embryo. In a chick embryo, all or most of the LS1-3 neural tube segments were removed and the quail tissue positioned in an incorrect position within the gap (i.e. an LS1 piece in place of LS3 and adjacent to LS4). St 29-30 chimeras were fixed, paraffin sectioned, and stained with a quail-specific antiserum. Motoneuron axons stained by the antiserum projected to their originally appropriate muscle targets. These observations suggest that motoneuron specification within a 1/3-1 segment neural tube piece is independent of the adjacent dorsal spinal cord and the presence of correct neighboring segments.

139.14

METABOLIC ENZYMES FOR QUINOLINIC ACID HAVE DIFFERENT AND FUNCTIONALLY SIGNIFICANT LOCALIZATIONS IN THE RAT MAIN OLFACTORY BULB.

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Quinolinic acid (QUIN) is an excitotoxin endogenous to the brain. Previous studies demonstrated that QUIN's synthetic enzyme 3HAO (3-hydroxyanthranilic acid oxygenase) and degradative enzyme QPRT (quinolinic acid phosphoribosyltransferase) are preferentially expressed in glial cells widely distributed throughout the brain, including the main olfactory bulb (MOB). Since 1) excitatory amino acids can influence growth cone motility, and 2) primary olfactory neurons (PON) are replaced throughout adult life, the existence of QUIN in MOB astrocytes could function to modulate the motility of new PON axons reaching the bulb.

We report that *QPRT and 3HAO have a complementary distribution just superficial and just deep, respectively, to the glomerular layer* (GL) where PON axons terminate. QPRT antibody staining is heaviest at the nerve layer-glomerular interface where PON axons enter the GL; 3HAO staining is heaviest in the external plexiform layer (EPL) just deep to the GL. This suggests a gradient of QUIN expression - high in the EPL, low in the nerve layer. If QUIN stops PON growth cones, a gradient of QUIN might function to restrict the ingrowth of new PON axons to the glomeruli. Supported by NIDCD-DC00347, NS 16102.

139.15

THE CORTICOSPINAL TRACT IN THE ABSENCE OF CNS MYELIN. B. B. Stanfield. Lab. of Clinical Science, NIMH, NIHAC, Poolesville, MD 20837.

Since myelination normally occurs relatively late in neural development and, in any given fiber pathway, well after axonal extension is complete, it has generally been assumed that myelin plays no role in the initial formation of axonal projections. Recently however it has been shown that CNS myelin can inhibit neurite extension *in vitro*, and thus the myelination of early developing pathways could influence the formation of later developing projections. One late developing pathway in which such interactions might occur is the corticospinal projection. Corticospinal axons have only begun to enter the cervical cord at a stage when myelination is already well under way in other spinal pathways, including the adjacent gracile and cuneate fasciculi. I therefore examined the disposition of the corticospinal tract in the dysmyelinated mutant mouse, jimpy (jp). Jimpy is an X-linked, juvenile-lethal mutation in which the lack of oligodendrocyte differentiation together with frank oligodendrocyte loss results in the virtual absence of myelin from the CNS.

To label the corticospinal tract I injected WGA-HRP or biocytin into the rostral neocortex of 15 to 19 day old hemizygous jimpy mice (jp/Y) and littermate controls (X/Y ♂'s or ?/X ♀'s). One day later the animals were perfused with fixative and the brains and spinal cords were processed appropriately. In both jimpy and normal mice the corticospinal fibers traverse the medulla along its ventral aspect in a well-defined pyramidal tract, decussate in several fascicles at the spinomedullary junction and extend down the spinal cord in a compact bundle in the ventral part of the dorsal funiculus, just beneath the ascending primary sensory fibers. Even in the virtual absence of central myelin, the trajectory of the corticospinal tract in jimpy appears to be entirely normal. Thus, while the neurite inhibiting properties of central myelin may prevent neurite extension and axonal sprouting and thereby help stabilize existing pathways within the mature CNS, the disposition of the corticospinal tract in jimpy suggests that CNS myelin does not play a critical role in pathway selection during the development of this projection.

139.16

IS THE PITUITARY CHEMOTROPIC TO GNRH AXONAL OUTGROWTH FROM PREOPTIC AREA (POA) GRAFTS IN HYPOGONADAL (HPG) MICE? M.J.Gibson, Y.Saitoh, and A.J.Silverman. Department Medicine, Mount Sinai Sch. of Medicine, New York,NY 10029 and Columbia Coll. Phys.& Surgeons, New York, NY 10032.

GnRH fibers consistently grow out of intraventricular (3rdV) POA grafts in hpg mice to the median eminence (ME) of the GnRH-deficient hosts. GnRH axons there terminate on capillaries of the pituitary-portal plexus; resultant stimulation of the hpg gonadotrophs leads to corrected reproductive function. In continuing studies to determine the factor(s) mediating this directed outgrowth, we are evaluating the role of the pituitary (PIT): (1) Hpgs were given 3rdV fetal co-grafts of PIT+POA, muscle+POA, or POA alone. After 1 mo, 2 hpg with POA had increased testes wt (54.9±1.3 mg) and GnRH innervation of the ME. In 3 mice with POA+2PITs testes wts were 15.6±4.5 mg. GnRH neurons in PIT co-grafts were often in large clusters with thick fascicles of axons but with only modest GnRH outgrowth in the ME. GnRH axons entered PIT co-grafts and were often in apposition to growth hormone cells. Muscle in co-grafts degenerated into a fibroblastic mass which the GnRH axons appeared to actively avoid. (2) Hpg mice were hypophysectomized (HYPOX) 1-2 wk before POA graft surgery. GnRH innervation was absent or sparse in the anterior and mid-ME, with some outgrowth in the post-infundibulum in 4 of 5 mice studied 1-4 wk after HYPOX. Together, these studies suggest that the pituitary may exert a chemotropic influence on GnRH axonal development.

139.17

TARGET SPECIFIC OUTGROWTH OF DIFFERENT TYPES OF VASOPRESSIN NEURONS TRANSPLANTED INTO VASOPRESSIN-DEFICIENT BRATTLEBORO RATS. H.A. Al-Shamma, and G.J. De Vries. Program in Neuroscience and Behavior, Univ. of Massachusetts, Amherst, MA 01003.

Suprachiasmatic nucleus (SCN) grafts in Brattleboro rats show specific outgrowth of vasopressin (VP) fibers into areas that are innervated by the SCN in non-deficient rats (Wiegand SJ, Gash DM, *J. Comp. Neur.* 265:562 '88; Boer GJ et al., *Neurosci.* 15:1087 '85). We compared the outgrowth of VP fibers from grafts of the SCN, bed nucleus of the stria terminalis (BST) and medial amygdaloid nucleus (MA) of fetal long evans rats. These grafts were placed into the lateral ventricle of adult Brattleboro rats. Eight weeks later, these rats were sacrificed, and immunocytochemically stained sections of their brains were studied using computerized image analysis. Grafts of the SCN showed very little outgrowth of VP fibers into the lateral septum (LS), which correlates with the absence of VP innervation from the SCN to the LS in non-deficient rats. In contrast, grafts of the bed nucleus of the stria terminalis (BST) showed significantly denser VP outgrowth into the LS ($p<0.05$), which correlates with the dense VP projections from the BST to the LS in non-deficient rats. MA grafts showed a similar pattern of outgrowth as did BST grafts. This is remarkable, since in contrast to the BST, the MA does not appear to send dense VP projections to the LS in non-deficient rats. However, the MA and BST supposedly share a common evolutionary lineage (Alheid GF, Heimer L, *Neurosci.* 27:1 '88) and are similar in many other respects (neurotransmitter content, steroid sensitivity, morphology etc.). Thus, our findings suggest that the VP neurons of these two nuclei may also follow similar cues to reach their target.

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS III

140.1

THROMBOSPONDIN PROMOTES AXONAL GROWTH IN SYMPATHETIC NEURONS. D.J. Osterhout and D. Higgins, Dept. of Pharmacology, State Univ. of New York, Buffalo, N.Y.14214

Thrombospondin (TSP) is an extracellular matrix protein which has been detected in both the central and peripheral nervous systems. We examined its effects on cultures of neurons dissociated from embryonic rat sympathetic (21d) ganglia. Neurons were plated in serum-free medium onto poly-D-lysine coated coverslips with or without adsorbed TSP. After 24 hours, TSP had caused a 6-fold increase in the number of neurons extending processes; it also increased the number of processes/neuron and the total neuritic length. Processes formed in the presence of TSP had the cytochemical characteristics of axons; dendrites were not observed. Maximal effects of TSP were obtained with precoating concentrations of 100 ug/ml; similar concentrations of fibronectin, fibrinogen or albumin failed to elicit significant process extension. The neurite-promoting activity of TSP was blocked by a monoclonal antibody (A4.1) directed to the stalk region of the molecule. These data suggest that TSP could participate in regulating axonal outgrowth in situ. (supported by NSF #8909373 and NIH GMO 7145)

140.2

NEURAL CREST CELLS UTILIZE THROMBOSPONDIN AS A MIGRATORY SUBSTRATUM IN SITU. L.J. BOYNE, K.S. O'SHEA, V.M. DIXIT*. Univ. of Michigan Medical School, Ann Arbor, MI 48109.

Thrombospondin (TSP) is a trimeric glycoprotein temporally and spatially located along the neural crest cell (NCC) migratory pathway. TSP supports NCC migration in vitro to a greater extent than fibronectin and that addition of anti-TSP antibodies inhibited migration on TSP. To determine if TSP is a substrate for NCC migration in situ, day 9 mouse embryos (plug+=day 1) were injected with 10 ul anti-TSP IgG unilaterally in the thoracolumbar region of the developing spinal cord. Control embryos were injected with 10 ul pre-immune serum or PBS. Embryos were allowed to develop for an additional 8 hours in whole embryo culture and then fixed, sectioned, and progress of NCC migration and morphology examined. Injection of anti-TSP antibodies but not PBS or pre-immune serum into the mesenchyme resulted in cell rounding and inhibition of cell migration. Consistant with their ability to migrate on TSP in vitro, NCC utilize TSP as a migratory substratum in situ. Supported by NIH grant HD-23867.

140.3

PROTHROMBIN mRNA IS EXPRESSED IN RAT BRAIN. M. Dihanich*, E. Reinhard and D. Monard. Friedrich Miescher Institute, 4002-Basel, Switzerland.

Glia-derived Nexin (GDN) is a serine protease inhibitor mainly expressed in the brain, but also in the peripheral nervous system during nerve regeneration. Its function in the brain is still largely unknown, but *in vitro* experiments suggest a role in the proteolytic balance regulating neurite outgrowth. Since thrombin, a serine protease of the blood coagulation system, antagonizes neurite outgrowth and is by far the best target protease for GDN *in vitro*, a thrombin-like protease might also interact with GDN in the brain. Investigation of rat brain mRNA isolated from various developmental states (E16, E20, P5, P10, P21, adult) by quantitative PCR, Northern analysis and *in situ* hybridization indicate that thrombin itself is expressed in the brain, especially during early development. A comparison of thrombin sequences cloned from both rat liver and rat brain shows that nerve cells produce the same enzyme as liver cells. Both neuronal and glial cell lines are capable of expressing prothrombin mRNA. Future experiments will investigate the regulation of thrombin expression and its possible role in nerve degeneration and regeneration.

140.4

REGENERATING RETINAL AXONS OF GOLDFISH RESPOND TO A REPELLENT GUIDING COMPONENT ON CAUDAL TECTAL MEMBRANES OF ADULT FISH AND EMBRYONIC CHICK

J. Vielmetter, J. Walter and C.A.O. Stuermer, Friedrich-Miescher-Lab./Max-Planck-Gesellschaft and *Max-Planck-Inst. f. Entwicklungsbiologie, Tübingen, FRG*

We applied the *in vitro* choice assay developed by F. Bonhoeffer for the retinotectal system of embryonic chick (Walter et al., '87) to the retinotectal system of adult goldfish (Vielmetter and Stuermer, '89). On a substrate of alternating rostral (R-)/caudal (C-) tectal membrane stripes of adult goldfish regenerating fish temporal (T-) axons accumulate on R-membranes.

Here we demonstrate that fish T-axons (like embryonic chick T-axons) avoid the C-membranes. C-membranes of adult fish -as those of embryonic chick- have a repellent influence on T-axons. This property is abolished when C-membranes are exposed to phosphatidylinositol-specific phospholipase C (PI-PLC). Then T-axons grow randomly over R-/C-stripes. In a cross species choice assay with R-/C-membranes of E9 chick, fish T-axons respond to the repellent properties of C-membranes. Here fish T-axons accumulate on chick R-membranes but cross freely chick R-/C-stripes after PI-PLC-treatment of C-membranes.

When offered to fish axons as the sole substrate fish and more so chick C-membranes have an outgrowth reducing effect on fish T-axons which is abolished after PI-PLC-treatment of these membranes. E16 chick C-membranes, no longer repellent for embryonic chick T-axons (Walter et al., '87), are less outgrowth reducing for fish axons than E9 chick C-membranes.

Thus, fish tecta posses a repellent guiding component for retinal axons that is related to the repellent component in chick tecta. Unlike chick, however, fish appear to express this axon guiding component lifelong.

140.5

GROWTH INHIBITION OF GOLDFISH OPTIC AXONS IN VITRO BY NICOTINE. Ronald L. Meyer and Thomas H. Hogan*. Developmental Biology Center, University of California, Irvine, CA 92717.

The activity rearrangement of optic fibers during the formation of the retinotectal projection implies the existence of retrograde activity mediated signal affecting growing fibers. Since transmitter release is good candidate for this, the capacity of neurotransmitters to regulation axonal growth was tested. Retinal explants were made onto laminin-polylysine coated coverslips and the outgrowing neurites, which filled several criteria for being optic axons, were monitored by timelapse videomicroscopy. A number of neurotransmitters (and agonists) including glutamate, serotonin and dopamine were added to the culture media without notable effect. TTX also had no effect. Nicotine at 50 μm, however, rapidly and strongly inhibited filopodial movement and growth. This inhibition could be antagonized by curare and a-bungarotoxin. Nicotine was equally effective when delivered locally to the growth cone with a micropipette. We speculate that nicotinic cholinergic receptors may be a component of a retrograde regulatory signal. This work was supported by EY06746.

140.6

ADDITIONAL PURIFICATION STEPS OF AN ACTIVITY FROM EMBRYONIC CHICK BRAIN THAT INHIBITS NEURONAL GROWTH CONE MOTILITY. D.W. Raible* and J.A. Raper. Dept. of Anatomy, Univ. of Penn. Sch. of Med., Philadelphia, PA 19104

Local environmental cues that guide growth cones to their appropriate targets may either promote or inhibit neurite outgrowth. We recently reported the development of a growth cone collapse assay that has made possible the search for molecules that inhibit neurite outgrowth. Neuronal growth cone collapsing activity from embryonic chick brain was enriched by column chromatography on heparin and hydroxylapatite (Raper, J.A., Kapfhammer, J.P., Neuron 2:21-29, 1990). We have now identified two additional purification steps that we hope will allow isolation and identity of the collapsing activity.

Heparin-purified collapsing activity is retained by the mannose-binding lectins *Lens culinaris* (lentil) agglutinin, Concanavalin A and *Pisum sativum* (pea) agglutinin. Growth cone collapsing activity elutes from *Lens culinaris* agglutinin upon incubation with methyl α-D-glucopyranoside and methyl α-D-mannopyranoside.

Collapsing activity enriched by heparin and hydroxylapatite chromatography can be further purified on a HPLC cation exchange column. The enriched activity binds to a sulfopropyl matrix in buffer containing 0.2 M NaCl and elutes in buffer containing 0.9 M NaCl. We are now combining these isolation steps in sequence so as to further purify the growth cone collapse activity.

140.7

EVIDENCE FOR THE DELINEATION OF NEURAL CREST MIGRATION PATHWAYS BY INHIBITORY BOUNDARIES. S. Schroeter, C. J. Lasky*, R. A. Oakley, C. A. Erickson*, and K.W. Tosney. Biol. Dept. and Neurosci. Program, Univ. of Michigan, Ann Arbor, MI 48109 and Zool. Dept., Univ. Cal., Davis, CA 95616.

We have demonstrated that tissues known to act as barriers to axon advance *in vivo* also express peanut agglutinin (PNA) binding epitopes (Oakley and Tosney NS Abstr. 347.7, 1988). We are characterizing the binding of PNA in relation to neural crest migration in the chick embryo to determine if PNA binding is also typical of tissues that exclude neural crest cells. We determine the distribution of neural crest cells using the HNK-1 antibody with both frozen sections and the whole mount staining method of Loring and Erickson (Dev. Biol. 121:220, 1987).

We find a differential binding of PNA within the posterior half of early epithelial somites prior to the invasion of the anterior half sclerotome by the earliest migrating neural crest cells. This suggests that PNA binding epitopes may inhibit migration in the posterior sclerotome and thereby contribute to the preferential migration through the anterior sclerotome.

We also find consistent HNK-1 labeling of the neural crest cells that enter the dorsolateral melanocyte pathway between the ectoderm and the dermamyotome using the whole mount method. These crest cells enter the dorsolateral path approximately 24 hours after the first crest cells have invaded the anterior sclerotome. We find that the delayed entry of the premelanocyte population is correlated with a diminution of PNA binding within the dorsolateral pathway. These results suggest that PNA binding epitopes may restrict neural crest migration in at least some pathways.

Supported by NIH grant NS-21308.

140.8

AN ANTIBODY AGAINST A HELISOMA LAMININ-LIKE PROTEIN INHIBITS OUTGROWTH FROM IDENTIFIED NEURONS IN VITRO. J. D. Miller* and R. D. Hadley. Dept Anat & Cell Biol, Med U of SC, Charleston, SC 29425.

Laminin is a potent stimulator of outgrowth from PNS and some CNS neurons in culture, and the laminin B chains are proposed to function as neurite outgrowth promoting (NOP) factors in some conditioned media (CM). We have identified, and raised a rabbit antibody against, a ~300 kD protein in the extracellular matrix (ECM) of the snail, Helisoma, that is antigenically related to the B chains of vertebrate laminin.

NOP activity of CM can be abolished either by immunoprecipitation of CM proteins with the anti-~300 kD antibody, or by addition of the anti-~300 kD antibody to CM (at 170 μg/ml). In 3 experiments, a total of 13 B5 neurons were cultured for 3-4 d in antibody-treated CM. No outgrowth from any B5 neuron was observed (100% inhibition) in these experimental plates, although the cells appeared healthy, as evidenced by the formation of veils surrounding the cell bodies. In control plates which were treated with non-immune IgG, 16 of 17 B5 neurons (94%) exhibited completely normal outgrowth, with neurites at least twice the soma diameter in length.

Immunoblots of Helisoma brain CM probed with the anti-~300 kD antibody, or an antibody against the B chains of vertebrate laminin, reveal a prominent ~300 kD protein, which comigrates with the ~300 kD protein in Helisoma ECM. Reduction of CM proteins with β-mercaptoethanol causes a decrease in the ~300 kD band, with a concomitant increase of a ~190 kD immunoreactive protein. Reduction of the ~300 kD ECM protein does not result in the appearance of an ~190 kD immunoreactive protein, suggesting that the laminin-related protein may be processed during the production of CM. An important question is whether such processing may be necessary for the NOP activity of the ~300 kD laminin-related protein.

140.9

SUBSTRATE ADHESIVENESS DOES NOT CORRELATE WITH NEURITE GROWTH AND FASCICULATION. V. Lemmon, G.J. Elmslie*, and M.L. Hlavin*. Departments of Neurosciences and Neurosurgery, Case Western Reserve University, Cleveland, OH 44106.

The differential adhesion hypothesis has had a major influence on the design and interpretation of axon growth and guidance investigations. A major prediction has been that axons prefer to grow on more adhesive substrates. We have compared the relative adhesiveness of different substrates and their ability to promote growth and fasciculation. Neurites were grown from chick retinal explants. The relative adhesiveness of different substrates was determined by squirting tissue culture media from glass pipettes at the neurite growth cones. The pulse duration required to blast a growth cone from the substrate was used as a measure of substrate adhesivity. Growth rates were measured using time-lapse video microscopy.

substrate	laminin	L1	poly-L-lysine
blast duration ms	74 ± 2	181 ± 8	179 ± 12
growth rate μm/hr	110±15	56 ± 5	10 ± 2
fasciculation	+	-	+++

These results indicate substrate adhesivity does not primarily determine neurite growth rate. In addition, fasciculation does not correlate with the relative adhesiveness of a substrate. Implications of these results will be discussed.

Supported by NIH Grant EY-5285 to V. Lemmon.

140.10

NCAM DEPENDENT NEURITE OUTGROWTH IS DOWN REGULATED DURING DEVELOPMENT AND INHIBITED BY REMOVAL OF POLYSIALIC ACID FROM NEURONS. P. Doherty, J. Cohen and F.S. Walsh. Depts. Experimental Pathology and Anatomy, UMDS, Guy's Hospital, London SE1 9RT, England.

Neurite lengths have been measured for chick retinal ganglion cells (RGCs) cultured on confluent monolayers of control 3T3 cells and 3T3 cells expressing transfected human NCAM (see Doherty et al., Nature, 343:464-466). NCAM in the monolayer stimulated neurite outgrowth from E6, but not E11 RGCs. This response could be specifically inhibited by removal of α 2-8-linked polysialic acid (PSA) from retinal neurons, treatment with heparin (250 μg/ml) or treatment with antibodies that react exclusively with chick (neuronal) NCAM. These data suggest that NCAM on the marginal endfeet of neuroepithelial cells in the optic tract may stimulate neurite outgrowth from RGCs in a time dependent manner, with this response controlled in part by a down-regulation of expression of PSA on neuronal NCAM.

140.11

LACK OF NEURITE OUTGROWTH IN HYDROCEPHALIC MUTANT RAT EMBRYO NEURONS. C.J.D'Amato, K.S.O'Shea and S.P.Hicks. Depts. of Pathology, and of Anatomy and Cell Biology, Univ. of Michigan Medical Center, Ann Arbor, MI 48109.

A recessive mutation that arose in long brother-sister mated Wistar albino rats was expressed in homozygous state principally as stenosis of the aqueduct with resultant prenatal hydrocephalus, many animals living to adulthood. A transient structural defect of formation of the basal lamina (BL) of the neuroepithelium of the midbrain-thalamic junction (MTJ) associated with delayed deposition of type IV collagen in that BL occurred during embryonic days 11-13. Normal collagen deposition and BL formation then resumed, but stenotic overgrowth of MTJ ensued. The current study tested whether neurons from 13th day mutant embryo brains were competent to extend neuritic processes on different extracellular matrix substrates. Neurons isolated from mutant and control embryo brains were plated ($5x10^5$ cells/ml) on petri dishes coated with laminin (25ug/ml), fibronectin (20ug/ml), type IV collagen (20ug/ml), or tissue culture plastic alone (control), and grown in medium composed of 50% BME, 25% fibronectin-depleted horse serum, and 25% HBSS. Neuron responses were recorded photographically at 4 hr intervals for 24 hrs. The length of neuritic processes was measured from the photomicrographs using a digitizing tablet interfaced with a microcomputer. Mutant embryo neurons were unable to extend neurites on any of the substrates, while controls formed elaborate neurites.

140.12

RETRACTION OF GROWING AXONS: CYTOSKELETAL CORRELATES AND ENHANCEMENT OF RESPONSE MAGNITUDE BY LAMININ. S. Finnegan Sloan,* E. Koenig and V. Lemmon. Dept. of Physiology, SUNY at Buffalo, Buffalo, NY 14214, and Center for Neurosciences, Case Western Reserve Univ., Cleveland, OH 44106.

Monoclonal antibody (mAb) 8A2 triggers a global retraction response in ganglion cell (RGC) axons regenerating from goldfish retinal explants *in vitro* which is arrested by cytochalasin D (*Soc. Neurosi. Abstr.*, vol. 15, p. 1027, 1989). The response is characterized by (i) retrieval of axoplasm from growth cones, (ii) aggregation into a distal mass, and (iii) bulk retrograde translocation of axoplasm, leaving evacuated residual strands distally. Induced global retraction may serve as a model of axon elimination during developmental maturation. The 8A2 retraction response is significantly more robust when axons are grown on laminin substratum as compared to that of polylysine, implicating membrane-cytoskeletal interactions as modulating factors. RGC axons label with antibodies to putative talin. When the column of distal axoplasm is disrupted by nocodazole, the distal aggregate mass becomes isolated and continues to translocate. This suggests that microtubules are probably passive components in the response, and that the force generating mechanism may be of an acto-myosin type intrinsic to the moving mass. Myosin II, putative myosin I, actin, myosin light chain kinase, calmodulin and putative talin are present in the aggregate mass.

140.13

MEROSIN, A LAMININ-LIKE MOLECULE, IS ASSOCIATED WITH NEURONAL PROCESSES IN SELECTED REGIONS OF THE ADULT RABBIT CNS. C. Portera-Cailliau*, T. Hagg, E. Engvall*, S. Varon, J.C. Louis, M. Manthorpe. Dept. Biol., M-001, UCSD, La Jolla, CA 92093

Merosin is a "laminin-like" molecule found selectively in peripheral nerve and muscle basement membranes (Leivo et al., *PNAS* 85:1544, 1989). Merosin, like laminin, possesses two 200 kD short "B_1" and "B_2" chains and one 400 kD long "M"-chain, which has about 40% sequence homology with the corresponding laminin "A"-chain. Like laminin it has potent in vitro neurite-promoting activity (Manthorpe et al., *Soc. Neurosci. Abstr.*, 16, 1990). We have previously described that anti-laminin polyclonal and anti-B_2 monoclonal antibodies react with a "laminin-like" molecule within rat CNS neurons (Hagg et al., *Neuron* 3:721, 1989). Here we used monoclonal chain-specific antibodies and a sensitive ABC immunochemical staining technique to compare the cellular distribution of B_2, A and M chains in the adult rabbit CNS. With all three antibodies immunoreactivity was detected in blood vessel basement membranes. As in rat, most neuronal cell bodies stained intracellularly with anti-B_2 antibodies. With antibodies to M-chain (merosin) no cell body staining was seen but punctate staining associated with neuronal fibers was observed in certain brain regions., e.g. rostral cingulate, insular, perirhinal, piriform and entorhinal cortices, molecular layer and hilus of the dentate gyrus, amygdala and hypothalamus. In addition, M-chain, but not B_2- or A-chain, antibodies stained 3rd ventricle-associated tanycytes. These results suggest the existence in the brain of multiple forms of "laminin-like" molecules with different chain compositions and different cellular distributions. Supported by NINCDS grants NS-16349, NS-25011 and NSF grants BNS-88-08285, BNS-86-17034.

140.14

MEROSIN, A LAMININ-LIKE EXTRACELLULAR MATRIX MOLECULE, STIMULATES NEURITE OUTGROWTH. M. Manthorpe, E. Engvall, S. Varon, D. Muir. Dept. Biol., Univ. Calif. San Diego, La Jolla, CA 92093

Merosin is a laminin-like molecule whose location in situ is restricted to peripheral nerve and muscle basement membranes (Lievo, PNAS 85: 1544, 1989) and to basement membranes and neuronal cells within central nervous system (Portera-Cailliau, Soc. Neurosci. Abstr. 16: 1990). In common with laminin, merosin has two 200 kD chains ("B1" and "B2") but has a 400 kD "M" chain which shares only about 40% sequence homology with the corresponding laminin "A" chain. Since the neurite-promoting activity of human laminin is postulated to reside within the A chain, we tested whether merosin, in which the "A" chain is replaced by a analogous "M" chain, also possesses neurite promoting activity. Microwells were coated with polyornithine for 1 hr, washed and treated for 2 hrs with serial dilutions of purified human laminin or merosin. Embryonic chick ciliary ganglion neurons were cultured in the wells for 4 hrs, the assay terminated by fixation of the neurons, and the percentage of neurons bearing neurites determined. Merosin expressed a specific neurite promoting activity having an ED_{50} of 800 ng/ml (50 μl/well) and was comparable to that of laminin in the same assays. The JG22 anti-chick β_1 integrin antibody inhibited the neuritic response of ciliary neurons in response to both laminin and merosin, suggesting that both proteins stimulate ciliary neurite outgrowth using integrin receptors. Supported by NINCDS NS16349, NS25011 and NSF BNS8808285, BNS8617034.

140.15

ANALYSIS OF AN ASTROCYTIC SURFACE MOLECULE THAT PROMOTES NEURITE OUTGROWTH. B.MITTAL and S.DAVID. Centre for Research in Neuroscience, MGH, McGill Univ., Montreal, Canada, H3G 1A4.

Laminin, N-cadherin and N-CAM are known to mediate neurite growth on cultured astrocytes. The present study was undertaken to identify other neurite outgrowth-promoting molecules on astroglia. A monoclonal antibody (mAb) 1A1 was generated using neonatal rat astrocytes.

By indirect immunofluorescence, the 1A1 molecule is found on a subpopulation of cultured astrocytes. Flat, type-1 astrocytes express the 1A1 molecule on their surfaces. Less than 5% of process-bearing, type-2 astrocytes are $1A1^{+}$. 1A1 immunoreactivity is not seen on oligodendrocytes or neurons, even in long term cultures. Fab fragments of mAb 1A1 inhibit neurite outgrowth from cerebellar neurons on astrocytes by 25%. Under reducing conditions, the immunoaffinity purified 1A1 molecule migrates as a major band at 60 kDa and a minor one at 30 kDa. Purified 1A1 molecule adsorbed on to nitrocellulose-coated substrata is able to promote neurite outgrowth from cerebellar neurons. This mAb, therefore, recognizes an astrocyte surface molecule that also contributes to nerve fiber growth in culture.

140.16

PURIFICATION AND CHARACTERIZATION OF A RECEPTOR FOR NEURITE OUTGROWTH FACTOR (NOF). N.Miki*, H.Taniura*, C-H.Kuo, H.Higuchi* and Y.Hayashi. Dept. of Pharmacol., Osaka Univ. Sch. of Med., Osaka 530, Japan.

A glycoprotein of about 82kDa solubilized from the gizzard muscles both binds to NOF (ligand blot) and inhibits the neurite promoting activity of NOF, suggesting that an 82kDa protein is a NOF-receptor. An 82kDa protein (NOF-receptor) was purified into a doublet band on an SDS-PAGE from gizzard muscle membranes and prepared an antibody against NOF-receptor. An NOF-receptor in the developing retinas exhibited the same physico-chemical properties as that of the gizzard muscles. The quantitative decrease of NOF-receptor in embryonic retinas was observed after 11-day embryos when measured by the inhibition assay, ligand blots and immunoblots, which was paralleled well with the reduction of NOF-induced neurite outgrowth of embryonic retinas. The antibody only stained the ganglion cell and optic fiber layers of the retinas of 8-day embryos, but the staining disappeared in the late developing retinas of 18-day embryos. The results suggest that the loss of response of the retinal neurons to NOF reflects a decrease in the NOF receptor molecules.

140.17

EXTRACELLULAR MATRIX MOLECULES ARE ASSOCIATED WITH PREPLATE CELLS IN EARLY NEOCORTICAL DEVELOPMENT.

A.M. Sheppard and A.L. Pearlman, Depts. of Cell Biology and Neurology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

The first postmitotic cells of embryonic neocortex form the preplate zone; they are divided by cortical plate formation into the marginal zone and subplate. We have previously shown that fibronectin (FN)-like immunolabeling is distributed along radial glia but is restricted to the preplate, marginal zone and subplate and is virtually excluded from the cortical plate. In this study we immunolabeled sections of murine cortex (E11-13) with antisera to laminin (abLAM), J1 and chondroitin sulfate proteoglycan (abCS). Labeling with abLAM resembles abFN but is less intense. Immunoreactivity to abJI does not appear until cortical plate formation; it is sparse and largely restricted to the subplate and marginal zone. Labeling with abCS is very prominent around the cells of the preplate and continues to be associated with them as they are displaced into the marginal zone and subplate. Thus CS and FN are the most prominent of several extracellular matrix (ECM) components in developing neocortex. ECM is distributed in the layers that contain preplate cells; regulation of its components may be important in establishing a framework for cortical plate formation and a pathway for early axons. (Supported by NEI grant EY00621).

140.18

The effects of substrate-bound proteins on the cyto-skeletal changes underlying growth cone advance. D. W. Burmeister, A. Buriani, R.J. Rivas, D.J. Goldberg, Dept. of Pharmacology, Columbia Univ. N. Y., N.Y. 10032.

Aplysia neurons form growth cones and rapidly extend neurites on poly-lysine (PL) substrates treated with substrate binding factors (SBF) from *Aplysia* hemolymph or conditioned medium. Growth cones form on PL without SBF, but advance slowly and have larger, more stable veils. Confocal fluorescence microscopy reveals differences in micro-tubule (MT) distribution on the two substrates. On SBF MTs are aligned along the growth cone axis, rarely cross each other, and remain parallel to the substrate, on PL, MTs curve, cross, run perpendicular to the neurite axis, and have a complex vertical distribution. Growth on PL and SBF occurs through the same sequence: filopodial and veil protrusion, engorgement of the actin rich veils with organelles and MTs from the central region, and the consolidation of the growth cone into the neurite. Acute application of SBF to growth cones on PL leads to the acceleration of engorgement before any other visible change. The engorgement effect of SBF is mimicked by application of cytochalasin B and is inhibited by application of colcemid. The growth and engorgement stimulation factor(s) in SBF are proteins found in high molecular weight fractions, and are active only when bound to the substrate. Our experiments demonstrate acute effects of substrate molecules on the growth cone cytoskeleton, and suggest that substrate molecules may exert their growth stimulatory effects by regulating the cytoskeletal changes underlying veil engorgement.

140.19

EVIDENCE THAT ACCUMULATION OF GM2 GANGLIOSIDE RE-INITIATES DENDRITE GROWTH ON PYRAMIDAL CELLS IN A NON-GANGLIOSIDE STORAGE DISEASE. L.A. Goodman, P.O. Livingston* and S.U. Walkley. Dept. Neuroscience, Rose F. Kennedy Center, Albert Einstein College of Medicine, Bronx, NY. 10461.

Several neuronal storage diseases are characterized by a dramatic alteration in neuronal morphology: renewed dendrite growth occurs on the axon hillock of mature pyramidal cells. Ectopic neurite growth first observed in a case of GM2 gangliosidosis suggested that gangliosides might function as neuritogenic agents (Purpura and Suzuki, *Brain Res. 116*:1). Although ectopic neurites are most abundant in ganglioside storage diseases, they also occur in some non-ganglioside storage disorders. In cortical samples from feline models of non-ganglioside storage diseases in which ectopic dendrites do occur, GM2 ganglioside is elevated (Siegel and Walkley, *Neurochem. Abstr. 21*:110). In the cortex of cats with the carbohydrate storage disease, α-mannosidosis, only a small proportion of pyramidal cells bears ectopic dendrites. Using a Golgi-EM technique with PAS staining, we established that neurite-bearing pyramidal cells, as well as certain intrinsic neurons, contain PAS-positive membranous inclusions, consistent with storage of sialic acid-containing glycolipids (e.g., gangliosides). The presence of membranous inclusions in those neurons suggests they contain the excess GM2 ganglioside. In contrast, pyramidal cells lacking ectopic neurites contain PAS-negative flocculent inclusions, consistent with storage of oligosaccharides. Using an anti-GM2 monoclonal antibody, we found that subpopulations of both pyramidal and intrinsic neurons contain GM2-like immunoreactivity. EM analysis of GM2-positive cells showed them to contain membranous inclusions. Thus, it is likely that the GM2-positive pyramidal neurons are the neurite-bearing cells. We propose that the re-initiation of dendrite growth on mature pyramidal cells is brought about by the accumulation of GM2 ganglioside. (NS18804)

140.20

EMBRYONIC HUMAN RETINAL NEURITE GROWTH ON CELLULAR AND ACELLULAR SUBSTRATA. N. Kleitman and J.M. Hopkins, The Miami Project to Cure Paralysis, University of Miami School of Medicine, 1600 NW 10 Ave., R48, Miami, FL 33136.

Rat, chick and goldfish differ in substratum preferences during retinal development (reviewed in Kleitman et al., J. Neurosci. 8:653, 1988). This led us to question whether the requirements for human retinal neurite outgrowth could be adequately predicted by animal models. Therefore, we investigated neurite outgrowth from explants of embryonic human retina. Human embryos, aged 8-12 weeks were obtained from elective abortions following protocols approved by the University of Miami Committee for Protection of Human Subjects. Retinas were dissected, separated from the pigment epithelium, cut into 8-10 explants (each containing an area adjacent to the optic nerve head) and plated on one of the following: type I collagen (either an air-dried collagen gel, ADC, or flatter, ammoniated collagen, AC), polylysine (PL), polylysine and laminin (PL-lam), or rat Schwann cells on AC. Extensive outgrowth was observed on ADC but not PL. Neurite growth on AC and PL-lam mostly occurred on the surfaces of flat cells emanating from the explants, but fascicles appeared to relate directly to the acellular substrata. Human retinal explants also extended neurites extensively on a sublayer of rat Schwann cells; neurites associated with Schwann cells in preference to the flat cells which migrated from explants onto the underlying AC. The neurons survived for several weeks in culture and reextended neurites after secondary explantation to fresh culture substrata. We are presently preparing human Schwann cells to test their influence on neurite growth and neuronal survival (Morrissey et al., this volume). Supported by The Miami Project to Cure Paralysis.

140.21

REGENERATION OF AXONS FROM ADULT HUMAN RETINA IN VITRO. J.M. Hopkins and R.P. Bunge, The Miami Project to Cure Paralysis, University of Miami School of Medicine, 1600 NW 10 Ave., R48, Miami, FL 33136.

In an effort to establish an in vitro model of regenerating adult human CNS neurons, we have investigated the potential for neurite growth from explants prepared from adult human retina. This approach is made possible by the fact that certain eye banks make available, for research, eyes donated for corneal transplantation. Eyes were removed within 2 hrs. post-mortem and stored on ice for 1.5-7.0 days. Retinas were then dissected out and cut into explants (1mm square), which were cultured at 37°C on various cellular and acellular substrata (see below) in an oxygen-rich, humidified atmosphere. Neurite outgrowth onto the culture substratum was observed only in the presence of Schwann cells, after a quiescent period of approximately six days in vitro. Of 52 explants cultured for 7 days or more on substrata containing Schwann cells, 43 were viable (showed evidence of intraretinal neurite growth in vitro) and 28 extended neurites beyond the explant border. Estimated rates of neurite growth on Schwann cell substrata reached a maximum of 0.22mm/day. Neurites did not grow beyond the border of viable explants onto acellular culture substrata composed of either polylysine, laminin, type-I collagen, or monolayers of adult human retinal glia. These results demonstrate that, under selected culture conditions, explants prepared from adult human retina harbor viable neurons and that Schwann cells promote and support regeneration of neurites from these retinal neurons in vitro, allowing systematic study of conditions favorable to axonal regeneration in the adult human CNS. (Supported by EY06073-03 [JMH] and the Miami Project to Cure Paralysis.)

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: FIBER GUIDANCE AND SYNAPTOGENESIS

141.1

PARTIAL CHARACTERIZATION OF NEURONAL INFLUENCE ON CELLS OF REGENERATING LIMBS. C. Eberhardt Maier and Robert H. Miller. Department of Neuroscience, Case Western Reserve University School of Medicine, Cleveland, OH 44106

The ability of urodele amphibians to regrow amputated limbs is known to be nerve dependent, and we have initiated in vitro studies to begin to characterize the required neuronal factors. Dissociated blastemal cells from regenerating newt limbs were cultured on fibronectin in the presence or absence of homogenized newt brain extract. After three days blastemal cells were treated daily for 3 or 6 days with fresh brain extract, culture media, and BrdU. Cells were fixed, permeabilized, and exposed to antibodies to BrdU to identify proliferating cells. The total number of cells and the percentage of dividing cells was established.

After both 3 and 6 days treatment with brain extract the percentage of dividing cells was double that seen in control cultures, suggesting that brain extract increases the number, but not the rate, of blastemal cells undergoing mitosis.

However, to provide any neurotrophic factors axons must be present in the blastema. We have begun studies to identify possible molecular mechanisms used for guidance of regenerating axons in regrowing limbs. Frozen sections of blastemas in predifferentiation and differentiation phases were labeled with antibodies against NCAM, L-1, laminin, and fibronectin then visualized by indirect immunofluorescence. Preliminary results indicate that in predifferentiation blastemas the majority of cells stained for the four antigens. The majority of cells also stained for NCAM and L-1 in the differentiation phase while fibronectin staining was significantly reduced. Interestingly, during the differentiation phase laminin staining was confined to specific cells and produced a linear pattern which was reminiscent of that for axons in silver stained sections of blastemas. This suggests that laminin may be a potential substrate for the regenerating axons of regrowing limbs.

141.2

DIFFERENTIAL DEVELOPMENT OF RAT TRIGEMINAL GANGLION CELLS. I.A. Scarisbrick, P.J. Isackson, D.L. Benson, E.G. Jones. Department of Anatomy and Neurobiology, University of California, Irvine, CA., 92717.

Trigeminal ganglion (Vg) neurons innervate the rat whisker pad vibrissae and brain stem nuclei in a highly somatotopic manner. Immunocytochemical localization of the cell surface antigens, HNK-1, which recognizes neural crest cell derivatives and neural cell adhesion molecule (NCAM), which recognizes all Vg cells, has been employed in the same or adjacent sections of staged embryos to assess the contributions of neural crest and placodal cells to the Vg cell population. HNK-1 immunoreactive Vg cells are arranged dorsomedially within the rostral ophthalmic and maxillary lobes but are virtually absent from the mandibular lobe. Placode derived cells are distributed throughout the Vg. By E14, all HNK-1 immunoreactive cells become parvalbumin positive.

On E12, NCAM immunoreactive neurites extend further through the maxillary arch mesenchyme than HNK-1 labeled neurites. Thus, placode derived axons may pioneer the pathway to the periphery. Crest and placode derived axons enter the neural tube by E12 and contribute fibers to the trigeminal tract. Mesenchyme of the presumptive whisker pad is intensely NCAM immunoreactive on E13 before contact with infraorbital nerve fibers. By E18, the overlying epithelium becomes NCAM immunoreactive and is contacted by nerve fibers. Crest derived axons remain confined to the connective tissue sheath of developing whisker follicles; placode derived axons are located within this and the external root sheath.

Neural crest and neuroepithelial components of the Vg are known to respond differentially to nerve growth factor and brain derived neuronotrophic factor in vitro. Therefore, we are examining the expression of mRNAs encoding these substances in vivo and correlating this with gangliogenesis, axon outgrowth and target differentiation.

Supported by The Easter Seal Research Institute of Canada.

141.3

POLARIZED OUTGROWTH OF CIRCUMFERENTIAL FIBERS FROM EXPLANTS OF AVIAN EMBRYONIC SPINAL CORD. H.O. Nornes, E. Knapik*, and S. Kroger*. Dept. of Anat. and Neurobiol., Colorado State Univ., Ft. Collins, Co 80523, and Max Planck Inst. for Devel. Biol., Tubingen, F.R.G.

The neuroblasts in the alar plate of the neural tube have a stereotypic pattern of axon growth. The axons of the circumferential neuroblasts extend ventrally along the marginal zone to the floor plate where the axons change direction and extend longitudinally. The floor plate has been shown to release a chemotropic factor (Tessier-Lavigne et al., Nature, 336:775-778). The objective of this study was to analyze the growth of circumferential fibers from the alar plate in the absence of basal and floor plates.

Explants of the dorsal 1/2 of the neural tubes of chick embryos were grown on basal lamina substrate. Embryonic spinal cords of 4-5 D embryos were dissected free of their external limiting membranes (ELM), cut into longitudinal strips (300 um x 2 mm) or squares (300 um) and placed ELM-side down onto basal lamina prepared from retina of 8-9 D chick embryos, and cultured in F-12 media supplemented with 10% fetal bovine serum.

There was a polarized growth of axons from both the longitudinal strips and squares of the alar plate. The majority of the axons grew out from the side of the explant that was originally facing the basal/floor plates. These observations indicate that this polarized growth is inherent to the alar plate.

(Supported by NIH grant #NS-21309-04 to HON)

141.4

EXPANSION OF SENSORY DERMATOMES FOLLOWING EXPERIMENTAL NEURAL LESIONS AND IN SUBJECTS WITH SPINA BIFIDA: TROPHIC INTERACTIONS OF AFFERENT NERVES AND THEIR CUTANEOUS TARGETS. B.L. Munger[1], T.E. Jones*[1], K. Morohunfola*[1], D. Feinberg*[1], A. Mauger*[2], and R. Saxod*[2]. [1]Dept. Anat., Penn St. Univ., Hershey, PA 17033 and [2]Lab. Biol. Anim., Univ. Scient. & Med., Grenoble, France.

The present study analyzes effects of neural lesions in chick embryos and opossum pups, and compares the results with human spina bifida. Serial sections of chick embryos (operated at Stage 14, incubated 8 days) and opossum pups (operated day 1, survival day 1-14) were stained with silver, and whole mounts of chick skin stained for cholinesterase. Lesions of spinal cord and dorsal root (DRG) or trigeminal (TG) ganglia produced skin devoid of sensory nerves, feathers, and hairs. Skin near lesions was hyperinnervated, epidermis thickened, and precocious hairs formed. Axons invaded aneural skin from residual hyperplastic DRG & TG, and new waves of feathers and hairs formed. Opossum TG lesions resulted in altered patterns of eyelashes and auricles failed to separate from the head. Serial neurological examinations in patients with spina bifida document equivalent expansion of sensory dermatomes in 65% of 77 patients. These changes result from trophic interactions of afferent nerves and their cutaneous targets similar to the role of afferent nerves in development of muscle spindles (Zelena, 1957). Supported in part by USPHS Research Grant NS 19462.

141.5

DEVELOPMENT OF THE NASAL INNERVATION IN RAT EMBRYOS. M.-C. Bélanger*, R. Marchand and L. Bertrand*. Lab. Neurobio., Hôp. Enfant-Jésus, 1401, 18e rue, Québec, Qc. Canada, G1J 1Z4.

The olfactory system, though well studied in respect to its morphogenesis, has not been fully explored regarding the development of its connections. The fluorescent tracer DiI can diffuse passively along axonal membranes in fixed tissues (Godement et al., 1987). By implanting crystals of DiI in the olfactory epithelium of rat embryos (ages from $E16_0$ to $E18_0$), we could follow the development of several nerves. After implantation in the olfactory epithelium, the following results were obtained. The olfactory nerve was always labeled. The olfactory nerve layer, slightly labeled at $E16_0$ displayed a bright fluorescence from $E16_8$. A few fibers had already penetrated deeper in the brain at $E16_8$. At $E18_0$, the mitral cell layer and the lateral olfactory tract were also labeled, suggesting a trans-synaptic transport of DiI. The nervus terminalis was never labeled farther than the olfactory bulb (OB) though its cell bodies were seen as soon as $E16_8$. The nasociliary nerve was labeled at $E17_8$, and a few cell bodies were labeled in the dorsomedial part of the trigeminal ganglion. DiI was also inserted in the vomeronasal organ (in $E16_0$ to $E17_8$ embryo). The vomeronasal nerve was strongly fluorescent. Fibers were seen in the accessory olfactory bulb, which was situated more caudally as the embryos grew older. As early as $E16_0$, the nervus terminalis was labeled in the telencephalic septum where, at $E16_{18}$ and $E17_8$, fluorescent cell bodies were present. The pterygopalatine ganglion was labeled as soon as $E16_0$. From $E16_8$ distinct cell bodies were fluorescent in the ventral aspect of the ganglion. A few cell bodies in the ventral portion of the trigeminal ganglion were also labeled in all animals. In the $E17_8$ embryo, fibers of the spinal root of the trigeminal nerve were seen from the level of the nucleus principalis to the spinal trigeminal nucleus. Those results illustrate the rich innervation of the embryonic nasal epithelia, as soon as $E16_0$. (Supported by MRC, FRSQ, FCAR).

141.6

DISTRIBUTION OF TWO HEPARIN-BINDING NEURITE PROMOTING MOLECULES, AMPHOTERIN (p30) AND HB-GAM (p18) IN DEVELOPING RAT BRAIN.
E. Castrén, P. Panula, J. Merenmies* and H. Rauvala*. Departments of Anatomy and Medical Chemistry, University of Helsinki, 00170 Helsinki, Finland.

Amphoterin is a 30 kD heparin-binding protein that promotes neurite outgrowth of newly formed neurons presumably by an adhesive mechanism. This 214 amino acid protein is rich in lysine residues and has a polyanionic carboxy terminus consisting only of glutamic and aspartic acids. HB-GAM (heparin-binding growth-associated molecule) also binds strongly to heparin and promotes neurite growth. This 136 amino acid polypeptide is also rich in lysine residues, but has two polycationic heads rather than one cationic and one polyanionic.

We have studied the distribution of amphoterin and HB-GAM and their respective mRNAs in developing rat brain. Both amphoterin and HB-GAM are unevenly distributed in the developing rat brain. Amphoterin is present in cortical neurons and HB-GAM is widespread in superficial cortical laminae. HB-GAM is also found in distinct laminae of the olfactory bulb. Amphoterin gene is expressed in all areas of developing brain with high concentrations in cortex. The expression of both proteins is developmentally regulated. Amphoterin content is very high just before birth, whereas HB-GAM peaks one week postnatally. mRNA concentrations for both proteins follow the same developmental pattern as the respective protein levels.

These results suggest that amphoterin and HB-GAM may have an important role in brain growth and maturation.

141.7

THE SEQUENCE OF MYELIN FORMATION IN THE BRAIN OF THE NORTH AMERICAN OPOSSUM. G. Ghooray and G.F. Martin, Department of Anatomy and Neuroscience Program, The Ohio State University, Columbus, Ohio 43210.

The aim of this study was to observe the sequence of myelin formation in the opossum brain by using myelin basic protein (MBP) immunohistochemistry and the Weigert technique. By postnatal day (PD) 26 the white matter of the spinal cord exhibited some MBP immunoreactive structures. MBP-like immunoreactivity (LI) was also present in the reticular formation, the spinal and mesencephalic tracts of V, the trapezoid body and lateral leminscus, the medial longitudinal fasciculi, the superior cerebellar peduncles, and the posterior commissure. In contrast, the above areas did not stain for myelin by the Weigert technique until PD 47. By PD 33, additional MBP-LI was also found in the brainstem as well as within the optic chiasm and tracts, the supraoptic decussation, the presumptive medial forebrain bundle, and the internal capsule. The Weigert technique showed no evidence for myelin in any of these areas until sometime between PD 54 and 68. MBP immunostaining developed rapidly between PD 26 and 40 during which time most of the tracts in the medulla and pons became immunoreactive. By PD 41, MBP-LI was present in most, if not all, of the appropriate areas in the spinal cord, medulla and pons as well as within many areas of the midbrain and forebrain. In the forebrain, immunostained areas included the fornices, the vertical limbs of the diagonal band of Broca, and the olfactory tracts. These structures were not stained by the Weigert technique until PD 68. By PD 62, MBP-LI was present in the ventral (olfactory) part of the anterior commissure, but the neocortical portion was not immunostained until PD 77. The ventral part of the anterior commissure was not stained with the Weigert technique until about PD 81. Although the sequential appearance of MBP immunostaining and Weigert staining appeared to be comparable, MBP-LI was present well before myelin could be demonstrated by the latter technique. (Supported by NS-25095).

SPECIFICITY OF SYNAPTIC CONNECTIONS

142.1

TRANSIENT EXPRESSION OF CELL-SPECIFIC LABELS ON GROWTH CONES AND DEVELOPING SYNAPSES IN THE GRASSHOPPER EMBRYO. H. Reichert and T. Meier*. Dept. of Zoology, University of Geneva, CH-1211 Geneva, Switzerland.

The construction of a functional nervous system requires a high degree of neuronal specificity for establishing the correct pattern of synaptic connections. Neuron-specific molecular labels may be involved in generating this specificity. We are studying such highly specific neuronal labels by using monoclonal antibodies to characterize molecules that are transiently expressed on the growth cones and developing synaptic terminals of identified neurons in the grasshopper embryo during the period of axonal outgrowth and synaptogenesis.

Several cell-specific molecular labels of this type have been found. The molecular label with the highest degree of specificity is expressed in two pairs of identified projection neurons, which have their cell bodies and dendritic arbors in the brain and project to the ganglia of the ventral nerve cord. During axonogenesis, the label becomes concentrated in the axonal growth cones of these neurons. Later during synaptogenesis, the label is predominantly expressed on the membrane of the axon collaterals and synaptic boutons of these neurons. This neuron-specific label is not expressed in any other of the approximately one million neurons in the nervous system, nor in any other cell in the developing embryo.

These findings suggest the existence of a molecular labeling system that is remarkably specific for individual cells and may be important for establishing precise synaptic connectivity. The isolation and characterization of these molecules are now in progress. (Supported by the Swiss NSF).

142.2

POSITIONAL INFORMATION CONTROLS AXON MORPHOLOGY AND SYNAPTIC CONNECTIVITY VIA INDEPENDENT MECHANISMS.
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We are using the insect peripheral nervous system to study the effects of positional information on sensory axon morphology and choice of synaptic partners. Each cercus of the first instar cockroach *Periplaneta americana* has two wind-sensitive filiform hair sensory neurons, one medial (M) and the other lateral (L). The L axon runs in the lateral tract of the cercal nerve, and forms a characteristic arborization within the terminal ganglion, where it forms a strong monosynaptic connection onto giant interneuron 3 (GI3) but does not synapse with GI2. The M axon follows the medial tract, has a different morphology, and evokes large monosynaptic EPSPs in GI2 and small EPSPs in GI3. We have bred a strain of cockroaches in which supernumerary hairs arise at different positions on the cercus. Supernumerary lateral neurons have the same axonal branching pattern and synaptic connectivity as the normal L neuron, while supernumerary medial neurons are similar to the M neuron, thus supporting the hypothesis that circumferential position controls both axon morphology and synaptic connectivity. Very infrequently, extra sensory neurons arise on the cercal midline (C neurons). In 4 out of 4 cases, the C axon follows the lateral tract of the cercal nerve, and has a arborization similar to that of L. However, the C axon always forms M-type synapses. We conclude that the circumferential position in which the neuron is born may control both its axon morphology and choice of synaptic partners, but via independent mechanisms. (Supported by NIH Grant NS07464 and the SERC, U.K.)

142.3

PERIPHERAL LOCATION OF CUES THAT SPECIFY MUSCLE AFFERENTS. P. Wenner and E. Frank. Dept. of Neurobiology, Anatomy and Cell Science, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15261

How are neurons specified during development to make their appropriate connections? Motoneurons appear to be committed to supply a particular target muscle at the time their axons exit the spinal cord. In contrast, muscle sensory neurons can innervate a variety of peripheral targets; some aspect of their peripheral projection then appears to specify their central connections with motoneurons. This peripheral influence might arise from the target muscle itself, or the sensory axons might receive cues directly from the motoneurons with which they grow.

To study the location of this peripheral cue, we made dorsoventral rotations of chick hindlimbs at the level of the knee at stages 20 - 22, before sensory or motor neurons have grown into the limb bud. This surgical manipulation forces motoneurons to innervate inappropriate (and often antagonistic) muscles (Whitelaw and Hollyday, 1983). Do muscle afferents supplying these muscles make central connections appropriate for the muscles themselves or for the motoneurons with which they grow?

Muscle sensory input to lumbosacral motoneurons was assessed using intracellular recordings from isolated spinal cords. In normal embryos, muscle afferents project strongly to motoneurons supplying their own muscle (homonymous inputs) but only weakly to functionally unrelated motoneurons. Some antagonistic motoneurons actually receive disynaptic inhibition. Preliminary results suggest that in embryos with rotated hindlimbs, muscle afferents make central connections appropriate for the muscle they supply, not for the motoneurons they grow with. Motoneurons supplying rotated muscles were less likely to receive homonymous monosynaptic input, and we have seen some cases of homonymous disynaptic inhibition. These results provide further evidence that muscle afferents are not specified to supply a particular muscle as they grow into the limb, and that their specification depends upon some signal in the rotated part of the limb. (Supported by NSF to EF)

142.4

A GENETIC ANALYSIS OF THE GENE *UNC-55* AND THE D MOTONEURONS IN *CAENORHABDITIS ELEGANS*. W. W. Walthall, H. Park* and E. A. Murry* Dept. of Biology, Georgia State University, Atlanta, GA. 30303

Fourteen hours after hatching in the nematode *C. elegans* a class of embryonic motoneurons, the DD cells, respecifies its pattern of synapses. At about the same time a related set of postembryonic motoneurons, the VD cells, complete differentiation and assume a pattern of connections equivalent to the initial pattern of the DD cells (White et al., Nature 271,1978). Morphologically and biochemically representative neurons from each class are indistinguishable. Eight genes have been identified by mutation that cause identical changes in both classes suggesting that they have similar genetic programs. However, the two classes of neurons have different patterns of synapses. Mutations in a single gene, *unc-55* (uncoordinated), suggest that its gene product is responsible for establishing the differences in target specificity that distinguish the two classes. In *unc-55* alleles the VD and DD motoneurons have identical patterns of synapses (Nawrocki and White, personal communication). We have expanded the collection of *unc-55* alleles from 3 to 10, and have found that placing the most severe allele, *jd1*, over the corresponding chromosomal deletion does not increase the severity of the uncoordination. Thus the null phenotype is not lethal and this is one criterion for establishing it. Another allele, *jd5*, is temperature sensitive: animals grown at 25°c are uncoordinated, animals grown at 15° are normal. Temperature shift experiments are underway to determine the critical period for the expression of the gene.

142.5

DEVELOPMENTAL INTERACTIONS BETWEEN CHEMICAL AND ELECTRICAL SYNAPSES IN IDENTIFIED LEECH MOTOR NEURONS. G.K. Bryan and W.B. Kristan, Jr. Biology Dept., Univ. of Calif. - San Diego, La Jolla, CA 92093.

We are investigating interactions in the development of synapses among identified motor neurons in the leech Hirudo medicinalis. The dorsal excitatory motor neuron, DE-3, excites dorsal longitudinal muscles, and the dorsal inhibitors, DI-1 and DI-102, inhibit the same muscles; they also inhibit DE-3. This neuronal inhibition is known to be important for two different leech behaviors, swimming and local bending. In addition, DI-1 is electrically coupled to DI-102. We want to know whether this coupling plays a role in the establishment of the inhibitory connection onto DE-3. We have previously investigated the morphological development of DE-3 from embryonic day 10 to the adult (Kristan and Jellies, Soc. Neurosci. Abstr., 1989, 15: 498) and have found an orderly development of the neurites. We characterize the development of electrical coupling between DI-1 and DI-102 by pairwise intracellular recording from them, then we fill the two cells with different dyes to characterize their morphological development. In a similar way we use pairwise recordings and dye fills of DE-3 with each inhibitor to characterize their chemically mediated inhibition and associated morphological development. These studies will show whether the electrotonic connections of DI-1 and DI-102 are established prior to their chemical inhibition of motor neuron DE-3. Supported by USPHS research grant NS25916.

142.6

SEGMENTAL ORGANIZATION OF VIth NERVE RELATED MOTONEURONS IN THE CHICK HINDBRAIN. R. Baker and D. Noden*, Dept. of Physiol. and Biophys., NYU Med. Ctr., New York, NY 10016, Dept. of Anat., New York State Coll. of Vet. Med., Cornell Univ., Ithaca, NY 14853.

Avian embryos have been studied with carbocyanine dyes from H-H Stages 16-35 in order to establish the early temporal and spatial relationship between motoneurons and extraocular muscle anlagen. All mesodermal muscle precursors are reported to originate from the prechordal plate between H-H 8-10 and later become aligned in a paraxial segmental array in the head mesenchyme surrounding the neural tube. Sixth nerve related motoneurons arise medially from neuroblasts in two segments of the hindbrain neuroepithelium - rhombomeres 5 and 6. At H-H 16 each rhombomere contains 3-6 cellular clusters that sends axons towards the common mesenchymal muscle precursor. Although the collective rootlets from r5 and r6 fasciculate beneath the hindbrain and travel together, they are distinct, and by H-H 18 each enters an individual muscle condensation. Frequently, 3 distinct terminal arborizations distinguish between muscle primordia of the lateral rectus, pyramidalis and quadratus that can only be recognized later following primary myotube specification. At this time, H-H 32-35, each innervated muscle condensation begins to diverge toward its scleral insertion site. Also, the accessory abducens motoneurons in r5 migrate to a ventro-lateral position in the medulla. Since the separate populations of abducens and accessory abducens motoneurons are placed in register with appropriate paraxial mesodermal units, these data demonstrate an unexpected early source of axial position signals intrinsic to the hindbrain r5 and r6 neuromeres. Thus, motoneurons from adjacent rhombomeres distinguish between peripheral musculature producing the different somatic motor behaviors of eye rotation and translation. We hypothesize that neuromeric specific phenotypes underlie positioning of the motoneuronal pools just as neural crest derived mesemchyme patterns all peripheral head muscles. Supported by grant NEI-02007 and DE-06632.

142.7

TOPOGRAPHICALLY SELECTIVE MOTOR REINNERVATION OF THE RAT'S SERRATUS ANTERIOR MUSCLE AFTER A NEONATAL LESION. M. B. Laskowski, M. DeSantis, A. Norton, and P. Berger. Dept. of Biol. Sci. & WAMI Program, Univ. of Idaho, Moscow, ID 83843.

The rat's serratus anterior (SA) muscle has seven sectors that are innervated by motor neurons of spinal segments C6 and C7; their axons form the long thoracic (LT) nerve. Normal development results in a topographic projection such that C6 motor neurons predominate over those of C7 in more rostral sectors of SA and vice versa for the most caudal sectors. We compared the sequence of events that take place in an optimal reinnervation paradigm with those that occur during normal development. Within 48 hrs of birth anesthetized rats had the LT nerve frozen where it passed between SA rostral sectors I and II. After recovery periods from 32 hrs to 70 days, the rats were reanesthetized and the C6 and C7 roots, the LT nerve and the SA muscle were dissected as a unit and superfused with Ringers. Intracellular recordings were made from muscle fibers in each sector of SA while stimulating by turns the C6 and C7 ventral roots. Sectors II through VII were reinnervated sequentially from rostral to caudal between 2 and 8 days after the lesion. For all time periods (6.5 to 70 days), the average target fields of the C6 versus C7 motor neurons were significantly different from each other, but not to as great an extent as in sham-operated and unoperated controls. The average target fields at one week reinnervation time (initial contact) were as precise as at 10 weeks. This means that topographic selectivity is established by motor neurons at an early stage of reinnervation rather than being a gradual reorganization from a random outgrowth to the muscle. Reinnervation of SA after a neonatal lesion resembles embryological development in that a topographically selective pattern is present from the earliest stages so far examined (E-17). They differ in that topographic selectivity is enhanced over a period of several postnatal weeks in normal development but does not improve following reinnervation. (Supported by NIH, NS 27024)

142.8

LACK OF TOPOGRAPHY IN MOTOR NEURON PROJECTIONS TO MUSCLE FIBERS IN THE NEONATAL RABBIT SOLEUS MUSCLE. K. S. Cramer and D. C. Van Essen, Division of Biology 216-76, California Institute of Technology, Pasadena, CA 91125

A topographic arrangement of projections from spinal motor neurons to muscle has been found in some, but not all, mammalian skeletal muscle. We have examined the topography of innervation in the neonatal rabbit soleus muscle with respect to the rostrocaudal position of the innervating motor neurons in the spinal cord. The extensive polyinnervation at postnatal days 4-5 allowed us to use the tension overlap method to assess whether neurons which are clustered in the spinal cord are more likely to innervate common muscle fibers than neurons which lie in different regions of the soleus motor pool. Using an *in vitro* preparation, we stimulated pairs of ventral root filaments together as well as separately, and measured the tetanic tension generated in each case. Tension overlap is defined as the degree to which tetanic tensions of pairs of filaments were not additive. Assuming that stimulation of all inputs to a fiber will not evoke more tension than stimulation of any single suprathreshold input, we used tension overlap to estimate the percentage of muscle fibers in each filament which were shared between the two filaments. We compared overlap for neighboring filaments to that for widely separated filaments along the rostrocaudal axis of the spinal cord.

Our results indicate that for 10 pairs of adjacent multiunit filaments which contributed 29 $\pm$ 4% of the maximum tension, the mean overlap was 44 $\pm$ 9%; for 10 pairs of distant filaments which contributed 28 $\pm$ 4% of the maximum tension, the mean overlap was 41 $\pm$8%. Thus, the overlap was not significantly different between these two groups. As expected, these overlap values were significantly higher than those found in singly innervated control muscles from older animals. These results suggest that the projection to the neonatal soleus muscle has little, if any, topographic organization.

142.9

Loss of Regenerative Specificity Coincides With the Development of Myelination in the Bullfrog Tadpole. M.L. Meeker and P. B. Farel. Dept. Physiol., Univ. N. Carolina Sch. Med., Chapel Hill, NC 27599

Transected lumbar ventral root axons are able to regenerate to the proper hindlimb region during the first third of larval (tadpole) life in the bullfrog, after which time regenerative specificity is lost (Farel and Bemelmens, 1986). Based on an analysis of reinnervation errors we have hypothesized that regenerating axons in older tadpoles have the capability of responding to guidance cues, but may be prevented from doing so by the development of non-neural structures in the hindlimb (Lee and Farel, 1988). The most likely such structure is the basal lamina secreted by Schwann cells and which surrounds all peripheral axons. The basal lamina forms a continuous tube that persists following axon transection, even though the distal axon and myelin degenerate. Regenerating axons entering the incorrect tube might be constrained from responding to guidance cues and thus forced to innervate the structure at the distal opening of the particular tube they had entered.

To test this hypothesis, we examined the development of myelination at the ultrastructural level. We found that the development of myelination and of the basal lamina around axons coincides with the loss of regenerative specificity. These findings are consistent with previous studies of cross-stage limb transplants which show that motor axons from older animals have the capability of responding to at least some guidance cues. Thus, motor axons may be capable of responding to guidance cues in the hindlimb at developmental stages when specific reinnervation is not typically expressed.

142.10

A COMPARISON OF IMMUNOSUPPRESSION TECHNIQUES AND THEIR APPLICATION TO THE PRODUCTION OF MONOCLONAL ANTIBODIES SPECIFIC FOR AXONS ON THE NASAL SIDE OF THE DEVELOPING RETINA. C. Vermeersch, C. Stechmann* and S. McLoon. Univ. of Minnesota, Minneapolis, MN 55455.

Monoclonal antibodies produced from immunizations with complex mixtures of antigens have allowed identification of numerous molecules important in developmental processes. A common problem in the application of this technology is the tendency of the immune system to recognize only a few immunodominant antigens. Consequently, it has been difficult to generate antibodies to minor antigens that may play an important role biologically. Several investigators have designed approaches to suppress the immune system to common antigens in order to favor the production of antibodies to the minor antigens. These techniques include injecting uninteresting antigens into newborn mice or killing activated immune cells with cyclophosphamide. The degree of immunosuppression achieved with different techniques and combinations of techniques was compared in this study.

The optimal immunosuppression technique was applied to the production of antibodies to axons from the nasal side of the developing chick retina. Hybridomas were produced from mice suppressed with temporal retinal tissue and immunized with nasal retinal tissue. The antibodies generated were screened by ELISA for recognition of protein from nasal but not temporal retina. Three antibodies to nasal retinal axons have been identified.

142.11

ROSTROCAUDAL POLARITY OF AVIAN OPTIC TECTUM AND ORDERED NERVE CONNECTION. Hiroyuki Ichijo*, Toru Matsuno* and Harukazu Nakamura. Dept. of Pathol. and Biol. Kyoto Pref. Univ. of Med. Kyoto 603 Japan.

Retinotectal projection is organized precisely in a retinotopic manner. Nasal retinal axons project to the caudal tectum, and temporal axons to the rostral tectum. We carried out rotation of the tectal primordium at an early stage of development to understand the mechanism of retinotectal map formation.

We used three marking systems. Quail tissue was used as a graft and transplanted into a chick embryo by rotating rostrocaudal axis 180°. Dio was applied to a graft locally to ascertain orientation of the graft. We could make sure of orientation of the graft. DiI was used to examine topographic relationship between the retina and the tectum.

Nasal retinal axons projected to the caudal side of the rotated tectum (originally rostral side of the tectal primordium), and temporal axons to the rostral side of the rotated tectum (originally caudal side). This pattern was the same as the normal projection. Our results showed plasticity of rostrocaudal polarity of the tectum primordium.

142.12

CELL BIRTH DATE DOES NOT PREDICT THE VIBRISSA FOLLICLE INNERVATED BY A GIVEN TRIGEMINAL PRIMARY AFFERENT NEURON. W.R. Bauer, C.M. Goddard*, N.L. Chiaia, G.J. Macdonald*, H.L. Enfiejian and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

The trigeminal (V) primary afferent neurons innervating different rows of mystacial vibrissa follicles in rats are organized in partially overlapping, rostrocaudally oriented bands that generally parallel the long axis of the ganglion. It has been proposed that, during development, vibrissa follicles are innervated in a caudal to rostral order. This, in turn, suggests that ganglion cells born early during gestation may innervate caudal follicles while cells born later might innervate rostral follicles. We tested this possibility directly by combining prenatal injections of [^{3}H]-thymidine with retrograde tracing from the C-1 and C-4 vibrissa follicles in adulthood. The V primary afferent neurons supplying the mystacial vibrissa follicles are born throughout the period of V ganglion neurogenesis (embryonic days 9-14) and those innervating the C-1 and C-4 follicles have essentially the same distribution of birth dates. Furthermore, primary afferent neurons born on a given day and innervating a given follicle are distributed throughout the band of all ganglion cells supplying that vibrissa. Thus, V ganglion cell birth date cannot be readily related to the organization of the sensory innervation of the mystacial vibrissae follicles. Supported by DE 07734, BNS 85 17537, and funds from the State of Ohio Research Challenge.

142.13

EFFECT OF FETAL DEAFFERENTATION UPON THE ORGANIZATION OF SOMATOSENSORY REPRESENTATIONS IN THE GRACILE, CUNEATE AND TRIGEMINAL NUCLEI OF ADULT RATS. H.P. Killackey, J.T. Wall, N.L. Chiaia, and R.W. Rhoades. Dept. of Psychobiology, Univ. of Calif., Irvine, CA 92717 and Dept. of Anatomy, Medical College of Ohio, Toledo OH 43699.

Prenatal forelimb removal in rat increases the portion of primary somatosensory cortex devoted to the representation of the hindlimb. We recorded from 3 adult rats that sustained left forelimb removals on embryonic day 16 to determine the extent to which this reorganization might reflect altered brainstem somatotopy. Receptive fields were mapped for unit clusters in the gracile, cuneate, and trigeminal nuclei at, and up to 2 mm caudal to, the obex. Data from the deafferented side of the brainstem were compared with those from penetrations on the intact side and with recordings from 2 normal adults. In each experimental animal, units with receptive fields restricted to the remaining forelimb stump and/or shoulder were recorded in the part of the brainstem normally containing the cuneate forepaw and forelimb representations. In addition, there were small reductions in the mediolateral extents of the portion of the brainstem devoted to the residual forelimb and shoulder representations and the cuneate nucleus as determined from cytochrome oxidase stained sections. The changes in somatotopy induced by fetal limb removal appear less extensive in brainstem than in cortex. Supported by DE 07734, BNS 85 17537, NS 21105, BNS 87 19311, and DE 08971.

142.14

CHROMOGRANIN A IN THE OLFACTORY SYSTEM OF THE RAT M. Gratzl*, G. Lahr*, C. Heiss*, A. Mayerhofer*, K. Schilling*, R.J. Parmer*, Ch. Pilgrim, and D.T. O'Connor*. Abt. Anatomie und Zellbiologie, University of Ulm, D-7900 Ulm, FRG; Univ. of California, San Diego, CA 92161, USA.

Olfactory bulbs of the rat contain chromogranin A (CGA) in amounts comparable to those of rat adrenal or hypophysis. In various brain regions including hippocampus CGA could not be detected by immunoblotting, while CGA mRNA was found in all brain regions examined, including the olfactory bulb. In situ hybridization histochemistry and immunocytochemistry revealed CGA synthesis in cell bodies of mitral and tufted cells in the external plexiform layer and in the periglomerular region of the olfactory bulb. Immunocytochemically, CGA was also detected in the axonal terminals of mitral and tufted cells (primary olfactory cortex and amygdaloid nucleus) but not in the olfactory glomeruli, where the incoming olfactory nerve fibers of the primary olfactory neurons establish synaptic contacts. Thus CGA in mitral and tufted cells is specifically transported into their axonal terminals but not into their dendrites. We propose that the rat olfactory system could serve as a model for the study of CGA function in signal transduction.

142.15

TRANSMITTER-MEDIATED INTERACTIONS BETWEEN CULTURED SYMPATHETIC AND HIPPOCAMPAL NEURONS OF THE RAT. M.D. Johnson, A.G. Yee and D.D. Potter. Dept. of Neurobiology, Harvard Med. Sch., Boston, MA 02115

When dissociated neurons of the superior cervical ganglion (SCG: hooded rats, 0-6 d old) were co-microcultured with cells of the hippocampal formation (HF) in the presence of NGF, transmtter-mediated interactions were frequently seen. In 23 of 52 trials, activity of an HF neuron resulted in a psp-like event in an SCG neuron; 91% of these effects were depolarizations that were usually less than 15 mV in amplitude. Bicuculline (10 μM) partially or completely blocked these events, reversibly, in 10 of 11 trials; this is evidence for GABAergic interaction. The similarity of the time course of these events to conventional nicotinic cholinergic epsp's in the SCG network raises the possibility that the two types of neurons were synaptically linked. In 18 of 51 trials, stimulation of an SCG neuron produced a rapid and/or slow, depolarizing and/or hyperpolarizing effect on a HF neuron. In 9 trials of atropine, 4 cases were completely blocked, 4 cases partially blocked and one case was not affected. The origin of the atropine-resistant interactions and the effects of norepinephrine and other transmitters are being investigated, as is the morphological basis for interaction between the two types of neurons. Supported by NS 02253.

142.16

THE DENDRITIC ORGANIZATION OF GABAERGIC PARVALBUMIN-CONTAINING NEURONS IN THE RAT HIPPOCAMPUS IS ALTERED BY PARTIAL DEAFFERENTATION

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Entorhinal afferents form synapses with dendrites of parvalbumin (PARV)-immunoreactive neurons in the rat fascia dentata. In this study we analyzed the postlesional effects of entorhinal deafferentation on the dendritic morphology of the postsynaptic PARV-positive cells. PARV-immunocytochemistry was performed 2,8 and 55 days after a unilateral entorhinal lesion. Two days after the lesion we observed an abnormal swelling of the distal tips of PARV-positive dendrites in the termination zones of the perforant path. The distal dendrites of immunoreactive neurons seemed to retract from these zones. 8 days after the lesion PARV-positive distal dendrites were reduced by 70% in the outer molecular layer and by 40% in the stratum lacunosum-moleculare. This reduction of PARV-positive dendrites in the entorhinal termination zones persisted 55 days after the lesion. In the electron microscope, we observed abnormal glial invaginations in the PARV-positive dendrites. Our results suggest a high neuronal specificity of this hippocampal input as sprouting of nearby afferents (e.g. commissural fibers) is not effective in restoring a normal pattern of PARV-positive dendrites.
(This study was supported by the DFG: SFB 45 and Fr 620/1)

142.17

DEVELOPMENT OF CORTICOSPINAL ARBORS IN THE POSTNATAL HAMSTER. R.Z. Kuang and K. Kalil. Dept. of Anatomy, Univ. of Wisconsin, Madison, WI 53706.

A previous study of corticospinal axon arbors showed that when one pyramidal tract is severed early in development contralateral corticospinal axons sprout into the denervated spinal cord, maintaining topographic and functional specificity. We wished to determine whether sprouting was an extension of early bilateral connections or occurred in response to injury, and how corticospinal arbors achieved specificity during development. DiI crystals were inserted into the hindlimb sensorimotor cortex of hamsters 3-6 days postnatal. After 2-6 day survival times animals were perfused with 4% paraformaldehyde, and the brains and spinal cord sectioned with a vibratome at 50µm. Sections were viewed under fluorescence microscopy to visualize DiI labeled corticospinal axon arbors. Results show that (1) axons grow out from the dorsal column with no significant waiting period (2) axons innervate only one side of the spinal cord with no transient bilateral connections (3) functional sensory and motor specificity of corticospinal arbors innervating the dorsal and ventral horn is apparent as soon as axons grow out (4) axons growing into the dorsal or ventral horn begin to branch immediately. These results show that specificity of adult corticospinal axon arbors is apparent from the onset of target innervation and does not occur by the paring away of diffuse or inappropriate branches. Second, injury induced sprouting does not occur by the maintenance of transient bilateral connections but arises de novo from signals similar to those present during development. (Supported by NIH grant NS-14428).

142.18

CHARACTERISTICS OF VISUAL CALLOSAL TRANSFER IN NORMALLY REARED KITTENS. C.Milleret* J.C.Houzel* and P.Buser *(1).(SPON : European Neuroscience Association). Lab de Neurophysiologie, Collège de France ;(1) Dept de Neurophysiologie comparée CNRS & UPMC,75005 Paris France.

While abundant data is available on the characteristics of the callosal transfer of visual information in the adult cat, surprisingly little is known in the kitten. The present study considers this transfer in animals aged from 2 to 9 weeks.

Fifteen kittens underwent a midsagittal section of their optic chiasm under Saffan anaesthesia. Two days later, they were again anaesthetized and paralyzed with flaxedil. Unit activities were recorded from the quasi total extent of visual cortical areas 17 and 18 and were tested for their responses to stimulation of the contralateral eye through slits and bars projected into the visual field.

From the analysis of 548 cells, it appeared that callosal transfer (observed in 71 cells) : 1) was already effective in the youngest animals tested, at an age when the first visual cortical responses are detectable according to classical data; 2) only concerned the central vertical meridian of the visual field; 3) mainly implicated cells at the 17/18 border (identified through Nissl or Cytochrome-Oxidase staining); 4) differed however from the adult through weaker visual responses and larger receptive fields.

Visual callosal transfer in kitten is thus very similar to that in the adult. Transient callosal projecting neurons described by anatomists do not seem to establish detectable functional connections with the contralateral visual cortex at least outside the 17/18 border.

ENDOCRINE CONTROL AND DEVELOPMENT I

143.1

NEURONS BORN DURING SONG LEARNING DO NOT CONTRIBUTE TO THE MAJOR EFFERENT PROJECTION OF AREA X. K. W. Nordeen, F. Sohrabji, and E. J. Nordeen. Dept. Psych., U. Rochester, Rochester, NY 14627.

Area X is a large, sexually dimorphic nucleus that has been implicated in avian song learning. During song learning in zebra finches, the number of new neurons added to this region is far greater in males (who sing), than in females. In this study we determined if the Area X neurons added during song learning form part of the efferent projection to the medial nucleus of the dorsolateral thalamus (DLM), and if this projection is sexually dimorphic. Male and female zebra finches received 2.5uCi/gm 3H-thymidine daily between 15 and 34 days posthatch. The birds were killed at 60 days, 5 days after receiving an injection of the tracer Fluorgold (Flg) into DLM. In both sexes the region corresponding to Area X was examined in autoradiograms for thymidine and retrogradely-labeled cells.

The density of cells projecting to DLM (Flg-labelled) was about 50% greater in males than in females. However, in neither sex did this sexually dimorphic pathway include neurons born during adolescence (thymidine-labeled). Hence, the majority of neurons added to Area X during song learning are not recruited into the Area X-DLM pathway and are likely to be interneurons. Furthermore, the sexually dimorphic projection to DLM probably consists of earlier-born neurons incorporated before or during the onset of song acquisition (<20 days). In fact, preliminary studies using the tracer DiI indicate that this pathway is present in males as early as 15 days.

143.2

CHARACTERIZATION OF A TRANSIENT PATHWAY LINKING AN AVIAN SONG NUCLEUS WITH THE VENTRICULAR ZONE DURING SEXUALLY DIMORPHIC NEURON ADDITION. F. Sohrabji, E. J. Nordeen, and K. W. Nordeen. Dept. Psych., U. Rochester, Rochester, NY 14627.

Area X is a sexually dimorphic region critical for avian song learning. Early estrogen (E2) exposure masculinizes this region by stimulating addition of neurons born during adolescence. Identifying putative proliferative zones and migratory paths for these neurons will aid in determining whether E2 stimulates their production or survival. Here we describe a transient pathway linking Area X to the ventricular germinal zone (VZ) during hormone-dependent neuron addition.

Brains of male zebra finches (12d, 25d, and >120d) were perfused and a crystal of dil placed into the lobus parolfactorius (LPO) either inside or outside of Area X. Following a 3 week incubation period, the position of labeled cells and fibers were traced in coronal sections.

At 12 days, injections of dil into Area X labeled fibers leading to cell bodies in the VZ dorsal to the lamina medullaris dorsalis. DiI injections outside of Area X labeled a distinctly different portion of the VZ. The VZ-Area X pathway was sparse at 25 days, and absent in adult birds. The morphology of labeled VZ cells and the orientation of their processes resembled those of avian radial glia (Alvarez-Buylla and Nottebohm, 1989). In support of this hypothesis, we have recently identified vimentin-positive fibers oriented similarly to the dil-labeled pathway. This Area X-VZ projection may reveal the source and migratory route of Area X neurons, thus facilitating identification of cellular processes underlying sex differences in the addition of Area X neurons.

143.3

THE RAT VISUAL CORTEX IS SEXUALLY DIMORPHIC BY WEANING AGE. P. Seymoure and J. M. Juraska. Dept. of Psychology, University of Illinois, Champaign, IL 61820.

Recently our laboratory reported sex differences (male > female) in laminar thickness in the binocular (Oc1B) region of the visual cortex of adult socially housed Long-Evans rats (Reid & Juraska, Neuro. Abs. 1989). In the present study cortical depth was measured in the monocular (Oc1M) and Oc1B regions of littermate pairs of 25-day old male and female Long-Evans rats to determine if sex differences in cortical depth were present before puberty. The weaning-age males had thicker layer I, layer II-IV, and total cortical depth than females in Oc1B. No sex differences were found in Oc1M layers. The possibility that sex differences in dendritic field size might account for the differences in layer thickness was explored by quantifying Golgi-Cox stained layer III pyramidal neurons from the visual cortex of 7 littermate pairs of weaning-age Long-Evans rats. Analysis of the dendritic tree revealed that females had a 12% greater length in the basilar tree and a 19% greater length of apical oblique branches than males. We are presently analyzing whether the differences in dendritic size vary between Oc1M and Oc1B. At this time, the relationship between the sex differences in the thickness of the upper layers and dendritic field size is unclear. Supported by NSF BNS 89-09164.

143.4

SEX DIFFERENCES IN THE ULTRASTRUCTURAL DEVELOPMENT OF THE RAT CORPUS CALLOSUM. J.H.Y. Kim and J.M. Juraska. Neuroscience Program & Dept. of Psychology, Univ. of Illinois, Champaign, IL 61820

Our previous research has demonstrated that 55 day old female Long-Evans hooded rats have more axons than male rats in the splenium of the corpus callosum (Juraska & Kopcik, Br.Res. 1988). This sex difference could be due to developmental differences in axonal outgrowth and/or withdrawal in the corpus callosum prior to 55 days of age. To investigate this, we examined the development of the corpus callosum at 15 and 25 days of age. First, gross size measurements of the splenium (posterior fifth) were obtained from brains cut midsagittally and Weil stained en block. No sex differences were found, but there was an increased area in 25 day old relative to 15 day old rats. Second, the splenial region was sampled with electron microscopy in a dorsal to ventral fashion. There were no sex differences in the number of axons at 15 days of age. At 25 days, females had significantly more unmyelinated axons than their male littermates, similar to the pattern previously seen in young adults. The number of myelinated axons increased in both sexes between 15 and 25 days. In females, the number of unmyelinated axons increased from 15 to 25 days of age. This latter result is startling enough that we are adding subjects to confirm that the corpus callosum can continue to add axons after 15 days of age. Supported by NSF BNS 89-09164.

143.5

DO DIFFERENCES IN NEURON NUMBER CONTRIBUTE TO THE SEX DIFFERENCES IN THE SIZE OF THE BINOCULAR AREA OF THE RAT VISUAL CORTEX? S.N.M. Reid and J.M. Juraska. Neuroscience Program & Dept. of Psychology, Univ. of Illinois, Champaign, IL 61820

We have shown that there are sex differences (M>F) in the overall thickness of the binocular area (the 17/18a border) of the hooded rat visual cortex (Reid & Juraska, Neuro. Abst. 1989). In order to investigate whether sex differences in neuron number are the basis for the differences in thickness, we first established the volume of each layer within the binocular area of each sex. The posterior cortex of nine littermate pairs of rats, socially housed to 90 days of age, was frozen sectioned at 40 microns and stained with methylene blue-azure II. The borders of the binocular area (Oc1B according to Zilles, 1985) were delineated and the boundaries of each layer were identified. Serial reconstruction of the area from 5-8 sections (both hemispheres) were made with a Eutectic imaging system. Male rats had a significantly greater volume overall (11.8%) and in each layer (I, II- III, IV, V, VI). No hemispheric differences were found. Currently, neuronal number and size are being estimated through the disector method (Sterio, J.Micro. 1983) on semi-thin sections obtained from the same animals that were used for the volume estimation. Supported by NSF BNS 89-09164 & HD07333.

143.6

NEONATAL EXPOSURE TO GONADAL HORMONES AFFECTS CATECHOLAMINE SYSTEMS IN THE DEVELOPING CORTEX OF THE RAT. J. Stewart, S. Kühnemann and H. Rajabi. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Canada, H3G1M8.

The effects of neonatal castration of male, and TP treatment of female rat pups on levels of monoamines and metabolites in the cerebral cortex were assessed using HPLC with electrochemical detection. In a first experiment, pups were killed at 0, 4, 10 and 21 days of age, and the anterior and posterior portions of cortex in each hemisphere were rapidly removed. At 21 days of age the levels of DA in anterior cortex were higher in males and TP-treated females than in females and GX males. However, dopaminergic activity developed earlier in females than in males and the gonadal hormone manipulations shifted the pattern of development to that of the other sex. In a second experiment, the effects of these same gonadal hormone manipulations on the uptake, metabolism and storage capacity of CA neurons in cingulate (CING), agranular insular (AID), parietal (PAR) and occipital (OC) cortex were estimated at 4 and 10 days of age by considering the difference between measured CA in animals pretreated with vehicle or reserpine and then given l-dopa (Coyle & Molliver, 1977). Again, the data indicated earlier development of CA neurons in females, especially in AID. DA was found to account for the group differences; when only DA was considered it was found that at 4 days of age females had the highest levels in every area with the exception of OC where GX males had equally high levels. These data suggest a mechanism that might account for sex differences in the development of specific cortical regions

143.7

PUBERTAL-RELATED INFLUENCES ON HYPOTHALAMIC NOREPINEPHRINE PROJECTIONS. S. Choi and C.K. Kellogg. Dept. of Psychology, Univ. of Rochester, Rochester, NY 14627.

Earlier studies have indicated that norepinephrine (NE) uitilization (following synthesis inhibition) was undetectable in the diencephalon until after day 14 and that NE turnover in the hypothalamus (HY) at 28 days was only one third adult rates. NE turnover rate in the cortex (CTX) was found to be adult-like at birth. During puberty, organisms undergo dramatic hormonal changes, and the events occurring during puberty may influence further development in NE projections to the HY. To test this hypothesis, male Long Evans rats were castrated at 14 days of age. NE and dopamine turnover were estimated in the HY and CTX at 28 (prepuberty), 42 (early puberty) and 70 (late adolescence) days. Catecholamine (CA) turnover was estimated by measuring the decrease in CA levels (using HPLC coupled to electrochemical detection) four hrs after administering a tyrosine hydroxylase inhibitor, alpha methyl-p-tyrosine (250 mg/kg). The rate of NE utilization increased in the HY in intact animals over age but changed little in the CTX. Juvenile castration retarded the development of NE utilization in the HY but not in the CTX. Whereas NE levels in the HY decreased approximately 57% in intact rats at 70 days, there was only a 33% decrease noted at this age in castrated rats, a magnitude of loss observed in both intact and castrated rats at 42 days. DA turnover in the HY was unaffected by castration. Full kinetic analyses are underway. In summary, NE projections to the HY show a delayed onset in function, compared to other NE projection systems, and pubertal events may influence development in this system. Supported by Grant MH31850.

143.8

REDUCTION IN THE VOLUME OF THE SEXUALLY DIMORPHIC NUCLEUS OF THE MEDIAL PREOPTIC AREA (SDN-MPOA) RESULTING FROM PRENATAL STRESS IS INDEPENDENT OF DEFICIENT MALE RAT COPULATORY PATTERNS. M. Kerchner, W. Grisham, and I. L. Ward. Psychology Department, Villanova University, Villanova, PA 19085.

Prenatal stress disrupts full masculinization of adult sexual behavior and reduces the number of neurons within the Spinal Nucleus Bulbocavernosus (SNB) and the Dorsolateral Nucleus (DLN) [Grisham, Kerchner, & Ward, unpublished]. However, there is no difference in cell number between subjects that ejaculate and those that fail to ejaculate. We now report the SDN-MPOA volume from the same animals for whom we previously counted SNB and DLN neuron number.

56 control males and 44 male offspring of pregnant female rats exposed to daily stress during the last week of gestation were studied. All males were screened as being either an ejaculator or nonejaculator. Brain sections were stained with thionin. A computer assisted digital image analysis technique was used to obtain morphometric data. In each animal, the average volume of the left and right SDN-MPOA was calculated.

The mean volume ($\times 10^{-3}$ mm^3) of the SDN-MPOA of stressed males was significantly smaller than that of control males [13.93 (±0.15) and 18.39 (±0.90) respectively ($p < .01$)]. There was no difference between ejaculators and nonejaculators, in either the control or stressed groups. We conclude that prenatal stress can lead to an incomplete masculinization of sexually dimorphic structures in the brain and spinal cord, but that these alterations need not be associated with abnormal sexual behaviors. (Supported by 2R01 HD-04688 from NICHHD and 2K05 MH00049 from NIMH.)

143.9

Early Experience Affects Spatial Learning Ability. L.A.Wilson and L. Nadel. Dept. of Psychology, Univ. of Arizona, Tucson, AZ 85721.
Handling and isolation of infant rats are forms of experience that alter structure and function of the nervous system. Handling of rat pups increases brain and body size, glucocorticoid receptor concentrations in the hippocampus, and produces reduced emotionality and improved learning ability on many tasks. Isolation of young rats from their mothers halts growth in the pups and increases the tendency of the pups to respond to stress with elevation of normally low levels of corticosterone. Emotional and social behavior is impaired in monkeys that have been isolated during infancy, however it is unclear what effect this manipulation may have on learning ability. Although it is not immediately obvious what these two manipulations have in common, one possibility is that both exert at least some of their effects by interfering with the late developing areas of the hippocampus. This brain area is in a dynamic phase of development during the time that handling and isolation produce their effects; it plays a crucial role in learning ability and is also an important link in the adrenocortical stress response system. Thus it was hypothesized that when rat pups were handled and/or isolated, they would exhibit enhanced hippocampal learning ability, and that they would respond to a novel environment with higher activity levels than controls. Litters of 8 Long-Evans rats were assigned to either handled(HO), isolated(IO), handled/isolated (HI) or control (CO) groups at birth. Activity levels were measured in an open field at 40 days of age and all animals were tested on a spatial and a nonspatial version of the Morris water maze task at 60-70 days. Activity levels did not differ between the groups, and none of the groups had any difficulty learning the nonspatial task. On the spatial task however, the HO animals required significantly longer to acquire the task than IO or CO, and HI animals learned the task more quickly.

143.10

EFFECT OF HYPOTHALAMIC LESIONS INDUCING PRECOCIOUS PUBERTY ON THE MORPHOLOGICAL AND FUNCTIONAL MATURATION OF THE LHRH CELL SYSTEM. M.P. Junier, A. Wolff*, G. Hoffman# and S.R. Ojeda. Div. Neuroscience, OR Reg. Prim. Res. Ctr., Beaverton, OR 97006, and #Dept. Physiology, Univ. Pittsburgh, Pittsburgh, PA.

We recently showed that preoptic area-anterior hypothalamic area (POA-AHA) lesions induce precocious puberty through the enhanced production of transforming growth factor α (TGFα). In the present study we examined the effect of these lesions on the morphological and functional maturation of the luteinizing hormone-releasing hormone (LHRH) neuronal system. Electrolytic lesions of the POA-AHA of 22-day-old female rats advanced the timing of puberty by 6 days. Immunocytochemical studies revealed that the lesion, although compromising the medial preoptic area, spared most LHRH neurons. The morphological characteristics of LHRH neurons at the light microscopy level, evaluated at the time of vaginal opening, were not different in animals undergoing precocious puberty than in the almost 1 week older intact controls. While TGFα mRNA levels increased within 4 days after the lesion, no changes in LHRH mRNA levels were detected by RNA blot hybridization of polyadenylated RNA to a LHRH cRNA probe. During the first four days after the lesion LHRH content in the POA-AHA and median eminence (ME) of lesioned animals was similar to that of age-matched controls. On the day of vaginal opening the LHRH content in the ME of lesioned rats was almost two-fold greater than control animals having vaginal opening at a normal age. Although these results do not rule out a change in LHRH gene expression induced by the lesion, they suggest that POA-AHA lesions advance puberty by precociously setting in motion the releasing mechanisms underlying the first preovulatory surge of gonadotropins. (Supported by NIH grants RR09988 and RR00163)

143.11

THE DEVELOPMENT OF CUTANEOUS CONTROL OF LORDOSIS. G.S. Benedict and C.L. Williams. Depts. of Psychology, Columbia Univ. and Barnard College, NY, NY 10027.

Previous work has demonstrated that male and female 6-day-old rats are capable of performing lordosis to rapid brushing on the sides combined with pressure on the rump. Priming with estradiol benzoate (EB) and progesterone (P) facilitates lordosis frequency, duration, and intensity (Williams, Behav. Neurosci., *101*, 1987). Kow and Pfaff (Brain Res., *101*, 1976) demonstrated that input from a region around the anterior curve of the hips is the important cutaneous region for the elicitation of lordosis in the adult female rat. The purpose of this study is to examine the effect of EB and P priming on lordosis responding to tactile stimulation and to compare the cutaneous control of lordosis in the neonate and the adult female. To examine the localization of cutaneous input for lordosis, subjects were denervated on the waist (a region which includes the anterior curve of the hips), on the midriff (a larger region including the waist), on the flanks, and on an extensive region containing the midriff and flanks. Denervated and sham-operated adult females received either 5 or 100 µg EB and 0.5 mg P, 40 and 4 hrs prior to lordosis testing, respectively, while neonates were tested either without hormone-priming or with 100 µg EB and 0.5 mg P. The results suggest: **1)** a high dose of EB increases the size and/or sensitivity of the cutaneous region critical for eliciting lordosis; **2)** lordosis in the infant does not appear to be under strong cutaneous control as it is in the adult; **3)** there is a sex difference in the cutaneous control of lordosis in infant rats -- female rats require more regionally specific input than male rats.

143.12

DEVELOPMENTAL EXPRESSION OF VASOPRESSIN IN HUMAN HYPOTHALAMUS: DOUBLE LABELING WITH IN SITU HYBRIDIZATION AND IMMUNOCYTOCHEMISTRY.
K Murayama, RB Meeker, S Murayama and RS Greenwood. Depts of Neurology and Pathology, Univ. of North Carolina, Chapel Hill, NC, 27599

Magnocellular neurons in human hypothalamus were studied by double labeling with in situ hybridization for vasopressin (VP) mRNA and immunocytochemistry for VP peptide. Frozen (20 um) or paraffin (8um) sections were cut from 10% buffered formalin-fixed brains from the files of Department of Pathology. The sections were hybridized by newly synthesized 27-mer (HVP-2) and 30-mer (HVP-1) oligonucleotide antisense probes complementary to sequences in Exon C of the human VP gene coding for glycoprotein (HVP-2) or VP neurophysin (HVP-1), double-stained with an anti-neurophysin II antibody (K.Dierickx, *Cell Tissue Res.* 184:15-27, 1977) using the avidin biotin complex or alkaline phosphatase-avidin method, and processed for autoradiography. Single cell silver grain densities were analyzed with an image analysis system (Bioquant IV).

Both probes (HVP-1, HVP-2) gave comparable patterns of hybridization. Positive cells for both in situ hybridization and immunocytochemistry were detected in supraoptic, paraventricular and intersupraoptico-paraventricular nuclei of hypothalamus as early as 19 weeks of gestational age. By 22 weeks gestation vasopressinergic neurons were found in the usual adult position in the hypothalamus. Cellular size grew larger as gestational age increased. No significant decrease in hybridization intensity was apparent in the 22 week fetal brain relative to adult levels. Our study shows that some human fetal supraoptic and paraventricular neurons express vasopressin and have migrated by the second trimester.

Supported in part by the Dept of Neurology Research Fund.

143.13

THYROXINE INDUCED CHANGES IN DISTRIBUTION OF A MULLER CELL ANTIGEN. D. Eastzer, G. Kirchbaumer* and S. Hoskins. Department of Biology, City College of New York, NY, NY 10031.

To study thyroxine regulated developmental changes in the *Xenopus* visual system, we used immunosuppression techniques to generate monoclonal antibodies to molecules expressed in the retina during metamorphosis. One antibody, IPS30, stains a subset of Müller cells, glial cells which span the thickness of the neural retina. The immunoreactivity is both developmentally regulated and spatially localized. Before metamorphosis, staining is seen in the ganglion cell layer and at the inner limiting membrane. During metamorphosis, the portion of the cell which spans the inner plexiform layer (IPL) is also stained.

To test the hypothesis that the apparent redistribution of the IPS30 antigen is caused by thyroxine, we blocked endogenous thyroxine synthesis in premetamorphic tadpoles using propylthiouracil. One eye of each tadpole was then injected with 3 ng of thyroxine in oil, the other eye receiving oil alone. At this dose, no systemic effects of thyroxine are seen. In 4 of 5 metamorphically-blocked tadpoles, the thyroxine treated retina showed more IPS30 staining in the inner plexiform layer. On average, 71% (range: 42%-89%) more cells with staining spanning the IPL were found in thyroxine treated eyes. One tadpole showed no staining in the IPL of either eye. Thus, the altered distribution of this Müller cell antigen during metamorphosis appears to be regulated by thyroxine acting at the level of the eye.

Supported by NIH R29 NS25042 and NSF BNS 8616730.

143.14

ALTERED CEREBELLAR PROTEIN SYNTHESIS IN PROPYLTHIOURACIL TREATED RATS. D.M. Jaworski*, N.C. Mills* and J. Hines Dept. of Biology, Texas Woman's Univ., Denton,TX, 76204.

Thyroxine alters the developmental expression of a number of genes. Central nervous system effects include altered cellular morphology, migration and synaptogenesis in addition to gross morphological alterations of cretinism -retarded skeletal growth, retention of infantile skull proportions and awkward motor coordination. The goitrogen propylthiouracil (PTU) (20 mg/kg s.c. to dams and pups) was used to induce hypothyroidism for investigation of altered cerebellar protein synthesis. A significant reduction in body, brain and cerebellum weights, as well as brain:body and cerebellum:brain weight ratios were observed. Histologically, cerebellar cortical stratification is also altered. Total RNA has been isolated from pooled cerebella of 1,10,20,30 and 60 day postnatal male rats and mRNA prepared using oligo (dT) cellulose. Protein profiles were generated by in vitro translation in a micrococcal nuclease treated reticulocyte lysate system and synthesized proteins were separated by weight using SDS-PAGE. In addition, the mRNAs are being used for Northern hybridization with cDNAs for apolipoprotein E (apoE) and transferrin (Tf). In control animals both apoE and Tf display time-dependent alterations in synthesis. It is anticipated that synthesis in hypothyroid animals will be both reduced and delayed.

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143.15

SEXUAL DIMORPHISMS IN VERTEBRATE MOTOR SYSTEMS: HORMONAL INFLUENCES ON MOTONEURONS AND THEIR TARGET MUSCULATURE IN A VOCALIZING FISH. A. Bass, M. Marchaterre and R. Brantley, Neurobiology & Behavior, Cornell University, Ithaca, N.Y. 14853.

In a vocalizing fish, the plainfin midshipman (*Porichthys notatus*), the fundamental frequency of sound communication signals is determined by the contraction rate of striated "sonic" muscles apposed to the lateral walls of their swimbladder; this rate is established in turn by the rhythmic discharge frequency of sonic motoneurons. Only large, nest-guarding (Type I) males are sonic; gravid females and non-reproductive juveniles are not known to generate sounds. Earlier studies have shown that differences in vocal behavior are paralleled by those in the dimensions of sonic motoneurons and sonic muscles. For example, absolute muscle mass is about 2500%, muscle fiber diameter is 200-300%, and motoneuron soma diameter is 100%, greater in vocalizing males. The muscle fibers of vocalizing males are also characterized by expanded peripheral and central zones of sarcoplasm filled with mitochondria, a highly branched sarcoplasmic reticulum, and myofibrils with widened Z-lines. Juvenile males and juvenile females were implanted with pellets of 11-ketotestosterone or testosterone (androgens). The muscle fibers of androgen- treated juveniles resembled those of vocalizing males: namely they had an expanded peripheral and central sarcoplasm filled with mitochondria. Their peripheral sarcoplasm was also characterized by dense "whorls" of endoplasmic reticulum, clusters of glycogen, and islets of myofribrils. There was also an average increase of 40-50% in motoneuron soma diameter. Together with data on androgen-binding in sonic muscle but not motoneurons, the results support the hypothesis that the development of sexual dimorphisms in the sonic pathway are ultimately dependent upon a cascade-like series of trophic events initiated by an expanded periphery. Support by NIH, NSF and NIMH.

143.16

SEXUAL POLYMORPHISMS AND ANDROGEN SENSITIVITY OF SOUND-GENERATING MUSCLE IN A VOCALIZING FISH. R. Brantley and A. Bass. Section of Neurobiology and Behavior, Cornell University, Ithaca NY 14853 and UC Bodega Marine Lab, Bodega Bay CA 94923

Two sexually mature male morphs exist in *Porichthys notatus*: "Type I" males which produce a "humming" vocalization believed to function as a mate call, and sneak-spawning "Type II" males which, like females, are not known to vocalize. Sounds are generated by the contraction of "sonic" muscles apposed to each wall of the swimbladder. Differences in vocal behavior are paralleled by those in muscle mass: Type I males possess larger muscles than either females or Type II males in both absolute mass (25-fold and 53-fold, respectively) and relative to body weight (11-fold and 7-fold). To test for a role of gonadal steroids in the development of these dimorphisms, both intact and gonadectomized juvenile males, juvenile females, and Type II males received subcutaneous pellet implants for 9 weeks of testosterone (T), 11-ketotestosterone (kT), 17B-estradiol (E2), or cholesterol (C). Relative sonic muscle mass was then determined. Responses by intact and gonadectomized groups did not differ, so the data were pooled. KT stimulated increases of 68-99% in all three classes of fish (PLSD $p<0.05$); T results were similar ($p<0.05$; Type II males not implanted with T). Neither E2 nor C showed any effect. We conclude that the dramatic increase in sonic muscle mass in Type I males at reproductive maturity is induced by androgens.

Natural sex dimorphisms in sonic muscle mass are paralleled by those in fiber number: Type I males have more sonic muscle fibers than either females or Type II males (means of approx. 41,648 fibers per side (n=11) vs. 8130 (n=4) and 14,056 (n=5) respectively ; Scheffe $p<0.01$). Given this, one hypothesis is that androgens stimulate sonic muscle enlargement in Type I males at least in part through their influences on muscle fiber number. Supported by NIMH, NSF, and NIH grants.

143.17

MATERNAL STIMULATION AFFECTS THE NUMBER OF MOTONEURONS IN THE SPINAL NUCLEUS OF THE BULBOCAVERNOSUS (SNB).
C.L. Moore, H. Dou and J.M. Juraska. Dept of Psychology, Univ. of Illinois, Champaign, IL 61820

During early postnatal development, male rats receive extensive stimulation from maternal anogenital licking (AGL). Previous work has shown that reduction of AGL by producing anosmia in the dam results in deficits in masculine sexual behavior of offspring, thus raising the question of how the stimulation affects the development of underlying neural mechanisms. In the present study, we examined the spinal cord of adult male rats that had either been raised normally or been raised by dams made anosmic through intranasal applications of zinc sulfate. The lumbar spinal cord was celloidin embedded, sectioned at 50 microns and stained with methylene blue. Motoneurons having visible nucleoli were counted in each section of the SNB motor column. In our preliminary data, we have found a significant ($t=2.9$, $df=13$, $p<.02$) 12% reduction in the number of motoneurons in the SNB of males raised by anosmic dams (n=6) compared to control males (n=9). SNB motoneurons innervate penile muscles that control sexual reflexes, and inadequate development of this neuromuscular system may underlie some reported deficits in sexual behavior. Supported by NSF RII 89-05498.

143.18

FIELD POTENTIAL ANALYSIS OF DESCENDING PROJECTIONS TO THE LUMBAR SPINAL CORD AND OF PROPERTIES OF ANDROGEN-SENSITIVE MOTONEURONS IN MALE RATS.
J. Tanaka & A.P. Arnold, Department of Psychology, UCLA, Los Angeles CA 90024-1563.

Recent anatomical evidence suggests that projections from the lateral vestibular nucleus (LVe) and gigantocellular reticular nucleus (Gi) innervate areas of the lumbar spinal cord near the spinal nucleus of the bulbocavernosus (SNB). To confirm this result electrophysiologically, we recorded and mapped averaged field potentials within the lumbar spinal cord of urethane-anesthetized male rats in response to electrical stimulation of the LVe or Gi, and compared this with the location of somatic action potentials elicited at the same levels by stimulation of SNB axons. In all the rats (n=7) tested, LVe stimulation elicited a negative field potential (latency 2.7 $\pm$ 0.4 ms; threshold 46 $\pm$ 7 μA). Gi stimulation also produced a negative potential (latency 2.4 $\pm$ 0.1 ms; threshold 32 $\pm$ 12 μA). LVe- and Gi-evoked responses were largest at sites 200-450 μm dorsolateral to the SNB somata, indicating that afferent input to the lumbar spinal cord from LVe and Gi occurs most strongly some distance away from the SNB somata, perhaps onto a population of interneurons.

Since SNB motoneurons are regulated by androgen, we compared antidromic somatic field potentials of SNB motoneurons in intact (n=14) and castrated (n=8) rats, but found no group differences in the latency or refractory period. When the SNB motoneurons were antidromically activated with double axonal pulses, long-latency inhibitory effects were observed suggesting the presence of recurrent or other inhibition evoked by stimulation of the motor nerve. Supported by NIH grant HD15021.

143.19

NORMAL DISTRIBUTION OF SEROTONIN AND SUBSTANCE P IN THE SEXUALLY DIMORPHIC CREMASTER NUCLEUS OF ANDROGEN-INSENSITIVE TESTICULAR FEMINIZED (Tfm) RATS. B.W. Newton & K.W. Chung* Depts. of Anatomy, U. of Arkansas for Medical Science, Little Rock, AR 72205 & U. of Oklahoma Health Sciences Center, Oklahoma City, OK 73190

King-Holtzman (K-H) Tfm male rats produce androgens, but they lack at least 85% of their androgen receptors. Consequently, two of their sexually dimorphic lumbar nuclei (male motoneuron number > female) and their muscle targets are distinctly feminized (spinal nucleus of the bulbocavernosus and dorsolateral nucleus; J. Neurobiol., 17:157,'86). In Sprague-Dawley rats the male cremaster nucleus (CN) has a larger number of motoneurons and a much greater serotonin (5HT) and substance P (SP) innervation than females. Previous data suggests that the SP and 5HT innervation of the male CN is predominately under muscle target vs. androgen control (Brain Res. 485:149,'89). However, no one has examined the Tfm CN and its 5HT and SP innervation. Normal male, female, and male Tfm K-H rats were perfused for the PAP technique and their CN immunostained for either 5HT- or SP-immunoreactivity (IR). Results reveal that despite the >85% reduction in androgen receptors, the 5HT- and SP-IR within the CN of male Tfm rats appears the same as normal male K-H rats, and is much greater than in normal K-H females. These data suggest that either androgens are not the principle factor which maintains the 5HT and SP innervation of the CN in male Tfm K-H rats or, that <15% of androgen receptors can maintain the 5HT and SP afferent input in male K-H rats. Supported by BRSG RR05350, Arkansas Caduceus Club (BWN), and Presbyterian Health Foundation (KWC).

143.20

ONTOGENY OF STEROID ACCUMULATION IN RAT LUMBAR MOTONEURONS. C.L.Jordan, S.M.Breedlove & A.P.Arnold[1], Psych. Dept., UC Berkeley and [1]UCLA, CA.

Androgens influence postnatal development of motoneurons in the spinal nucleus of the bulbocavernosus (SNB), for example by regulating neuromuscular synapse elimination and growth and retraction of dendrites. We used steroid autoradiography to measure the ontogeny of steroid accumulation in these motoneurons as part of an attempt to determine where steroids act during the postnatal period. Male rat pups were castrated 2 days prior to s.c. injection of ^{3}H-testosterone (300uCi/100g BW) on postnatal days (P)7, 14, and 21, and sacrificed 1.5 hr later. At least 40 SNB cells were examined from each animal (n=3/grp). Based on the Poisson criterion, virtually all SNB motoneurons accumulated testosterone at both P14 (83$\pm$3.2%, mean$\pm$SEM) and P21 (84$\pm$8.9%) whereas very few SNB motoneurons accumulated androgen at P7 (9$\pm$7.8%). There was also an overall increase in the density of labelling between P14 and P21. At P14, 46% of SNB motoneurons had 3 or more times the number of background silver grains whereas 75% of the SNB motoneurons exceeded this criterion at P21. Since SNB motoneurons begin to accumulate androgen during the second week of life, androgen may act directly on SNB motoneurons to regulate synapse elimination and dendritic development. Supported by HD15021, NS08686, and the Sloan foundation.

143.21

GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IMMUNOREACTIVITY IS INCREASED IN THE HYPOTHALAMUS OF ANDROGEN-INSENSITIVE (*Tfm*) MICE. K.L. Olsen, J.C. Speh and R.Y. Moore. Dept. Microbiology, George Washington Univ., Washington, DC 20037; Dept. Neurology, SUNY-Stony Brook, NY 11794.

Mice with the testicular feminization mutation (*Tfm*) have a reduced number of androgen receptors. As a result of this *X*-linked recessive gene defect, these *XY* males are insensitive to their testicular androgens and develop a female external phenotype.

Using the *Tfm* mouse, GFAP was identified as a protein that may be important in hormone-regulated functions (Olsen et.al., *Neuroendocri.*, 50:392,1989). Silver-stained 2D gels showed that GFAP concentration was higher in the hypothalamus of *Tfm* mice compared to wild-type males. In this study, we used immunohistochemistry to localize this increase in GFAP.

Nineteen mice (*Tfm*-9; wild-type-10) were perfused and coronal sections through the hypothalamus were cut and stained for the presence of GFAP immunoreactivity. The increased staining in the *Tfm* extends from the caudal medial preoptic area through the anterior hypothalamic area into the retrochiasmatic area. This is predominantly periventricular in location. There are no consistent differences in other regions, including regions such as the hippocampus with heavy GFAP staining. The difference between the *Tfm* and wild-type mice appear to be an increase in astrocytic GFAP rather than in the number of astrocytes but this remains to be established.

143.22

MATERNAL SEPARATION EVOKES CRF RELEASE AND HPA AXIS ACTIVATION IN NEONATAL RAT PUPS. C. Pihoker, M.J. Owens, C.M. Kuhn and C.B. Nemeroff. Depts. of Psychiatry and Pediatrics, Duke Univ. Med. Center, Durham, NC 27710

Stress activates the hypothalamo-pituitary-adrenocortical (HPA) axis, with release of the hypothalamic neuropeptide corticotropin-releasing factor (CRF) stimulating pituitary ACTH secretion, which in turn promotes adrenal glucocorticoid release. The neonatal rat has been shown to be relatively stress hyporesponsive, during which time the hormonal response to certain stressors is diminished or absent. We have investigated the response of 10 day rat pups to a major stressor, maternal separation. Pups were removed from their dams for 2-24 hr, then sacrificed by decapitation. Trunk blood was collected and plasma assayed for corticosterone. Brains were rapidly removed and frozen; the median eminence was dissected and assayed for CRF concentration by RIA. Pups subjected to maternal separation exhibited a significant reduction in median eminence CRF concentration, accompanied by a dramatic rise in serum corticosterone. These results are similar to those observed in adult rats subjected to stress, with the decrease in CRF content reflecting its release from median eminence nerve terminals. Thus, release of CRF and subsequent activation of the HPA axis occurs in neonatal rats subjected to maternal separation. Supported by: The MacArthur Foundation, NIMH MH-19109, MH-42088, NIH DK-41777.

143.23

ESSENTIAL ROLE OF THYROID HORMONES IN MATURATION OF OLFACTORY RECEPTOR NEURONS IN POSTSUCKLING RATS: A KNOB COUNT AND OMP STUDY. M. Paternostro and E. Meisami, Physiol. Dept., Univ. Illinois, Urbana, IL 61801.

We have shown that growing hypothyroid rats show a 40% reduction in the normal increase in olfactory epithelial (OE) surface area and olfactory neuron (ORN) number during the suckling period and no growth post-weaning. Rats allowed to recover from hypothyroidism at 25 d, by withdrawal of PTU, show, by 90 d, complete compensation of OE surface area and total ORN number. To examine the maturation of ORNs, we determined the surface density of ORN dendritic knobs in (1 μ) sections of nasal septum in normal and hypothyroid rats. Dendritic knobs are characteristic of mature ORNs (1 knob/ORN). The surface density of knobs increases postnatally, reaching $60x10^3$ (knobs/sq mm) in 90 d rats; the density was 45% and 70% of this value in newborn and 25 d normal rats. By 25 d, hypothyroid pups had 25% reduction in this parameter, compared to controls. After weaning, no more increase in knob density occurred in the hypothyroid rats, resulting in a 45% deficit by 90 d. The recovering rats, however, showed marked growth in density, nearly catching up with normal rats by 90 d. Immuno-histochemical localization of olfactory marker protein (OMP) in sections of septal OE confirmed the results obtained by knob count.
Supp.: NIH Training Grant to M.P. & UICU Res. Board.

143.24

INSULIN-LIKE GROWTH FACTOR 1 (IGF-1) STIMULATES LHRH RELEASE FROM THE PREPUBERTAL FEMALE MEDIAN EMINENCE IN VITRO. **J.K. Hiney*, S.R. Ojeda*, and W.L. Dees. Dept. of Veterinary Anatomy, Texas A&M University, College Station, Texas 77843 and *Division of Neuroscience, OR Regional Primate Res. Ctr., Beaverton, OR 97006.**

Insulin-like growth factor 1 (IGF-1), is a polypeptide that functions in growth and development. Studies using the chimpanzee (Copeland, K.C. et al., *J Clin Endo Met* 55:1198, 1982), and the rat (Handelsman D., Endo, 120:491, 1987) revealed serum IGF-1 increases prior to and during puberty. In the present study we have examined the ability of IGF-1 to affect the release of LHRH from the median eminence (ME) of 30 day old female rats *in vitro*. The ME's were incubated in Krebs Ringer Bicarbonate Glucose Buffer (KREBS) then followed by KREBS containing doses of IGF-1 ranging from 1-200ng/ml. Results indicate that IGF-1 significantly induced LHRH release in a dose dependent manner, with a minimal effective dose of 10 ng/ml and a maximal effective dose of 100 ng/ml. Experiments to verify the specificity of this effect indicated that IGF-1 is ten times more potent at stimulating LHRH release than IGF-2 or Insulin. These results indicate that IGF-1 can induce LHRH release in the prepubertal female rat and suggest that IGF-1 may play a role in the onset of female puberty.

143.25

IMMUNOCHEMICAL AND MORPHOMETRIC CHARACTERIZATION OF NEUROPHYSIN NEURONS IN PARAVENTRICULAR CULTURES. M. Morris, B.A. Bennett and H. Xu*. Physiology and Pharmacology, Bowman Gray Sch. of Med., Wake Forest University, Winston-Salem, N.C. 27103.

Immunochemical and neurophysiolgical studies show that hypothalamic neurophysin (NP) cells are viable in culture. Experiments were performed to further characterize these peptidergic cells. Dissociated cultures of the paraventricular (PVN) region from neonatal rats were fixed and stained immunochemically with NP, vasopressin (VP) and oxytocin (OT) antisera. The NP antisera recognizes both VP and OT-NP while the peptide antisera are specific for the amidated peptides or the C-terminal extended forms. A morphometric analysis was performed to determine the size distribution of the NP cells and the density of the specific cell types. The NP positive cells showed a herterogeneity in size and shape. 38% of the NP cells were between 100 and 150 μm^2, 48% were larger than 150 μm^2 and the remainder were less than 100 μm^2 (n = 50 cells). The cell diameter and process length were 14.8 ± 1.4, 16.5 ± 0.7 and 20.3 ± 0.8 μm (diameter) and 84 ± 26.3, 243 ± 40.6, and 261.2 ± 43.2 μm (process length) for the small, medium and large cells, respectively. The immunochemical data showed that there was little or no staining when the specific VP and OT antisera were used. The majority of the peptide cells were immunopositive for the extended peptide forms. There were 34.8 ± 2.8 VP cells and 102 ± 17.1 OT cells/culture well (n = 4). This represented 14 and 41% of the total NP positive cells which averaged 248 cells/culture. These results demonstrate that PVN cultures contain a heterogenous population of neurophysin cells, reminiscent of the in vivo organization. There are differences, however, in the nature of the peptide cells since there is evidence for incomplete processing of the vasopressin and oxytocin precursor in the cultured cells.

143.26

"Regulation of the homeotic gene Ultrabithorax by ecdysone in the Drosophila larval central nervous system" M. A. Glicksman and J. W. Truman, Zoology, Univ. of Washington.

The adult CNS of Drosophila is made up of remodeled larval neurons as well as adult-specific cells that are born during larval and early pupal stages. After the adult-specific cells are born, they are then arrested in their development until metamorphosis when they mature into functional neurons. We are interested in the role of homeotic genes in regulating the changes that occur in the CNS at metamorphosis. Using antibodies against the Ultrabithorax (Ubx) proteins, we have examined the time course of expression of Ubx in the post-embryonic nervous system. Ubx shows two levels of protein expression, high levels in neurons of embryonic origin and low levels in post-embryonic neurons. At the start of metamorphosis, the post-embryonic neurons shift to the high level of expression. Studies with ecdysoneless mutants and hormone treatment of larval nervous systems in tissue culture show that ecdysteroids control this change in level of protein expression.
(Supported by NIH F32HD07079)

143.27

CO-LOCALIZATION OF ECDYSTEROID RECEPTORS (ER) AND 29 K-PROTHORACICOTROPIC HORMONE (PTTH) IN THE NEURO-ENDOCRINE COMPLEX OF MANDUCA SEXTA. H.-J BIDMON*, W.E. STUMPF, W.E. BOLLENBACHER+, and N.A. GRANGER, DEPT. OF CELL BIOLOGY & ANATOMY, +DEPT. OF BIOLOGY, UNIV. of NC, CHAPEL HILL, NC 27599.

Ecdysteroids regulate insect development and metamorphosis and their production is controlled by the neuropeptide, PTTH. In vitro incubation of brains (BR), subesophageal ganglia (SEG), corpora allata (CA) and prothoracic glands (PG) in the 20-hydroxyecdysone agonist ^{3}H-ponasterone A (4 nM, spec. act. 178 Ci/mmol), followed by thaw mount autoradiography was used to study the distribution and expression of ER during larval-pupal developopment. ER are found in neurons of the pars intercerebralis, pars lateralis and certain other regions in the BR and SEG on day 0, 3.5 and 4-6 of the last larval stadium, while ER in the PG are present on day 0, days 3-9 and after pupation. Between days 3 and 5 the number of neurons having ER increases as well as the number of ER per cell. Certain neurons like the ventral unpaired motoneuron in the SEG exhibit ER only at distinct times during development, e.g. days 5-6. The identification of the PTTH neurons in the BR with a PTTH monoclonal antibody revealed that the PTTH neurons contain nuclear ER. The presence of ER in PTTH neurons suggests that these cells respond to specific intercerebral ecdysteroid concentrations as part of the feedback regulation of ecdysteroid synthesis.

ENDOCRINE CONTROL AND DEVELOPMENT II

144.1

CO-LOCALIZATION OF TACHYKININ AND ENKEPHALIN IMMUNOREACTIVITY IN CELLS OF THE RAT HYPOTHALAMUS.

C. A. Priest, P. Popper and P. E Micevych. Department of Anatomy and Cell Biology and the Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1763.

Regulation of lordosis behavior by the ventromedial hypothalamus (VMH) involves the interaction of estrogen with several steroid-sensitive, reproductively relevant neuropeptide circuits, including those which contain enkephalin (M-ENK) and the tachykinin, substance P (sP). Recently, cells in the VMH which contain sP or M-ENK have been shown to concentrate estrogen (Akesson and Micevych, J. Neurosci. Res., 19:412, '88; Akesson and Micevych, Submitted), and estrogen induces expression of M-ENK, but not of sP. Because distributions of M-ENK immunoreactive and sP immunoreactive cells overlap, we hypothesized that the differential regulation of M-ENK in sP cells would represent an important component of the mechanism by which estrogen modulates lordosis. To test this hypothesis, we determined whether M-ENK and sP immunoreactivity were co-localized in cells of the VMH. Adult Long-Evans rats received 100 µg colchicine i.c.v., were perfused 48 h later with 4% paraformaldehyde and brains were processed for histochemistry. Immunoreactivity was localized using a rat monoclonal antiserum to sP (Sera Labs) labeled by a biotin-avidin conjugated Texas Red reaction combined with use of a rabbit polyclonal antiserum to M-ENK (Incstar) which was visualized using an antirabbit IgG conjugated to fluorescein isothiocyanate. M-ENK and sP immunoreactivity were co-localized in cells of the ventrolateral part of the ventromedial nucleus. Additionally, doubly-labeled cells were found in the medial preoptic area, dorsomedial hypothalamus, lateral hypothalamus, and in a band extending from the ventrolateral part of the ventromedial nucleus toward the fornix. The co-localization of M-ENK and sP immunoreactivity provide evidence for a common neuroanatomical substrate for the regulation of lordosis behavior by cells of the VMH. Supported by NS 21220.

144.2

TACHYKININ IMMUNOREACTIVE NEURONS ACCUMULATE ESTROGEN IN SEXUALLY DIMORPHIC NUCLEI OF THE RAT LIMBIC SYSTEM.

T.R. Akesson and P. E Micevych. Dept of VCAPP, Washington State University, Pullman, WA 99164 and Dept of Anatomy and Cell Biology and the Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1763.

Gonadal steroid induction of mechanisms regulating reproductive function have been proposed to involve activation of a network of intercommunicating neurons in the hypothalamus and limbic system. In addition to their ability to concentrate estrogen, subsets of neurons which form parts of this network have been further characterized by identification of neuropeptide content. In an earlier study we combined the techniques of steroid autoradiography with immunohistochemistry to demonstrate a population of estrogen-concentrating, substance P-immunoreactive (sPir) neurons in the ventromedial nucleus of the hypothalamus (VMH). Present results extend the localization of doubly labeled neurons to include the encapsulated part of the bed nucleus of the stria terminalis (BSTenc) and the posterodorsal division of the medial nucleus of the amygdala (MeApd). The results further indicate that while numbers of estrogen-concentrating sPir cells were similar between sexes in the VMH, males were found to have numbers of doubly-labeled neurons in both the BSTenc and MeApd that greatly exceeded female numbers. These findings are consistent with the possibility that estrogen-concentrating sPir cells convey steroid sensitive olfactory information to the hypothalamus and thus represent a component of the network which regulates reproductive function.

To address the question of whether or not gonadal steroids modulate tachykinin levels, intact males (n=6) and ovariectomized females receiving 4 mm silastic implants of estradiol benzoate (n=6) rats were compared to orchidectomized (n=6) or ovariectomized (n=6) rats. Gonadectomized rats were found to have greatly reduced immunodectable levels of sP in cells of the BSTenc and MeApd but not the VMH. In contrast, expression of mRNA coding for preprotachykinin has been shown to be unaffected by ovariectomy. This raises the possibility that estrogen may affect sP levels postranscriptionally. Supported by HD 22869 and NS 21220.

144.3

ANDROGENS REGULATE EXPRESSION OF CALCITONIN GENE-RELATED PEPTIDE IN THE SNB MOTONEURONS THROUGH THEIR TARGET MUSCLES.

P.Popper, C.Ulibarri and P.E Micevych. Dept. of Anatomy and Cell Biology, Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Previous work showed that castration of adult male rats increases the steady state levels of calcitonin gene-related peptide (CGRP) and αCGRP mRNA in the motoneurons of the spinal nucleus of the bulbocavernosus (SNB). In the present experiments we tested the hypothesis that castration-induced up-regulation of CGRP expression is the result of feed-back of the bulbocavernosus (BC) and levator ani (LA) muscles on the SNB. We hypothesized that lack of androgens induce a soluble, CGRP-inducing factor by reducing BC activity. Muscle extracts were prepared from the BC of gonadally intact and castrated male Long Evans rats. Extract or buffer (50 µl/treatment) was injected in the BC of intact male rats for 10 days at 48 hours intervals. The activity of the BC was reduced by injecting 1 cc of bupivacaine in the BC of intact rats every 12 hours for 10 days. A group of rats whose pudendal nerves were cut (PDNX) 10 days prior to their killing was also included in the experiments to account for the effect of nerve damage. Alternate section through the SNB were processed for CGRP immunoreactivity and in situ hybridization with a probe complementary to the 3'-end of the αCGRP mRNA, respectively. The effects of different treatments were compared to buffer and sham treated rats. Anesthesia significantly increased the number of SNB motoneurons containing αCGRP mRNA and CGRP and increased the steady state levels of αCGRP mRNA. Treatment with extract prepared from the BC of castrated rats increased the number of SNB motoneurons containing αCGRP mRNA and the level of αCGRP mRNA. PDNX did not increase the number of SNB motoneurons expressing αCGRP mRNA but slightly increased the steady state levels of αCGRP mRNA. These results suggest that the increase of CGRP and αCGRP mRNA steady state levels in the SNB of castrated rats may be explained, at least in part, by the effect of castration-induced decreased muscle activity on the SNB motoneurons. Supported by NS 21220.

144.4

PREPROCHOLECYSTOKININ mRNA IN SPINAL CORD AND BRAINSTEM MOTONEURONS OF THE RAT.

L.A. Abelson and P.E Micevych. Dept. of Anatomy and Cell Biology, Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

Preprocholecystokinin (pCCK) mRNA was localized to the motoneurons of the rat brainstem and lumbar spinal cord with in situ hybridization. A ^{35}S-labelled single-stranded cRNA probe complementary to the entire coding sequence of the prepro-CCK mRNA was used. The probe was transcribed from a 535 bp prepro-CCK cDNA inserted into a pGEM riboprobe vector (gift from Dr. J. Dixon, Purdue University, IN). Adult male Long Evans rats were perfused with 4% paraformaldehyde in 0.1M Sorensen's phosphate buffer. Tissue was removed and cryoprotected in 15% sucrose. 30 µm sections were cut through the lumbar cord and brainstem on a sliding microtome and collected onto silanated slides. Tissue pretreated with RNase A as well as tissue hybridized with a ^{35}S-labelled sense-strand CCK probe were used as controls. pCCK mRNA was localized to motoneurons with the antisense probe, but no specific labelling was noted on sections pretreated with RNase A or after hybridization with the sense-strand. Motoneurons of each motor column in the caudal lumbar spinal cord contained pCCK mRNA; the ventral motor pool, the dorsolateral nucleus, the spinal nucleus of the bulbocavernosus, the retrodorsolateral nucleus and the lateral motor nucleus. In the brainstem pCCK mRNA was localized in motoneurons of the facial nucleus, abducens nucleus and the motor trigeminal nucleus. Motoneurons, however, do not appear to be labelled when antibodies directed against CCK-8 are used, thus only speculative considerations about this discrepancy are possible. Currently, we feel that the greater sensitivity of in situ hybridization methods may account for this discrepancy.

144.5

DEVELOPMENT OF THE INHIBITORY EFFECT OF CHOLECYSTOKININ ON LORDOSIS IN RATS IS CONTROLLED BY PERINATAL ESTROGENS. C. Ulibarri and P.E Micevych. Department of Anatomy and Cell Biology and the Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1763.

Female rats are more sensitive than males to the inhibitory effects of infusions of cholecystokinin (CCK) into the ventromedial nucleus of the hypothalamus (VMN). Neonatal castration of male rats prevents the defeminization of this effect of CCK on lordosis behavior. Androgenization of females with 100 μg of testosterone propionate (TP) on postnatal day 5 reduces the response to CCK. The present research investigated the role of estrogenic component of the defeminization of the response to CCK.

On the day of birth male rats were either given sham surgeries, castrated, implanted with either a 4 mm Silastic capsule of aromatase inhibitor 1,4,6 - androstatrien-3,17-dione (ATD), or a 1 mm capsule of the antiestrogen tamoxifen. Females received either sham surgeries, or were implanted with a 4 mm capsule of TP. Capsules were removed 10 days later. As adults the rats were castrated and implanted unilaterally with a cannulae directed at the VMN. Forty-eight hours after injections of 5 μg estradiol benzoate the animals were tested in a counterbalanced design for their response to infusions of 0, 5, 50, or 100 ng CCK/0.3 μl artificial CSF.

ATD-treated males, neonatally castrated males and control females showed similar high levels of lordosis behavior and inhibition of that behavior by CCK. TP-treated females and control males showed low levels of lordosis and therefore no response to CCK. Tamoxifen-treated males showed virtually no lordosis behavior. The high dose of tamoxifen may have been estrogenic and thereby increased the level of defeminization.

These results suggest that estrogens are involved in the defeminization of the response to CCK. Specifically, perinatal estrogens may reduce adult sensitivity to the inhibitory effects of CCK on lordosis behavior.

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144.6

DEVELOPMENT OF RAT PITUITARY AND HYPOTHALAMIC ANDROGEN RECEPTOR (AR) AND AR mRNA. E.W. Rodriguez, L.H.Burgess, D.B. Lubahn*, F.S. French*, E.M. Wilson*, R.J. Handa. Dept. of Cell Bio., Neurobio. and Anat., Loyola Univ., Stritch Sch. of Med., Maywood, IL 60153 and Lab. Reprod. Biol., Univ. of North Carolina, Chapel Hill, N.C. 27599

Androgens play an important role in the neonatal organization and adult function of the hypothalamo-pituitary-gonadal (HPG) axis. To investigate how androgens affect HPG development, we examined the ontogeny of AR and AR mRNA in Sprague/Dawley rats of various ages. AR were measured by the in vitro binding of ^{3}H-dihydrotestosterone to cytosol preparations of hypothalamus (HT), preoptic area (POA), hippocampus (HIP), and anterior pituitary (AP). AR was detected in HT, POA, and AP as early as postnatal day (PND) 1. Significant increases were detected on PND 12 and adult levels were reached by PND 20 (9.3±0.6, 6.8±0.3, 25.8±2.8 fmol/mg prot.; respectively). We examined AR mRNA from AP, hypothalamus-preoptic area (HPOA), and HIP using two ^{32}P-labelled fragments of rat cDNA (rAR-1 and rAR-2) and a ^{32}P-labelled cRNA transcribed from a Tha1 fragment of rAR-1. Northern blot analysis revealed a single 10 kb hybridization band in adult AP, HPOA, and HIP. A similar band was seen in neonatal HPOA and AP and was first detected on embryonic day 20-21 in HPOA and on PND 7 in AP. These data show that AR and AR mRNA are present in the neonatal HPOA and AP and thus may be involved in the organization of the HPG axis.

144.7

CHROMATOGRAPHIC CHARACTERIZATION OF RADIOACTIVITY IN CELL NUCLEI FROM THE BRAINS OF NEONATAL MACAQUES AFTER ADMINISTERING ^{3}H-TESTOSTERONE. R.W. Bonsall and R.P. Michael, Department of Psychiatry, Emory University School of Medicine, Atlanta, Georgia 30322 and the Georgia Mental Health Institute, Atlanta, Georgia 30306.

During the neonatal period in male macaques, the testes produce adult-like plasma levels of testosterone. To investigate the mechanism of testosterone's uptake by the brain during this stage of development, 2 male and 2 female neonatal cynomolgus monkeys, gonadectomized 2-4 days after birth, were injected s.c. 3 days later with 500 μCi [^{3}H]testosterone ([^{3}H]T). After 60 min, brains were removed and dissected into blocks containing the hypothalamus and preoptic area (HYP), amygdala (AMG), hippocampus (HIP) and midbrain (MB). Samples of cerebral and cerebellar cortex and the entire pituitary gland were also taken. Purified nuclear pellets were prepared by centrifugation through 2 M sucrose, extracted into ether and analyzed by high performance liquid chromatography. Concentrations of radioactivity in nuclei from HYP were 10-20 times higher than those in cerebral cortex ($P<0.001$). In HYP, 49.9 ± 6.5% of the extracted radioactivity was in the form of [^{3}H]estradiol, 16.5 ± 2.1% was in the form of unchanged [^{3}H]T and 10.1 ± 1.6% was in the form of [^{3}H]dihydrotestosterone. Nuclear levels of radioactivity were also significantly higher than those in cerebral cortex in midbrain and amygdala ($P<0.05$). In pituitary gland, concentrations of radioactivity were similar to those in HYP, but 77.7 ± 13.3% was in the form of [^{3}H]T and less than 4% was in the form of [^{3}H]estradiol. No significant differences were observed between male and female neonates. Results suggested that testosterone in the neonatal male primate may interact with cell nuclei in the brain and pituitary gland and that aromatization is active at this stage of development. (USPHS grant MH 40420)

144.8

An Organ Culture System for the Study of CNS Metamorphosis in Drosophila melanogaster

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The central nervous system (CNS) of holometabolous insects becomes extensively remodelled during metamorphosis by the addition of adult- specific neurons, and the modification or elimination of larval neurons. Previous studies in the tobacco hornworm moth, Manduca sexta, have shown that the molting hormone, 20-OH-ecdysone is intimately involved in regulating the cellular and molecular events that take place in the CNS during metamorphosis, including the differentiation of adult-specific neurons. We have designed a tissue culture system in order to study the role of 20-OH-ecdysone in Drosophila CNS metamorphosis. Isolated larval nervous systems can be grown in vitro, with or without 20-OH-ecdysone, for periods up to several days. Using this system, we have found that many of the changes that occur in the CNS during the first 24 hours of normal metamorphosis are regulated (directly or indirectly) by 20-OH-ecdysone, and can be reproduced within 48 hours of growth in vitro. Larval nervous systems cultured in the presence of 20-OH-ecdysone show an increase in cell proliferation, partial disintegration of the perineural sheath, and neuropilar expansion (in particular in the leg neuropiles and the optic lobes) due to the outgrowth of adult specific neuronal processes. In addition, we are using this system to analyze the hormonal regulation of changes in the morphology of some of the existing larval neurons that express SCP-like immunoreactivity which normally become modified during metamorphosis. (Supported by NIH Training Grant HD07183 (TAA) and NIH Grant NS13079 (JWT)).

144.9

ONTOGENY OF THE SEXUALLY DIMORPHIC MALE NUCLEUS IN THE PREOPTIC/ANTERIOR HYPOTHALAMUS (POA/AH) OF FERRETS AND ITS MANIPULATION BY GONADAL STEROIDS

J.A. Cherry, M.E. Basham*, C.E. Weaver*, R.W. Krohmer and M.J. Baum, Biology Department, Boston University, Boston, MA 02215

A sexually dimorphic nucleus exists in the dorsal region of the ferret POA/AH, called the male nucleus of the POA/AH (MN-POA/AH) because it is found only in males. The MN-POA/AH is present in males as early as embryonic day 37 (E37) of a 41-day gestation, and its volume increases until postnatal day 56 (P56). No nucleus is present in the dorsal POA/AH of females at any age. Densities and average somal areas of cells in the dorsal POA/AH are similar in both sexes at E33, before the MN-POA/AH can be visualized. However, at E37 and E41 dorsal cells are greater in density and/or somal area in males than in females. Previously, it was shown that the dorsal POA/AH nucleus could be created in adult females whose mothers were implanted sc with testosterone (T), and prevented from forming in adult males whose mothers were ovariectomized and implanted sc with the aromatase inhibitor, ATD, on day 30 of gestation. These treatments also sex-reverse dorsal POA/AH morphology in animals sacrificed on E41, confirming that the dorsal POA/AH nucleus seen in males at E37 and E41 is the same structure present in adult males. Thus, the MN-POA/AH is potentially functional during much of the period between E28 and P20 when behavioral sexual differentiation occurs in the male ferret. Supported by MH09841, HD21094, and MH00392.

144.10

INDEPENDENT EFFECTS OF AN AROMATASE INHIBITOR ON ESTRADIOL LEVELS AND THE DEGREE OF MASCULINIZATION OF THE ZEBRA FINCH SONG SYSTEM.

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Department of Psychology, UCLA, Los Angeles, CA 90024-1563.

In an attempt to block the masculine ontogeny of the zebra finch song system hypothesized to be caused by early estrogenic action, 100ug of the aromatase inhibitor 4-hydroxyandrostenedione (4-OHA) or vehicle were administered daily to zebra finch chicks for the first 20 days after hatching (Experiment 1). Males in both experimental and control groups were castrated at Day 20. At 60 days the animals were killed and their brains were sectioned at 50um and stained with thionin for light microscopic analysis. In males 4-OHA increased neuronal soma area in MAN ($p<.001$) and HVc ($p<.001$), and HVc volume ($p=.002$). In females 4-OHA increased neuronal soma area in HVc ($p<.001$) and the volume of nucleus rotundus ($p=.04$).

In Experiment 2 we measured plasma estradiol (E_2) and testosterone (T) levels in six day old zebra finch chicks that had received daily injections of 100ug 4-OHA or vehicle for the first five days after hatching. 4-OHA did not alter levels of T or E_2 in males. 4-OHA-treated females had E_2 levels that were equivalent to those found in males (2.3X that of control females, $p=.006$), and T levels that were no different from those in control females

These results show (1) that 4-OHA hypermasculinized males without affecting E_2 levels early in development, and (2) that females are only modestly masculinized by 4-OHA that raised E_2 levels early in development to the levels seen in males. Supported by NIH grant DC00217.

144.11

AROMATASE ACTIVITY IN DISCRETE FOREBRAIN NUCLEI OF EMBRYONIC MALE AND FEMALE FERRETS: DISTRIBUTION AND REGULATION BY ANDROGEN. C.E. Weaver* and M.J. Baum, Department of Biology, Boston University, Boston, MA 02215.

Previous studies have failed to demonstrate consistent sex differences or androgenic regulation of aromatase activity (AA) in the developing ferret brain. These negative results may reflect the use of large blocks of neural tissue in those studies. In the present study AA was measured in punches of discrete forebrain regions from male and female ferrets taken on embryonic day 35 (E35) from mothers which had been treated over days E28-E35 with the androgen receptor antagonist, Flutamide, or vehicle. This is a period when the biosynthesis of estrogen promotes the development of a sexually dimorphic nucleus in the dorsal preoptic/anterior hypothalamic area (POA/AH), and controls aspects of behavioral differentiation in males. In both sexes AA was significantly higher in the medial POA/AH and medial amygdala than in the lateral POA/AH and lateral amygdala, respectively. AA in the bed nucleus of the stria terminalis (BNST) and ventromedial nucleus of the hypothalamus was significantly higher in males than in females. Flutamide significantly reduced AA only in the male BNST. Very low levels of AA were measured in parietal cortex. These results show that sexually dimorphic, androgenic regulation of AA does occur selectively in fetal ferret brain, though not in the POA/AH which is the site of a structural dimorphism. (Supported by: HD21094 and MH00392)

144.12

AROMATASE ACTIVITY IN THE BRAINS OF NEONATAL GRAY SHORT-TAILED OPOSSUMS (MONODELPHIS DOMESTICA). Barbara H. Fadem, Neil J. MacLusky and Michael J. Walters. Department of Psychiatry, UMDNJ-New Jersey Medical School, Newark 07103 and Division of Reproductive Science, Toronto General Hospital, Toronto, Ontario, Canada.

In developing eutherian mammals, presence of neural aromatase activity is correlated with critical periods for brain sexual differentiation. Marsupials are useful for studying hormonal organization of the brain since they are born neurally and reproductively undeveloped. For the present report, aromatase activity in brain tissue homogenates obtained from 1, 4, 8, 16 (approximately equivalent to postnatal day 1 in rats) and 60 day old gray short-tailed opossums was assayed by the tritiated water release method (Roselli et al., Endocrinology 114:192, 1984). Control samples contained either no tissue, or the same quantities of tissue homogenate in the presence of the specific aromatase inhibitor, 4-hydroxyandrostenedione (4-OHA).

Aromatase activity was low but detectable on postnatal day 1 (8.4 $\pm$ 2.7 fmol/mg protein/hr) and increased to levels similar to those seen in adult male gray opossums by postnatal day 16 (77.77 $\pm$ 3.5 fmol/mg protein/hr). This finding suggests that the aromatization of testosterone to estradiol may, as in eutherian mammals, be involved in sexual differentiation of the marsupial brain.

Supported by NSF grant BNS 8616514 to B.H.F.

144.13

EXPRESSION AND REGULATION OF ESTROGEN RECEPTOR mRNA IN THE DEVELOPING RAT FOREBRAIN. R.C.Miranda, & C.D.Toran-Allerand Dept. Anat. & Cell Biol., Columbia Univ. P&S, New York, N.Y., 10032.

The estrogen receptor (ER) is an important ligand-modulated regulator of gene expression. The expression and developmental regulation of ER mRNA was studied by non-isotopic (digoxigenin) *in situ* hybridization histochemistry, using a 48 base oligonucleotide probe, complementary to a sequence in the region of the estrogen-binding domain of rat uterine ER cDNA. Hybridization signal was limited to cytoplasm and proximal processes; nucleus and background were unstained. Controls demonstrated probe specificity. During development, in intact brain and in organotypic cultures, mRNA was observed in all regions previously reported to be ER containing, such as the medial and periventricular preoptic area, bed nucleus of the stria terminalis, septum, nuclei of the diagonal band, and cerebral cortex. ER mRNA was also observed in regions not traditionally considered targets of estrogen (striatum & thalamus). The spatial distribution of ER mRNA in the cerebral cortex showed age-related changes. ER mRNA was observed in primitive cerebral cortex as early as E16. By E20, there was intense staining in the emerging cortical plate, with lower levels of staining in the plexiform layer and ventricular zone. With maturation of the cortical plate, staining appeared to be restricted to the upper third of the cortex and to the large neurons of its deepest layer. In all ER-containing regions, mRNA expression persisted through P21, declining to low or undetectable levels from P28 onwards. Ovariectomy of adult female rats dramatically increased ER mRNA expression in all ER containing regions, including the cerebral cortex. The results demonstrate developmental regulation of ER mRNA expression and provide further evidence that in the adult, estrogen down-regulates ER mRNA expression. *(Supported by NIH grants HD08364 and AG08099 and NSF BNS8700400 and AHAF.)*

144.14

CO-LOCALIZATION OF ESTROGEN RECEPTOR mRNA AND PROTEIN IN DEVELOPING RAT BRAIN. C.D. Toran-Allerand[1], N.J. MacLusky[2], R.C. Miranda[1], and R.B. Hochberg*[3]. Dept. Anat. & Cell Biol.[1], Columbia Univ. P&S, New York, NY 10032, Div. Reprod. Sci.[2], Univ. Toronto, Toronto, Canada, M5G 1L4, and Dept. Ob. & Gyn.[3], Yale Univ. Sch. of Med., New Haven, CT 06510.

Neurons expressing estrogen receptor mRNA have been reported in both estrogen receptor and non-receptor CNS regions. ^{125}I-estrogen autoradiography (2200 Ci/mmol) of P12 forebrain was followed by non-isotopic (digoxigenin) *in situ* hybridization histochemistry with a 48 base oligonucleotide complementary to a sequence of rat uterine estrogen receptor cDNA to study if the mRNA is translated into the encoded protein. Many neurons of the hypothalamus/POA, septum/diagonal band and cerebral cortex and in presumed non-estrogen targets (thalamus, striatum) co-expressed nuclear silver grains (receptors) and blue/purple cytoplasmic hybridization signal. All neurons showing receptors co-localized mRNA, confirming probe specificity. mRNA expression while very extensive **did not** always co-localize with ligand binding, perhaps reflecting regional, intercellular or temporal differences in receptor content. Strong hybridization signal with few or no nuclear silver grains during development may also herald initiation of receptor synthesis or its decline, particularly in regions (cerebral cortex) with transiently high receptor levels. Co-expression of estrogen receptor mRNA and protein during development in a distribution transcending neuroendocrine boundaries, as identified by ^{3}H, documents the greater sensitivity of ^{125}I and suggests that these neurons may comprise an extensive, interacting neural system with broader functional significance. Supported by NIH grants HD08364 and AG08099 (DT-A) and CA3799 (RBH); and AHAF and NSF BNS 8700400 (DT-A).

144.15

ESTROGEN RECEPTORS IN HIPPOCAMPAL AND NEOCORTICAL, TRANSPLANTS DURING DEVELOPMENT. J.A. O'Keefe, E.B. Pedersen*, A.I. Castro, R.I. Handa. Dept. of Cell Biology, Neurobiology, and Anatomy, Loyola Univ. Stritch School of Medicine, Maywood, IL 60153.

Previous investigations have demonstrated a transient rise in estrogen receptor (ER) levels in the rodent hippocampus and neocortex during the first postnatal week. The function of these receptors is largely unknown as is the mechanism by which they are regulated. To further study ER development, we grafted fetal (E14-15) hippocampal or frontal cortical tissue into frontal cortical lesion cavities made in postnatal day (PND) 0 rats. Accordingly, the grafted tissue was one week younger than the host. At two and four weeks post transplantation surgery (corresponding to theoretical donor age PND 7 and 21), the grafts, a region of the adjacent cortex and the endogenous hippocampus or opposite homotypic cortex of the host were assayed for ER content. ER levels were measured by the *in vitro* binding of [3H]-estradiol to cytosolic preparations. Radioinert moxestrol was used to determine non-specific binding. ER levels in hippocampal grafts at theoretical age PND 7 (4.52 $\pm$ 0.72 fmol/mg protein; n=12) were significantly higher than those found in the adjacent host (PND 14) cortex (1.3 $\pm$ 0.29) and the endogenous hippocampus (1.58 $\pm$ 0.19) [ANOVA; Dunnett T3 post hoc comparison test; $p<0.05$]. At theoretical donor age PND 21 (host age PND 28), ER concentration in hippocampal transplants had decreased to levels that were comparable to host levels. A similar transient elevation in ER levels which corresponded to the donor developmental timetable was observed in neocortical grafts. These data suggest that developmental alterations in hippocampal and neocortical ER levels are intrinsically determined by the genetic age of the tissue. (Supported by AA 06487, NS 13230 and a Potts Foundation Award.)

144.16

EARLY POSTNATAL MATERNAL DEPRIVATION AND CORTICOSTERONE ADMINISTRATION INCREASE BEHAVIORAL REACTIVITY TO NOVELTY AND AFFFECT GLUCOCORTICOID RECEPTORS IN THE HIPPOCAMPUS OF RATS STUDIED AS ADULT. E. Merlo Pich.*, G. Biagini*, M. Zoli*, F. Benfenati, L.F. Agnati* and K. Fuxe. Inst. of Human Physiology, University of Modena, Modena, Italy and Dept. of Histology, Karolinska Institutet, Stockholm, Sweden.

The effect of neonatal administration of corticosterone and of repeated episodes of maternal deprivation on the levels of glucocorticoid receptor (GR) immunoreactivity (IR) in the hippocampus have been studied in male rats. Pups were repeatedly injected with corticosterone (10 mg/kg/1 ml sesame oil every 48 h., s.c.) or segregated from the mother for 5 h/day from day P2 to day P6. On day P45 the effects of novelty were assessed in the rats using an exploratory two-compartment test (Merlo Pich & Samanin, Pharmachol Res 21:295 1989). Rats were then killed and brain sections (40 µm) were processed for indirect immunoperoxidase method using a mouse monoclonal antibody against rat liver GR. Microdensitometric and morphometric analyses were carried out by IBAS II image analyzer. A high significant reduction of GR specific immunoreactivity was found in the CA1 area of hippocampus of rats treated with corticosterone, whereas significant increase of GR-IR was found in maternal deprived rats. Both groups showed an increase reactivity to novelty when compared with control animals. These results suggest that early maternal deprivation induces behavioral changes which are similar to those obtained in rats administered with corticosterone in the same period of life, but with different effects on GR levels in hippocampus, an area involved in mediating the negative feedback regulation of plasma glucocorticoids.

144.17

THE STRESS NON-RESPONSIVE PERIOD PERMITS NATURALLY OCCURRING CELL DEATH IN THE DEVELOPING DENTATE GYRUS. E. Gould, C.S. Woolley, D.L. Miller*, G.M. Begany*, R.E. Brinton and B.S. McEwen The Rockefeller Univ. N.Y.,N.Y. 10021, Univ. Southern California, L.A., CA., 90033

The granule cells of the dentate gyrus appear to be a unique population of neurons in that they display both birth and death in adulthood. Sloviter and coworkers (1989) have demonstrated that, in the adult, dentate gyrus granule cells are dependent on glucocorticoids for their survival. Our laboratory has demonstrated that short term adrenalectomy (ADX) results in massive cell death in the granule cell layer of the adult dentate gyrus. In order to determine whether cell death occurs naturally during the developmental period during which glucocorticoid levels are very low, i.e. the stress non-responsive period (SNRP), we examined Nissl stained brain sections from rats at ages E18,E19,P2,P4,P6,P8,P15 and P40. Quantitative analysis revealed degenerating cells in the dentate gyrus at all ages examined with the peak in the density of pyknotic cells, accompanied by a decrease in the density of healthy cells, at P6, the midpoint of the SNRP. This phenomenon is sensitive to glucocorticoids as administration of corticosterone to neonatal rats significantly attenuated cell death at P6 and removal of circulating glucocorticoids by ADX at P15, toward the end of the SNRP, extended the period of cell death. These results suggest that low levels of glucocorticoids which occur during the SNRP permit naturally occurring cell death in the developing dentate gyrus.

144.18

A TESTOSTERONE RELATED SEXUAL DIMORPHISM IN THE DENTATE GYRUS OF THE RAT. **R.L. Roof and M.D. Havens*. Dept. of Psych. and Neuroscience Program, Univ. of Wyoming, Laramie, WY 82071.**

The hippocampus is thought to be involved in spatial processing, a skill for which sex differences have been found. To determine whether these sex differences might be related to hippocampal structure, a morphometric analysis was conducted.

Male and female rats (n=24) were treated with either testosterone (150 ug) or the oil vehicle on postnatal days 4 and 6. Nissl stained sections, 300 um apart, (6 per animal), containing the dorsal hippocampus were included. Medial-lateral (width) and dorsal-ventral (thickness) measures of the right and left pyramidal and granule cell layers were made with the aid of a microprojector and a digitizing pad interfaced with a computer. Mean granule cell area was also determined by tracing the somata with camera lucida at 1000x.

The granule cell layer was wider and thicker in control males than in control females. This sex difference was of greater magnitude in the right hemisphere than in the left. The size of this cell layer in testosterone treated females was intermediate between control males and females. In control males, treated males and treated females, the right granule cell layer was wider than the left. Since mean granule cell area did not differ between the groups, the sex and hormone effects were likely due to increased numbers of cells. No sex or hormone effects were seen on the measures taken in the pyramidal cell layer.

Width of the right granule cell layer was found to correlate (r=.86) with Morris water maze scores obtained from the same animals in an earlier study (Roof, 1989).

These data suggest a testosterone related sexual dimorphism in the number of granule cells in the dentate gyrus of the rat, which may be related to sex differences in spatial performance.

144.19

ESTRADIOL REGULATES SYNAPSE DENSITY IN THE CA1 REGION OF THE HIPPOCAMPUS IN THE ADULT FEMALE RAT. C.S. Woolley and B.S. McEwen, Rockefeller Univ., NY, NY 10021

Recently, our laboratory has demonstrated a positive correlation between circulating levels of estradiol (E) and progesterone (P) and the density of dendritic spines on hippocampal CA1 pyramidal cells both with administration of E and P to ovariectomized (OVX) animals and as E and P levels fluctuate naturally during the estrous cycle. As dendritic spines have been shown to be postsynaptic sites, which very often, if not always, contain synapses, these results imply that the density of synapses may also be regulated by ovarian steroids. In order to determine whether synapse density in the hippocampus is sensitive to estradiol, we compared the density of synapses in the stratum lacunosum-moleculare of the CA1 region of the hippocampus, estimated according to Beaulieu and Colonnier (J. Comp. Neurol., 289:178, 1989), in 3 OVX adult female rats which received estradiol (OVX+E) to that in 3 OVX rats which received oil vehicle (OVX+O). Synapse density on postsynaptic elements identified as dendritic spines was significantly higher in OVX+E animals compared to OVX+O. In contrast, synapse density on postsynaptic elements identified as dendrites was unchanged by estradiol treatment. These results demonstrate that the density of synapses on hippocampal dendritic spines is sensitive to estradiol and imply that synapse density on spines may fluctuate naturally as ovarian steroid levels vary during the estrous cycle.

144.20

GLUCOCORTICOID RECEPTOR IN THE DEVELOPING ANIMAL: EFFECT OF ADRENALECTOMY ON HIPPOCAMPAL BINDING CAPACITY AND mRNA LEVELS. D.M. Vázquez, J.F. López, M.I. Morano and H. Akil. Mental Health Research Institute and Department of Pediatrics, University of Michigan, Ann Arbor, MI 48109-0720.

An important component of the adrenocortical response is the glucocorticoid negative feedback which "turns off" the hypothalamic and pituitary secretion. The hippocampus glucocorticoid (GR) and mineralocorticoid (MR) receptors have been implicated in the modulation of this particular mechanism. The developing animal exhibits a stress hyporesponsive period during the first two weeks of life and a gradual adrenocortical response appears after postnatal day 14. In order to investigate the emergence of the glucocorticoid negative feedback mechanism; we have studied the GR binding capacity (Bmax) and the GR and MR mRNA levels in response to 12 hour and 48 hour adrenalectomy (ADX) in adult (A), 14 (D14) and 18 day old (D18) male rats. Analysis of the the Bmax was carried out using saturating amounts of ^{3}HDexamethasone, RU26938 and Corticosterone. mRNA levels were quantitated by solution hybridization using a 456bp GR cRNA and a non-homologous 544bp MR cRNA. An increase in Bmax was seen on the 12 hr. D14 ADX group when compared to SHAM controls (D14 SHAM=52.2±5.45 (±SE); D14 ADX=227.4±16, $p \leq 0.05$). Increases in Bmax of the 48 hr. ADX A and D18 animals was also seen (A SHAM =132.3 ± 8.8 ; A ADX= 379.10±6.2; D18 SHAM=115.75±4.9; D18 ADX=464.47±5.0, $p \leq 0.05$). In addition, it appears that up-regulation of the GR and MR mRNA levels occurs on the D18 48 hr ADX animal. This implicates an enhanced gene response in the D18 animal which is not present in the adult. Supported by NIMH MH09720 and MH422251.

144.21

TIMING AND DURATION OF DHT TREATMENT ALTERS MOTONEURON NUMBER, MORPHOLOGY, AND SPECIFICITY IN A SEXUALLY DIMORPHIC RAT SPINAL NUCLEUS. L.A. Goldstein and D.R. Sengelaub. Program in Neural Science, Dept. of Psychology, Indiana University, Bloomington, IN 47405.

The development of the sexually dimorphic spinal nucleus of the bulbocavernosus (SNB) is regulated by androgens. Females treated with testosterone (T) or estrogen (E) and dihydrotestosterone (DHT) combined (E+D females) from embryonic (E) day 16 through postnatal (P) day 5 have masculine numbers of SNB motoneurons. Treatment with E alone does not masculinize the SNB; treatment with DHT alone from E17-22 (ST-D) results in a feminine SNB and significantly altered motoneuron morphology and connectivity. To determine if masculinization of the SNB involves the interaction of E and DHT or results from a longer exposure to DHT alone, the number, morphology, and connectivity of SNB motoneurons in females treated with DHT from E16-P5 (LT-D) were examined.

At E22, LT-D females have SNB motoneuron numbers identical to E+D and normal females, but far fewer than normal males, indicating that T is essential for masculine prenatal development. After E22, motoneuron number declines precipitously in normal females but remains stable in LT-D and E+D females, who do not differ from normal males at P10. In adulthood, HRP histochemistry reveals that the connectivity, dendritic length, and soma size of SNB motoneurons in LT-D females are identical to normal males but differ significantly from ST-D females. These data suggest that the altered connectivity in ST-D females is not simply a hormone-specific effect, but the result of a truncated hormone exposure. Thus, DHT can fully masculinize SNB morphology and connectivity if given during the appropriate period of development. Therefore, while T may be required prenatally, DHT may be involved postnatally in the masculinization of the SNB. (Supported by NIH NS24877)

144.22

ANDROGENIC REGULATION OF CONTRACTILE PROPERTIES IN SEXUALLY DIMORPHIC MUSCLES OF FROGS (*Xenopus*). A.A. Herrera and M. Regnier. Biological Sciences, University of Southern California, Los Angeles, CA 90089.

During mating male frogs use forearm flexion to maintain a prolonged loose grip on females for several hours. They are also able to tighten their grip briefly but rapidly in response to sudden movements by the female (Hutchison & Poynton, Behavior 22:41,1964). Flexor muscles of the male forearm are sexually dimorphic and androgen sensitive. To test the effects of androgens on muscle contraction we compared male frogs that were either castrated or castrated and given testosterone implants. Tension measurements were made from the androgen sensitive flexor carpi radialis. At 8 weeks, time to peak twitch tension was not different but twitch relaxation times were significantly longer in muscles of androgen treated frogs ($P<0.01$). These results suggest that androgens enhance the maintainence of tension without compromising the ability to develop tension rapidly. An interesting finding was that tension per unit cross sectional area of fiber was greater in castrates than in androgen treated frogs. We are testing whether this involves androgenic regulation of particular fiber types, conversion of fiber types, or differences in synaptic efficacy. We are also investigating the time course of androgen effects. Supported by NIH grant NS27209.

144.23

DIFFERENTIAL SENSITIVITY TO ANDROGENS WITHIN SEXUALLY DIMORPHIC MUSCLES OF MALE FROGS (*Xenopus*). M. Regnier and A.A. Herrera. Biological Sciences, University of Southern California, Los Angeles, CA 90089.

Forearm flexor muscles in male frogs vary in size and contractility in response to seasonal changes in levels of circulating androgens. To understand the androgenic regulation of muscle properties, males were either castrated or castrated and given testosterone implants. Cryostat cross sections of the androgen sensitive flexor carpi radialis muscle (FCR) were used to measure myosin ATPase activity (pH=10). Adjacent sections were used to measure fiber cross sectional area and for quantitative measurements of succinate dehydrogenase (SDH) activity. Preliminary analysis showed that the pattern of myosin ATPase staining was not affected by androgen levels. At 8 weeks, average fiber size was 45% smaller in castrates without androgen implants. Fibers that stained darkly for myosin ATPase atrophied twice as much as lighter fibers (56% vs. 24%) regardless of location within the muscle. Total SDH activity decreased dramatically but activity per unit cross sectional area of muscle fiber was unchanged. We are currently investigating whether some motor units in the FCR are more sensitive to androgens than others and also determining the time course of androgen effects. Supported by NIH grant NS27209.

144.24

PROGESTIN RECEPTOR CELLS IN ESTROGEN PRIMED POST-NATAL MALE AND FEMALE MOUSE CORTEX: EVIDENCE FOR A SEXUAL DIMORPHISM. P.J. SHUGHRUE, W.E. STUMPF and M. SAR*. Cell Bio. and Anat., Univ. of NC, Chapel Hill, NC 27599.

Autoradiographic studies were performed to determine whether and, if so, where progestin (P) receptor (R) cells are located in cortex, and whether there is evidence for a sexual dimorphism during early postnatal brain development. Eight (4 male/4 female) 8-day postnatal mice, treated with 100μg/100g bw estradiol for 72 hr, were sc injected with 0.32μg/100g bw of (Z)-17β-hydroxy-17α-(2-[^{125}I]iodovinyl 4-estren-3-one (^{125}I-P) 2200ci/mM. Two hr after injection brains were frozen and 4μm cryostat sections processed for thaw-mount autoradiography. After 8-60 days of exposure, cells with a nuclear retention of ^{125}I-P were concentrated in lamina VI of the male and female lateral cortical regions, while additional cells were scattered throughout the remaining regions and laminae. Competition studies with unlabeled R5020 prevented nuclear concentration of ^{125}I-P. A sex comparison revealed that the number of PR cells was significantly greater in female cortex at several brain levels evaluated. The results demonstrate that the developing cortex contains PR cells and that a sexual dimorphism in labeled cell number is present in cortical cells.

144.25

EFFECT OF CASTRATION AND TESTOSTERONE REPLACEMENT ON THE GnRH NEURON SYSTEM IN PUBERTAL DJUNGARIAN HAMSTERS. S.M. Yellon and C.P. Haase*. Div. Perinatal Biology, Depts. Physiology, Pediatrics and Anatomy, Loma Linda Univ. Sch. of Med., Loma Linda, CA, 92350.

At puberty (25 days after birth), testes growth and peak gonadotropin secretion in Djungarian hamsters are correlated with increased numbers of GnRH-stained cells in the medial preoptic area (MPOA) and diagonal band of Broca (DBB) regions of the brain compared to cell numbers in prepubertal males (age 15 days). The developmental increase in number of GnRH cells was hypothesized to depend upon gonadal steroid feedback. Males were castrated at 12 days and implanted with a blank or testosterone-filled silastic capsule (5 mm, s.c.). At age 15 and 25 days, five hamsters from each castrate group and from testes-intact controls were anesthetized and killed by intracardiac perfusion. Brain sections (60 μm) were processed for GnRH immunocytochemistry (LR1 courtesy of Dr. R. Benoit). GnRH-stained somata had smooth contours and were predominantly unipolar or bipolar, irregular-shaped or multipolar somata were rare. In testes-intact controls, the total number of unipolar GnRH cells in the MPOA and DBB was increased at 25 days (75 $\pm$ 7) compared to 15 day males (39 $\pm$ 2, $p<0.05$). At 15 or 25 days, the number of unipolar GnRH somata was not different among castrates (blank or testosterone-treated) and controls ($p>0.05$). However, as in testes-intact males, more GnRH cells were found in 25 day castrates whether implanted with a blank or testosterone capsule compared to similarly treated males at 15 days. Bipolar GnRH cell numbers in the MPOA and DBB were the same irrespective of age or treatment (about 55 perikarya). The data do not support the hypothesis that gonadal steroid feedback affects the pubertal increase in numbers of unipolar GnRH cell bodies, rather development of the GnRH neuron system in the male Djungarian hamster at puberty is independent of the testes or testosterone feedback. (Supported by NIH HD22479)

144.26

HYPOTHALAMIC SOMATOSTATIN mRNA EXPRESSION IN DWARF MICE: DEVELOPMENTAL TIME COURSE OF RESPONSE TO GROWTH HORMONE DEFICIT. D.L. Hurley* and C.J. Phelps. Department of Neurobiology & Anatomy, University of Rochester Medical Center, Rochester, NY 14642. (Present address: Department of Anatomy, Tulane University Medical Center, New Orleans, LA 70112)

Immunocytochemical expression of somatostatin (SS) neuropeptide shows severe reduction in growth hormone (GH)-deficient adult dwarf mice, isolated to pituitary-regulatory neurons in anterior periventricular hypothalamus (PeN) (Phelps and Hoffman, 1987), and likely in response to absent GH stimulatory feedback. In order to examine the possible developmental onset of the SS deficit at a transcriptional level, brains of Ames dwarf (df/df) and normal littermate (Df/?) mice were examined for SS mRNA using *in situ* hybridization histochemistry at intervals from 1 day to 6 months of age. Heterozygous Ames dwarf (Df/df) breeders were a gift from Dr. Andrzej Bartke. ^{35}S-labeled SS antisense RNA probe was synthesized with SP6 RNA polymerase from a rat SS cDNA provided by Dr. Richard Goodman, and hybridized to 20-30 µm coronal brain sections using standard conditions and stringent washes. Autoradiography revealed strong hybridization signals by 7 postnatal days in regions of the PeN, thalamus, hippocampus, and cortex, as previously described (Bendotti *et al.*; 1989). Strong signal was also noted in neurons of the medial basal hypothalamus. Through 21 days of age, signals in all SS-expressing areas were comparable for df/df and Df/? mice. By 6 months of age, dwarfs showed markedly fewer SS mRNA-positive neurons in PeN alone, particularly in dorsal regions of the nucleus. Signal in non-GH controlling regions, such as cortex, remained comparable between dwarf and normal. The results indicate that SS deficit in GH deficiency in Ames dwarfs occurs at the transcriptional level, as in adult Snell dwarfs (O'Hara *et al.*, 1989) and following hypophysectomy (Rogers *et al.*, 1988). Further, the results suggest that the adult SS deficit is the result of a regressive developmental sequence in response to critical periods of GH deficiency. Supported by NIH grant NS25987 (CJP)

MOTOR SYSTEMS: DEVELOPMENT AND PLASTICITY I

145.1

METABOLIC VARIABILITY OF MUSCLE FIBERS IN SELF-REINNERVATED MOTOR UNITS IN THE CAT TIBIALIS ANTERIOR MUSCLE. G.A. Unguez, S. Bodine-Fowler, D.J. Pierotti, R.R. Roy and V.R. Edgerton. Dept. Kinesiology and Brain Research Inst., UCLA, LA., CA 90024 and Div. Orthopaedics & Rehab., UCSD, SD, CA 92039.

The succinate dehydrogenase (SDH) activity of muscle fibers belonging to a motor unit (MU) following 6 months of self-reinnervation (SR) was studied. Nerve branches innervating the anterior compartment of the tibialis anterior in both limbs were cut near the muscle and resutured in 7 adult cats. MUs were functionally isolated via ventral root teasing, physiologically characterized and glycogen depleted. Two of the four MUs analyzed were classified as fast fatigue-intermediate and two as fast fatigue-resistant (Burke et al *J Physiol* 234:723, 1973). SDH activity was determined histochemically (Martin et al *Am J Physiol* 255:C43, 1988) in a sample of MU fibers from the cross-section containing the largest number of MU fibers. All sampled MU fibers stained dark for myosin ATPase (pH=8.75). The fatigue index (FI), sample size (n), mean (X) and range in SDH activity, and coefficient of variation (CV) for the 4 MUs were:

MU	FI	(n)	X(OD/min)	Range	CV%
1	0.99	13	0.008	.006-.009	12
2	0.91	36	0.017	.009-.029	30
3	0.74	37	0.014	.005-.032	51
4	0.48	37	0.010	.005-.018	40

The mean CV for SDH of non-depleted fibers was 40% with a range of 32-45%. The interfiber variability within MUs 1 and 2 was less than that observed in non-depleted fibers, whereas a similar or greater variability was seen in fibers from MUs 3 and 4. Within MUs 3 and 4, MU fibers taken from two distinct regions showed different SDH activities, i.e., fibers with high and low oxidative capacity. These data indicate that after 6-months of SR, SDH variability among MU fibers is the same or greater than that found in normal MUs suggesting that SDH activity of fibers of a MU can be influenced but is not under complete control by the motoneuron. (SUPPORTED BY NIH GRANT NS16333).

145.2

HISTOCHEMICAL PROFILES OF MOTOR UNITS OF THE CAT TIBIALIS ANTERIOR AFTER 6 MONTHS OF ELECTRICAL INACTIVITY. D.J. Pierotti, R.R. Roy, J.A. Hodgson*, and V.R. Edgerton. Brain Research Institute and Kinesiology Department, UCLA, LA, CA, 90024-1568.

The effects of electrical inactivity on the succinic dehydrogenase (SDH) and myosin ATPase activity of fibers in tibialis anterior (TA) motor units (MU) of adult cats were studied. Electrical inactivity was produced in 10 cats by spinal isolation (SI), i.e., spinal cord transection at T12-T13 and L7-S1 and bilateral dorsal rhizotomy between the two transection sites. Two 24-hr EMG recording sessions were used to verify that the muscles in the lower limb were electrically silent. One motor unit from each hindlimb was isolated using ventral root isolation techniques, physiologically typed, and glycogen depleted as described by Bodine et al. (*J Neurophysiol* 57 :1730, 1987). Optical density measurements from glycogen-stained frozen sections were used to classify fibers as depleted (unit) or non-depleted (non-unit). Myosin ATPase was determined using a modification of the technique of Weisberg et al. (*Circ Res* 51:802, 1982). SDH activities were determined using quantitative histochemical technique of by Martin et al. (*J Histochem Cytochem.* 33:1053, 1985). The coefficient of variation (CV) of cross-sectional area (CSA) ranged from 29 to 68% for unit fibers and from 31 to 58% for non-unit fibers. In all MUs analyzed, the ranges in CV in SDH and ATPase activities were 12 to 28% and 4 to 17% respectively. These ranges were 25 to 69% and 8 to 27% in non-unit fibers. Compared to fibers in control MUs (Martin et al. *Am J Physiol.* 255:C43, 1988), SI MU fibers showed a larger degree of variability in fiber CSA and a similar degree of variability in SDH activity. The variability in ATPase activity was lower than either SDH or CSA in SI MUs. These data suggest that the variability in mean SDH and CSA observed among fibers within normal units persists in units after 6 months of electrical silence. Therefore, electrical activity cannot be the sole determinant of these MU properties. (SUPPORTED BY NIH GRANT NS16333).

145.3

SPATIAL DISTRIBUTION OF MOTOR UNITS: A GENERAL PATTERN ? A. J. Dekhuijzen, R. R. Roy, and V. R. Edgerton, Brain Research Institute and Department of Kinesiology, UCLA, Los Angeles, CA 90024-1568.

The spatial distribution of the muscle fibers of motor units from published papers including Bodine, S.C. et al, (*J. Neurophysiol.*, 57:1730, 1987) was analyzed. The power (C) was calculated from the number of muscle fibers (N) as a function of radius (r): $N \sim r^C$. A power of 2 reflects an even distribution of fibers within the territory of a unit, while a value >2 suggests a higher density of fibers toward the periphery of a unit and C<2 reflects a reduction in density toward the periphery. The mean ($\pm$ SD) power for 14 normal motor units from rats and cats was 1.8 $\pm$ 0.2. After denervation-reinnervation in the neonatal rat, units had a power of 2.2 $\pm$ 0.8 (n=7). The mean power for reinnervated units was not significantly different ($p>0.05$) from normal. However, the variance of normal units was significantly less then reinnervated units ($p \leq 0.05$). In addition, the spatial distribution of fibers of units modelled by Monte Carlo methods reflecting a random pattern were studied. Of 14 normal and 7 reinnervated units, 13 and 4 respectively, were not significantly different ($p>0.05$) from Monte Carlo generated units. SUPPORTED BY NIH GRANT NS16333.

145.4

CHRONIC CHANGES IN VENTRAL CORD FIELD POTENTIALS EVOKED BY STIMULATION OF RETICULOSPINAL NEURONS AFTER PARTIAL SPINAL CORD LESIONS IN THE RAT. J.H. KIM, Y.-G. PARK, and D.M. Shim, The Miami Project to Cure Paralysis, University of Miami School of Medicine, Miami, FL 33136.

Reticulospinal neurons (RtN) project to all levels of spinal cord and terminate mainly ipsilateral in the ventral horn (VH). Some of these RtN send collaterals across the midline at the level of termination and make synaptic contacts with contralateral VH neurons. Stimulation of RtN produces mono- and di-synaptic EPSPs in motoneurons. The goal of this study was to investigate chronic changes in RtN evoked field potentials in the spinal cord after controlled spinal cord lesions (SCLs). A total of 20 female rats were used. Under sterile conditions, SCLs were made at the T5 level using a #11 blade sparing only one ventral quadrant (VQ). The electrophysiological study was made from 1 day to 4 weeks after the lesion. RtN evoked field potentials were recorded bilaterally in the VH at L2/3 level using a glass microelectrode filled with 2 M NaCl (1.5-1.7 M Ohm). The gigantocellular reticular nucleus ipsilateral to the spared VQ was stimulated using a microelectrode. In some animals field potentials were monitored just before and right after the SCL. In an intact spinal cord, RtN stimulation produced very consistent field potentials bilaterally. The amplitude of the fields generated in the VH contralateral to RtN stimulation, however, was about one third of those recorded ipsilaterally. Following the lesions sparing the left VQ, field potentials evoked by left RtN were completely abolished in the right side VH whereas the field potentials remained intact in the left VH. The RtN evoked field potentials in the right VH gradually returned within 7 to 10 days after SCL. The time course of the returning evoked potentials coincided with the restoration of right hindlimb locomotion. Supported by The Miami Project.

145.5

POSITION AND FIBRE TYPE DEPENDENT SELECTIVITY IN NEUROMUSCULAR SYNAPSE FORMATION. A. W. Everett*, D. R. Brown* and M. R. Bennett, Neurobiology Research Centre, The University of Sydney, Sydney, Australia, 2006.

Using glycogen depletion procedures we have shown that motor axons to the glutaeus muscle of the toad (*Bufo marinus*) distribute their terminals according to the origins of the axons along the neural axis. Those glutaeus axons arising most rostrally innervate muscle fibres located in the ventral portion of the muscle, near nerve entry, while caudal axons innervate fibres mostly towards the opposing dorsal surface. The terminals of 'intermediate' axons are found mostly in the middle regions of the muscle. We found the same projection was re-established in adult muscle following two experimental procedures: firstly, when the muscle nerve was cut and regenerating axons were forced to reinnervate the muscle via entirely novel pathways through the dorsal surface; and secondly, when the muscle was reinnervated by a greater than normal number of axons that were mostly foreign but segmentally appropriate. The distribution of terminals, at least of rostral and intermediate axons, was clearly unrelated to the arrangement of fibre types: the majority of the muscle, excluding the very dorsal surface layers, consisted of a mosaic of type 1 and 2 amphibian fibre types both before and after nerve cut. In addition, the regenerated axons did not synapse randomly with the available selection of muscle fibres in their particular territory, but instead displayed the same fibre type bias seen normally, with rostral units consisting of mostly type 1 fibres and caudal units mostly type 2. The findings provide evidence for a clear distinction between at least two determinants that influence the innervation of muscle - positional cues and fibre types, with the former taking precedence over the latter.

145.6

Determining the Postnatal Growth Period of Corticospinal Axons in Cats Using MAP-1B Immunoreactivity. J.M. Alisky and D.L. Tolbert, Dept. of Anat. & Neurobiol. and Surgery (Neurosurg.) St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Growing and mature axons have different microtubule associated proteins (MAPs). Growing corticospinal (CS) axons in rats have been demonstrated immunocytochemically using monclonal antibodies to MAP-1B (J. Neurosci. 9:1712-1730). We used MAP-1B immunoreactivity to determine the growth period for CS projections in cats. From 1-21 postnatal day (PND), CS axons were strongly immunoreactive for MAP-1B in the pyramidal tract and spinal cord. The lateral corticospinal tracts (LCSTs) at all levels were immunoreactive. There was strong immunoreactivity in the anterior corticospinal tracts (ACSTs) only in the cervical levels but some ACST axons were labeled in lower levels. Immunoreactive axons were present in the dorsomedial portion of dorsal columns in cervical spinal cord levels. At 28 PND, immunoreactivity of CS axons was markedly decreased in the pyramidal tract and spinal cord. Within the context of this overall decrease, immunostaining of the LCSTs in thoracic and lumbar levels was slightly greater compared to cervical levels. At 38 and 46 PND, CS axons were not immunoreactive in the cervical levels and only lightly reactive in thoracic and lumbar levels. We conclude that most of the postnatal growth of CS axons occurs in the first 3-4 weeks after birth. By 6 weeks after birth, the growth period is virtually complete, ending rostrally before caudally.

We wish to thank Richard Vallee and George Bloom for their gift of anti- MAP-1B monoclonal antibody (PNAS 82:5404-5408). We also thank Thomas Schoenfeld and James Hammarbach for their helpful suggestions. Supported by NIH Grant N5-20227.

145.7

ADAPTATIONS OF DIAPHRAGM TO PROLONGED COMPENSATORY LOADING. G.C.Sieck and W.Z.Zhan. Dept. Biomed. Engineering, USC, Los Angeles, CA 90089.

Compensatory loading (CL) of the left hemidiaphragm (DIA) in hamsters was induced by paralysis of the opposite side for 2 or 6 weeks. CL doubled the transdiaphragmatic pressures generated during eupnea and increased integrated DIA EMG activity by 60%. Proportions of type I or II fibers were unaffected by CL. The cross-sectional areas of type I fibers were unchanged whereas the size of type II fibers increased throughout the 6 weeks of CL. Succinate dehydrogenase (SDH) activity (quantified microdensitometrically) of both type I and II fibers increased after 2 weeks and remained higher. Isometric twitch contraction time (direct muscle stimulation) decreased after 2 weeks and then increased by 6 weeks. Half relaxation time decreased throughout the 6 weeks of CL. Maximum tetanic tension increased after 2 weeks and then decreased by 6 weeks. The DIA also became more fatigable, but only after 6 weeks. In conclusion, the effect of CL on DIA mechanical properties are time-dependent but do not correlate with morphological adaptations. Changes in fatigability do not correlate with adaptations in fiber SDH activity.

145.8

ADAPTATIONS OF THE MEDIAL GASTROCNEMIUS MUSCLE TO INACTIVATION. W.Z.Zhan and G.C.Sieck. Dept. Biomed. Engineering, USC, Los Angeles, CA 90089.

The effects of 2 weeks of inactivity on the physiological, morphological, and histochemical properties of the medial gastrocnemius (MG) muscle were studied in hamsters by blockade of the sciatic nerve with tetrodotoxin. The proportions of type I and II muscle fibers (classified based on ATPase activity) were unaffected by inactivity. The cross-sectional area (CSA) of type I fibers increased while the CSA of type II fibers decreased. Fiber succinate dehydrogenase (SDH) activities (quantified microdensitometrically) of both type I and II fibers decreased with inactivity. Isometric contractile properties of the MG were studied using direct muscle stimulation. Twitch contraction and half relaxation times increased after 2 weeks of inactivity. Muscle tensions decreased at all frequencies of stimulation. Fatigue resistance to repetitive activation improved. We conclude that the mechanical changes induced by inactivity are most likely due to the increased relative contribution of type I muscle fibers to the total area of the MG. The improvement of fatigue resistance is not correlated with changes in fiber SDH activity.

145.9

MOTOR-UNIT ACTIVITY PATTERN IN THE DEVELOPING RAT SOLEUS MUSCLE. T. Eken*, G. Elder* and T. Lømo* (SPON: European Brain and Behaviour Society). Institute of Neurophysiology, University of Oslo, 0162 Oslo 1, Norway.

The fibre-type composition of the rat soleus muscle undergoes marked changes during postnatal development, from 57% slow-twitch Type I fibres at day 18 to 90% at day 224 (Elder & McComas, *J Appl Physiol* 62:1917–1923, 1987). Can these changes in fibre type be explained by changes in motor-unit activity pattern?

We implanted electrodes for single-unit (Hennig & Lømo, *Nature* 314:164–166, 1985) and gross EMG registrations chronically in soleus muscles of 1–5 week old rats. The rats were allowed to move freely in a cage, and the recordings were made during spontaneous movements. In selected cases the movements were also recorded on video tape.

The modal firing frequency of single motor units did not change appreciably from 10 days to adult, ranging from approximately 20 to 30 Hz in all groups. The distribution of interspike intervals was broader in the younger rats compared to adult rats, and the bistable firing behaviour characteristic of adult soleus motor units (Eken & Kiehn, *Acta Physiol Scand* 136:383–394, 1989) was not observed. Gross EMG recordings indicated a two-stage development of the adult activity pattern: During the second week, the activity changed from phasic to irregular tonic. This tonic activity gradually changed into the adult type, which is characterised by single units often firing with a stable frequency for periods lasting up to several minutes.

It is concluded that changes in firing frequencies are not likely to be responsible for the observed changes in fibre-type composition. However, during the first weeks the animal steadily increases the amount of muscle activity, which is an important parameter for the induction or maintenance of slow contractile properties in the adult (Eerbeek, Kernell & Verhey, *J Physiol* 352:73–90, 1984; Eken & Gundersen, *J Physiol* 402:651–669, 1988; Westgaard & Lømo, *J Neurosci* 8:4415–4426, 1988).

145.10

SEGMENTAL ORGANIZATION OF THORACIC PREGANGLIONIC NEURONS IN CHICK AND RAT EMBRYOS. E.B. Ezerman[1], J.C. Glover[2] and C.J. Forehand[1]. [1]Dept. of Anatomy and Neurobiology, Univ. Vermont Coll. Med., Burlington, VT 05405 and [2]Inst. Physiology, Univ. Oslo, Oslo, Norway.

In the adult rat, an individual midthoracic spinal segment provides sympathetic preganglionic innervation to paravertebral ganglia at levels both rostral and caudal to the segment; however, individual preganglionic neurons send their axons either rostrally or caudally in the sympathetic chain (Forehand & Rubin, *Soc. Neurosci. Abstr.* 12:1056, 1986). We have explored preganglionic projections in the embryonic rat and chick to determine whether directional specificity is a property of the early outgrowth of these fibers.

Preganglionic projections were labeled with fluorescein and rhodamine conjugated dextran amines (FDA, RDA) in isolated spinal cord/sympathetic chain preparations of 9-10 day chicken and 17-19 day rat embryos. Rostrally and/or caudally projecting preganglionic neurons within a single segment were labeled by placing RDA and FDA on the sympathetic chain, one on either side of a single ganglion. The sympathetic chain rostral and caudal to the labeling site was avulsed prior to labeling. The preparations were maintained alive for 10-16 hours prior to fixation and vibratome sectioning. In some cases, DiI was used to label adjacent segmental sympathetic chain ganglia on either side of the spinal cord in fixed preparations.

In both chick and rat embryos, as in the adult rat, spinal segments provided innervation to both rostral and caudal ganglia, while individual preganglionic neurons projected only in the rostral or caudal direction. Moreover, preganglionic neurons with rostral projections occupied the rostral 2/3 of the spinal segment, while those with caudal projections occupied the caudal 2/3 of the segment. Supported by NIH and a NATO Grant for international collaboration.

145.11

THE NUMBER OF FAST AND SLOW MOTOR UNITS DURING POSTNATAL DEVELOPMENT IN THE RAT SOLEUS MUSCLE. W. J. Thompson and S. H. Astrow. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

During the first 2 weeks of postnatal life the rat soleus contains about 1200 fast and 1500 slow fibers. Glycogen depletion experiments indicate that the fibers are organized into fast and slow motor units as early as postnatal day 8; however, this technique cannot be used to determine the number of such fast and slow motor units in individual muscles. Twitch unit size (given by the fraction: unit twitch force/muscle twitch force) and the twitch rise time provide no unambiguous way to identify slow and fast motor units at 17-18 days; these parameters form a continuous distribution extending from units with fast rise times and large unit sizes to units with slow rise time and small unit sizes. However, the ratio of the twitch force to the tetanic force generated by a unit, a value which can be determined for each single unit isolated in a muscle, can be used to predict a unit's fiber type identity in the 17-18 day rat soleus. In such muscles, motor units can be divided into two groups which differ significantly in their twitch/tetanus ratios (Mann Whitney U test). Units with high twitch/tetanus ratios have more rapid rise times, suggesting they contain fast fibers. This prediction has been verified using glycogen depletion. Nine units have been depleted, and all units with ratios ≥ 0.38 have been found to be fast and all units with ratios ≤ 0.35 have been found to be slow, even though some of the units have similar twitch rise times. Based on the fraction of units which have ratios characteristic of fast units, 38% of the units in soleus at 17-18 days are fast. Since the total number of motor units in the muscle is roughly 24, these results suggest an estimate of 9 fast and 15 slow units in soleus at 17-18 days.

145.12

MOTOR UNIT TRANSFORMATION IN A MOUSE FAST-TWITCH MUSCLE K. Condon and W. J. Thompson. Dept. Zoology, Univ. of Texas, Austin, TX 78712.

The mouse extensor digitorum longus (EDL) muscle undergoes a progressive loss of slow fibers during early adult life, from ca. 160 fibers at 18 days of age to 0-9 fibers at 3-6 months. Immunocytochemistry using antibodies to myosin isoforms shows many fibers which express slow myosin also co-express fast 2a myosin during the transition, suggesting that slow fibers are being transformed to fast type 2a. Based on the response to denervation, this transformation is innervation dependent. Two explanations can be offered for this transformation: 1) this muscle possesses no slow motor neurons and the slow fibers, innervated by fast motor neurons, are converted to fast; 2) the slow fibers are selectively innervated by slow motor neurons but these slow motor neurons transform to fast and in turn convert the fibers they innervate. The first explanation predicts mixed motor units, the slow fibers being distributed among the fast units based upon their relative frequency (approximately 5-6 fibers per unit). The second explanation predicts slow motor units in varying stages of transformation to a fast 2a type. Individual motor units in C57BL/6J mice between the ages of 20-30 days were isolated from teased ventral roots and identified using the glycogen depletion technique. Biasing our sample to units of slow twitch rise time, we found 3 units where greater than 90% of the fibers stained for slow myosin. Each of these units contained fibers transforming to 2a. Thus, slow fibers in mouse EDL appear to be selectively innervated and their loss results from the transformation of slow units to type 2a, analogous to the reverse transformation known to occur in rat soleus.

145.13

THE EFFECTS OF SPINAL TRANSECTION ON THE MOTOR CYCLICITY IN NEONATAL RATS. M. Dyer, E. A. Strauss, and C. R. Almli. Devel. Neuropsychobiol. Lab., Dept. of Psychol., Wash. Univ., St. Louis, MO, 63130.

Cyclic, spontaneous movement (CSM) patterns have been demonstrated in newborn rat pups. This study determined if fore- and hindlimb CSM were decoupled after midthoracic spinal cord transection.

Under cryoanaesthesia, a midthoracic spinal transection was performed (aspiration) in neonatal rats. After surgery, pups were placed in an incubator ($37 \pm 1^{\circ}C$) until recovery from anaesthesia and then returned to the dam for 24 hours. At 24 hours post-surgery the pups were video-taped for 10 minutes while in the incubator. Tapes were computer-analyzed for counts of movements of fore- and hindlimbs. Cyclicity patterns were measured with spectral analyses (FFT).

The present data indicate that spinal transection in rat pups may alter the cyclicity and phase of the hindlimbs. (Conducted under NIH Guide for Care and Use of Laboratory Animals).

146.1

ONTOGENIC DEVELOPMENT OF SENSORIMOTOR REFLEXES IN THE OPOSSUM, MONODELPHIS DOMESTICA. G. Cassidy *, D. Boudrias* and T. Cabana, Dép. de Sciences biologiques, Université de Montréal, C.P. 6128, Succ. "A", Montréal, Qc. Canada H3C 3J7.

The ontogenesis of sensorimotor reflexes can be studied in Monodelphis domestica, a marsupial born very immature. At birth, the forelimbs perform a rhythmic, alternate movement resembling swimming, which enables the newborn to climb on the mother's belly and reach a nipple; the hindlimbs are merely more developed than buds and do not move independent of the trunk. A number of reflexes were tested in the adult animal and, daily, in newborn opossums up to 55 days postnatal (PND), when the expression of the reflexes studied resembled the adult pattern. The following sequence in the appearance and maturation of reflexes could be described: forelimb grasp, body righting, forelimb hopping, hindlimb grasp, chin placing, dorsal then lateral forelimb tactile placing, hindlimb hopping, air righting, dorsal then lateral hindlimb tactile placing and, finally, visual placing. Rooting which is present in neonatal opossums, disappears around 32 PND. This behavioral sequence generally matches the sequence of limb and neural (spinal cord and brain structures involved in the control of these behaviors) development and maturation, as judged from histological sections and preliminary tracing studies.

146.2

MORPHOLOGY, DISTRIBUTION AND QUANTITATIVE DEVELOPMENT OF NEURONS IN THE WHITE MATTER OF THE HUMAN MOTOR CORTEX. G.Meyer* and P.Wahle. Dept.Anatomy,Fac.Medicine, La Laguna /Tenerife, Spain, and MPI Biophys.Chemie,Dept.Neurobiology, Göttingen,FRG.

In the adult human brain,neurons in the white matter (interstitial neurons) are especially numerous under the motor cortex. They are mostabundant in the vicinity of layer VI, where they show a columnar alignment, but they are also present in the white matter several cms below the gray matter. The Golgi method and MAP 2-immunohistochemistry, carried out on autopsy brains,reveal that most neurons in the white matter have a pyramidal morphology, displaying apical and basal dendrites and dendritic spines. The multipolar, nonpyramidal NADPH diaphorase-positive neurons form only a small fraction of the white-matter population. Cell counts in 30 Nissl-stained brains from pre-and perinatal ages until old age show a sharp decrease of cell density due to both cell death and dilution in the expanding white matter, until the first postnatal months.This decrease is more significant in the superficial than in the deep white matter. After the age of 10 years cell density remains stable throughout life.

146.3

AFFORDANCES IN GAP CROSSING : DEVELOPMENTAL CONSIDERATIONS K.G. Holt*, L.Fetters, I.Chazan*, J.Meehan* and B. Nelson*. Dept Physical Therapy, Boston University

Adults have been shown to body-scale to leg length in their perception of climbable stairs and in freely adopted cadence of walking . The purpose of this study was to determine the accuracy with which adults and children perceive and act when faced with the task of crossing gaps, as might be experienced in crossing a stream. Leg lengths of nine year-olds change more rapidly than at any other age, and they might be expected to be less sensitive to the action capabilities their bodies afford. Subjects were asked whether various gaps between two platforms were crossable and then to attempt the crossing. Two setups of the gaps were presented for the actual crossing: as discrete widths (DIS) and as a continuous V-shape (CON). Pi (π) values defined as the ratio of perceived (π_{PER}) or actual crossability (π_{DIS} and π_{CON}) to leg length were calculated. Results indicated that nine year-olds and adults have the same body-scaling relationships for perception (π_{PER}= 1.4), and continuous action (π_{CON}=1.5) and are significantly different for discrete action (π_{DIS}= 1.4 and 1.3 respectively). Children tended to overestimate the gaps they were able to cross, while adults tended to underestimate. Both groups increased the actual size of the gap they could cross on successive trials. CON setup was more ecologically valid than DIS setup These results suggest that despite rapid increases in growth children are able to quickly adapt to their new dimensions and new tasks. It is suggested that the paradigm may be used to diagnose children whose motor development may be delayed.

146.4

MODIFICATION OF THE HUMAN BICEPS SPINAL STRETCH REFLEX: PRELIMINARY STUDIES ON PROLONGED CONTROL AND FOLLOW-UP PERIODS. R.L. Segal, P.A. Catlin*, C. Barker*, P. Broadbent*, A. Cockerham*, L. Reaver* and S.L. Wolf. Div. of Phys. Ther., Emory Univ. Sch. of Med., Atlanta, GA 30322

This study examined the effect on biceps EMG of a prolonged control period before training (subject = 5) and prolonged follow-up period after training (subjects = 4) of the biceps spinal stretch reflex (SSR) in normal humans. Each subject had 4 baseline sessions without feedback of biceps SSR magnitude. Then, subjects were randomly assigned to one of two sequences: 1) control (8 sessions as in baseline)-Training (8 sessions with feedback of SSR magnitude and operant conditioning) or; 2) Training-Follow-up (Follow-up = 8 sessions same as baseline). For subjects in sequence 1, the biceps SSR decreased during the first two control sessions, then remained relatively stable with a mean decrease of 22.7%. For subjects in sequence 2 (at present all uptrainers), the SSR was uptrained a mean of 25% with an average decrease of only 7% during the 8 follow-up sessions. These results suggest that a more prolonged baseline period than previously used is necessary before initiation of training. In addition, there appears to be some persistence of the training effect during follow-up for uptrainers in this study. Interestingly, we also monitored a similar (but more variable) increase in EMG for the synergist brachioradialis during biceps uptraining, but the decrease in follow-up was more substantial (-39%).

146.5

PRE-TREATMENT WITH DSP-4 SLOWS BEAM-WALKING RECOVERY IN THE RAT. L.B. Goldstein, A. Coviello, G.D. Miller and J.N. Davis. V.A. & Duke Medical Centers, Durham, N.C. 27705

Treatment with amphetamine hastens the recovery of beam-walking in rats after a unilateral lesion of the sensory-motor cortex. We have hypothesized that the amphetamine effect is mediated through central noradrenergic neurons since selective a2-adrenergic receptor antagonists facilitate recovery while a2-adrenergic receptor agonists are detrimental. To further test this hypothesis, we carried out an experiment in which one group of rats were pre-treated with DSP-4 and a second group with saline. Two weeks after pre-treatment, rats were trained at the beam-walking task and then randomly assigned to undergo a unilateral sensory-motor cortex suction ablation or sham operation: 1. DSP-4/Lesion (n=14), 2. Saline/Lesion (n=19), 3. DSP-4/Sham (n=6), 4. Saline/Sham (n=8). DSP-4 pre-treatment slowed recovery in lesioned rats (ANOVA (3,43) $p<.0001$; Fisher LSD, $p<.05$) but did not interfere with beam-walking in sham operates (Fisher LSD, $P>.05$). DSP-4 pre-treatment resulted in similar decreases in cerebral cortical norepinephrine in both sham-operated and cortical lesioned rats. These data are consistent with the hypothesis that norepinephrine influences motor recovery after unilateral cortex lesions.

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146.6

L-DOPA INDUCED LOCOMOTION IN NEONATAL DECEREBRATE RATS. T. Iwahara*, C. Van Hartesveldt, E. Garcia-Rill and R. D. Skinner. Dept. of Anatomy, University of Arkansas for Medical Sciences, Little Rock, AR and Dept. of Psychology, University of Florida, Gainesville, FL.

Neonatal rats were anesthetized with penthrane and a precollicular brainstem transection performed. At 0-3 days, L-DOPA (100mg/kg s.c.) induced continuous, long-lasting air stepping consisting of four-limb walking with stronger alternation in the forelimbs compared to the hindlimbs. At 6-8 days, continuous air stepping was characterized by better agreement between forelimb and hindlimb step cycles. By 14-16 days, the hindlimbs showed faster stepping than the forelimbs. The first evidence of consistent galloping was observed in this age group. In some cases, the forelimbs were held extended and only the hindlimbs walked or galloped. At 20-22 days, walking (forelimbs) and galloping (hindlimbs) became episodic. Each age group was tested for overground locomotion. Only in the 20-22 day group was weight-bearing overground stepping observed. In general, the duration of the effect of the same dose of L-DOPA decreased with age. Animals in each group which received a mid-thoracic spinal cord transection showed forelimb alternation but no hindlimb stepping. These results suggest that L-DOPA induces stepping by activating brainstem and/or cervical spinal centers via an as yet unknown mechanism. The pattern of the development of gait (forelimb to hindlimb gradient) at various ages was similar to that observed in intact neonatal rats (Sickles et al, ISDP, 1989).

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146.7

THE FUNCTIONAL SIGNIFICANCE OF NEURONAL AND GLIAL CHANGES OCCURRING IN THE ADULT RAT PHRENIC NUCLEUS DURING AGING. H.G. Goshgarian and X.-J. Yu*. Dept. of Anatomy, Wayne State Univ. Sch. of Med., Detroit, MI 48201.

Morphometric analysis of the ultrastructure of the phrenic nucleus in young adult rats (9-10 weeks) and older adult rats (9-10 months) showed that there is a significant increase in the number of double synapses in the phrenic nucleus of older rats (27.5 $\pm$ 2.4) as compared to younger animals (20.25 $\pm$ 1.2). Moreover, astroglial process retraction from in between adjacent dendrites during aging results in a significant increase in the length (1.73 $\pm$ 0.1μm) and percentage (6.56 $\pm$ 0.4) of dendrodendritic membrane appositions in the older animals as compared to the length (1.42 $\pm$ 0.1 μm) and percentage (5.05 $\pm$ 0.4) of appositions in the younger rats. Quantitative electrophysiological analysis of phrenic nerve activity during a respiratory reflex known as the "crossed phrenic phenomenon" (CPP) in both young and older adult rats revealed that there was a significant increase in the mean integrated area of the phrenic nerve compound action potential in older rats (45.3 $\pm$ 15.3 mm^2) as compared to measurements taken from younger rats (11.8 $\pm$ 1.6 mm^2). Moreover, the duration of the reflex was significantly longer in older rats (36.4 $\pm$ 3.7 sec) as compared to the duration in younger animals (15.81 $\pm$ 3.1 sec). These results suggest that there is a correlation between the morphological changes and the enhanced expression of the CPP.

146.8

TRANSCRANIAL MAPPING OF THE CORTICAL HOMUNCULUS. W.J. Levy, V. Amassian, J. Cadwell, M. Kitagawa*, U. Schmid*, M. Traad*. Dept. of Neurological Surgery, Univ. of Pittsburgh, Pittsburgh, PA 15261.

The motor homunculus is a well-described and well-characterized organizational feature. However, its representation varies substantially between individuals, and more significantly in the presence of disease. In this sense, the traditional description is oversimplified. These variations are of scientific interest, and can aid or hinder surgery for lesions in the vicinity. We have developed a method for the use of a magnetic coil (Cadwell figure of eight using an MES-10 stimulator) to stimulate designated scalp sites. The contralateral EMG is recorded from surface electrodes over the biceps, triceps, APB, and ADM muscles. Maps are then constructed of EMG amplitude versus stimulation site. These maps are superimposed on an MRI scan of the subject, done with marking stereotaxic coordinates and cortical stimulation. This method is encouraging for the development of a transcranial cortical mapping system. Such a system would improve the knowledge of plasticity in the motor cortex with injury, and the management of patients with lesions in the area.

146.9

DIFFUSE NERVOUS SYSTEM INJURY ALTERS CYCLIC, SPONTANEOUS MOVEMENTS OF NEWBORN, FULLTERM AND PRETERM RATS. C.R. Almli and M. Dyer. Developmental Neuropsychobiology Laboratory, Washington University Medical School, St. Louis, MO 63110.

Cyclic, spontaneous movement (CSM) patterns are displayed by fullterm, preterm and fetal rats. The present study determined the effects of diffuse nervous system injury on CSM patterns of neonatal rats born vaginally (fullterm) or delivered by caesarian section at preterm gestational ages (Preterm).

Following delivery at 21-22 days (fullterm), 21 days (preterm), or 20 days (preterm) gestational age, the"neonates" were housed in an incubator (37 $\pm$1^{o}C) for 24 hours. Diffuse nervous system damage was produced by oxygen deficiency. Neonates were videotaped for 10 minutes pre-treatment, at 1-hour post-treatment, and at 19-hours after treatment. Movements were analyzed with computers for counts and durations of body segment movements (mouth, head, limbs, and trunk) and complex movements (ambulation, body position change, and face brushing). Segment and whole body movements were analyzed for cyclicity with spectral analysis (FFT).

Diffuse nervous system injury alters movements and movement patters of fullterm and preterm rats. The treatment severely disrupted CSM patters for all age groups. (Conducted under NIH Guide for Care and Use of Laboratory Animals).

DEVELOPMENT AND PLASTICITY—VISUAL SYSTEM: RETINA AND OPTIC NERVE

147.1

THE CHARACTERIZATION OF THE RIBBON SYNAPTIC STRUCTURE IN MICE WITH RETINAL DEGENERATION. Grant W. Balkema and Katherine Bachman*, Department of Biology, Boston College, MA 02167.

A monoclonal antibody, B16, has been produced that recognizes a protein determinant associated with the ribbon synaptic structure in the photoreceptor terminal. We have found this antigen in every species that we have examined (fish, frog, lizard, mouse, rat, rabbit, cat, and monkey).

We examined the emergence of this synaptic protein in developing mouse retina from birth (P_0=postnatal day zero) through P_{21}. Mouse retinas were lightly fixed with PLP (2% paraformaldehyde/lysine/periodate), frozen, sectioned at 8μM and placed on glass slides. Sections were incubated with the B16 primary antibody and visualized with a fluorochrome conjugated secondary antibody. Using the light microscope we first detected peri-nuclear labeling on days P_3 -P_4. By days P_4-P_5 a thin, faint line in the outer plexiform layer (OPL) could be detected. At day P_7 the OPL was well developed and formed a sharp line. These results are in agreement with previous ultrastructural studies.

The early postnatal development in the rd/rd mouse was similar to its wild-type control through P_7. The rd/rd mouse failed to develop well defined ribbons. In the normal mouse (and all other species) the ribbon structure has a characteristic arch shape with the top of the arc pointing towards the outer nuclear and the open end pointing towards the inner nuclear layer; the ribbon in the rd/rd mouse was punctate. We also observed labeled debris at the retina/pigment epithelium border in sections from rd mice days P_4-P_7. The ribbons in the rd/rd mouse persist as the photoreceptor outer segments degenerate, although they are drastically reduced in number. (Supported by the NSF and the NEI.)

147.2

EMERGENCE OF THE PHOTORECEPTOR MOSAIC FROM A PROTOMAP OF EARLY-DIFFERENTIATING CONES IN THE PRIMATE RETINA Kenneth C. Wikler and Pasko Rakic. Section of Neuroanatomy, Yale University School of Medicine, New Haven, CT 06510

In the adult rhesus monkey retina about 90% of all cones are red/ green- sensitive as revealed by opsin-specific antisera (Wikler and Rakic, J. Neurosci., 1990). Although the general gradient of neurogenesis proceeds from the center to periphery in the monkey retina, a subset of peripheral cones are generated in advance of neighboring photoreceptors (LaVail et al., J. Comp. Neurol., 1990). We have used the red and green opsin- specific polyclonal antibody, 4942A (Lerea et al., Neuron, 1989), to assess whether precociously- generated cones in the periphery express their photopigment in advance of later- generated adjacent cones.

Eight monkey retinae from embryonic (E) day 64 to term were flat-mounted and processed for immunocytochemistry. Between E80 and E121, the distribution of 4942A- immunoreactive cells revealed two distinct patterns of red/green- sensitive cones. As in the adult, more centrally located retinal areas contained about 90% red/green- sensitive cones. In contrast, in peripheral regions of the same retinae, only about 10% of morphologically-identified cones were immunoreactive. These early-maturing, red/green- sensitive peripheral cones were stationed evenly throughout the embryonic retinal surface among still undifferentiated, but already postmitotic cones. The distribution of a sub-population of early immunoreactive cones paralleled that of peripherally located cones labeled by early fetal injections of 3-H thymidine. By E140, the proportion of 4942A- labeled cones reached the mature level of 90% across the entire retina. We suggest that precocious red/green cones may induce the differentiation of about ten surrounding, later-generated immature cones. The emergence of cell phenotypes in the photoreceptor mosaic, prior to the establishment of their synaptic connectivity in the outer plexiform layer (Nishimura and Rakic, J. Comp. Neurol., 1985), supports the concept of a protomap proposed in other regions of the central nervous system (Rakic, Science, 1989) and appears similar to the mode and sequence of development of pioneer cells described in the photoreceptor mosaic in drosophila (Ready, TINS, 1989).

147.3

GENESIS OF RETINAL GANGLION CELL SUBTYPES IN THE MONKEY D.H. Rapaport[1], J.T. Fletcher[1*], M.M. LaVail[2] and P. Rakic[3] [1]Dept. Anatomy, Univ. of Sydney, [2]Dept. Anatomy, U.C.S.F., [3]Sect. Neuroanatomy Yale Univ.

The retinal ganglion cell layer (GCL) contains several types of cell which can differ on soma size. Displaced amacrine cells are generally smaller than ganglion cells and P(primate)α, Pβ and Pγ tend to be large, medium and small respectively. Determination of the birthdates of different types and subtypes of cell could suggest the influences which operate in shaping their fate. Retinas from monkeys injected with ^{3}H-thymidine *in utero* and allowed to survive to maturity were analyzed. Analysis was performed in peripheral retina since central cells are largely of homogeneous size.

The mean diameter of somas in the GCL born on E38 was 10.5μm, and increased monotonically to 14.5μm at E56 before dropping to 9.2μm at E70. The initial increase is due partly to an expansion of the range of diameters. All neurons in the GCL born on E38 or E40 are between 6-14μm dia., the maximum increases to 19μm at E50 and 22μm at E56. The increasing frequency of labeled cells ≥20μm is accompanied by a decrease of labeled cells in the 15-19μm dia. range (from 46% of population at E50 to 30% at E56). By E70 the density of labeled GCL cells dropped considerably and all labeled cells were ≤10μm.

Although distinct size groups of ganglion cells are not readily apparent in the primate the data suggest that different types of ganglion cells are born at different times. Specifically, that Pγ cells are generated early, Pβ cells somewhat later, and Pα cells are last to be born. The absence of labeled cells >10μm at the last stage of generation of cells in the GCL suggests that those small cells which are labeled may be amacrine cells. If this is the case then displaced amacrine cells would be born last among the GCL population, but before cells of the same phenotype in the INL (LaVail et al., submitted).

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147.4

THE DENSITY OF BETA CELLS AFFECTS THE SIZES OF ALPHA CELLS IN CAT RETINA. S.J. Ault, K.G. Thompson, Y. Zhou, and A.G. Leventhal, Anat. Dept., Univ. of Utah Sch. of Med., Salt Lake City, UT 84132

In recent years, the retinal distributions, morphologies, and development of alpha and beta cells have been studied in detail. Experiments have been carried out in normal retinae and in retinae depleted of retinal ganglion cells in the neonate. Indirect evidence from these studies has led to the idea that class specific interactions mediate retinal ganglion cell development. We have now tested this hypothesis directly by examining the morphologies of alpha cells in regions of mature cat retina selectively depleted of beta cells as a result of lesions of visual cortex at birth.

We find that, in beta cell poor regions, alpha cells are present in normal numbers and are distributed normally in a 'mosaic-like' pattern. However, they are clearly larger than normal. Alpha cell size was observed to vary as a function of beta cell density; alpha cells in regions of central retina depleted of beta cells were nearly the same size as their normal peripheral counterparts. Thus, the 'coverage' of central retina by alpha cells appeared to be abnormally high. These results suggest that the sizes of cat retinal ganglion cells depend upon overall cell density; the extrinsic determinants of retinal ganglion cell size appear not to be class specific. On the other hand, the nonrandom, territorial distribution of alpha cells across the retina may be determined by class specific mechanisms. Supported by EY04951.

147.5

VARIATIONS IN RETINAL LAMINAR THICKNESS MAY PREDISPOSE DIFFERENTIAL STRETCH DURING RETINAL GROWTH. A.D. Springer and B. Lia. Dept. of Cell Biol. and Anat., New York Med. Coll., Valhalla, NY 10595 and Dept. of Psych., Univ. Calif., Davis, CA 95616.

Thickness of the neuroblast (NBL), ganglion cell (GCL), inner nuclear, (INL) and outer nuclear layers (ONL) was determined from plastic sections in developing goldfish retina. At stage 23, prior to any retinal differentiation, the neuroblast layer was 1.5 times thicker at the temporal edge of the embryonic fissure than it was at the temporal periphery or nasal to the fissure. Differentiated layers were observed at stage 25. The GCL, INL, and ONL were 1.5, 1.4, and 1.3 times thicker, respectively, temporally than nasally. By 56 days posthatch these values were 2.2, 1.7, and 1.7 respectively.

E35 cat retina has a GCL 2.5 times thicker temporal to the optic disc than elsewhere. Combined with the NBL, overall retinal thickness is also greatest in this zone at a time when cell size and packing density is relatively uniform throughout the retina. Differential retinal expansion after this stage has been documented (Mastronarde et al., JCN, '84; Lia et al., Sci., '87).

If the retina is thought of as an elastic sheet (Kelling et al., Vis. Nsci., '89), the thickened region would be subject to disproportionately less stretch than the surrounding area as the retina is stretched by growth of the eye. Thus, the thickened cell layers on the temporal side of the early retinal anlage may predispose the vertebrate retina to differential retinal stretch and contribute to the generation of cell density gradients.

147.6

CALCIUM HOMEOSTASIS IN GROWTH CONES OF GOLDFISH RETINAL GANGLION CELL NEURITES. V.P. Bindokas and A.T. Ishida. Dept. Animal Physiology, University of California, Davis CA 95616.

We have found that single retinal ganglion cells of goldfish (*Carassius auratus*) grow extensive neuritic processes in primary cell culture (Ishida and Cheng, submitted). Since cytoplasmic calcium levels appear to affect neurite outgrowth in a wide variety of cells, we have studied $[Ca]_i$ (free intracellular calcium concentration) in growth cones of single goldfish retinal ganglion cells by ratio imaging analysis of fura-2 spectra. In saline containing 5 mM K^+, $[Ca]_i$ averaged over growing tips is greater than in the somata. Between the distal and proximal regions of growth cones, $[Ca]_i$ ranges from ca. 40 nM to several μM; these gradients persist for tens of seconds, even though positions of high $[Ca]_i$ shift over time. In high-K^+ salines, growth cone $[Ca]_i$ increases, and appears to be regulated largely by 3 mechanisms: (1) The increase results largely from influx of external Ca^{++} through L-type Ca-channels: this influx is blocked by Co^{++}, enhanced by BAY K 8644, and antagonzied by nifedipine. (2) $[Ca]_i$ appears to be buffered in a Na^+-dependent manner: in salines containing 120 mM Na^+ and 23 mM K^+, plus 0.3 μM TTX, $[Ca]_i$ initially rises ca. 5-fold over basal $[Ca]_i$, and thereafter declines by 30% (i.e. to ca. 3-fold over basal) within 5 min. After replacing Na^+ by n-methylglucamine, the $[Ca]_i$ increase elicited by 40 mM K^+ declines by ≤ 3% over 7 min. (3) $[Ca]_i$ also appears to be buffered in a Na^+-independent manner: $[Ca]_i$ decreases upon return to control Na^+-free saline, consistent with the possibility that retinal ganglion cells also possess an ATP-driven Ca^{++} pump.

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147.7

DEVELOPMENT OF A SPECIFIC GANGLION CELL TYPE IN EMBRYONIC CHICK RETINA. S.M. Fraley. Dept. Ophthalmology, New York Medical College, Valhalla N.Y., 10595.

The dendritic development of displaced ganglion cells was studied using an in vitro method for labeling the optic nerve stump retrogradely with horseradish peroxidase (HRP).

The cells were identified in flatmounts and sections of flatmounts by soma position within the retinal laminae. Displaced ganglion cells were observed at the earliest developmental stage studied (E9) and were characterized by a round or elliptical soma containing 3-5 proximal dendrites. The developmental sequence from E9 to the time of hatching (E21) consisted of soma enlargement, considerable dendritic elongation and branching, as well as elimination of short side branches and processes from the dendrites and soma, respectively. At all assessed stages the dendritic field of the displaced ganglion measured as one of the largest compared to other labeled ganglion cells. Thus, it appears that the major morphological features of displaced ganglion cells are evident early in retinal development. Supported by NIH EY0720603.

147.8

DENDRITIC DEVELOPMENT OF ABNORMALLY PROJECTING RAT RETINAL GANGLION CELLS (RGCs). E.N. Yamasaki & A.S. Ramos. Lab. Mol. Pharmacol. II, IBCCF, UFRJ, Rio de Janeiro, 21944 RJ, Brazil and Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. of Med., Baltimore, MD USA 21201.

Interaction with a target is critical for survival of mammalian RGCs, but it is less clear whether this interaction plays a role in maturation of their dendritic trees. To make inferences about the role of target nuclei in regulation of dendritic development we have compared the morphology of three populations of RGCs with distinct patterns of axonal projection: topographically correct projection to caudal superior colliculus (CSC), topographically incorrect projection to CSC or transient projection to inferior colliculus (IC). To identify RGCs, rhodamine-labeled latex microspheres were injected in IC or CSC. Retinae were dissected and the detailed morphology of RGCs was then revealed by intracellular injections of Lucifer Yellow. Different classes of neurons projected to IC and CSC: topographic targeting errors were most frequent for class III (50% of 70 cells at P3–P30) while class II cells projected to IC (100% of 23 cells at P3–P12). They were morphologically indistinguishable from correctly projecting cells. Dendrite and soma diameters as well as dendritic complexity of aberrant cells were within the normal range for each class. Moreover, as occurred in correctly projecting cells, their dendritic trees displayed a large number of transient filaments which increased in number during the first postnatal days. These results support the notion that dendritic development of RGCs is independent of the pattern of axonal projections but regulated by intraretinal cues. (CNPq & FINEP/Brazil; FINEP/Maryland)

147.9

LOCALIZATION OF SPECIFIC mRNAs IN DIFFERENTIATING RETINAL CELL TYPES. P.S Jones and A.J. Aguayo. Center for Research in Neurosciences, Montreal General Hospital and McGill University, Montreal, Quebec, H3G 1A4.

In situ hybridization was used on developing rat retina to examine the localization and time course of expression of specific mRNAs. At E14 GAP-43 message was localized primarily in the inner layer of the posterior retina, near the optic disc. By E20 reactivity had spread throughout the inner surface of the retina. After birth this reactivity decreased, reaching the adult signal intensity by P25. This pattern of reactivity is consistent with GAP-43 being expressed mainly in retinal ganglion cells undergoing active axonal growth.

Opsin message was first observed postnataly, and was restricted to cells in the outer retina. The opsin hybridization signal increased after P3 and attained adult intensity and distribution by P25. These observations closely follow the development of photoreceptor cells in the rat.

These observations indicate that GAP-43 and opsin mRNA levels reflect the differentiation and maturation of two distinct cell types in the developing rat retina.

147.10

DEVELOPMENT OF RETINAL CELL NUMBER, DISTRIBUTION, AND TYPE DURING THE PROTRACTED DEVELOPMENT OF THE CALIFORNIA MOUSE. A.A. Dos Santos, D.A. Schaefer*, and D.R. Sengelaub. Program in Neural Science, Dept. of Psychology, Indiana University, Bloomington, IN 47405.

The California mouse (Peromyscus californicus) and the Syrian hamster (Mesocricetus auratus) are closely related cricetid rodents with virtually identical eye size, retinal cell number, type, and distribution. This similarity is interesting in that developmental duration and rate, two factors hypothesized to be important in producing species differences in retinal organization, are strikingly different between these rodents. Gestation, closed eye period, and attainment of sexual maturity and adult body size are significantly longer or delayed in the mouse. We are studying the protracted development of the California mouse to determine how formative events in retinogenesis are orchestrated in the production of its hamster-like eye.

Neurogenesis of the mouse retinal ganglion cell layer (RGCL) is twice that of the hamster (12 vs. 6 days), but peak cell numbers in the RGCL do not differ. The morphology of 3H-thymidine labeled cells at adulthood indicates that, like the hamster, ganglion cell generation precedes that of displaced amacrine cells. Perinatal cell death accounts for a decline of at least 40% in the RGCL and is complete by postnatal day 14. Like the hamster, this death is greatest in peripheral retina, and center/periphery differences in cell density begin to emerge during this period of differential death. Significant and symmetrical eye growth occurs postnatally, and this growth likely also contributes to the production of the adult cell distribution. At adulthood however, differences in center/periphery cell densities are apparent for only the early-generated cohorts suggesting that retinal growth alone cannot account for the creation of the retinal topography. Thus, despite its protraction, the events and consequences of retinal development in the California mouse are similar to those seen in the hamster, resulting in a similar retinal morphology.

147.11

EXPRESSION OF THE COLLAGEN IV GENE DURING DEVELOPMENT OF THE MOUSE RETINA. P.V. Sarthy, M. Fu* and J. Huang*. Department of Ophthalmology, University of Washington, Seattle, WA 98195.

In a recent study we showed that laminin B1 mRNA was expressed by both non-neural cells and retinal ganglion cells (RGC) during development of the mouse retina. Since collagen IV is usually associated with laminin in the basal lamina, we examined collagen expression in the developing mouse eye by immunostaining and *in situ* hybridization.

Collagen immunostaining was detected as early as embryonic day 12 (E-12). At E-12 and E-15, collagen was found in the lens, the hyaloid vessels which lie between the lens and the retina, and the internal limiting membrane (ILM) of the retina. At E-17 and subsequent stages, immunostaining was no longer detected in the retina except at the far periphery. The lens and optic vessels were, however, stained. The *in situ* hybridization experiments showed that at E-12, labeled cells were present only in the lens and the HV. By E-17, the density of labeling in these structures decreased dramatically and only faint labeling was observed at later stages in development. No labeled cells were observed in the retina at any stage during development. Northern blot analysis showed that a 6 Kb collagen IV transcript was present in the eye. These findings establish that collagen IV is present at the ILM only at early developmental stages (E-12 to E-17) when most axonal growth occurs in the retina. Furthermore, retinal collagen is derived from either the lens or, more likely, the HV. Hence, during development of RGC axons, it appears that both collagen and laminin are present in the ILM when there is maximal axonal growth. At later developmental stages, however, laminin alone remains in the matrix. *[Supported by EY03664 and EY01730]*

147.12

RETINAL PROJECTIONS AND AXONAL MORPHOLOGY IN THE DEVELOPING VISUAL SYSTEM OF THE QUOKKA, *SETONIX BRACHYURUS*. SA Dunlop, WM Ross & LD Beazley. Dept. Psychology, University of Western Australia, Nedlands 6009.

During development in the marsupial quokka, there is an excess of optic axons compared to retinal ganglion cells (RGCs) labelled by injecting HRP into the visual centres (Braekevelt et al., *Dev Brain Res* 25:117-125, 1986). To investigate the source of additional axons, we examined animals during the peak of axon numbers (P40-50). First, we looked for projecting cells from the opposite retina and from the brain by injecting one eye with HRP or by placing HRP-gels into the optic disk. Uninjected retinae were wholemounted or cut as wax sections; brains were cut as vibratome sections. Very few retino-retinal or efferent projecting cells were observed. Second, we examined axonal morphology in the optic nerve by labelling small groups of axons in the retina with HRP either *in vitro* (Dunlop, *J Comp Neurol* 293:425-447) or *in vivo*. Nerves were cut as longitudinal vibratome sections. Optic nerves from additional animals were prepared for EM and individual axons reconstructed from serial sections. HRP-labelled axons often bore short side-branches both behind the eye and at the chiasm; such side-branches were also seen in our EM reconstructions and would have been included in optic axon counts. Some HRP-labelled axons executed loops before rejoining the main fascicle while others took sinuous trajectories across the nerve. We assume that axons with unusual pathways would have been counted more than once during quantitative analysis of EM sections. A considerable number of growth cones were also seen in both the HRP and EM sectioned nerves suggesting that some RGC's had not reached the brain. We conclude that there are several sources of excess axons over RGCs in the developing visual system of the quokka.

147.13

FASCICULATION, DEFASCICULATION AND DISTRIBUTION OF PIONEERING AXONS IN THE RETINOFUGAL PATHWAY OF EMBRYONIC HAMSTERS. S.Jhaveri, R.S.Erzurumlu and G.E. Schneider. Dept. Brain & Cognitive Sciences, M.I.T., Cambridge, MA 02139.

The lipophillic tracer DiI was placed on the optic nerve head of fixed hamster embryos, aged E11 through E16. Labeled axons were examined in flat-mount preparations of the brainstem.

Tightly bundled optic nerve fibers defasciculate and undergo a spatial reorganization prior to reaching the chiasm. Upon crossing the midline, a group of fibers emerges to form the leading edge of the developing optic tract. These pioneer axons, tipped by large growth cones, course distinctly separate from each other. Successive waves of "follower" axons, each tipped by a large growth cone, are interdigitated between pioneers, but do not necessarily contact them. Axon fasciculation occurs in regions where fiber density is high.

A smaller contingent of ipsilateral axons emerges from the chiasm at about the same time as the contralateral fibers. Leading axons on the ipsilateral side keep pace with the growth of their contralateral counterparts.

Pioneer axons fan out to span the entire LGB. Further caudally, leading axons may slow their growth rate: by E13.5, the separation between pioneers and followers is not as distinct as at earlier times. Support:NIH grants EY05504, EY00126, EY02621.

147.14

OLIGODENDROCYTES IN THE MATURING OPTIC TRACT OF POSTNATAL HAMSTERS STUDIED WITH THE RIP ANTIBODY. G.E.Schneider, R.S.Erzurumlu, S.Jhaveri, & B.Friedman. Dept. Brain & Cogn.Sci., M.I.T., Cambridge, MA 02139; Regeneron, Tarrytown, NY.

The monoclonal antibody "Rip", specific for rat oligodendrocytes (Friedman et al., *Glia*, '89), was used to study the developing optic pathway of postnatal hamsters. At P3 a sparse distribution of pre-ensheathing oligos is seen in the tract (OT) between chiasm and LGB, and also along the tectal midline. Two days later, such cells are present in the OT as far as the pretectum, whereas in the chiasm, axon ensheathment by Rip-positive cells has begun. By P7 pre-ensheathing oligos can be detected in the stratum opticum of SC and within the LGB. OT axon ensheathment is evident to the level of the ventral LGB. From P14-21, increasing fiber ensheathment is found throughout the OT, but Rip-positive elements remain sparse in the upper part of the superficial gray of the SC.

Our findings are consistent with a possible role of oligo-derived proteins in inhibiting axon growth (Caroni & Schwab '89): Appearance of pre-ensheathing oligos corresponds to the time (after P3) when OT regrowth after transection of the brachium of SC fails; sprouting within the SC can occur at the ages studied, but it is restricted to the upper part of the superficial gray. Support: NIH grants EY05504, EY00126, EY02621.

147.15

RETINOTECTAL AXONS CROSS TO THE WRONG SIDE FOLLOWING DISRUPTION OF TECTAL MIDLINE CELLS IN THE HAMSTER. D.-Y. Wu, G.E. Schneider and S. Jhaveri, Dept. Brain & Cognitive Sci., M.I.T., Cambridge, MA 02139.

A group of radial cells in the tectal midline of developing hamsters expresses GFAP and vimentin. To investigate the possibility that these cells serve as a barrier to retinal axon growth (Wu et al. *SN Abstr.* '89) we undercut the midline in 1-day-old hamsters using a hooked tungsten wire inserted from a point off the midline in the left SC (disrupting midline cells without damaging the overlying pial surface) and the right eye was also removed. Animals were sacrificed on P3-P14, 18 hr. after an injection of HRP into the left eye. Adjacent series of brain sections were processed for visualizing the HRP or for immunolocalization of vimentin or GFAP.

In the region of damage, midline cell processes had lost their pial attachments, although the pia itself remained intact, and retinotectal axons from the left eye crossed the dorsal midline abnormally from the right to the left side. This crossing was not detected in areas where midline cells were intact from their ventricular attachment to the pia.

We conclude that during development, midline cells may serve to preserve the laterality of the retinotectal projection. Support: NIH grants EY00126, EY05504, EY02621, MH15761.

147.16

THE PATTERN OF GANGLION CELL FASCICLES IN THE PIGMENTED AND ALBINO RAT. W.L. Holcomb*, D.M. Murakami, I.S. Westenberg[1], and C.A. Fuller, Dept. of Animal Physiology, University of California, Davis, CA, 95616; [1]Dept. of Psychology, Glendale College, Glendale, AZ 85302.

This study compares the organization of ganglion cell fascicles in the retinal nerve fiber layer of the pigmented and albino rat. In order to control for variation due to different genetic backgrounds, we examined a specific strain (Westenberg Long-Evans) which is congenic except at the albino locus (albino *c/c*; pigmented *c/+*). In a previous study we described the fascicle organization in the normally pigmented rat. Light microscopy was used to examine the HRP labeled fascicles in the wholemounted retina. The distribution of fascicles reflects the distribution of ganglion cell bodies in that there is a region of high fascicle density in superior temporal retina located 0.6 mm from the optic disc. In addition, the mean diameter of fascicles in superior temporal retina was smaller when compared to fascicles in other retinal regions. Fascicle density was similar in the pigmented and albino strains; however, isodensity lines failed to exhibit the high density region of specialization in the albino retina. Mean fascicle diameter was smaller in superior temporal retina for both the pigmented and albino rat. Samples of fascicles from the entire retina did not reveal any difference in the distribution of fascicle diameter between the two groups; however, the mean diameter of fascicles in superior temporal retina appears to be reduced in the albino. This study supports others which conclude that the region of central specialization is underdeveloped in the retina of hypopigmented mammals. (Supported in part by NIH MH41477.)

147.17

FIBER ARRANGEMENTS IN THE OPTIC STALK OF NORMAL AND ALBINO RATS.

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The rat optic stalk was studied by the anterograde DiI labelling technique in order to answer, first whether a retinotopy exists in the optic stalk, and second, whether the ventral temporal fibers in albinos take an abnormal course causing them to enter the wrong tract.

In rats aged E16.5 and E18.5 (plugged date= E0), a small DiI granule was put onto one retinal quadrant and the position of labelled fibers was traced with confocal laser microscopy in serial transverse sections. By E18.5 fibers from the ventrotemporal crescent have already reached the tract (Chan et al., 1990 ENA Meeting Abstracts). Just behind the eye, at both ages, fibers from each retinal quadrant cluster together and occupy distinct parts of the stalk. Across the stalk from anterior to posterior, fibers from lower nasal, upper nasal, upper temporal and lower temporal retina lie sequentially. As the fibers are traced towards the optic chiasm, fibers from one retinal quadrant begin to mingle extensively with those of neighbouring ones. The lower temporal fibers spread gradually from the posterior to the anterior parts of the stalk. At the optic chiasm, there is virtually no recognizable map at all. In albino rats, no discernable difference in the fiber arrangement in the stalk can be observed at either age.

We conclude that fibers in the post-optic stalk are retinotopically arranged, but that this order is lost gradually as the fibers pass centrally. Retinotopic maps in the targets are not simply formed by an orderly arrangement of fibers in the stalk. Second, the albino abnormality is not simply due to a misrouting of lower temporal fibers as they leave the eye.

147.18

DO LATE-ARRIVING OPTIC AXONS ALL TAKE A CROSSED CHIASMATIC COURSE? B.E. Reese[1], R.W. Guillery[1], C.A. Marzi[2]*, and G. Tassinari[2]* Dept. Human Anatomy, Univ. Oxford, OX1 3QX, U.K.[1] and Ist. Fisiologia Umana, Univ. Verona, 37134 ITALY[2]

We have examined the relative positions in the optic tract of the crossed and uncrossed optic axons arising from the temporal retina. Since position within the optic tract reflects time of axonal arrival during development (Walsh and Guillery, J. Neurosci. 5: 3061, 1985), we have used axonal position as an indicator of age and have related this to the chiasmatic pathway choice of the axons. Some adult cats were monocularly enucleated, to reveal the position of surviving crossed and uncrossed axon classes within the tract, while others had HRP injected or implanted to label axons in either the deep or the superficial parts of the tract selectively. The retrograde transport of the HRP then showed the distribution and the morphology of the ganglion cells giving rise to early (deep) or late (superficial) arriving axons.

The deep parts of the optic tract contain fine and medium, crossed and uncrossed axons arising from mainly medium-sized cells in the contralateral nasal and the ipsilateral temporal retina; there is a clear line of decussation. In contrast, the superficial parts of the tract contain mainly fine diameter axons arising from small cells in the whole contralateral retina, and a small proportion of large diameter axons arising from large, alpha cells in the whole contralateral retina and also in the ipsilateral temporal retina.

The likelihood that axons from the temporal retina will project contralaterally, therefore increases as development proceeds. However, the presence of a few large uncrossed axons near the surface of the tract complicates the picture of a simple time-dependent signal that guides temporal axons into an uncrossed path and that weakens with age.

147.19

DEVELOPMENT OF PERMANENT RETINAL PROJECTIONS TO SOMATOSENSORY AND AUDITORY THALAMIC NUCLEI IN HAMSTERS: A DiI STUDY. P.G. Bhide, W.C. West* & D.O. Frost, Neurology, Mass. General Hospital East, Charlestown, MA 02129.

In normal adult hamsters, the optic tract divides into superficial and internal tracts (SOT and IOT, respectively) at the level of the ventral lateral geniculate nucleus. Only SOT axons have collaterals that arborize in the visual thalamic (dorsal lateral geniculate, LGd) nucleus (Schneider & Jhaveri, *Neurosci. Abstr.*, 9:809, 1983). During development, retinal ganglion cell (GC) axons in the IOT emit transient collaterals to LGd and to the thalamic somatosensory (ventrobasal, VB) nucleus (Langdon et al., *Neurosci. Abstr.*, 13:1023, 1987). Permanent retinal projections to VB and the thalamic auditory (medial geniculate, MG) nuclei were induced in newborn Syrian hamsters by ablation of the superior colliculus and transection of the somatosensory and auditory lemniscal pathways (Frost, *J. Comp. Neurol.*, 203:227, 1981).

It is not known if the permanent retino-VB projection arises exclusively by the stabilization of the normally transient retino-VB collaterals from IOT or if thalamic SOT axon collaterals (normally restricted to LGd) also extend to VB following surgery. It is also not known which GC axons project to MG. We examined these issues by labeling individual GC axons with DiI in paraformaldehyde-fixed brains from 6-, 8-, 15-, and 21-day-old hamsters operated at birth to produce retino-VB and retino-MG projections. DiI was placed in the optic disc or the optic nerve contralateral to the novel projections and allowed to diffuse for 3 mo. The DiI label in the thalamus was photoconverted to a DAB reaction product. Labeled retino-VB and retino-MG axons were reconstructed from serial sections. In operated hamsters, by the end of the first postnatal week, both SOT and IOT axon collaterals have entered the VB. Some of these collaterals have "simple" growth cones while others have developing terminal arbors. A single SOT or IOT collateral may have growth cone-bearing branches or developing arbors in both the LGd and VB. By the end of the 2nd postnatal week growth cones are no longer present. By the end of the 3rd postnatal week all collaterals elaborate adult-like terminal arbors. The retino-MG projection, which is not present in normal hamsters at any age, also arises from both SOT and IOT axons and has a similar developmental history. Therefore, it appears that the permanent retino-VB projection is formed both by the stabilization of normally transient (IOT) axon collaterals and by the sprouting of new ones (from SOT); the retino-MG projection arises by the sprouting of both SOT and IOT collaterals.

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147.20

RETINAL GANGLION CELLS IN HAMSTERS WITH RETINAL PROJECTIONS TO THE VENTROBASAL AND MEDIAL GENICULATE NUCLEI. A. Irons, C. Métin* and D. Frost. Univ. P & M Curie, Paris, France & Mass. General Hospital, Boston, MA

Retinal projections to the ventrobasal (VB) and medial geniculate (MG) nuclei were induced in newborn Syrian hamsters by ablation of the dorsal lateral geniculate nucleus (LGd) and superior colliculus and transection of the somatosensory and auditory lemniscal pathways. When the hamsters were ≥12 wk old, a polyacrylamide gel containing 35% HRP was placed in the optic tract on the side of the brain containing the retino-VB and retino-MG projections. After 48-72 h, whole mounted retinae were processed with the Hanker-Yates technique to reveal labeled retinal ganglion cells (GCs). Contralaterally, GCs were found throughout the retina. Their density was 10-950 GC/mm^2, compared with 300-4,000 GC/mm^2 in normal hamsters. The total number of labeled GCs was ca 10,000, 15-20% of normal. GC soma diameters were normal: 7-23 μm with a peak in the distribution at ca 13-14 μm. As in normal hamsters, maximum GC densities occured in an elongated inferotemporal to superonasal zone located just above the optic disk, whereas minimal densities were observed in the inferior periphery; GC diameter tended to increase with decreasing density. The best filled GCs could be classified as belonging to one of the 3 types distinguished in normal hamsters (Métin, C.M. & Frost, D.O., *Neurosci. Abs.*, 15:1207, 1989). Ipsilaterally, GCs were normally distributed in the temporal crescent and soma diameters were normal: 9-23 μm with a peak in the distribution at ca 15-18 μm. These data show a dramatic decrease in the number and density of GCs in neonatally operated hamsters with retino-VB and retino-MG projections, but no obvious changes in GC soma size or morphological type. Therefore it is possible that the mix of GC types projecting to VB or MG in operated hamsters is similar to the mix projecting to LGd in normal hamsters. Support: NIH - EY03465; March of Dimes - 1-1148.

147.21

SOMATIC AND SYMPATHETIC INNERVATION OF THE RAT CORNEAL LIMBUS. R. E. Kingsley and C. F. Marfurt. SBCME & NWCME, Indiana University School of Medicine, Notre Dame IN 46556.

This study is part of a larger comparative study which is directed at examining the normal innervation of the cornea in various species. Based upon retrograde HRP methods, we previously reported that in the rat there is a normal sympathetic innervation of the cornea by axons whose cell bodies reside in the superior sympathetic ganglion. The results reported here are based upon electron microscopic observations of the rat cornea in both normal and lesioned tissue.

We used adult Sprague-Dawley male rats in this study. In all of our experiments, we anesthetized the animals with 50mg/kg pentobarbital. In some animals, we removed the trigeminal ganglion while in others we performed a superior cervical sympathectomy. After a degeneration period of three to six days, we removed the corneal tissue under pentobarbital anesthesia and immediately placed it in a cacodylate buffered (pH 7.4) solution of 2% glutaraldehyde and 2% formaldehyde. After one hour of aldehyde fixation, we processed the tissue conventionally for transmission electron microscopy.

In the corneal limbus, we saw a number of large nerve bundles which contain both myelinated and unmyelinated axons. These bundles are collected together by Schwann cells which express a well defined extracellular matrix. We only observed these well defined bundles in or near the limbus. Axons in the corneal stroma are isolated and always unmyelinated.

Within the axon bundles we saw synaptic-like profiles which contain densely packed clear core vesicles. These profiles did not disappear with trigeminalectomy. With sympathectomy we also saw synaptic-like profiles which contain dense osmiophilic granules.

REGENERATION: MOLECULAR CORRELATES

148.1

AXOTOMY INDUCED CHANGES IN CGRP IMMUNONREACTIVITY IN TROCHLEAR MOTOR NEURONS. X.H. Wang, P.G. Iannuzzelli, R.G. Baker and E.H. Murphy. Department of Anatomy, Medical Coll. of PA, Phila, PA 19129 and Dept. Physiol. & Biophysics, NYU Med. Ctr., New York, NY 10016.

In many neurons the level of transmitter\peptide production following axotomy decreases. Some trochlear nucleus motoneurons (TMNs) contain the peptide Calcitonin Gene-Related Peptide (CGRP). We examined the distribution of CGRP immunocytochemically in trochlear motoneurons (TMNs) in the adult cat. CGRP+ cells were counted and the number compared with counts of TMNs in adjacent Nissl stained sections. The trochlear nucleus of the cat contains no interneurons and approximately 1000 TMNs, of which approximately 30% CGRP+. We used quantitative densitometry to classify the intensity of the CGRP in each neuron as light (L), intermediate (I) or dark (D). The distribution was D=1%; I=25% and L=75%.

One week following axotomy of the IVth nerve, the number of CGRP+ cells increased dramatically: almost all cells were CGRP+. The distribution of intensity of staining also differed from normal: D=60%; I=20% and L= 20%. In animals 1 to 6 weeks postoperative, the number of CGRP+ cells and the percentage of these cells which were classified D decreased with increasing survival time, but remained above normal at 6 weeks (percent of neurons CGRP+ =40%, percent of CGRP+ cells classified as D = 40%). The changes in the percent cells classified as D,I or L indicate that axotomy increases CGRP expression. The increased number of CGRP+ cells following axotomy presumable reflects an increased production of CGRP or may indicate that axotomy induces CGRP expression in some neurons which do not normally express CGRP. Thus, the behaviour of CGRP in axotomized neurons differs from that found for most peptides and may indicate a role for CGRP in regeneration. Supported by NS24707 to EHM and EY20007 to RGB.

148.2

AXOTOMY INDUCED CHANGES IN CYTOCHROME OXIDASE ACTIVITY IN TROCHLEAR MOTOR NEURONS. P.G.Iannuzzelli, X.H. Wang, B. Ju*, R.G. Baker and E.H. Murphy. Department of Anatomy, Medical Coll. of PA, Phila, PA 19129 and Dept. Physiol. & Biophysics, NYU Med Ctr., New York, NY 10016.

When the IVth nerve is cut just distal to the anterior medullary velum, approximately 50% of trochlear motor neurons (TMNs) die. The surviving neurons regenerate and re-innervate their target, the superior oblique (SO) muscle. Regeneration may result in a change in the neurons' oxidative metabolic machinery. Cytochrome oxidase (CO) activity is an indicator of oxidative energy metabolism. In this study, we determined CO levels in control and axotomized TMNs. Density of CO staining in the trochlear nucleus, revealed histochemically, was measured by quantitative image analysis at intervals from 3 days to 6 weeks following unilateral IVth nerve axotomy. At 3 days, no difference was detectable between the control and experimental sides. CO levels in the axotomized nucleus were slightly decreased at 1 week, and this decrease was more marked at 3 weeks. By 6 weeks, (2 weeks after regenerating axons reach their target), CO levels in the axotomized nucleus were still lower than normal, but the difference was less marked than at 3 weeks, suggesting some restoration following re-innervation of the target. This decrease in CO levels following axotomy cannot be attributed to decreased excitatory input to TMNs since axotomy-induced synaptic stripping is restricted to inhibitory synapses on TMN somas. The data indicate that oxidative energy metabolism decreases during regeneration and that restoration of normal levels may be delayed for several weeks after the regenerating axons reach their target. Thus, regenerative growth either requires less oxidative energy than normal neuronal function or a different form of energy. Supported by NS24707 to EHM and EY20007 to RGB.

148.3

IS C-FOS PROTO-ONCOGENE INDUCED IN FACIAL MOTONEURONS FOLLOWING PERIPHERAL AXOTOMY? C. Evinger[1], G.A. New[2] and K.J. Jones[2]. [1]Dept. of Neurobiology & Behavior, SUNY-Stony Brook, NY 11794 and [2]Dept. of Cell Biology & Anatomy, Chicago Med.Sch., North Chicago, IL 60064.

Many different stimuli have been shown to induce the expression of c-fos mRNA and protein in brain. The general time course of the effect is rapid, with maximal mRNA levels reached by 30' and protein levels at 3 h. In this study, we tested the hypothesis that peripheral axotomy will induce the expression of c-fos in motoneurons. Adult hamsters were anesthetized and subjected to axotomy of the right facial nerve at its exit from the stylomastoid foramen, with the left side serving as internal control. As a positive control in separate animals, bicuculline was used to chemically induce seizure activity. At 3 h post axotomy or seizure, the animals were sacrificed by intracardiac perfusion with 4% paraformaldehyde in PBS. Standard immunocytochemistry procedures were accomplished using a panel of commercially available polyclonal antibodies to c-fos. In the positive seizure control animals, the nuclei of neocortical neurons exhibited intense c-fos staining. In contrast, in neither normal nor axotomized facial motoneurons was c-fos detectable with immunocytochemistry. We are currently examining this question at the mRNA level, and in other species, as well as extending the time course for possible c-fos induction.

148.4

IMPAIRMENT OF NERVE REGENERATION BY ACRYLAMIDE (AC) INHIBITS THE AXOTOMY-INDUCED EXPRESSION OF PHOSPHORYLATED NEUROFILAMENT EPITOPES IN NEURONAL PERIKARYA. B.G. Gold, D. Austin*, N. Sternberger and L. Sternberger*. Center Res. Occ. Environ. Toxicology, Oregon Health Sci. Univ., Portland, OR 97201 and Dept. of Anatomy, Univ. Maryland Sch. Med., Baltimore, MD 21201.

Abnormal expression of heavily phosphorylated neurofilament (pNF) epitopes in neuronal perikarya is observed in a variety of disorders (e.g., Alzheimer's disease). Little is known about how this response is regulated. However, the appearance of pNF epitopes in sensory neurons following axotomy suggests that expression of at least some of these epitopes represents a neuronal perikaryal response to injury. Moreover, continued expression of pNF epitopes appears to depend upon sustained neuritic outgrowth following axotomy. In the present study, we asked whether inhibition of neuritic outgrowth by AC prevents the induction of pNF epitopes in sensory neurons. Seven-week old rats underwent a bilateral sciatic nerve crush and 10 ml of 0.1M AC (a concentration previously found to inhibit nerve outgrowth following crush) was injected subperineurially just proximal to the crush site via a glass micropipette; the right nerve was injected with saline. Animals were perfused with 4% paraformaldehyde at 7 days. Sections (10 mm) of L4 and L5 dorsal root ganglia (DRG) were stained with antibody 2-135 (directed against a nonphosphorylated NF epitope) or 7-05 (directed against a pNF epitope) using the peroxidase-antiperoxidase method. Antibody 7-05 demonstrated modest to intense staining in 31-36% of neuronal perikarya in DRG from saline-injected crushed nerves. Fewer (9-12%) cell bodies showed staining and only a rare perikarya was strongly stained in DRG from AC-injected crushed nerves; axons were intensely stained. Antibody 2-135 demonstrated intense staining in all perikarya and axons from both AC- and saline-injected nerves. These findings suggest that expression of pNF epitopes in neuronal perikarya is regulated by growth processes in the axon and/or interactions between elongating neurites and supporting elements in the distal stump. Supported by NS 26265.

148.5

LOCALIZATION OF THROMBOSPONDIN IN MOUSE SCIATIC NERVE FOLLOWING NERVE CRUSH OR TRANSECTION.
J.R. Hoffman, K.S. O'Shea, and V.M. Dixit*
Univ. of Michigan, Ann Arbor, MI 48109.

Thrombospondin (TSP) is a 420 kDa component of the extracellular matrix associated with outgrowing nerve processes in the embryo, but its role in the adult nervous system is unknown. This study examines the pattern of TSP localization during nerve regeneration. Female CD-1 mice were anesthetized, the sciatic nerve was crushed or transected at the mid-thigh level. After 1-90 d, frozen sections were cut and processed to localize TSP. In the adult sciatic nerve, TSP was present in the connective tissue sheaths, the Schwann cells and axoplasm. With nerve injury, Wallerian degeneration proceeds to form empty Schwann cell tubes as the axon degenerates. TSP levels in the endoneurium remained at higher than normal levels in excess of 14 d, but gradually declined. There was also an increase in TSP in association with nerve outgrowth in the axoplasm and endoneurium.

During nerve regeneration, TSP was present in higher than normal levels, but disappeared from the endoneurium if regeneration was prevented.
Supported by NIH grant HD-23867.

148.6

DIFFERENTIAL REGULATION OF CALCITONIN GENE-RELATED PEPTIDE (CGRP) IN REGENERATING MOTOR AND SENSORY SYSTEMS.
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Axotomy of the rat facial nerve has previously been shown to increase CGRP-immunoreactivity in parent motoneurons[1]. To further characterise the regulation of CGRP in regenerating neurons we have studied the levels of both CGRP-peptide and CGRP-mRNA in regenerating motor and sensory systems.

Axotomy of the rat facial nerve leads to a biphasic *increase* in CGRP in the facial motor nucleus with a first rapid, 3-fold increase around day 3, a decrease to normal levels at day 9 and a second, late peak during the time of reinnervation (day 21). The CGRP-mRNA levels reveal a similar time course with the 2 peaks appearing approximately one day earlier. Quite in contrast to that, axotomy of the sciatic nerve slowly *decreases* CGRP-levels in the sensory, L5 dorsal root ganglia reaching basal levels of approximately 30-50% by day 9. Once again CGRP-mRNA levels decrease 1-2 days earlier.

These data show that changes in CGRP content following axotomy and during regeneration reflect altered levels of CGRP-mRNA expression. The strikingly different patterns of CGRP expression in the DRG and facial nucleus suggest the presense of distinct and opposing regulating signals in the regenerating motor and sensory systems. This differential regulation of CGRP in motor versus sensory systems may reflect different yet unknown functions during nerve regeneration.

[1] Streit W.J., F.L.Dumoulin, G.Raivich, and G.W.Kreutzberg, *Neurosci. Lett.* 101:143-148, 1989.

148.7

IMMUNOCYTOCHEMICAL LOCALIZATION OF THY-1.2 ON ADULT AND EMBRYONIC MOUSE RETINAL GANGLION CELL NEURITES IN VITRO.
C. A. Bates and R. L. Meyer. Developmental Biology Center; UCI; Irvine, CA 92717

Thy-1 is a cell surface glycoprotein which is a member of the Ig superfamily and thus may be involved in intercellular adhesion. It has been suggested that Thy-1 interacts with astrocytes to inhibit neurite outgrowth (R. Morris, 1989). In culture, embryonic day 15 (E15) mouse retinal explants extend many long neurites on astrocyte monolayers while adult retinal explant neurites are less numerous and shorter. We have looked at the presence of Thy-1 on neurites of retinal ganglion cells in adult and E15 retinal explants grown on laminin using a Mab to Thy-1.2. Retinal segments were explanted one week after optic nerve crush in adults or at E15. In adult explants, outgrowth was first observed at 24 hours., at which time all neurites extending from the explant were Thy-1.2 positive. E15 explants showed no Thy-1.2 staining 24 hours after explantation. Nor did E15 explants show any Thy-1.2 staining up to 7 days post explantation. We are currently extending our investigations to adult explants grown on astrocyte monolayers. Supported by NS26750 (RLM) and HD07029 (CAB).

148.8

GAP-43 UPREGULATION IN REGENERATING RETINAL GANGLION CELLS IN THE RAT.
B. C. Wouters and J. J. Norden. Department of Cell Biology, Vanderbilt University, Nashville, TN 37232.

Several reports have shown that retinal ganglion cells (RGC) in rats may be induced to regrow axons through peripheral nerve shunts following injury (eg, Richardson et al., *Nature* 1980 **284**, 264-65). We have used this paradigm to investigate the role which the growth-associated protein GAP-43 may play in this regrowth.

Sciatic nerve shunts were grafted onto optic nerves of adult rats following complete transection. Using electron microscopy and the retrograde transport of horseradish peroxidase, we have demonstrated the viability of our preparation and confirmed that rat RGCs axons regrow through the peripheral shunts. At 4-5 weeks post grafting, we show using ^{35}S-methionine that the regrowing RGCs increase their synthesis and transport of GAP-43 by 130%-192% above levels observed in normal control adult rat RGCs or in RGCs whose axons have been transected but not grafted. Immunohistochemistry of retinas using our polyclonal antibody to GAP-43 reveals that this response is localized to a subpopulation of the retinal ganglion cells. We conclude that GAP-43 is upregulated during axon growth through peripheral nerve grafts. We are currently determining whether this upregulation is mediated by translational or transcriptional mechanisms. (Supported by NIH grant NS25150 to J. J. Norden.)

148.9

THE EXPRESSION OF GAP-43 mRNA IS REGULATED BY AN AXONALLY-TRANSPORTED FACTOR
L. I. Benowitz, C. O'Brien, N. I. Perrone-Bizzozero, N. Irwin and C. J. Woolf. Harvard Medical School, McLean Hospital (Belmont, MA, 02178), and University College London (UK).

In view of the likelihood that GAP-43 (B-50, F1, neuromodulin) is causally involved in the formation of neuronal connections, we are investigating the mechanisms that control its expression. Axonal regeneration in the peripheral nervous system (or in the CNS of lower vertebrates) is accompanied by a marked increase in the synthesis of this membrane phosphoprotein[1,2]. We previously found that either crushing the sciatic nerve or treating it with vinblastine (100 µM, 20 min, to disrupt axonal transport) greatly increased GAP-43 immunostaining in the dorsal horn of the spinal cord[3]. To verify that these changes were due to an enhanced expression of GAP-43 in the primary sensory neurons, dorsal root ganglia were dissected 1-30 days after sciatic nerves were crushed or treated with vinblastine, and the RNA was probed on Northern blots with a labeled GAP-43 cDNA[4]. Both treatments caused a several-fold induction of GAP-43 mRNA within the first week. Measurements of nerve conduction verified that vinblastine treatment did not physically injure the axons. We conclude that the expression of GAP-43 is normally inhibited by a retrogradely-transported factor that arises from the periphery (or from the glial environment), and that removal of this factor induces the expression of GAP-43 (and perhaps other growth-associated proteins) in mature neurons. *Support: NIH NS 25830, the MRC (UK), Wellcome Trust and International Spinal Res. Trust.*

[1]Skene, Ann. Rev. Neurosci. **12**:127-156, 1989; [2]Benowitz & Routtenberg, TINS **10**: 527-532, 1987; [3]Woolf *et al*, Neurosci. 34: 465-478, 1990; [4]Neve *et al*, Molec. Brain Res. 2: 177-183, 1987.

148.10

RUBROSPINAL NEURONS INCREASE GAP43 AND TUBULIN mRNA AFTER CERVICAL BUT NOT AFTER THORACIC AXOTOMY
W. Tetzlaff, B.J. Tsui* and J.K. Balfour*. Depts. of Anatomy and Pathology, University of Calgary, Calgary, Alberta, T2N 4N1

We have shown previously that axotomized rubrospinal neurons increase the expression of mRNAs for tubulins, actin and GAP43. These studies were performed with hemi-sections of the spinal cord at cervical level C3/4. Rubrospinal neurons have been reported to regenerate into peripheral nerve transplants implanted at cervical level but not into those implanted at lower thoracic level. We have therefore investigated whether a lesion at lower thoracic level (T10/12) fails to induce the regeneration associated changes in gene expression. Axotomized rubrospinal neurons were retrogradely filled with Fast Blue in order to restrict the analysis to axotomized i.e. labelled neurons. This technique was combined with in situ hybridization for the study of mRNA expression in individual neurons. Neither at 7 days nor at 14 days post thoracic lesion was there an increase in GAP43 or tubulin mRNA expression. Thus, thoracic lesions do not seem to induce the regeneration associated genes. These data support the view that the failure to regenerate into the permissive environment of a PNS transplant is related to the failure to express these genes.

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148.11

AXOTOMIZED CORTICOSPINAL NEURONS DO NOT INCREASE TUBULIN AND GAP43 mRNA EXPRESSION. M.A. Bisby, W. Tetzlaff and .S. Alexander*. Dept. of Physiology, Queen's University, Kingston, Ontario K7L 3N6 and Depts. of Anatomy and Pathology, University of Calgary, Calgary, Alberta, T2N 4N1.

Mammalian CNS neurons generally have a limited capacity for regeneration, perhaps due to inhibitory molecules in the CNS environment, and/or defects in the response to injury. When provided with the permissive environment of a nerve transplant, some CNS neurons can regenerate into the transplant, but corticospinal neurons do not. This difference may be associated with differences in the response to injury, and so we have studied the expression of regeneration-associated genes for GAP43 and embryonic tubulin isotypes in rat corticospinal neurons following pyramidal lesions.

Axotomized cortical neurons were identified by retrograde labelling with fluorogold applied to the lesion site, and combined with in situ hybridization using specific probes for GAP43 and tubulin mRNAs. There was no increase in mRNA levels, and in some animals total tubulin mRNA levels decreased by 30%. This contrasts with our findings for rubrospinal neurons lesioned at C3, where both GAP43 and tubulin mRNAs increased. The inadequate response to injury may account for the failure of corticospinal axons to regenerate, even if offered a permissive environment.

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148.12

DIFFERENTIAL REGULATION OF S100ß AND mRNAs CODING FOR S100-LIKE PROTEINS (42A AND 42C) AFTER LESION OF RAT SCIATIC NERVE AND DURING DEVELOPMENT. M. De Leon[1], L. J. Van Eldik[2], and E. M. Shooter[1]. [1]Dept. of Neurobiology, Stanford University School of Medicine, Stanford CA 94305, and [2]Depts. of Pharmacology and Cell Biology, Vanderbilt University, Nashville TN 37232.

The present study analyzes changes in S100ß (a protein that stimulates neurite extension and neuronal survival) and 42A and 42C (S100-like proteins whose mRNAs are induced in PC12 cells by NGF) during nerve regeneration and development. Distal segments of rat sciatic nerve were examined by Northern blots at various times after a cut or crush injury, and it was found that S100ß, 42A, and 42C mRNA showed a differential regulation during regeneration. As reported before, 42A and 42C mRNA levels increased in the distal segment of axotomized nerves. In contrast, S100ß mRNA levels significantly decreased during the first week after injury and returned to normal levels during the second week. The decrease in S100ß mRNA levels was reflected by a corresponding decrease in S100ß protein levels. S100ß mRNA was present both in sciatic nerve and brain. However, 42A and 42C mRNAs were more restricted to sciatic nerve, with little found in either embryonic or adult brain. These three proteins, with a high degree of sequence similarity, are differentially regulated during both sciatic nerve regeneration and development, and thus may play distinct roles in these processes.

148.13

REGENERATION OF TRANSECTED SPINAL CORD OF TADPOLE STUDIED BY 2-D GEL ANALYSIS AND IMMUNOCYTOCHEMISTRY. H.S. Yin and M.M. Chiu*, Dept. of Anatomy, Col. of Med. National Taiwan Uni.,Taipei, Taiwan, R.O.C.

The spinal cords of bullfrog tadpoles at stage IV were transected at the ninth spinal segment. The cords within 4 mm of the scar were dissected at various times following surgery and prepared for two-dimensional gel electrophoresis(2-D) and immunocytochemical staining. The analysis of 2-D gels revealed that the expression of at least 30 proteins was decreased initially and then gradually returned towards control levels by 28 day post-operation except that 3 proteins persisted at the lower expression levels. Among the former β-tubulin was identified by immunoblotting of 2-D gels. Six proteins of the cord increased amounts of their expression until 28 days. Immunostaining of paraffin sections of the scar area by the anti-200K neurofilament antibodies showed that the normal configuration of the neurofilament in the cord atrophied after transection and then was restored by 40 days. This was also supported by a quantitative assay of ELISA using the same antibodies. These results indicate that the morphological regeneration is accompanied by the biochemical regeneration in lesioned spinal cords of tadpoles.

148.14

A25, A NERVE DAMAGE ASSOCIATED PROTEIN(S) IS PRODUCED AT A COLD-BLOCK. G-S. Perng[*1], D.L. Wilson[1,2], and G.W. Perry[3], Departments of Physiology and Biophysics[1], and Biology[2], University of Miami, Miami, and Center for Complex Systems[3], Florida Atlantic University, Boca Raton, FL.

Previously, we have seen the appearance of a specific polypeptide(s) in crushed optic and sciatic nerves of frogs. The polypeptide, designated A25, appears within a few hours after crush specifically at the crush site, and is not produced normally in terminals. A25 is most likely produced from post-translational processing of a normally fast-transported protein.

To begin to address the mechanism of production of A25, especially whether physical damage to axons is necessary for its production, we have investigated whether A25 is produced at the site of a cold-block placed on a frog sciatic nerve in vitro. Labelled fast-transported proteins, including presumably the precursor to A25, accumulate proximally to a cold-block. Analysis of segments of nerve proximal to the cold-block by 2D-gels, shows that A25 is present in the proximal nerve segment. However, if distal nerve segments containing labelled, fast-transported proteins are kept cold in the in vitro paradigm, A25 does not appear. This indicates that cold alone does not produce A25. Little, if any, physical damage to axons occurs at a cold-block site since rewarming the nerve allows passage of the accumulated fast-transported proteins. Furthermore, A25 does not appear at a crush site which is maintained in the cold. Preliminary evidence also suggests that when the cold-block is released, that is the nerve is allowed to warm-up to room temperature, then A25, which was produced at the cold block is carried orthogradely with other labelled fast-transported proteins.

We conclude from these data that physical damage to axon is not necessary to produce A25. Possibly then, the accumulation of fast-transport vectors proximal to the cold block results in production of A25, perhaps through mixing or fusion of different vectors which are normally kept separate.

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148.15

INCREASE IN ACETYLCHOLINE RECEPTOR ALPHA-SUBUNIT MESSENGER RNA LEVELS IN HINDLIMB MUSCLES OF SPINAL CORD INJURED CATS. S. Sayers*, T. Khan, M. Dauzvardis, R. Hauser* and K. Burket*. Neuro-Regeneration Lab., RR&D Center, VA Hospital, Hines, IL 60141.

Peripheral nerves contain neurotrophic substances which may influence gene expression in the muscles which they innervate. Transection of the sciatic nerve was found to cause an increase in acetylcholine receptor (AChR) alpha-subunit messenger RNA (mRNA) levels in rat hindlimb muscles (Goldman, D., et al., Ann. NY Acad. Sci., 505:286, 1987). We conducted a study to determine whether upper motor neuron damage would produce an increase in mRNA levels in hindlimb muscles similar to that observed after transection of the sciatic nerve.

Following a 20-week survival period, total RNA was prepared from the soleus and extensor digitorum longus (EDL) muscles obtained from 8 cats; 2 cats received complete transection of the spinal cord at the T8-T9 level, 2 cats sustained a contusion injury, and 4 normal cats served as controls. Total RNA was slot-blotted onto membranes which were subsequently hybridized with ^{32}P-labeled riboprobe transcribed from the cDNA clone BMA 407-12 (provided by Dr. James Boulter, The Salk Institute) which codes for the alpha-subunit of the AChR. The membranes were subjected to autoradiography, the autoradiographs were scanned, linear regression lines were plotted, and the slopes of the lines were compared.

The level of alpha-subunit AChR mRNA in the soleus muscle was found to be increased by 40% in cats which received spinal cord transections and by 10% in cats which sustained contusion injuries as compared to normal cats. In contrast, alpha-subunit AChR mRNA levels in EDL muscle was increased by 14% in spinal cord transected cats and by 30% in spinal cord contused cats as compared to normal cats. Our preliminary data suggests that spinal cord injury causes AChR alpha-subunit mRNA levels to increase in both the soleus and EDL muscles of the cat, a condition similar to that observed after sciatic nerve transection. Funding: VA, Rehab. R&D Service, Rehab. R&D Grant #B423R.

148.16

REGULATION OF uPA mRNA IN MOUSE SCIATIC INNERVATED MUSCLE. **Daniel Hantaï, Riichiro Suzuki, Bokka R. Reddy, Jasti S. Rao and Barry W. Festoff, Department of Neurology, University of Kansas Medical Center, Kansas City, KS 66103, and Neurobiology (151), DVA Medical Center, Kansas City, MO.**

Plasminogen activators (PAs) play important roles in fibrinolysis as well as in modifying the extracellular environment during embryogenesis, inflammation, and neoplasia. Previous studies (J. Cell Biol. 103: 1415-1421, 1986 and PNAS 87: 2926-2930, 1990) showed eight to ten fold increase in the activation of urokinase (uPA) in muscle after denervation. Present studies further explore neural regulation of PA activities in muscle at the transcriptional level. The nerve crush paradigm was used and both muscle contraction and nerve simulation, and the return of choline acetyltransferase (CAT) activity was used to monitor reinnervation. Total RNA was extracted from contralateral and sciatic nerve innervated muscles from 1-43 days after crush. Total RNA was hybridized with ^{32}P random prime labeled cDNA mouse uPA probe by slot blot and Northern hybridization. uPA mRNA increased from day 5 to 12 in the crushed samples. No increase of the uPA mRNA in the contralateral muscle was seen during these time points. These results suggest that uPA is tightly regulated by nerve at transcriptional level and may play an important role during muscle reinnervation.

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148.17

RELATIONSHIP OF INSULIN-LIKE GROWTH FACTOR I mRNA CONTENT IN MUSCLE TO REGENERATION OF NEUROMUSCULAR SYNAPSES. G.W. Glazner*, D.M. Niedzwiecki and D.N. Ishii Physiology Dept., Colorado State Univ., Ft. Collins, CO 80523.

Insulin-like growth factor I (IGF-I) mRNA content in muscle is developmentally correlated with synaptogenesis at the NMJ, and increases after denervation. We tested the hypothesis that IGF-I mRNA content in gastrocnemius muscle (GM) correlates with regeneration and reestablishment of synapses. Sciatic nerves in adult rats were crushed on the left but not right sides. IGF-I mRNA content increased specifically in the left GM, correlating with its renewed capacity to accept innervation. IGF-I mRNA content peaked with the arrival of regenerating sciatic axons at the muscle. Thereafter, gene expression slowly returned to baseline, consistent with our previously published hypothesis for neuromuscular feedback inhibition of IGF mRNA content. These data support the hypothesis in which (i) elevated IGF-I levels support regeneration of synapses, (ii) innervation provokes an inhibitory signal on IGF-I mRNA levels which is relieved upon nerve crush, and (iii) reinnervation reestablishes the inhibitory signal which down-regulates IGF-I mRNA. (Supported by NINDS grant PO1 NS 28323)

ONTOGENY OF DOPAMINERGIC SYSTEMS

149.1

ROLE OF NIGROSTRIATAL DOPAMINE SYSTEMS IN THE EXPRESSION OF CALMODULIN-DEPENDENT ENZYMES IN DEVELOPING RAT STRIATUM. J. W. Polli* R.L. Kincaid and M.L. Billingsley. Department of Pharmacology and Center for Cell and Molecular Biology, Penn State University College of Medicine, Hershey, PA 17033 and Section on Immunology, NIAAA, Rockville, MD 20852.

The possible role of nigrostriatal dopamine (DA) systems in the transsynaptic regulation of calmodulin-dependent enzymes (CaM-BPs) was studied in developing rat striatum. High levels of CaM-BPs are found in striatum, and develop in parallel with innervation by DA. Unilateral hemi-transections of nigrostriatal DA neurons were performed on postnatal day 7 (PND7). On PND21, striata were removed and CaM-BPs determined using immunoblots, CaM overlays and enzyme assays. Absence of immunoreactive tyrosine hydroxylase (TH) confirmed successful lesions. Dennervated striata expressed levels and activity of calcineurin, CaM kinase II and CaM-phosphodiesterase similar to paired control striata. Chemical ablation of developing DA neurons was performed on PND 8 and 15 using 6-hydroxydopamine. Although TH immunoreactivity was absent in lesioned animals on PND 28, there were no significant changes in CaM-BPs. Developing rats (PND3-24) were chronically treated with haloperidol (1.0 mg/kg/day). Again, no significant changes were seen in CaM-BPs on PND24. These results suggest that CaM-BP expression in developing striatum is not under transsynaptic regulation by DA

149.2

STATHMIN IS A MAJOR NEURONAL PHOSPHORYLATION SUBSTRATE ASSOCIATED WITH DEVELOPMENTAL AND FUNCTIONAL REGULATIONS. H. Chneiweiss°, J. Cordier°, V. Doye, J. Koppel, L. Beretta and A. Sobel. INSERM U153 and °INSERM U114, 75005 PARIS, FRANCE

Stathmin is an ubiquitous cytoplasmic phosphoprotein (M_r≈19kDa pI≈6.2-5.6) most likely acting as a relay integrating the various intracellular regulatory pathways triggered by diverse extracellular signals. Among all tissues examined, stathmin is most abundant in brain, where its expression peaks around the neonatal period, both at the protein and mRNA levels. It is mostly concentrated in neurons where, at least in primary cultures of mouse embryonic neurons, it is located mainly in the cell body and in one neurite, also labeled for proteins known as axonal markers.

In PC12 cells, phosphorylation of stathmin is stimulated by NGF, in a way probably associated to the early steps of its neuronal differentiating activity.

In mouse embryonic neurons in culture, phosphorylation of stathmin was stimulated both by forskolin and by the tumor promoter TPA, as well as by vasoactive intestinal peptide (VIP), whose receptors were previously characterized on the same neuronal cultures. Furthermore, the VIP-induced protein phosphorylation pattern indicates that this physiological peptide may act both through cAMP-dependent and independent mechanisms. Finally, the effects of VIP and the combined effects of forskolin and TPA on the various phosphoforms of stathmin and of other related proteins indicate an interdependence of their phosphorylation sites, in a way substantiating the proposed integrating role of stathmin.

Altogether, our observations indicate that regulations of stathmin phosphorylation and expression are related to both "developmental" and "functional" regulations of neurons.

149.3

DOPAMINE D-2 RECEPTOR AUTORADIOGRAPHY WITH ^{3}H-YM-09151-2 REFLECTS BRAIN AND BEHAVIORAL ONTOGENY. A.S. Unis, J.G. Vincent* and B. Dillon*. Dept. of Psychiatry, Univ. of Washington, Seattle, WA 98195.

Using the technique of in vitro receptor autoradiography to slide-mounted tissue sections, we studied the suitability of ^{3}H-YM-09151-2 as a ligand for labeling D-2 receptors in day 21 and adult F344 rat brains. Assay conditions were modified from Terai et al. (1989) for tissue slice binding. Alternate sections were incubated with 1 μM haloperidol to determine non-specific binding. Autoradiographs were analyzed using computer-assisted microdensitometry.

Specific ^{3}H-YM-09151-2 binding accounted for 70-80% of the total bound ligand and reached equilibrium after a 1 hour incubation. Scatchard analysis revealed a Kd of 870 pM for adult and day 21 animals. The apparent B_{max} was lower in 21 day-old F344 rats than in adult animals (16.9 fmol/tissue section versus 6.8 fmol/tissue section) and was 66% of the expected value after correcting for differences in brain weight. Autoradiographs for adult and day 21 sections demonstrated high grain densities in the striatum and olfactory tubercle. Diffuse specific binding was also observed in the cortex. These autoradiographic data reflect the expected marked increase in D-2 receptors during ontogeny and are in agreement with our previous work demonstrating an absence of locomotor "hyperactivity" in 21 day-old F344 rats.

149.4

THE EFFECT OF ASCORBIC ACID ON THE DEVELOPMENT OF DOPAMINE NEURONS IN CULTURE. H. H. Kalir and C. Mytilineou, Dept. of Neurology, Mt. Sinai Sch. of Med., New York, NY 10029

Rat embryonic brain contains high levels of ascorbic acid (AA), a major water soluble antioxidant. We have investigated the effect of AA on the development of dopamine (DA) neurons. Dissociated midbrain cells from embryonic day 14 rats were plated in serum-containing medium in the presence or absence of $2x10^{-4}$ M AA and maintained for 2 weeks in vitro. The initial AA level was 1.74ug/mg protein, and dropped sharply in the untreated cultures to below our detection level of 1ng by 7 days in vitro (DIV). In AA treated cultures, there was an initial drop of AA levels followed by a rise on days 7 through 14 to the initial embryonic cells' level. The development of DA neurons was monitored by measuring the DA and DOPAC levels with HPLC, [^{3}H]DA uptake and staining for tyrosine hydroxylase (TH). DA and DOPAC levels increased in both groups, but AA treated cultures had higher levels of both DA and DOPAC on DIV 7 and 14. [^{3}H]DA uptake was not affected by AA treatment. TH staining on DIV 8 showed 20% more TH+ cells in AA treated cultures. These results demonstrate that (1) AA levels are depleted in cultured cells but can be restored by addition of AA in the medium, and (2) DA neurons can survive and grow in the absence of detectable levels of AA. We then examined whether the presence of cellular AA would affect 6-OHDA neurotoxicity. On DIV 12, when AA levels are undetectable in untreated and high in treated cultures, 6-OHDA exposure produced similar damage to DA neurons in both groups. Supported by NIH grant NS-23017.

149.5

DOPAMINE D1 AND D2 AGONISTS HAVE OPPOSITE EFFECTS ON STRIATAL DOPAMINE RELEASE IN DEVELOPING RATS. D.E. Walters and S.G. Howard. MRRC, Dept. Pharmacology and BRI, Univ. California, Los Angeles, CA 90024-1759.

To determine the role of dopamine (DA) D1 and D2 receptor subtypes in mediating striatal DA release during development, a dialysis probe was implanted into the striatum of rats at 4 ages and perfused with a Ringer's solution. The DA contained in the perfusates was then measured by high pressure liquid chromatography with electrochemical detection following subcutaneous injection of DA agonists and antagonists selective for D1 and D2 receptor subtypes, respectively. The D1 agonist SKF 38393, 10mg/kg, significantly increased DA in perfusates from 35-36-day-old and adult rats. Pretreatment with the D1 antagonist, SCH 23390, 0.5mg/kg, completely blocked the SKF 38393-induced increase in DA levels in the perfusates. SCH 23390 alone did not significantly alter the DA levels at any age studied. The D2 agonist LY 171555, 0.05mg/kg, and the D2 antagonist sulpiride, 10mg/kg, significantly decreased and increased, respectively, the DA content of the perfusates at each age. Pretreatment with sulpiride blocked the effect observed when each drug was given alone. The results suggest that D1-mediated DA release develops between 21 and 35 days of age and that D2-mediated inhibition of DA release is functional by 10 days of age.

Supported by USPHS grant HD29512.

149.6

SELF-MUTILATION INDUCED BY COMBINED D1 and D2 RECEPTOR STIMULATION FOLLOWING SUBSTANTIAL ACUTE DEPLETION OF DOPAMINE IN WEANLING (BUT NOT INFANT) RAT PUPS.
C.A. Moody and L.P. Spear, Dept. of Psychology and Center for Developmental Psychobiology, SUNY-Binghamton, NY 13901.

The behavioral responses to single or combined administration of the D1 agonist SKF38393 and the D2 agonist quinpirole were examined in infant, postnatal day 10 (P10) and weanling (P21) Sprague-Dawley rat pups depleted of dopamine (DA) via pretreatment with alpha-methyl-tyrosine (AMT) (80% DA depletion) or AMT plus reserpine (approx. 99% DA depletion). AMT pretreatment had little impact on D2 agonist-induced behaviors (locomotion, sniffing, wall climbing) at either age. More extensive DA depletion induced by AMT/reserpine attenuated D2 responses at P10, while inducing a different pattern of D2 responses (coprophagia and oral stereotypies) at P21. D1 agonist-induced grooming was not blocked by DA depletion at either age. Combined D1 and D2 receptor stimulation induced marked synergism at P21 which varied with depletion state, inducing licking in non-depleted weanlings, licking and probing in AMT-treated pups, and self-mutilatory behavior in weanlings pre-treated with AMT/reserpine. Selective stimulation of each receptor subtype also elicited atypical responses (coprophagia: D1, D2: oral stereotypies: D2) in depleted weanlings, thus this self-mutilatory response may be related to a rapid depletion-induced increase in D1/D2 receptor responsivity.

149.7

ONTOGENY OF RAT STRIATAL D_2 DOPAMINE RECEPTOR mRNA LEVELS. S. X. Xu and Ian Creese. Center for Molecular and Behavioral Neuroscience, Rutgers, The State University of New Jersey, Newark, NJ 07102

We present here the developmental characteristics of D_2 dopamine (DA) receptor mRNA levels in the striata of male Fischer 344 rats during ontogenesis. Three [^{32}P]-labelled oligonucleotides, derived from the coding region of the rat D_2 DA receptor cDNA, were used together as a probe to measure the levels of mRNA for the D_2 DA receptors in rat striatum by Northern hybridization. These probes do not differentiate between the short and long isoforms of the rat D_2 DA receptor mRNA. The lowest and highest mRNA levels were found in the striata at one day and one month post partum, respectively. The ratios of the optical density of the D_2 DA receptor mRNA to total 28S mRNA in striatum were 0.23, 0.26, 0.39, 0.52, 0.71 and 0.33 at days 1, 7, 14, 21, one month, and 4 months (adult), respectively. There were significant differences in D_2 DA receptor mRNA ratio between days 1, 7, 14 or 4 months vs. one month of age ($p < 0.05$, Dunnett t test). This decrease in mRNA levels in adulthood is similar to the results that were found previously measuring the development of D_2 DA receptors using radioligand binding (Pardo et al, 1977). However, mRNA levels increased only 3 fold over the first month of life vs. an eight fold increase in receptor binding. We also observed that the expression of ß-actin mRNA decreased gradually as the rats developed.

Supported by MH 00316, MH 44211 and DA 04612

149.8

EFFECT OF IRREVERSIBLE DOPAMINE RECEPTOR INACTIVATION ON LOCOMOTOR ACTIVITY IN THE RAT PUP. C. A. Crawford*, S. A. McDougall*, and A. J. Nonneman. Dept. of Psychology, Univ. of Kentucky, Lexington, KY 40506.

The role of dopamine (DA) receptors in the ontogeny of locomotor activity was assessed using the irreversible DA antagonist, EEDQ. In two experiments, 16-day-old rat pups received a single i.p. injection of EEDQ (7.5 or 15 mg/kg) after DA receptors were left either unprotected or protected by a combinatin of sulpiride (100 mg/kg) and/or SCH 23390 (1 mg/kg). Quinpirole (0.1 mg/kg), SKF 38393 (15 mg/kg), NPA (1 mg/kg) or vehicle were then administered (i.p.) to these rat pups at 24, 48, 96, and 192 hrs after EEDQ treatment. Locomotor activity was then assessed, with activity quantified as the mean number of photobeam interruptions during a 20-min testing period. Results indicated that EEDQ given to non-protected pups produced an increase in locomotor activity at both 24 and 48 hrs after treatment. NPA and quinpirole increased the activity of non-protected and SCH 23390-pretreated rat pups, but these agonists did not increase the activity of pups with intact D-2 receptor systems (sulpiride pre-treated pups). SKF 38393 did not affect locomotor activity.

NEURAL PLASTICITY IN ADULT ANIMALS II

150.1

LONG-TERM INTRAVENTRICULAR ADMINISTRATION OF NGF TO RATS WITH PARTIAL FIMBRIAL TRANSECTIONS. E. O. Junard, P.A. Lapchak, C. N. Montero* and F.F. Hefti. Andrus Gerontology Center, U.S.C., Los Angeles, CA, 90089.

Adult rats with unilateral partial fimbrial transections (Montero and Hefti J. Neurosci. 8: 2986-2999, 1988) were injected intraventricularly twice weekly (during 5 months) with 1 µg of mouse beta-NGF through chronically implanted cannulas. These lesions resulted in a topographically gradual loss of AChE-positive and NGF receptor-positive fibers in the hippocampus. Fiber density was strongly reduced in the temporal portion, whereas only slight reductions were observed in the septal portion. Long-term, but not short-term (1 month), NGF treatment increased the fiber density in the septal part of the hippocampus on the side of the lesion. The increase was most pronounced in the dentate gyrus. In some animals, stained fibers appeared to invade areas of the dentate gyrus not normally receiving a large amount of cholinergic innervation. As evidenced by Nissl and thioflavin histochemistry, long-term NGF treatment did not produce pathological changes in the brain. Immunoblot experiments failed to reveal the presence of antibodies against mouse beta-NGF in the serum of rats given chronic intraventricular NGF injections. These results indicate that long-term NGF treatment affects fiber outgrowth in the hippocampal formation, but does not initiate pathological changes within hippocampal neurons.

150.2

MITOGENIC STIMULATION OF AGED PRIMATE SCHWANN CELLS *IN VITRO*. M.F.D. Notter, J.T. Hansen and D.M. Gash. Dept. of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642.

We have previously shown that primate peripheral nerve induces neuronal differentiation and maintains the neuronal phenotype of primate adrenal medullary cells in coculture *in vitro* as well as in cograft *in vivo*. Schwann cells from peripheral nerves are thought to be the source of the neurite promoting factor(s); however, studies with these cells are hampered by the poor yields and low mitotic rate of Schwann cells from aged animals. Therefore the present study was undertaken to define an appropriate mitogen to obtain cultures enriched for Schwann cells for transplantation.

Explants of sural nerve from aged Rhesus monkeys were established on collagen coated coverslips in F14 medium. After Schwann cell outgrowth was inititated, the medium was supplemented with one of the following substances: acidic fibroblast growth factor (aFGF) 100 ng/ml; basic FGF 100 ng/ml; interleukin (IL-1) 1 ng/ml; platelet derived growth factor (PDGF) 10 ng/ml; transforming growth factor ß1 (TGFß1) 10 ng/ml; Schwannoma conditioned medium (SCM) 1:1; or rat Schwann cell conditioned medium (rSCCM) 1:1. Cultures were scored for DNA synthesis by autoradiography for ^{3}H thymidine incorporation or by immunofluorescence for bromodeoxyuridine incorporation. A three to fourfold increase in DNA synthesis in Schwann cells was induced by IL-1, acidic and basic FGF, PDGF and Schwannoma conditioned medium. However, a more dramatic response was seen with rSCCM which enhanced DNA synthesis sixfold over control cultures while TGFß1 induced an eightfold increase in the mitotic rate of these cells.

These data indicate that rat Schwann cells release a factor that stimulates growth in Schwann cells from other species and that TGFß1 may be an important mitogen for obtaining enriched cultures from aging animals.

Supported in part by NIH NS 25778.

150.3

AXONAL ATTEMPTS AT REGENERATION IN THE EXCITOTOXICALLY LESIONED ADULT CNS, ROLE OF NEUROGLIAL INTERACTIONS. I. Dusart, S. Marty* and M. Peschanski, INSERM U 161, 2 rue d'Alésia, 75014 Paris France.

Many afferent fibers deprived of target-neurons by an excitotoxic lesion exhibit during several months morphological alteration resembling regenerative growth cones (Peschanski and Besson, JCN, '87). These attempts at regeneration can be successful if new target-cells are provided via neurotransplantation (Peschanski and Isacson, JCN, '88). The present study analyzes the evolution of the cellular content of a neurodegenerative lesion (induced by kainic acid-KA) of the somatosensory thalamus, in the search for mechanisms at work to support long-term axonal survival and regenerative attempts.

Three periods can be defined: (i) During three weeks after KA, there is a profuse leakage of the blood-brain barrier, a disruption of the glia limitans and a proliferation of macrophages/microglia. Few, if any, reactive astrocytes are present. There are conspicuous signs of a demyelinating process. (ii) Between three weeks and three months, numerous reactive astrocytes and some oligodendrocytes appear. In the same time, many Schwann cells -migrating along blood vessels- invade the parenchyma. Peripheral glia ensheath and myelinate central axons. (iii) Later, reactive astrocytes rebuild a glia limitans, containing Schwann cells and other elements, apparently tending to eliminate them.

The present results support the hypothesis that Schwann cells are called into a neurodegenerative area of the brain parenchyma and replace oligodendrocytes injured by toxic agents either blood-borne or released by macrophages. It is our contention that Schwann cells may transitorily support axonal survival and regenerative attempts in the CNS. (supported by MRT 106728)

150.4

BENZODIAZEPINE EFFECTS ON RECOVERY OF FUNCTION LINKED TO TRANS-NEURONAL MORPHOLOGICAL EVENTS. J.S.Sims, T.A.Jones, R.L.Fulton*, L.E.Shapiro*, M.D.Lindner, and T.Schallert. Inst.for Neuroscience,Univ.Texas,Austin,TX 78713.

Diazepam chronically disrupts recovery from the otherwise transient behavioral effects of unilateral damage to the anterior medial cortex if it is administered continuously during a sensitive 12-96 hour postoperative period. Sensorimotor asymmetries caused by the cortical lesion failed to recover within a 100 day post-drug period. Sedative and motor effects were ruled out. A benzodiazepine (BZ) receptor antagonist (RO 15-1788) enhanced recovery. Recovery was not affected by the non-BZ anxiolytic gepirone, a $5HT_{1A}$ receptor ligand.

Diazepam increased atrophy of subcortical brain areas in the ipsilateral hemisphere, including the striatum, substantia nigra pars reticulata and VM/VL thalamus, without affecting the size of the lesion or atrophy in primary thalamic afferent nuclei. A time course analysis of these morphological changes was carried out. The data suggest that multi-level degenerative events may contribute to the detrimental effects of BZ/GABAergic agents on recovery.
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150.5

LOCAL INJECTION OF INTERLEUKIN-1, TNF OR LPS INCREASES ω_3 (PERIPHERAL TYPE BENZODIAZEPINE) BINDING SITE DENSITIES IN THE RAT BRAIN. F. Bourdiol*, S. Toulmond*, A. Serrano*, J. Benavides and B. Scatton. Synthélabo Recherche (L.E.R.S.), 31, av. Paul-Vaillant Couturier, 92200 Bagneux (France).

The presence of high densities of ω_3 (peripheral type benzodiazepine) binding sites on astrocytes has provided the basis for the use of ω_3 sites to index the glial reaction to brain injury (Benavides et al, Ann. Neurol. 24, 708, 1988). Previous studies have suggested a role for interleukin-1 (IL-1) in the astroglial reaction to injury (Giulan et al, J. Neurosci. 8, 2485, 1988). In the present study, we have examined the effects of local injection of IL-1 and other immune mediators in the rat cerebral cortex and striatum on the density of ω_3 sites in these regions. Intracortical or intrastriatal infusion of IL-1 (5,10 and 20 units) caused a marked increase (+ 80 % at 20 units) in the density of ω_3 sites around the injection site at 7 days post-infusion. Significant increases in ω_3 site densities were also observed in striatal homogenates one week after the local injection of TNF-α (+ 80 % at 3 units). The local injection of E. Coli LPS, a bacterial endotoxin known to stimulate IL-1 and TNF production by microglial cells in culture, also resulted in significant increases (+ 170 % at 200 ng) in ω_3 site densities in striatal homogenates. In contrast, the intrastriatal injection of IL-2 or of the chemotactic peptide FMLP failed to alter ω_3 site densities. No change in the density of two binding sites associated with neurons (^{3}H-TCP and ^{3}H-Ro15-1788) was observed after instrastriatal injection of IL-1, TNF-α or LPS. These results suggest that the increase in ω_3 site densities observed after brain injury may involve the activation of microglia or monocytes, release of IL-1 or TNF and the promotion, by these cytokines, of the astroglial reaction.

150.6

CONTEMPORANEOUS OBSERVATION OF SYNAPTIC AND GLIAL POSITION IN LIVING MICE. S.L. Pomeroy, Departments of Anatomy and Neurobiology and of Pediatrics, Washington University School of Medicine, St. Louis, MO 63110.

The relationship between synapse remodelling and glial cell movement on the surface of parasympathetic ganglion neurons has been evaluated directly in living mice. When viewed independently in vivo, synapses and glia change their position over the course of weeks to months (Purves et al., 1987, Science, 238:1122-26; Pomeroy and Purves, 1988, J. Cell Biol. 107:1167-75). EM observation shows that synaptic boutons on ganglion cells tend to cluster near glial cell nuclei (op. cit.). To test whether glia and synapses move together, the positions of glial nuclei and synaptic boutons on the same cell were monitored over several weeks.

Salivary duct ganglion neurons in anesthetized adult male mice were viewed by low-light-level video microscopy after staining synaptic boutons with a styryl pyridinium dye. Video images of focal planes through the entire depth of the cell were digitized and stored, and the animals were allowed to recover. This procedure was repeated after 1 month, and the images obtained over this interval compared.

When synaptic boutons moved to occupy a different part of the neuronal surface, glial nuclei were found to move to the same region. The persistence of this association supports the idea that glia may have a role in synaptic maintenance and remodeling. (Supported by USPHS NS27773.)

150.7

PROGRESSIVE CHANGES IN THE PATTERN AND INTENSITY OF ASTROCYTE HYPERTROPHY IN THE BRAINS OF KINDLED RATS. M. Khurgel, N.W. Milgram, R.J. Racine and G. O. Ivy. Life Sci. Div., Univ. of Toronto at Scarborough, Ont. M1C 1A4 & Psychology Dept., McMaster Univ., Hamilton, Ont. L8S 1B9.

We have previously shown that repeated seizure activity causes both neural damage and astrocyte hypertrophy (AH). We now report the development of AH following kindling in the absence of neuronal death. Rats with electrodes in the amygdaloid complex (AC) underwent a) a standard kindling procedure (SK), b) a rapid kindling procedure (RK), c) stimulation which was subthreshold (ST) for afterdischarges or d) no stimulation. Rats were sacrificed at 1d, 1wk, 1mo or 2mo and the brains processed for AH using immunoreactivity for anti-GFA. Control rats display AH only at the site of electrode implantation regardless of survival time. In contrast, by 1 wk both ipsi- and contralateral pyriform-AC areas display AH in the stimulated groups, although the precise pattern of AH varies. By 1 mo, the AH pattern was unchanged in the ST group but had spread to include the entire AC and pyriform regions in the SK and RK groups and had increased in intensity; this effect was especially dramatic in the basolateral nucleus. Thus, subthreshold stimulation of AC triggers an AH response by 1wk which does not appear to increase over time, whereas kindling causes a progressive increase in AH in anatomically related brain regions. These results suggest that progressive functional changes occur after the termination of kindling.

150.8

INTRAAMYGDALOID INFUSIONS OF CLONIDINE RETARD LOW-FREQUENCY KINDLING. M. R. Pelletier and M. E. Corcoran. Department of Psychology, University of Victoria, Victoria, BC, Canada.

Considerable evidence indicates that noradrenaline (NA) delays the kindling of seizures provoked by stimulation at limbic and neocortical sites. Recently several laboratories have reported that systemic injections of the α-2 agonist clonidine slow the rate of amygdaloid kindling by delaying emergence out of the early stages of partial seizure. To begin to localize the critical α-2 receptors that delay epileptogenesis, we examined the effects on kindling of intraamygdaloid infusions of clonidine.

Male rats were prepared with chemitrodes implanted bilaterally in the amygdala. To reduce the number of infusions administered, we rapidly kindled seizures with 60-sec trains of unilateral square-wave stimulation at 3/sec and delivered at a high intensity at 48-hr intervals. Ten min before each stimulation the rats received ipsilateral infusions of 0.5 µl of saline or of clonidine in concentrations of 1 mM, 100 µM, or 1 µM.

Rats receiving infusions of saline developed generalized seizures in a mean of 2.7 stimulations, comparable to previous results (Corcoran & Cain, Brain Res., 1980). Kindling proceeded in clonidine-treated rats at the following rates: 1 mM, 4.4 stimulations; 100 µM, 5.0; 1 µM 4.2 (each $p<0.05$). Clonidine's prophylactic effects were produced by delaying the progression out of partial seizure during the early stimulations. A significant shortening of durations of afterdischarges and generalized behavioral seizures was observed only in the rats receiving 100 µM of clonidine. Administration of clonidine to already kindled control rats had no effect on seizure duration or intensity.

Intraamygaloid clonidine thus produces a small but significant retardation of kindling, and it is a genuine prophylaxis. Systemically administered clonidine may be acting in the pyriform lobe to delay limbic kindling.

150.9

DIFFERENTIAL EFFECT OF D-AMPHETAMINE ON SEIZURES INDUCED BY PICROTOXIN AND BICUCULLINE. R.D. Kirkby and L. Kokkinidis. Dept of Psychology, University of Saskatchewan, Saskatoon, Saskatchewan, S7N 0W0.

We have demonstrated that amphetamine (amph), administered twice daily, facilitates ongoing amygdaloid kindling (short-term effect). Under this condition, rats with prior amph experience (14 days, twice daily) show a further kindling acceleration (long-term effect). Moreover, several researchers have reported bidirectional transfer between electrical kindling and PTX- or PTZ- induced convulsions or "chemical" kindling. In addition, amph withdrawal has transiently exacerbated PTZ-induced seizure expression. Collectively, these findings indicate that electrical kindling and "GABAergic" seizures share common mechanisms, which are subject to the influence of amph.

In the present investigation, a single intraperitoneal (i.p.) administration of d-amph sulphate (7.5mg/kg) shortened latencies to generalized motor seizures induced 12 hours later by PTX (i.p., 10mg/kg) in adult, male Swiss mice. Chronic (14-day) amph pretreatment (2 i.p. injections per day, 7.5mg/kg) failed to alter either the PTX-elicited convulsions or the seizure-facilitating effects of the single amph administration. By contrast, neither the chronic nor the single amph treatment affected (+)bicuculline-induced (BIC; i.p., 5mg/kg) seizures.

BIC does not readily kindle convulsions, and differs from PTX and PTZ with respect to mechanism of GABAergic antagonism and subsequent generation of behavioral and electrographic seizure activity. Whereas BIC blocks postsynaptic GABA receptors, PTX and PTZ block GABA-related chloride ionophores. It is therefore possible that withdrawal from amphetamine enhances both electrical kindling of the amygdala and seizures induced by PTX or PTZ via transient and subtle changes to GABA-mediated chloride ionic currents.

150.10

SHORT AND LONG TERM EFFECTS OF KINDLING AND CHRONIC IMIPRAMINE ON TYROSINE HYDROXYLASE REGULATION IN THE TEGMENTO AMYGDALOID PATHWAY. V.Leviel*, N.Emma*, B.Guibert*, N.Faucon Biguet*, C.Pasqualini*, G.Machek* and R.Naquet. Lab.Physiol.Nerveuse CNRS 91198 Gif sur Yvette, France

We observed previously that repeated electroconvulsive shocks (ECS) produced an initial activation of tyrosine hydroxylase (TH) activity in the tegmento amygdaloid pathway, followed by a long term inhibition (1 month). Moreover, the long term decrease occurred likely through modifications of TH gene expression. In order to determine if the antidepressive effect of ECS could be related with these particular biochemical modifications, chronic treatments with imipramine (5 and 20 mg/Kg/day, 20 days) were realized on the rat. The same modifications, i.e. an initial increase and a long term decrease were obtained in the same central structures. In addition, a similar analysis of TH regulation was conducted on another model of experimental epilepsy, the kindling phenomenon, which is not considered as an animal model of depression but is known to be antagonized by imipramine. In opposite with the results obtained after ECS treatment, TH activity was decreased the day after the last of a series of ten kindled seizure and increased 30 days later both in ventral tegmental area (VTA) and amygdala (AC). Thus, kindling induces opposite effects on TH regulation than do imipramine and repeated ECS. These results suggest first, that the monoaminergic control of AC by neurons originating from VTA could be implicated in the antidepressive mechanism initiated by ECS and imipramine. Second, that some particular biochemical modifications consecutive to kindling could participate to the development of the depressive syndrome.

150.11

TYROSINE HYDROXYLASE AND CHOLINE ACETYLTRANSFERASE ENZYMES EXHIBIT SIMILAR DOWN REGULATION IN RESPONSE TO AXOTOMY M. Weiser, T. Wessel, H. Baker, U.J. Kang and T.H. Joh Lab. Molec. Neurobiol. Cornell Univ. Med. Coll., Burke Rehab. Center, White Plains, NY 10605.

Axons of dopaminergic neurons in the substantia nigra travel rostrally in the medial forebrain bundle to synapse within the striatum. Cholinergic neurons in the horizontal limb of the diagonal band send axons rostrally which terminate within the olfactory bulb. To determine whether neurons expressing different neurotransmitter synthesizing enzymes have comparable responses to axotomy, the presence and distribution of tyrosine hydroxylase (TH) immunoreactivity in the substantia nigra and choline acetyltransferase (ChAT) immunoreactivity in the horizontal limb of the diagonal band were examined in Sprague-Dawley rats. One week following unilateral axotomy there was a prominent decrease ipsilaterally in both TH and ChAT immunoreactivities in comparison to the control side. Cell bodies of the neurons on the lesioned side were more rounded, more lightly stained and appeared fewer in number. In addition, a marked decrease was observed in the immunoreactivity of the ramifying dendritic processes of neurons of both phenotypes. *In situ* hybridization using a specific [^{35}S]-labelled cDNA for TH demonstrated a corresponding down regulation in the mRNA for this protein. Thus, we hypothesize that neurons of two different transmitter phenotypes exhibit similar responses to axotomy. Supported by MH44043 and AG08702.

150.12

CHANGES IN CORTICAL DOPAMINE CONCENTRATION AFTER EARLY OR LATE LESIONS OF FRONTAL CORTEX IN RAT. Deborah Christie and M. Bloomfield, (SPON: European Brain and Behaviour Society). Dept. of Experimental Psychology and University Laboratory of Physiology, University of Oxford, (U.K.)

It is commonly assumed that early brain damage causes fewer deficits than equivalent damage experienced as an adult. The greater plasticity shown to exist in the infant brain may make it more vulnerable to brain damage than the adult. Prefrontal cortex in all mammals receives a convergent input from Mediodorsal thalamus and the ventral tegmentum. Unilateral lesions of this area produce a transient polymodal contralateral neglect where animals fail to attend to stimuli presented in the contralateral field. After a 3 month recovery period animals with early (postnatal day 1) or late (3 months) lesions were tested on spatial alternation, non-visual search and visual search task. Compared to a group of age matched non-operated controls the degree of recovery measured by turning behaviour, was shown to be dependant upon both the age of lesion and the task used (Christie, D., & Cowey, A. *Neurosci. Abs.*1988). The initial contralateral neglect can be reduced by treatment with the DA agonist Apomorphine or increased with the DA antagonist Spiroperidol. Following the completion of behavioural testing the concentration of dopamine in the unlesioned and lesioned cortex was measured. The cortical DA concentration in adult lesioned animals was identical to the controls. In the neonatally lesioned animals there was a significant increase in DA concentration in the cortex surrounding the lesion. This was correlated with the individual animals' turning behaviour. It is suggested that there are different mechanisms underlying the recovery and subsequent control of animals behaviour depending on the age at which frontal cortex is removed.

150.13

TIME-DEPENDENT EFFECTS OF TRIGEMINAL MANIPULATION IN BASAL GANGLIA: ANATOMICAL CHANGES AND NEUROCHEMICAL EFFECTS ASSESSED BY IN VIVO DIALYSIS. H. Steiner, F. Adams*, R.K.W. Schwarting, F. Boix*, H.-T. Weiler*, S. Morgan* and J.P. Huston. Inst. of Physiol. Psychol. I, Univ. of Düsseldorf, Universitätsstr. 1, D-4000 Düsseldorf, F.R.Germany.

Unilateral removal of vibrissae (URV) induces neuronal changes in nigrostriatal (NS) and tuberomammillary-striatal projections in relation to recovery from asymmetries in facial scanning induced by URV. New data indicate that NS projections to the striosomes and to the striatal matrix are differentially influenced by URV. During the time when rats express behavioral asymmetries (1-3 days after URV), an asymmetry was found in the nigro-matrixal subset of crossed NS projections (more HRP labeling in the projection to the CPU ipsilateral to URV). In contrast, during a period after behavioral recovery, an opposite asymmetry was found (more HRP labeling in the crossed projection to the CPU contralateral to URV); this asymmetry was restricted to the nigro-striosomal subset. These results are indicative of a functional dissociation of nigrostriatal projections to striosomes and matrix.

Trigeminal stimulation also affected the striatal dopamine (DA) transmission (assessed by in vivo dialysis) and behavior: After unilateral mechanical stimulation of the vibrissal area of the snout, asymmetries in extracellular DA and metabolites (increased levels in CPU ipsilateral to stimulation) were found, concomitant with contraversive asymmetries in facial scanning.

150.14

TIME COURSE OF RECOVERY FROM COPULATORY BEHAVIOR DEFICITS FOLLOWING MPOA INJECTIONS OF 6-OHDA

T.Bazzett, L.A. Lumley, V.P. Markowski, D. Bitran, R. W. Warner E. M. Hull. Dept of Psychology, SUNY at Buffalo, Buffalo, NY 14260

We have shown that dopaminergic influence at the medial preoptic area (MPOA) is important for the regulation of male rat sexual behavior. A previous study using 6-OHDA to destroy DA terminals in the MPOA produced no copulatory deficits in male rats tested as soon as 3 days following 6-OHDA injection. The current experiments were undertaken to determine: 1) Whether earlier testing would reveal behavioral deficits 2) If so, when recovery occurred 3) What mechanisms could account for the recovery.

Using 20 male Long Evans rats we tested the effects of 6-OHDA injections into the MPOA on male copulatory behavior. Tests were conducted at 30 min and again at 24 hours after injections. A separate group of 20 males was tested at 4 hours and again at 28 hours after 6-OHDA injection.

6-OHDA animals at 30 min exhibited fewer ejaculations compared with their own scores and controls at 24 hours. However, they were not significantly different from controls at 30 min post lesion. Behavioral deficits 4 hours after injection were statistically significant when compared with vehicle injected animals at 4 hours and with 6-OHDA injected animals at 28 hours. Furthermore, a CA challenge by AMPT that had no effect on intact male rats, produced copulatory behavior deficits in rats that showed no deficits several weeks after 6-OHDA injections.

The results of these studies show that MPOA injections of 6-OHDA impair copulatory behavior. However, these deficits are rapidly reversed within 24 hours. One mechanism underlying behavioral recovery appears to be increased DA metabolism at remaining DA terminals.

Supported by NIMH grant MH-40826 to EMH.

150.15

CHANGES IN THERMAL PLASTICITY OF RAPHE NEURONES AFTER MICROCUT LESIONS AND NEUROTRANSMITTER APPLICATIONS.
P. Hinckel, M. Rüsing*, K. Schröder-Rosenstock*. Physiologisches Institut, Universität Giessen, Aulweg 129, and Zentrum für Neurologie und Neurochirurgie, Abt. Klinische Neurophysiologie, Am Steg 28, D-6300 Giessen 1, Federal Republic of Germany.

Neurones in the nucleus raphe magnus (NRM) have been shown to belong to the thermoafferent system. In cold-acclimated guinea-pigs average maximum activity and average basic spike rate of warm-responsive NRM units were significantly larger than in normal-acclimated animals.

After interruption of the dorsal and rostral afferents and efferents of the NRM by microcuts in cold-acclimated guinea-pigs basic spike rate was significantly reduced (to the basal level in normal-acclimated intact animals), whereas the maximum spike rate was not changed.

Microiontophoretic application of serotonin on warm-responsive NRM neurons caused a similar increase of peak activity and basic rate, whereas substance P caused an increase of peak activity but not of basic rate. Application of both substance P and serotonin together induced a prolonged peak activity increase in all tested warm-responsive NRM units.

It is possible that the observed cold-adapted peak activity increase in NRM neurons is generated by neuronal interactions at the lower brain-stem level in which this transmitter complex is involved.

mRNA REGULATION: GENERAL

151.1

INDUCTION OF A HEAT SHOCK GENE (hsp70) IN THE RABBIT SPINAL CORD AFTER HYPERTHERMIA: AN IN SITU HYBRIDIZATION STUDY.
I.R. Brown and S.J. Rush*. Department of Zoology, University of Toronto, Scarborough Campus, West Hill, Ontario, Canada, M1C 1A4.

We have previously reported that hyperthermia induces the expression of a heat shock gene in the rabbit brain (Sprang and Brown, Molec Brain Res, 3, 89-93, 1987). Striking regional and cell type differences in the pattern of induction of hsp70 mRNA were noted. Tissue injury also induces a rapid induction of hsp70 mRNA in the mammalian brain (Brown et al., Neuron, 2, 1559-1564, 1989). In the present study in situ hybridization with 35S-labeled antisense or sense riboprobe was employed to investigate the effect of fever-like temperatures on hsp70 gene expression in the rabbit spinal cord where delineation of white and gray matter is distinct. Constitutive expression of hsp70 mRNA was detected in large motor neurons. Within 1 hr after hyperthermia a massive induction of hsp70 mRNA was noted in fibre tracts in the spinal cord, a pattern consistent with a strong glial response to heat shock. Little induction was noted in the large motor neurons which exhibited constitutive expression. Supported by grants from MRC (Canada).

151.2

IDENTIFICATION OF LOCUS COERULEUS-ENRICHED PROTEINS BY SUBTRACTION HYBRIDIZATION.
P. Tinsley III, K. Saijoh, B.S. Duman, and E.J. Nestler. Lab. of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale School of Medicine, New Haven, CT 06508.

We have used subtraction hybridization and cloning procedures to identify proteins enriched in the locus coeruleus (LC) that could contribute to the distinct anatomical, electrophysiological, and pharmacological characteristics exhibited by these neurons.

"Subtracted" bovine LC cDNA was generated by hybridization with excess biotinylated mRNA from bovine cerebellum and then used to probe an LC cDNA library containing >10^6 recombinants. 150 positive clones were identified initially, of which the first 50 have been studied further. About half of these show enrichment in the LC compared to cerebellum by differential hybridization; the regional distribution of 15 of these clones have been studied to date by northern blotting.

Clone 21 (mRNA 3.9 kb, insert 1 kb) is present in the LC at 10-fold greater levels than any other brain region or peripheral tissue analyzed. Clone 38 (mRNA 3.2, insert 2.3 kb) is also enriched (by >3-fold) in the LC compared to all other brain regions and tissues analyzed. These two clones do not correspond to known proteins enriched in the LC. A number of other clones show interesting distributions in brain, although they are not enriched uniquely in the LC. For example, clone 9 appears to be a thalamus-enriched protein.

Current studies are aimed at sequencing these various clones and studying the regulation of their expression by psychotropic drug treatments known to influence the functional state of LC neurons. Characterization of proteins highly enriched in the LC will provide important information concerning the molecular basis of some of the unique functional properties of these neurons.

151.3

ANALYSIS OF mRNA SHOWING REGION-SPECIFIC EXPRESSION IN NONHUMAN PRIMATE CORTEX.
K. Chandrasekaran, M.F. Matocha, J. Stoll, S.P. Wise and S.I. Rapoport. Lab. of Neurosci., NIA, Bethesda, MD 20892 and Lab. of Neurophysiology, NIMH, PO Box 289, Poolesville, MD 20837.

Alzheimer's disease preferentially afflicts the phylogenetically recently elaborated association neocortices and their connections to phylogenetically older brain regions (Rapoport, S.I. Rev. Neurol, Paris, 144: 79,1988). To understand the molecular basis of this selective vulnerability, we have begun to identify mRNAs that show specific expression in association neocortical regions of the Rhesus monkey. A cDNA library was made using mRNA prepared from the frontal pole. 10,000 plaques in the frontal pole cDNA library were screened by differential hybridization. 10 cDNA clones were identified that hybridized with the total cDNA probe prepared from the frontal pole but not with the total cDNA probe prepared from the primary visual cortex.These cDNA clones, in Northern blot analyses, showed higher expression of mRNA in frontal and dl prefrontal regions as compared to the primary visual cortex. Sequencing and homology search with the GenBank were done on two of the clones. One clone showed homology to the mitochondrial cyt.oxidase subunit I gene, while the second clone did not show a homology with the sequences in the GenBank. Other clones are being sequenced and compared to identify the nature of the gene products.

151.4

SUBCELLULAR LOCALIZATION OF MRNA: ISOLATION AND CHARACTERIZATION OF MRNA FROM AN ENRICHED PREPARATION OF HIPPOCAMPAL DENDRITIC SPINES.
M.E. Chicurel[1], D.M. Terrian[2], and H. Potter[1]. [1] Program in Neuroscience, Dept. Neurobiology, Harvard Medical School, Boston, MA 02115. [2] Dept. Anatomy and Cell Biology, East Carolina Univ. School of Medicine, Greenville, NC 27858-4354.

Differential targetting of cellular components underlies the establishment and maintenance of cell polarity. The intracellular targetting of proteins is well established. More recently, evidence for mRNA targetting in several cell types has also been obtained. We are interested in analyzing the mRNA population within a specific neuronal cell compartment: the CA3 dendritic spine. Poly-A plus RNA was isolated from a preparation of rat hippocampal mossy fiber synaptosomes which includes the postsynaptic CA3 spines and their endogenous ribosomes. Electrophoresis and transfer of this putative spine RNA and of total hippocampal poly-A plus RNA to nitrocellulose filters was followed by hybridization to radiolabelled cDNA obtained from each mRNA preparation. The results revealed significant differences in labelling patterns. Some species were more abundant in the spine mRNA; some were more abundant in the total hippocampal mRNA. We have also used probes for known genes to assess the presence of specific RNAs in this subcellular compartment. Once again, comparisons of these signals to those obtained with total hippocampal mRNA indicate interesting differences, consistent with the conclusion that our spine mRNA preparation contains a specific subset of hippocampal mRNA species. Supported by NIH grant GM35967, AFOSR 89-0531; MEC is a Howard Hughes fellow.

151.5

PANCREATIC RIBONUCLEASE mRNA IN RAT BRAIN

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Pancreatic ribonucleases form a group of homologous proteins which are secreted in some organs of mammals.In past years,a ribonuclease strictly homologous to bovine pancreatic RNase has been purified from ox brain. On the other hand a ribonuclease activity, not yet characterized, has been detected in rat brain by histochemical and biochemical methods (Histochemistry,88:587,1988).

We have performed in situ hybridization experiments using -as a probe- a ^{35}S cDNA specific for the pancreatic ribonuclease mRNA, in order to study the presence and cellular localization of the secretory enzyme in the rat brain.

Emulsion autoradiography of brain sections reveals that the gene of the pancreatic RNase is highly expressed in neurons. The expression pattern appears higher in stratified regions such as the hyppocampus, the olfactory bulb, the cerebral and cerebellar cortices. In comparison the density of labeling in the glial cells of the white matter -corpus callosum- is very low. Experiments are in progress in our laboratory to try to understand the functional significance of the presence of a secretory ribonuclease in the brain.

151.6

A SPECIFIC INCREASE IN APP-695 mRNA LEVELS IS CORRELATED WITH AMYLOID PLAQUE PATHOLOGY IN THE CEREBRAL CORTEX OF ALZHEIMER'S DISEASE. J.S. Jacobsen, B. Beer, A.J. Blume and M.P. Vitek, Molecular Neurobiology Group, CNS Research, Lederle Laboratories, American Cyanamid Company, Pearl River, NY, 10965.

We have measured the expression of several similar but non-identical amyloid precursor protein (APP) mRNA forms associated with Alzheimer's disease (AD) in order to determine whether the expression of either one or all of the transcripts correlate with the observed amyloid plaque pathology. An S1 nuclease protection strategy employing an antisense probe derived from APP-770 cDNA was used to qualitatively detect mRNA forms including APP-770, APP-751 and APP-695, as well as our recently described 365 amino acid amyloid precursor related protein mRNA variant (APRP-365). Cyclophilin mRNA also was measured in order to permit one to quantitatively compare the relative abundance of APP mRNA forms between samples.

A comparison of frontal, temporal, parietal and occipital cortex (listed in order of decreasing plaque pathology) from AD and age-matched normal brain reveal the following observations on the relative abundance of APP mRNA forms: **(1)** some but not all APP forms increase in AD, **(2)** not all brain regions display the same uniform increase in APP levels, **(3)** APP-770 accounts for the largest "fold-increase" while APP-695 the largest molar increase, **(4)** an increase in APP-695 correlates with plaque densities, but APP-770 does not, and **(5)**, total APP levels roughly parallel plaques densities. When analyzed as a percent of total APP mRNA, the data reveal that plaque pathology is best correlated with **(1)** an increase in APP-695, rather than APP-770, and **(2)**, a decrease in APP-751 and APRP-365 forms.

These observations suggest an imbalance in the APP mRNA forms containing or lacking the Kuintz protease inhibitor (KPI) insert is potentially significant in the etiology of AD. Here we show a specific increase in the KPI-lacking APP mRNA form in AD. This pattern of expression coincides with those brain regions most involved with amyloid plaque formation.

151.7

IMMUNOREACTIVE POU-PROTEINS OF RAT SENSORY NEURONS AND CELL LINES. **J.N.Wood and P.R.Coote* Neuroimmunology Department, Sandoz Institute for Medical Research, 5 Gower place, London WC1E 6BN**

POU-proteins are a class of homeobox-containing proteins that play a role in tissue-specific transcriptional regulation. The distribution of transcripts for one such protein, Brn-3, is similar to the pattern of susceptibility to the toxic effects of the plant neurotoxin capsaicin [He et al.(1989) Nature 340, 35-42, Ritter and Dinh (1988) J. Comp. Neurol. 271, 79-90]. In order to compare the expression of capsaicin-sensitivity with that of Brn-3 protein at the single cell level, we raised specific rabbit antisera to a POU-domain peptide, and to a unique peptide present in Brn-3. Immunocytochemical analysis demonstrated that all DRG neurons in culture exhibit punctate nuclear staining associated with chromatin, the nucleolus and the nuclear membrane, using either POU or Brn-3 specific antisera. Western blots using POU-domain antisera define a number of immunoreactive proteins with molecular weights corresponding to the known POU proteins Oct-1, OTF2A and 2B. In addition, several other immunoreactive putative POU proteins, including a ubiquitous band of molecular weight 145 Kd are present in dorsal root ganglia, and neuronal (N18, ND7, ND8) endothelial (CPAE) and fibroblast (NIH-3T3) cell lines. Anti-Brn-3 peptide antisera define 3 candidate Brn-3 proteins on western blots of DRG extracts that also exhibit immunoreactivity towards POU-domain antisera, of molecular weights 67,60 and 58 Kd. It is concluded that both capsaicin-sensitive and insensitive sensory neurons express Brn-3-like immunoreactivity, and that novel putative POU proteins may be identified with these sera.

151.8

IN SITU HYBRIDIZATION OF GAP JUNCTION mRNA IN THE NEONATAL RAT BRAIN. A. Matsumoto (1), Y. Arai* (1), A. Urano (2) and S. Hyodo* (2). (1) Dept. Anat., Juntendo Univ. Sch. Med., Hongo, Tokyo, (2) Ocean Res. Inst., Univ. Tokyo, Nakano, Tokyo, Japan.

Gap junctions are considered to play an important role in intercellular communication. Possible participation of gap junctions is presumed in the neuronal organization of developing brain. We studied the cellular distribution of the mRNA for gap junction protein in the brain of rats at postnatal day 2 by using in situ hybridization. A complementary DNA specific for the mRNA for rat liver gap junction protein (connexin 32) (gift from Dr. D.A. Goodenough) was applied to in situ hybridization on cryostat and paraffin sections of the middle level of the brain. Autoradiographic signals for connexin 32 mRNA were found to distribute in various regions of brain such as parietal cortex, hippocampus, thalamus/striatum and hypothalamus. These signals were localized on neurons, glial cells and ependymal cells. The level of expression of connexin 32 mRNA on cryostat sections was much higher than that on paraffin sections. These results indicate that connexin 32 mRNA may be expressed in neural substrates in the neonatal rat brain and that the process of paraffin embedding may result in degradation of connexin 32 mRNA in tissues. (Supported by grant of Ministry of Education, Culture and Science of Japan).

151.9

REGULATION OF mRNAS ENCODING GAP JUNCTION PROTEINS IN ASTROCYTES AND C6 GLIOMA CELLS. C.C.G. Naus[1], S. Caveney*[2], J. Bechberger*[1], D. Belliveau[1], D. Zhu*[2] and G.M. Kidder*[2]. Depts. of Anatomy[1] and Zoology[2], University of Western Ontario, London, Canada, N6A 5C1.

Although gap junctions are present in the mammalian central nervous system, the cellular specificity and function of these junctions remains unclear. To begin to understand the significance of these junctions in neural systems, the expression of the gene coding for the gap junction protein, connexin43, has been investigated in astrocytes and C6 glioma cells, using a combination of Northern blotting, dye-coupling, and immunocytochemistry. While both connexin43 and connexin32 gap junction mRNAs are present in brain, only connexin43 mRNA is detectable in astrocytes. In contrast, the level of expression of connexin43 is dramatically reduced in C6 glioma cells. In cultures of primary astrocytes, the injection of carboxyfluorescein into a single cell resulted in the rapid spread of dye into surrounding astrocytes. In contrast, similar spread of dye was rarely noted in cultures of C6 glioma cells. To examine the role of gap junctions in these cells, C6 cell cultures were transfected with a full-length connexin43 cDNA. The level of connexin43 mRNA in these cells increased. We are presently monitoring the effect of increased connexin43 expression on the phenotype of these glioma cells.

151.10

AFFERENT ACTIVITY REGULATES GLIAL GENE EXPRESSION IN THE RAT HIPPOCAMPUS. E.R.Torre, R.A.Tomasulo, and O.Steward, Dept. of Neuroscience, Univ. of VA, Charlottesville, VA 22908

Message RNA for glial fibrillary acidic protein (GFAP) increases dramatically in the rat hippocampus within 48 hours after a unilateral electrolytic lesion of the entorhinal cortex (EC) (Steward et al, J.Neurosci. in press). The initial increase in message is not limited to the denervated area, but occurs throughout the hippocampus bilaterally. At longer postlesion intervals the increases in GFAP mRNA are restricted to zones containing degeneration debris. The increases in GFAP mRNA in zones not containing degeneration debris could occur in response to changes in neuronal activity, due either to the burst of afferent activity which accompanies the lesioning procedure, or to the reduction of activity which follows the lesion. Using 35S-labelled riboprobes for GFAP mRNA for dot blot hybridization we measured GFAP message in each hippocampus 24 hours after manipulations designed to increase or decrease activity in the perforant path. 25 Hz stimulation of the angular bundle (AB) increased GFAP message to 349% of control values in the ipsilateral hippocampus and 171% in the contralateral. This compares to 440% ipsilaterally and 144% contralaterally following electrolytic lesions. 0.1 Hz stimulation led to modest increases (208% ipsilaterally and 120% contralaterally). A knife lesion of the AB, which we assume to produce a smaller burst of afferent activity than an electrolytic lesion, led to large increases ipsilaterally (366%), but no change contralaterally (107%). Injections of tetrodotoxin into the EC led to small decreases in GFAP mRNA. We conclude that bursts of afferent activity can trigger transcription of the GFAP gene, and that the stimulation which accompanied electrolytic destruction of the EC contributed to increasing the mRNA. Supported by NSF BNS8818766 to OS. RT received postdoctoral training grant NS07199.

151.11

CHARACTERIZATION OF PLASMINOGEN ACTIVATOR AND PLASMINOGEN ACTIVATOR INHIBITOR RELEASE FROM SCHWANN CELLS TRANSFECTED WITH THE SV-40 LARGE T ANTIGEN GENE. T.K. White*, R.S. Compton* and M.B. Clark, Univ. of Maryland, Baltimore.

We have assessed plasminogen activator (PA) and plasminogen activator inhibitor (PAI) release and regulation in a permanent Schwann cell (SC) line prepared by transfecting primary SCs with the SV-40 large T antigen gene (Tennekoon et al., 1987, J.C.B. 105:2315). Using SDS PAGE and zymography, we have demonstrated that the transfected cell line (tSCs) and primary SCs show identical profiles for PAI activity and very similar PA profiles. Using anti-tPA IgGs, we have demonstrated that the tSCs, like primary SCs, release tissue type PA. Furthermore, Northern analysis indicates that both cell types contain identical levels of tPA mRNA. The changes that occur in the tPA activity profile in tSC cells following denervation of SC + NC cell cultures are similar to those previously reported for primary SCs under similar conditions. Most notably, we observed the appearance of a 92 kDa species with 1 day post-denervation, as well as the gradual disappearance of a 25 kDa species. Thus, transfected SCs and primary SCs exhibit virtually identical PA/PAI responses to denervation, and the tSC line will be an appropriate model for future studies in which we will further characterize neuronal regulation of SC PA and PAI at the protein and mRNA levels. (Supported by PVA-SCRF grant NBR 645 and NIH NS24252 to MBC).

151.12

DIFFERENTIAL CONTROL OF LAMININ B1 AND B2 CHAIN GENE TRANSCRIPTION IN PRIMARY ASTROCYTES. V. P. Kedar, H. Haleem-Smith*, J. R. Wujek and Y. Yamada* Lab. of Molecular Biology, NINDS, NIH, and Lab. of Dev. Biol. and Anomalies, NIDR, NIH., Bethesda, MD 20892.

Laminin, a large extracellular matrix protein, consists of three individual subunits: A chain, B1 chain and B2 chain. The whole molecule stimulates extensive neurite outgrowth from many neuronal cell types *in vitro*. Embryonic glial cells transiently express laminin during the development of the midbrain and optic nerve in rodents (Letourneau et al., Dev. Biol., 1988, 125: 135; McLoon et al., J. Neurosci., 1988, 8: 1981). Astrocytes, which stimulate neurite outgrowth *in vitro*, synthesize and secrete only the B2 chain of laminin (Wujek et al., Dev. Brain Res., 1990, in press). We have investigated the molecular mechanisms that regulate expression of laminin B1 and B2 chain gene transcription in primary astrocytes and in a hepatoma cell line (HepG2). We made plasmid constructs containing the 5' flanking region of both the B1 (-1900 to +1) and B2 (-830 to +106) laminin chain genes fused to a structural part of the chloramphenicol acetyltransferase (CAT) gene. We examined the promoter activity by transfecting the constructs into primary rat astrocytes and HepG2 hepatoma cells. Both the astrocytes and the HepG2 cells exhibited significant CAT activity when transfected with the B2 construct. In contrast, the astrocytes did not exhibit any CAT activity after transfection with the B1 construct whereas the HepG2 cells exhibited significant activity. These results suggest that astrocytes regulate B1 and B2 laminin chain gene expression by differential recognition of gene promoters.

151.13

TRANSCRIPTIONAL REGULATION OF NERVE GROWTH FACTOR INDUCED GENE A (NGFI-A) IN SCHWANNOMA CELLS. C. Matheny and J. Milbrandt. Department of Pathology, Washington University School of Medicine, St. Louis, MO 63108.

NGFI-A, an early response gene, encodes a zinc-finger protein that was identified by virtue of its induction by NGF in PC12 cells. In contrast, in JS1 cells, a rat Schwannoma cell line that expresses low affinity NGF receptors, NGFI-A is expressed constitutively. To determine the enhancer elements important for transcription in Schwann cells, a series of 5'nested deletions of the 5' flanking region of NGFI-A (ranging from -532 to -52 nucleotides(nts)) were cloned in front of a promoter-less CAT (chloroamphenicol acetyl transferase) gene and transfected into JS1 cells. Experiments with these deletion constructs indicated that an enhancer element must be contained within the 194 nucleotides between -532 and -339. Interestingly, the NGFI-A gene is rapidly induced, more than twenty fold, in both JS1 Schwannoma cells and primary Schwann cell cultures treated with cycloheximide (CHX). This suggests that NGFI-A is under stringent transcriptional control by repressor proteins. Nuclear run-off experiments indicated that the effect of CHX occurs at the transcriptional level. Currently stable transfectants of JS1 cells containing the NGFI-A/CAT deletion constructs are being analyzed for CAT gene expression to localize the sequence elements involved in the CHX induction (or derepression) of NGFI-A.

STAINING, TRACING AND IMAGING TECHNIQUES III

152.1

NOVEL MODIFICATIONS OF ACETYLCHOLINESTERASE (AChE) PHARMACOHISTOCHEMISTRY. C. Mathes, P. L. Di Patre and L. L. Butcher. Lab. of Chemical Neuroanatomy, Dept. of Psychology, UCLA, Los Angeles, CA 90024-1563, U.S.A.

Available methods for AChE histochemistry do not allow visualization of both somata and processes in striatum (Str), forebrain nuclei (FN) and their terminal fields in the same brain. To obtain good staining of AChE-positive neuronal bodies, pretreatment with diisopropylfluorophosphate (DFP) is commonly used. In order to demonstrate neuronal somata and fibers, we combined DFP-pretreatment with a new and highly sensitive technique (Tago et al., *J. Histochem. Cytochem.*, **34**: 1431, 1986), which affords clear visualization of axons in the rat brain. Sprague-Dawley rats were treated with DFP, 1.8 mg/kg i.m. and sacrificed 6, 12, 24 or 48 h after treatment. Brain sections were incubated in iso-OMPA 30 uM for 30 min, then for 15 min in diluted Karnovski-Roots (K-R) medium prepared according to Butcher et al. (*Naunyn-Schmied. Arch. Pharmacol.*, **285**: 31; 1974); the stain was enhanced with diaminobenzidine-nickel ammonium sulfate (DAB-NAS) (Tago et al., 1986). Intense staining of somata in Str and cholinergic FN was obtained with K-R medium diluted 1:1, enhanced with DAB-NAS. By diluting K-R medium 1:10 and increasing the concentration of DAB-NAS by two, it was possible to see fibers in the cortex, hippocampus and fimbria. Visualization of AChE-reactive fibers was previously unattainable after DFP-treatment; with this method it becomes feasible to use the same brain for AChE-histochemical analyses of somata and fibers on adjacent sections. [Support: NS 10928].

152.2

FLUORESCENT MITOCHONDRIAL STAINS AS VITAL PROBES OF METABOLIC ACTIVITY IN THE RAT HIPPOCAMPAL SLICE PREPARATION. S. R. Quartz[2], R. J. Adams and T. J. Sejnowski[2], Salk Institute, La Jolla, CA 92037 and [2]UC, San Diego, La Jolla, CA 92039.

Distribution of the cationic mitochondrial fluorescent dyes rhodamine 123 and 2-(4-dimethylaminostyryl)-N-methylpyridinium iodide is governed primarily by the Nernst equation. Since mitochondrial membrane potentials have been reported to be -150mV *in situ* (Farkas et al., Biophys. J. 56, 1989), these dyes serve as vital stains of mitochondria. We have utilized these properties of the dyes as probes of metabolic activity in conjunction with confocal microscopy. In agreement with the histochemical localization of cytochrome oxidase (CO) (Kageyama & Wong-Riley, Neuroscience 7, 1982), we observe the following: 1) low to moderate staining in principal cell layers; 2) low staining of proximal apical dendrites of CA1 pyramidal cells; 3) intense staining of outer molecular layer, dentate gyrus; 4) intense staining of stratum oriens, CA3; 5) low average staining of stratum lucidum, CA3, under low magnification. However, under higher magnification we observe intensely stained mossy fibers and their synaptic extensions, suggesting that average intensity under low magnification may not be an accurate indication of activity. In contrast to CO localization, we have not found general intense staining in stratum moleculare (SM) of CA1, although, in agreement with those studies, we have observed highly reactive dendritic processes, presumably of interneurons, extending throughout the region. The lack of general staining in SM may be due to the finding that synaptic mitochondria are less active than nonsynaptic mitochondria (Leong et al., J. Neurochem. 42, 1984).

Recent studies report a 50% higher ATP content in cultured astrocytes than in cortical neurons (Hertz et al., Neurochem. Res. 13, 1988). In agreement with this finding, we observe intense staining in both protoplasmic and fibrous astrocytes. We have determined that these cells are astrocytes according to general morphological characteristics, distribution patterns, and by double-labelling with immunofluroescent markers for GFAP. These results suggests that these dyes may provide a simple *in vitro* assay for the study of astrocyte function.

152.3

CYTOCHROME OXIDASE HISTOCHEMISTRY LABELS DISPLACED GANGLION CELLS THAT PROJECT TO THE ACCESSORY OPTIC SYSTEM (AOS) IN THE CHICK. Debora L. Nickla*, Michael D. Gottlieb, Gonzalo Marin*, Ximena Rojas* & Josh Wallman. *Biology Department, City College, City University of New York, New York, NY 10031.*

In birds, cells of the isthmo-optic nucleus (and ectopic neurons) send axons to the retina. We have previously reported that cytochrome oxidase (COx) histochemistry labels these axons and three types of cells in the inner nuclear layer (INL) of the chick retina (ARVO, 1990). One type, an amacrine cell, is clearly contacted by centrifugal terminals. Another type is the classical large oval displaced ganglion cell (DGC) that is known to project to the nucleus of the basal optic root (nBOR) of the AOS. The third type (round) could be large amacrine cells, or alternatively, the smaller DGCs reported to project to other targets. We here report that both types of COx positive cells (oval and round) are indeed DGCs that project to the AOS.

Rhodamine beads were bilaterally injected into the nBOR of the AOS of 3 week old chicks. After 8 days, the retinas were fixed, sectioned and reacted for COx (Wong-Riley protocol).

We observe two types of double labeled cells in the inner INL that correspond to those described above : (**1**) large (30 um) and oval (**2**) small (approximately 15 um) and round. This supports the notion that two types of DGCs project to the AOS. Furthermore, COx staining of DGCs, as well as that of centrifugal axons and their amacrine cell targets, provides additional support for a functional connection between the centrifugal system and the AOS.

(Supported by EY 02727).

152.4

INCREASED GFAP EXPRESSION IN AGED RAT BRAIN REVEALED BY QUANTITATIVE IMMUNOHISTOCHEMISTRY. MN Gordon, DG Berg*, CM Flores* and DG Morgan. Andrus Gerontology Center, U Southern Cal, LA, CA 90089-0191.

Two methods of immunohistochemical staining were compared as quantitative techniques to assess antigen levels in vibratome tissue sections. The amplified method used the avidin-biotin complex technique, horseradish peroxidase (HRP) and diaminobenzidine (DAB) as the chromogen, while the nonamplified method used a secondary antibody directly coupled to HRP. Brain sections from young (5-6 mo) and old (24-28 mo) Wistar rats were stained for glial fibrillary acidic protein (GFAP) using a monoclonal primary antibody. DAB-reacted sections were quantified using a computer-assisted densitometry system (Technology Resources) and a CCD camera with gain and blacklevel settings fixed by the user. Using the amplified technique, no differences in the area of the section occupied by reaction product were observed in three brain regions as a function of age. Using the nonamplified technique, significant increases of 90%, 80% and 40% in the section area occupied by reaction product were obtained in cerebral cortex, striatum, and hippocampus, respectively; no differences in the average density value were observed. These findings are in complete accord with the age-related increases in GFAP detected by immunoassay and solution hybridization measurements of GFAP RNA, both regionally and in terms of the fold increase. Supported by NIA AG07892, the Greenwall Award from AFAR and an Est. Invest. Award from the Am Heart Assn to DGM.

152.5

HIGH RESOLUTION IMMUNOHISTOCHEMISTRY OF TYROSINE HYDROXYLASE AND GLIAL FIBRILLARY ACIDIC PROTEIN IN SEMITHIN PLASTIC SECTIONS. S. Chen and D.E. Hillman. Dept. Physiol. & Biophys., New York Univ. Med. Ctr., New York, NY 10016.

We have successfully demonstrated immuno-labeling of tyrosine hydroxylase (TH) and glial fibrillary acidic protein (GFAP) on one micron plastic sections revealing the fine structure of Bergmann glia and dopamine cells of the substantia nigra. Rats were perfused with 4% paraformaldehyde and 0.1% glutaraldehyde for 10 minutes. Five-hundred micron, vibratome sections were collected in 0.1M phosphate buffer, slowly dehydrated with ethanol and embedded in Durcupan. The curing temperatures were kept below 58 °C. One micron sections were mounted on glass slides and dried overnight at 55 °C. Etching with a 1:1 alcohol dilution of saturated ethoxide for 20-30 minutes exposed the tissue surface from the soft plastic sections. Following a thorough rinsing in absolute alcohol, the sections were rehydrated to a phosphate buffered saline. Immuno-histochemical staining was performed for either GFAP or TH using the Vector ABC kit. Labeling was intense highlighting of glial and neuronal projections on a relatively clear background. Because the sections are thin, different antibodies can be applied to adjacent serial sections for labeling the same cell (Stensaas et al., Soc. Neurosci. Abst. 1989). Supported by USPHS NS-13742 from NINCDS.

152.6

INTRACELLULAR SEGREGATION OF PARVALBUMIN. Andressen C.*, Plogmann D.*, Celio M.R., Institute of Histology and general Embryology, University of Fribourg, Pérolles, CH-1700 Fribourg and Institute of Anatomy, University of Kiel, Olshausenstr. 40, D-2300 Kiel.

The Ca^{2+} binding protein Parvalbumin (PV) occurs in a subpopulation of neurons in the brain (Celio M.R. (1990) Neuroscience 35/2:375-475). In general the whole cell is stained by PV-antibodies, and this phenomenon is at the origin of it's excellent reputation as a marker. Sometimes, however, PV is restricted in occurrence to certain domains of a given neuron. In this study, using a combination of techniques to examine two different regions of the brain, we uphold the concept of intracellular segregation. In the deep cerebellar nuclei (DCN) PV occurs only in the axons, whereas the cell bodies are devoid of immunoreactivity. Colchicine application increases the concentration of PV in the perikaryon to a detectable level. As a further proof that DCN-neurons synthetize PV we can localize in them PV- mRNA by in-situ hybridisation. In the hippocampus PV is normally seen only in interneurons. However, using strong fixatives (2.5 % glutaraldehyde) and semithin cryo-sections (0.5 um thick), PV is revealed in the apical dendrite of pyramidal cells and in-situ hybridisation shows the presence of PV- mRNA in their cell bodies.

In conclusion PV, particularly in long-axon neurons, seems to be concentrated in those domains in which important movements of Ca^{2+} take place.

152.7

IMMUNOCYTOCHEMICAL DIFFERENTIATION OF GLUCOSEPHOSPHATE ISOMERASE ALLOTYPES. H.J. Li*, M.Chen*, T.G.Goodwin* and P.K.Law. Depts. of Neurol. and Physiol./Biophys., Univ. of Tennessee, Memphis, TN 38163.

The genetic polymorphs of glucosephosphate isomerase (GPI) differentiated electrophoretically have been used as markers to identify cell genotypes in chimeric mice and myoblast transplantation. However, such approach does not allow histological localization of the isozymes. The histochemical method for GPI staining does not differentiate between the allotypes. Antisera against GPI-1CC and GPI-1BB raised in rabbit lack specificity in tissue section staining . We have developed a method to produce specific antibodies that could differentiate between the two GPI allotypes. We first purified the GPIs from mouse muscles with cellulose phosphate ion exchange chromatography. About 50 g of mouse skeletal muscle was homogenized in 250 ml of 10 mM Tris buffer, pH 7. After centrifugation, the supernatant was concentrated with Amicon ultrafilter (XM 50) to about 20 ml final volume. It was then loaded into a column (2.5 X 60 cm) of activated cellulose phosphate, and 7 mM F-6-P buffer solution was used as a specific GPI elution. The enzyme isolated with this procedure was found to be homogenous in agarose gel and in SDS polyacrylamide gel electrophoresis. About 100 µg of purified GPI-1CC emulsified with Freund's complete adjuvant was injected intraperitoneally to a C57BL/6J-gpi-1b/b female mouse. A boost with the same dose of the antigen in Freund's incomplete adjuvant was given after three weeks. Another boost with the same dose of the antigen without adjuvant was given two weeks thereafter. Two weeks after the last injection, the samples of immunized mouse sera were tested with ELISA. Some of them showed high titer and specificity against GPI-1CC but not against GPI-1BB. These sera had been used successfully for mouse muscle section staining. The goat anti-mouse immunoglobulins peroxidase conjugated was used as second antibody in the section staining. We found that muscle sections from C57BL/6J-gpi-1b/b mouse were stained negatively, but muscle sections from C57BL/6J-gpi-1c/c mouse were stained positively. There were a lot of negative staining central nuclei in muscle sections of the C57BL/6J-gpi-1b/b dystrophic mice. (Supported by USPHS NS 20251 and NS 26185)

152.8

A SIMPLE TECHNIQUE FOR THE LOCALIZATION OF NEURONAL SURFACE ANTIGENS IN FORMALIN-FIXED HUMAN BRAIN D.T. Stephenson and P.D. Kushner, ALS Research Center, Pacific Presbyterian Medical Center, San Francisco, CA 94115.

Immunocytochemical analyses of human CNS tissue are limited by processing techniques considered necessary to optimize both tissue preservation and antigenicity. We have devised a simple technique that employs routine, formalin-fixed postmortem tissue. Blocks (1 x 1 x 1/2 cm^3) of formalin-fixed brains are first extensively washed in cold, buffered saline (up to 6 hours) and equilibrated through cold, graded sucrose solutions: 12% aqueous sucrose, 1 day; 15%, 1 day; 18%, 1-3 days. Blocks are then mounted, flash-frozen (in isopentane cooled with liquid nitrogen), cryosectioned, and immunostained with standard techniques. Gradual alcohol dehydrations (15 to 100% in 10% increments) are essential for the detection of surface antigens in plastic-mounted sections. The monoclonal antibody (MAb) *Tor* 23, which recognizes an epitope present at the surface of rare mammalian CNS neurons, was primarily utilized in the development of this technique, although all other MAbs tested thus far appear to work equally well. Significantly, *Tor* 23 staining is similar in all tissues, stored 3 days, 6 months or 3 years in formalin, although tissue frankly oxidized is not suitable for any marker. This protocol expands tremendously the number of cases available for any immunocytological investigation, a fact important in the study of neurological disorders, where tissues are frequently hard to come by. Supported by the State of California.

152.9

IMMUNOCYTOCHEMICAL DETECTION OF MONOAMINE OXIDASE A AND B IN CLONAL CELL LINES OF NEURONAL ORIGIN. M-C. Holst, M.S. Sutphin* and T.D. Buckman*. Nutritional Sciences, UCLA School of Public Health, Los Angeles, CA 90024.

Both forms of the enzyme monoamine oxidase (MAO A and B) appear to play a central role in the metabolism of monoamine neurotransmitters in the mammalian CNS. In primary brain cultures and clonal cell lines of neuronal origin only the A form, which appears first in development, is generally expressed. An exception to this is the report by Nagatsu et al. in 1981 (Neurochem. Internat. 3:137) of appreciable levels of activity identified as B type MAO on the basis of the effects on enzyme activity of the specific MAO A and B inhibitors, clorgyline and deprenyl in two neuroblastoma cell lines (NCB20, mouse neuroblastoma x fetal hamster brain hybrid, 80% MAO B, 20% MAO A; NCB140-3, x glioma, 75% MAO A, 25% MAO B). We have prepared polyclonal antibodies to MAO A and B which show a high degree of specificity on the basis of Western Blot analysis. Immunocytochemistry with these confirms the presence of both MAO A and B in these two neuroblastoma hybrids where staining was found both in the cell soma and neuritic processes. The PC12 pheochromacytoma cell line which, on the basis of biochemical evidence appears to have only MAO A, does not give any staining with our antibody to MAO B. Supported by NIH grant NS-25797.

152.10

DISTRIBUTION OF FOS IMMUNOREACTIVITY FOLLOWING STIMULATION OF THE PERFORANT PATH IN RATS. C. L. Mitchell, L. M. Grimes[1] and M. I. Barnes*. LMIN, NIEHS/NIH, Research Triangle Park, NC 27709 and [1]Curriculum in Toxicology, Univ. of North Carolina, Chapel Hill, NC 27514.

Depending on the stimulation parameters, unilateral stimulation of the perforant path (PPS) elicits wet dog shakes (WDS) only or WDS followed by behavioral seizures. We compared the distribution of fos immunoreactivity in rats exhibiting only WDS vs. those exhibiting profound behavioral seizures. Fischer-344 rats were anesthetized with pentobarbital 1 or 2 hrs following the initiation of stimulation, perfused with 4% paraformaldehyde and 0.2% glutaraldehyde for subsequent analysis of reactivity to antibody to fos protein. Areas stained in both the WDS and S groups were: dentate granule cells, CA1 and CA3 pyramidal cells, subiculum, amygdalohippocampal area, and layers II and VI of the cerebral cortex. Areas stained in the S group but not the WDS group were: all layers of the cingulate cortex, layer IV of the cerebral cortex, zona incerta, central and cortical nuclei of the amygdala, piriform cortex, subthalamic nucleus, caudate-putamen, medial geniculate nucleus of the thalamus and ventromedial nucleus of the hypothalamus. We conclude that analysis of fos immunoreactivity following PPS can aid in determining pathways activated by such stimulation.

152.11

rab3Ap DISTRIBUTION IN RAT BRAIN. B. Tavitian, K.L. Moya, A. Zahraoui* and A. Tavitian*. INSERM U334, S.H.F.J., C.E.A., 91406 Orsay, and INSERM U248, 75010 Paris, France.

Some members of the *ras* superfamily are associated with secretory vesicles, and some have been shown to mimic the effects of neuronotrophic factors. rab3Ap is a *ras*-like GTP binding protein found predominantly in the nervous system. Using a monospecific immune serum we examined the distribution of rab3Ap in the adult rat brain.

Cell bodies and major fiber bundles such as the corpus callosum, anterior commissure and optic tract were devoid of staining, while certain areas of neuropil were darkly stained such as the striatum, lateral geniculate, superior colliculus and regions of the cortex. The hippocampus exhibited a striking pattern with dark staining in the stratum oriens, stratum radiatum and the dentate gyrus molecular layer. The pyramidal cell layer was not stained in CA1 and CA3, while in CA2, this layer contained dense immunoreactivity surrounding blank profiles of cell bodies.

This pattern is consistent with a synaptic localization of rab3Ap and suggests that the protein may serve a specialized function in particular pathways.

152.12

ON THE USE OF C-FOS AS A CELLULAR ACTIVITY MARKER IN THE STUDY OF VISUAL PATHWAYS.

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C-fos, a proto-oncogene protein, is rapidly and transiently induced in response to a variety of stimuli. To test whether c-fos can be used as a high resolution activity marker for visual pathways tracing, c-fos immunoreactivity has been studied in rat visual cortex after unilateral pharmacological stimulation (intravitreal injection of picrotoxin), electrical stimulation of the LGN, and physiological (optokinetic) stimulation.

Picrotoxin induced moderate number of c-fos immunoreactive cells in layers II-III-IV and VI of the hemisphere contralateral to the stimulated eye and only a few in layer VI in the ipsilateral cortex. Electrical stimulation of the LGN induced c-fos in a large number of cells located in all layers except layer V of the ipsilateral visual cortex while very few immunoreactive cells were found in the contralateral hemisphere, located mostly in layers II and VI. Optokinetic stimulation did not increase c-fos immunoreactivity in the visual cortex but it induced c-fos immunoreactivity in several midbrain structures (superior colliculus, accessory optic system, and nucleus of the optic tract). We conclude that c-fos can be used as a high resolution activity marker in polysynaptic tracing of visual pathways.

152.13

ESTABLISHMENT OF OPTIMAL PARAMETERS FOR IN SITU HYBRIDIZATION WITH ALKALINE PHOSPHATASE CONJUGATED OLIGONUCLEOTIDE PROBES. J. Harper, Slippery Rock Univ., PA, and F. J. Denaro, TTUHSC. Alkaline Phosphatase conjugated probes have recently been used to identify mRNA of neurotransmitters and enzymes (Denaro, JF, The Histo.Chem. Soc., Orlando, FL, 1989). In the present study a series of hybridization experiments were conducted to establish the optimal parameters for maximum signal to noise. The major points to be investigated and which will be discussed are: 1. Tissue fixation, 2. Hybrization temp. & time, 3. Probe concentration, 4. Wash buffer, and 5. Digestion of unhybridized probe. These experiments reveal that excellent results can be obtained by using any one of a number of protocols. This makes the technique of in situ hybridization with Alk Phos conjugated probes adaptable to a number of experimental situations. For example, one may combine it with immunocytochemistry and one can choose between frozen or paraffin sections. The combination of a radio labeled probe with a colorimetric one is also possible. While standardization is desired to make such techniques readily available to the laboratory, one should not lose sight of the varied research application inherent in the different approaches.

152.14

CYTOARCHITECTURAL RELATIONSHIPS BETWEEN OUABAIN BINDING AND mRNA FOR ISOFORMS OF THE SODIUM PUMP CATLYTIC SUBUNIT IN RAT BRAIN

Michael L. Brines, Barbara I. Gulanski, Maureen Gilmore-Hebert*, Adam L. Greene*, Edward J. Benz, Jr.*, and Richard J. Robbins. Neuroendocrine Program, Yale University School of Medicine, 333 Cedar Street, New Haven, CT 06510

We compared the cell-type expression of the α-subunits of the sodium pump in rat brain using in situ hybridization and ^{3}H-ouabain autoradiography. These techniques allowed us to compare adjacent sections for localization of mRNA and active α2/α3 pumps. The perikarya of many neurons possessed high levels of α1 and α3 transcripts, compared to glial-enriched areas which appeared virtually unlabelled. In contrast, α2 probe was observed to hybridize strongly only over large pyramidal neurons and meningeal cells, and was diffuse elsewhere. A complex and varied regional pattern of α1 and α3 transcripts was noted. Large neurons of the olfactory bulb and piriform cortex possessed high levels of α3 transcripts, but low levels of α1 mRNA. In frontal cortex, pyramidal neurons of layers 2-3 were enriched in α1 mRNA while those in layer 5 exhibited high levels of α3 transcripts. In the hippocampus, all three mRNAs were present in the principal neurons. Dentate granule cells had a high α1/α3 mRNA ratio, which was reversed for CA1 pyramidal neurons. In the cerebellum, Purkinje and Golgi cells contained high levels of α3 message, while the granule cells appeared to express only α1 mRNA. Ouabain binding correlated most closely with the regional distribution of α3. It was highest in the olfactory cortex, hippocampus, and cerebral cortex, and lowest over perikarya, white matter and choroid plexus. In the cerebellum, ouabain bound at highest density in the granule cell layer, the inner third of the molecular layer in the basket region, and the deep cerebellar nuclei, but was low in the outer 2/3 of the molecular layer. Thus, mature α3 sodium pump appears localized to the axons of Purkinje, Golgi and basket cells, and not to their apical dendrites. These results suggest that in some cells translated α3 protein is predominantly inserted into axon terminals. Many neurons observed with dense α3 message are characterized by long axons. The α3 isoform may be specialized for vectorial transport along these axons into distant synaptic membrane.

152.15

QUANTITATIVE, NON-RADIOACTIVE DETECTION OF PREPROENKEPHALIN mRNA WITH DIGOXIGENIN-UTP LABELED cRNA PROBES. M.E. Lewis, E. Robbins, S.L. Meyer and D.S. Grega[1]. Cephalon, Inc., West Chester, PA 19380 and [1]Boehringer Mannheim Corp., Indianapolis, IN 46250.

Non-radioactive detection of mRNA with *in situ* hybridization histochemistry has emerged as an important new technology for the study of gene expression in brain and other tissues. Quantitative *in situ* hybridization studies have generally relied upon counting of autoradiographic grains in the emulsion overlying cells containing hybridized, radioactively-labeled probe. However, such studies have been plagued by the nonlinear relationship between grain density and radioactivity, and, in addition, weeks of exposure to the emulsion are necessary. To begin to explore the quantitative potential of non-radioactive *in situ* hybridization histochemistry, we have carried out studies using a digoxigenin-labeled cRNA probe prepared using the linearized plasmid pYSEA1, which terminates at the end of a 935 bp preproenkephalin cDNA insert. Detection of probe hybridized to striatal tissue sections was carried out enzymatically following complex formation with an antidigoxigenin-alkaline phosphatase conjugate. Striatal neurons were intensely labeled (mean OD = 0.308 ± 0.006; S/N ratio =7:1), while neocortical neurons exhibited less signal (mean OD = 0.179 ± 0.003; S/N ratio = 4:1), as expected. We are presently exploring the use of the digoxigenin-labeled probe for solution hybridization assays for the quantitation of preproenkephalin mRNA.

We gratefully acknowledge Drs. S. Sabol and K. Yoshikawa as the source of the pYSEA1 plasmid, and Dr. F. Baldino, Jr. for comments.

NEUROGLIA AND MYELIN I

153.1

ASTROCYTES - A HETEROGENEOUS FAMILY

Lars Rönnbäck and Elisabeth Hansson, Dep. of Neurology and Inst. of Neurobiology, Univ. of Göteborg, Göteborg, Sweden.

Data are accumulating that astrocytes are a heterogeneous class of cells both in terms of morphology, surface properties and in their distribution within the central nervous system. Using primary astroglial cell cultures from various brain regions it has been shown that these cells are heterogeneous with differences in surface receptors. The number of β-adrenoceptors per astrocyte and/or their sensitivity vary from one brain region to another. High-affinity uptake of amino acids also vary in different brain regions. The uptake of glutamate was found to be highest in striatum compared to cortex, brain stem and cerebellum. The GABA uptake showed the greatest intensity in brain stem astrocytes compared to cortical astrocytes. Taking such a heterogeneity into account, especially with respect to the expression of membrane receptors astroglia may be able to respond selectively to changes in their humoral environment which may provide new therapeutic approaches.

153.2

ISOLATION OF NEURONS, ASTROCYTES AND OLIGODENDROCYTES FROM BRAIN TO DETERMINE CELL SPECIFICITY OF GAP JUNCTION GENE EXPRESSION. D.J. Belliveau, J.F. Bechberger* and C.C.G. Naus. Dept. of Anatomy, University of Western Ontario, London, Canada, N6A 5C1.

The expression of gap junction genes for connexin32 and connexin43 have been described in the mammalian central nervous system. Cell specificity of gap junction expression has been demonstrated to some extent by the use of antibodies (Dermietzel et al. PNAS 86: 10148-52, 1989). Using previously described cell isolation techniques, we have separated neurons, astrocytes, and oligodendrocytes from adult Spraque Dawley rats (Farooq and Norton, J. Neurochem. 31: 887-95, 1978; Snyder et al., J. Neurochem. 34: 1614-21, 1980). Total RNA extracted from the cell isolation was analyzed using Northern blot analysis and cDNA probes specific for connexin32 and connexin43. Neurons and astrocytes expressed connexin32 and connexin43 mRNA respectively. Connexin32 was the major gap junction mRNA expressed in oligodendrocytes but a low level of connexin43 mRNA was detected and this was thought to be from an endothelial cell contaminant. RNA extracted from cultured endothelial cells confirmed the presence of connexin43 in these cells. The presence of connexin32 in neurons and oligodendrocytes and connexin43 in astrocytes was supported by the use of cell cultures. Similar cell isolation experiments during early postnatal development are underway to examine the developmental expression of gap junction genes in these various cell types.

153.3

DISTRIBUTION OF mRNA'S CODING FOR LIVER AND HEART GAP JUNCTION PROTEINS IN THE RAT CENTRAL NERVOUS SYSTEM.

P.E Micevych and L.A. Abelson. Department of Anatomy and Cell Biology and the Laboratory of Neuroendocrinology, Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024-1763.

The present study examined the distributions of connexin43 mRNA and connexin32 mRNA in the central nervous system (CNS) of the rat using in situ hybridization histochemistry. Connexin32 forms direct cell-cell channels in the liver, and connexin43 does in the heart. Generally, the distribution of cells labelled with connexin32 mRNA did not overlap with the population labelled by connexin43 mRNA, thus implying a regional or cellular specificity in the expression of connexins in the CNS. Cells containing connexin43 mRNA were uniformly distributed throughout the gray matter of the neuraxis. Several areas had a higher concentration of cells that express connexin43, such as the supraoptic and paraventricular nuclei of the hypothalamus, anterior cortical amygdaloid nucleus, the reticular part of the substantia nigra, lateral habenula, mesencephalic trigeminal nucleus, Purkinje cell layer of the cerebellum, facial nucleus, prepositus hypoglossal nucleus, and dorsal cochlear nucleus. Connexin32 mRNA was detected in discrete cell groups of the gray matter, including cells in layer 2 of the neocortex, piriform cortex, pyramidal and granule cell layers of the hippocampus, granule cell layer of the dentate gyrus, islands of Calleja, olfactory tubercle, lateral thalamic nuclei, lateral habenula and Purkinje cells of the cerebellar cortex. A significant population of cells in white matter tracts which were labelled with the connexin32 riboprobe were determined to be oligodendrocytes. These results indicate that neurons and glial cells may express mRNA's that code for liver and heart connexins. Many of the areas in which connexin mRNA's were demonstrated have electrically coupled cells, morphologically distinct gap junction plaques, and/or have immunologically identifiable connexin proteins. These results indicate that cell-cell coupling may be a widespread phenomenon in the mammalian CNS. Supported by NS 21220.

153.4

PRIMARY CULTURES OF GUINEA PIG ASTROCYTES: HIGH YIELD FOLLOWING DISPASE DISSOCIATION.

P.THAKRAN, M.P.LEUSCHEN, A.CHATTERJEE, R.M. NELSON Jr. Joint Division of Newborn Medicine, University of Nebraska Medical Center, Omaha, NE 68198

Primary monolayer cultures of neonatal guinea pig astrocytes were analyzed for yield and purity using a neutral protease Dispase (Collaborative Res. Inc, Bedford, MA.) dissociation and 3 different substrates. The method involved dissociation of cortices from 1-3 neonatal (0-48 hr) guinea pigs per experiment employing 5-6 extractions with Dispase(Frangakis,M.V.,Neurochem.Res.9:1689, 1984) during a maximum total dissociation time of 120 min. The cell suspension was passed through a 105 μ nylon mesh to remove meningeal remnants. Average cell yield was 15 to 20 X 10^7 cells/brain with initial viability of 97.7% cells excluding trypan blue. The cells were cultured in Dulbecco's Modified Eagle's Medium containing D-Glucose 4.5 g/L, sodium pyruvate 110 mg/L, Insulin 100 U/L, 100 X Vitamins 5ml/L (Gibco), penicillin 100,000 U/L,streptomycin 100 mg/L, amphotericin B 250 ug/l, supplemented with 20% fetal calf serum (FCS) for the first 48 hrs and 10% FCS subsequently. Three substrates were tested, collagen, poly-L-lysine and Matrigel™ (Collaborative Res, Inc). No significant difference in astrocyte yield was seen between collagen and poly-L-lysine (with an initial seeding density of 2.8 X 10^5 cells/cm^2 in 24-well plates, a saturation density in the third week of 3.7 X 10^5 cells/cm^2 and 2.9 X 10^5 cells/cm^2 was obtained on collagen and poly-L-lysine respectively). Contamination with oligodendrocytes was high on Matrigel™. Astrocytic purity was characterized by morphological response to 1 mM dbCAMP and immunohistochemistry (Vectastain ABC system, Vector Labs) with the astroglial marker,glial fibrillary acidic protein. This highly reproducible method produces high cell yield and viability for the primary culture of guinea pig astrocytes.

153.5

THE EFFECTS OF AXOLEMMA-ENRICHED FRACTIONS ON Po GENE EXPRESSION IN PRIMARY AND TRANSFORMED SCHWANN CELL CULTURES. R.M. Knight, B.L. Attema*, and G.H. De Vries. Dept. of Biochem. and Mol. Biophys., Med. Coll. of Va. Richmond VA 23298.

Primary Schwann cells (PSC) and transformed Schwann cells (TSC) were treated with axolemma-enriched fraction (AEF) and changes in Po gene expression were determined by changes in mRNA levels. AEF (40 ug/ml) caused a two fold increase in Po mRNA levels in the PSC and TSC cultures. Alkaline phosphatase treatment of AEF had no effect on the increase in Po mRNA induced by AEF, while trypsin treatment of AEF abolished the AEF induction of TSC Po mRNA. A myelin-enriched fraction did not increase Po mRNA levels in TSC. AEF isolated from non-myelinated splenic nerve was as potent as AEF isolated from myelinated axons in increasing Po mRNA in PSC and TSC. Inhibition of transcription via α-amanitin caused a 36 fold decrease in Po mRNA levels, while PSC treated simultaneously with α-amanitin and AEF had Po mRNA levels which were increased 11 fold over cultures treated with α-aminitin alone. This data indicates that the signal for the induction of Po mRNA is not restricted to myelinated axons and that AEF induced increases in Po mRNA may be due to increased Po mRNA stability. (Supported by NIH NS10821 and NIH NS15408).

153.6

5-BROMODEOXYURIDINE REDUCES THE EXPRESSION OF Po IN SCHWANN CELLS. S.S. Scherer*, L.G. Wrabetz, and J. Kamholz. Dept. Neurol., Univ. Penn. Sch. Med., Phila., PA 19104

The thymidine analogue 5-bromodeoxyuridine (BUdR) is incorporated into DNA and reversibly delays cellular differentiation in a variety of cell types. We added 0.5 to 50 μM BUdR to secondary Schwann cells (SC) that had been previously expanded with glial growth factor and forskolin until they were able to proliferate without these mitogens. After one week in BUdR, almost all of the SC nuclei could be stained with a monoclonal antibody against BUdR, and this staining was markedly diminished by the addition of a 5-fold molar excess of thymidine to the medium.

Untreated SC expressed nerve growth factor receptor (NGFR), as shown by staining with a monoclonal antibody, and contained the mRNA for both NGFR and the major myelin protein, Po, as shown by Northern blotting. After one week in BUdR, SC no longer expressed NGFR or Po, unless thymidine had been present in the medium. Futhermore, no Po mRNA was detected in BUdR-treated SC even after the addition of 4 μM forskolin, in marked contrast to the robust increase in untreated SC.

To determine whether BUdR has a cis- or a trans-acting effect on Po gene expression, SC were transiently transfected with a construct containing the Po promoter coupled to the chloramphenicol acetyl transferase (CAT) gene that had been previously shown to promote Po expression in a cell-specific manner (Lemke et al., Neuron 1: 73, 1988, construct pPCAT HA16). The BUdR-treated SC had as much CAT activity as the untreated SC, implying that BUdR had a cis-acting effect, probably as a consequence of its substitution for thymidine in crucial portions of the Po regulatory region. (SSS and LGW are supported by the C.A. Dana Foundation Program in Neuroscience).

153.7

AN INTERMEDIATE FILAMENT-ASSOCIATED PROTEIN, IFAP-300kD, AS A MARKER FOR HUMAN ASTROCYTOMAS. G.D. Pappas, R.P. Glick*+, D. Shao*, D. Johnson-Seaton*, and H.-Y. Yang. Depts. of Anatomy and Cell Biology, and Neurosurgery+, Univ. of Illinois at Chicago, IL 60612.

Intermediate filament (IF) proteins represent a group of cytoskeletal cell markers that can be used for immunotyping of human astrocytomas. For example, glial fibrillary acidic protein (GFAP), an IF structural protein specifically expressed in astrocytes, has been widely used as a marker for astrocytic tumors. In addition, vimentin, another IF structural protein that has been found in immature astrocytes and in some mature astrocytes, has been shown to be present in astrocytomas. However, the diagnostic usefulness of these two proteins is limited since they are normal IF constituents both in mature and reactive astrocytes. A 300kD intermediate filament-associated protein (IFAP) has been identified in baby hamster kidney (BHK) cells. (Yang, et al., J Cell Biol., 100:620, 1985) This IFAP is vimentin-associated, and has been found in many cell lines, including the C6 rat glioma cells. In the present study, the immunoreactivity of IFAP-300kD has been revealed by immunofluorescence in all human astrocytomas examined (including well-differentiated astrocytoma, anaplastic astrocytoma and glioblastoma multiforme) as well as in all primary cultures of some of these tumors, but not in normal human brain tissue. The ontogeny of IFAP-300kD has also been studied in the CNS of rats. The results show that this IFAP normally is only expressed in radial glia. It was not detected in $GFAP^+$ astrocytes in adult rat brain, with or without vimentin. Also it was not detected in reactive astrocytes induced by stab wound, nor in neonatal rat astrocytes, either in situ or in primary culture, although all these astrocytes are vimentin/GFAP-containing. These results suggest that IFAP-300kD can be a marker for human astrocytomas. It is also important to note that, since this IFAP is not present in reactive astrocytes, it may be used clinically to define the boundary of the human astrocytomas. (Supported by NIH grant NS26395)

153.8

GLUTAMATE-INDUCED CHANGES IN INTRACELLULAR Ca ARE OSCILLATORY IN TYPE-I BUT NON-OSCILLATORY IN TYPE-II CORTICAL ASTROCYTES. A. Jensen* and S.Y.Chiu. Neuroscience Training Program & Dept. of Neurophysiology, University of Wisconsin, Madison, WI 53706.

A mixture of type-I and type-II cortical astrocytes was obtained from neonatal rat cortices following dissociation and plating for 1-4 months. Image analysis of glutamate-induced responses in intracellular Ca was performed on cells loaded with the Ca indicator fluo-3. Following each experiment, the same cells were double labeled with antibodies for correlating Ca repsonse with astrocyte phenotype (type-I is GFAP+ and A2B5- , type-II is GFAP+ and A2B5+). In response to bath application of 30, 100 and 500 μm glutamate, all type-I cells which responded (75-85%) did so in an oscillatory manner. In contrast, all type-II cells which responded (85-100%) exhibited a slow and maintained increase in [Ca]i; this kind of response was seen both in cell soma and the fine proceses. Interestingly, in type-II cells, the glutamate-induced increase in [Ca]i was markedly reduced in 13 out of 16 cells by 100 μm nifedipine, a blocker of L-type calcium channels. In Ca-free bath, the % of cells responding to glutamate is reduced to 76% and 9% for type-I and -II respectively, relative to control with Ca present. Depolarization with 70 mM K+ increased [Ca]i in all type-II cells, but no increase was seen in type-I cells. These findings are consistent with the hypothesis that the [Ca]i increase observed in type-II cells is via voltage-gated calcium channels activated by glutamate-induced depolarization through ionotropic glutamate receptors and that the type-I response is mediated via the second messenger-linked metabotropic glutamate receptor.

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153.9

GENERATION AND RELEASE OF 1-METHYL-4-PHENYLPYRIDINIUM ION (MPP^+) BY PRIMARY CULTURES OF ASTROCYTES. E.Y. Wu*, B.M. Borgeson*, I. Irwin*, L.E. DeLanney, J.W. Langston, and D.A. Di Monte. California Parkinson's Foundation and California Institute for Medical Research, San Jose, CA 95128.

The neurotoxic effect of MPTP is due to its conversion to the fully oxidized pyridinium metabolite, MPP^+. This metabolic activation occurs via the MAO B-dependent generation of a dihydropyridinium intermediate, $MPDP^+$. Because MAO B is found in astrocytes, it has been proposed that MPP^+ is generated within these cells, released, and then taken up by dopaminergic neurons. To test this hypothesis, the mechanism of generation and release of MPP^+ was studied in primary cultures of glia cells. Addition of MPTP (250 μM) to these cultures resulted in the production of three metabolites, MPP^+, $MPDP^+$ and MPTP N-oxide. MPP^+ and $MPDP^+$ together accounted for approximately 90% of the overall biotransformation of MPTP after incubation for 24 hours. Although presumably generated within the cells, $MPDP^+$ was found predominately in the incubation medium. Interestingly, the concentration of $MPDP^+$ in the medium did not increase over time, being 4.2 μM and 4.7 μM at 8 and 24 hours, respectively. In contrast, the extracellular level of MPP^+ increased from 2.0 μM at 4 hours to 5.6 μM and 14.6 μM at 8 and 24 hours, respectively. No evidence of cytotoxicity was found in astrocyte cultures exposed to MPTP for 24 hours. These results are consistent with the conclusion that MPP^+ may be generated directly in the extracellular space via the autoxidation of $MPDP^+$.

153.10

PREFERENTIAL GLIAL UPTAKE OF RUBIDIUM IN LEECH CNS AS DETERMINED BY X-RAY MICROPROBE ANALYSIS. A. J. Saubermann, C. Castiglia*, and M. F Foster. Dept. of Anesthesiology, SUNY at Stony Brook, Stony Brook NY 11794-8480.

Glial cells are thought to play an important role in K homeostasis by clearing, and transiently holding, K from extracellular space (ECS) during neuronal activity and then returning K to adjacent neurons. We have used Rb as a K tracer to determine if glial Rb uptake occurs before adjacent neuron uptake. Determination of such a lag is critically important for confirming the K cycle hypothesis and for obtaining a means to study mechanisms of neuronal K repletion. Ganglia dissected from Macrobdella decora were perfused in either 4 or 20 mmol Rb substituted leech Ringer's solution (LRS) following an equilibration period of 20 min in normal LRS (4 mmol K). Ganglia were perfused for measured time periods ranging from 15 sec to 10 min, rapidly frozen in Freon 22, cryosectioned and subjected to x-ray microanalysis in the frozen hydrated and dried state. Rb, K, Na, and Cl mass fractions were determined (mmol/kg wet wt and dry wt). Following 30 sec of perfusion with 4 mmol Rb LRS, glial cells had exchanged 7% of their K for Rb and <1% in adjacent neurons. Whereas, glial cells in ganglia stimulated with increased ECS Rb (20 mmol LRS) for 32 sec exchanged 44% of their K for Rb, and neurons 13%. After 10 min of 4 mmol Rb LRS, 61% of glial K was exchanged with Rb and only 48% of neuronal K was exchanged. However, after 10 min of 20 mmol Rb LRS, 85% of the glial cells' K was exchanged for Rb compared to 88% of the neurons' K. The faster and, hence, preferential glial Rb uptake rate compared to adjacent neurons supports the K cycle hypothesis and offers a means to study mechanisms of K repletion. (supported by NIH NS21455)

153.11

RECURRENT SPREADING DEPRESSION INCREASES GFAP STAINING.

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Reactive gliosis (RG) that is seen after virtually every type of brain injury, may modulate the ultimate severity of associated tissue damage, and yet the molecular mechanisms of the process remain undefined. In other eukarytoic cells a rise in K^+_o or pH_i are proposed as signals capable of triggering proliferation and anabolism. Spreading depression (SD) is a stereotypic perturbation of brain with K^+_o and astrocytic pH_i changes which we have begun to use in order to define the ionic concomitants which may be necessary for RG.

SD was induced in halothane anesthetized rats (n=20) by application of KCl to parietal cortex for 3 hrs; equimolar NaCl was applied to the contralateral side. SD was confirmed by DC recording of SD made in frontal cortex. Animals were allowed to recover for 48 hrs and then their brains were processed for quantitative immunohistochemical analysis of GFAP staining because this is one, important defining characteristic of RG. SD (13-37 SDs; n=14) correlated with a significant ($p<0.01$; student's t test corrected with Bonferroni correction) increase in GFAP staining compared to controls (n=3). If SD was prevented (n=3), no significant increase in GFAP occurred.

These results show that SD can be used to study RG and that RG can be prevented. If this is also true for conditions associated with irreversible neuronal injury, it may be possible to prevent or mitigate any inhibition of regeneration or worsening of ultimate neural deficit dependent on RG.

153.12

IMMUNO-ULTRASTRUCTURAL LOCALIZATION OF SODIUM CHANNELS IN DEMYELINATED RAT SPINAL CORD

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Myelinated fibers exhibit spatial heterogeneity of the macromolecular organization of the axon membrane, with voltage-sensitive sodium channels present in much higher density within the axolemma at nodes of Ranvier than within internodal axon membrane. Demyelination of myelinated fibers leads to conduction block, which may result, in part, from the dispersion of sodium channels along the axon membrane from the nodal axolemma.

The dorsal columns of adult rat spinal cords were experimentally demyelinated by injection of ethidium bromide, and then irradiated to retard remyelination. The lesioned areas were processed for sodium channel localization utilizing antibody 7493 and HRP immunocytochemistry, which has been previously shown to densely immunostain nodal axon membrane.

Most regions of demyelinated axon membrane did not exhibit sodium channel immunoreactivity; however, occasional focal sites of sodium channel immunostaining were observed. Usually, these discrete regions were associated with glial processes. When the demyelinated axons became ensheathed by oligodendrocytes or Schwann cells, the axon membrane displayed moderately intense 7493 immunostaining. These data suggest that, following demyelination, there is a reorganization of sodium channels along the axon membrane.

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153.13

SODIUM CHANNEL EXPRESSION IN ASTROCYTES FROM RAT OPTIC NERVE IN VITRO

J.E. Minturn, J.A. Black, K.J. Angelides[1]* H. Sontheimer, B.R. Ransom, and S.G. Waxman. Dept. Neurology, Yale Univ. Sch. Med., New Haven, CT, 06510 and [1]Dept. Physiol. and Molec. Biophys., Baylor Coll. Med., Houston, TX 77030.

Astrocytes cultured from neonatal rat optic nerve can be classified into two subtypes, based on morphology and ability to bind antibody A2B5. The presence of sodium channels in astrocytes cultured from rat optic nerve was demonstrated by indirect immunofluorescence with polyclonal antibody 7493. Astrocytes cultured from postnatal day 7 (P7) optic nerves exhibited sodium channel immunostaining on both cell subtypes ($A2B5^+$ and $A2B5^-$) up to 6 days in vitro (DIV). In addition, both cell types showed Na^+ currents when examined by whole-cell voltage-clamp. Interestingly, the cell subtypes differed with respect to Na^+-current inactivation. After 6 DIV, the $A2B5^-/GFAP^+$ cells exhibited a loss of sodium channel immunostaining, while the $A2B5^+$ astrocytes continued to react to 7493. This staining pattern persisted up to 28 DIV (latest time point examined). Astrocytes cultured from P0 rat optic nerves, in which the vast majority are $A2B5^-/GFAP^+$, exhibited sodium channel staining in a pattern similar to the P7 astrocytes. After 6 DIV, the $A2B5^-$ astrocytes showed loss of 7493 immunoreactivity, while the rare (<1%) $A2B5^+$ astrocytes continued to express sodium channels reactive to 7493. The reduction of sodium channel immunoreactivity in $A2B5^-$ but not $A2B5^+$ astrocytes from both P0 and P7 optic nerves after a similar latency (~6 DIV) suggests that the loss of immunostaining may result from the absence of neuronal associations in the culture environment, rather than an intrinsic biologically-timed loss of sodium channel expression. An analysis of the time-course of Na^+-current expression and its correlation to immunostaining is in progress.

(Supported in part by grants from NIH, VA, and NMSS)

153.14

SODIUM CHANNELS IN THE CYTOPLASM OF SCHWANN CELLS

J.M. Ritchie[1], J.A. Black[2,3], S.G. Waxman[1,2,3] and K.J. Angelides[4]. Depts. of Pharmacology[1] and Neurology[2], Yale University School of Medicine, New Haven, CT 06510, PVA/EPVA Neuroscience Center[3], VAMC, West Haven, CT 06516, and Dept. of Physiology and Molecular Biophysics, Baylor College of Medicine, Houston, TX 77030.

It has become clear that Schwann cells in culture express voltage-gated sodium channels. However the physiological and pharmacological studies carried out to date provide little data about localization, within Schwann cells, of sodium channels. In the present study, immunoblotting, ultrastructural immunocytochemistry and saxitoxin (STX) binding experiments were used to study the distribution of sodium channels in Schwann cells.

Polyclonal antibody 7493, which is directed against purified sodium channels from rat brain, specifically recognizes a 260 kDa protein, corresponding to the alpha subunit of the sodium channel in immunoblots of crude glycoproteins from rat sciatic nerve. Electron microscopic localization of sodium channel immunoreactivity within adult rat sciatic nerves reveals heavy staining of the axon membrane at the node of Ranvier, while internodal axolemma displayed a lack of immunoreactivity . Perinodal Schwann cell processes also exhibit immunoreactivity, localized both within the Schwann cell cytoplasm and the plasmalemma. STX binding was studied both in intact rabbit cultured Schwann cells and after homogeneization. Saturable binding of STX was significantly higher in homogenized Schwann cells (410±37 fmole/mg protein) than in intact Schwann cells (214±21 fmole/mg protein). Moreover, the equilibrium dissociation constant was higher for homogenized preparations (1.77±0.37 nM) than for intact Schwann cells (1.06±0.29 nM). These data suggest the presence of an intracellular pool of sodium channels in Schwann cells.

[Supported in part by grants from the NIH, NMSS, and VA]

153.15

REACTIVE ASTROCYTES AND DiBcAMP-TREATED ASTROCYTES HAVE DIFFERENT SURFACE MARKERS

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Epithelioid astroblasts can easily be maintained in culture, but it would also be desirable to have *in vitro* models of mature protoplasmic and reactive astrocytes. An essential step to develop such models is the description of differential markers for these astrocytes. Antibodies to a dodecapeptide of the extracellular domain of the human EGF receptor (EGFR) bind to rat astrocyte plasma membranes, and discriminate between newborn rat astroblasts and resting and reactive astrocytes from adult animals. Anti-peptide A (residues 513-524 of EGFR) intensely stained cultured astrocytes. In Western blots of membranes from cultured polygonal astrocytes (astroblasts) and C6 glioma cells, the antibody recognized bands of apparent molecular weigth 115, 87 and 68 and much more weakly with a 170 kD band. A similar reaction pattern with an additional 50 kD band was observed with membranes from the same cells after assuming star-shaped morphology brought about by 48 hours treatment with di-butyryl-cAMP. Parallel blots with membranes from adult rat brain showed basically a 87 kD band and, in membranes prepared from tissue 15 days after an open injury, an additional band of 91 kD. These results suggest that the surface of diBcAPM-treated astroblasts is very similar to that of the untreated cells and that both are inaccurate models of mature protoplasmic and reactive astrocytes.

(Supported by a grant from the Spanish Science Research Council).

153.16

REGULATION OF MICROGLIAL PRODUCTION OF SUPEROXIDE ANION AND INTERLEUKIN 1 BY INTERFERON.

C. Colton, J. Yao* and D. Gilbert, Depart. Physiol. Biophy., Georgetown Univ. Med. School., Washington DC. 20007 and Lab. of Biophys., NINDS, NIH, Bethesda, MD, 20892.

Recent evidence supports the idea that microglia are CNS specific macrophages and share morphological, biochemical and functional similarities to other tissue macrophages, including the production of superoxide anion and Interleukin 1 (IL-1). We have examined the effect of interferon (IFN) on microglial production of the superoxide radical anion (O_2-) and IL-1 using a primary culture of microglia from neonatal rat cerebral cortices. Microglia were treated for 24 or 48 hours with α IFN or γ IFN and assayed for O_2- or IL-1. IL-1 increased in a dose dependent fashion, with maximal IL-1 levels at 100 U/ml of α IFN. γ IFN was less effective, with maximal IL-1 production at 1000 U/ml γ IFN. Pretreatment with 1000 U/ml of either γ IFN or α IFN was also shown to enhance O_2- production in microglia stimulated to release O_2- by opsonized zymosan (OPZ) or phorbo myristate acetate (PMA) compared to untreated, stimulated controls. Non-stimulated production was not altered.

153.17

A NOVEL ANTIGEN DEVELOPMENTALLY REGULATED IN OLIGODENDROCYTES. M.M. Daston and N. Ratner. Dept. of Anatomy and Cell Biology., Univ. of Cincinnati-College of Medicine, Cincinnati, OH 45267.

We have examined the cellular localization within the CNS of a novel protein, p30, in order to gain insight into its possible function(s) in the development of the nervous system. P30 was initially described as a protein present in the CNS of young rats, isolated based on its ability to promote adhesion and neurite outgrowth of embryonic brain cells in culture (Rauvala and Pihlaskari, 1987, J. Biol. Chem., 262:16625-16635).

Immunostaining of tissue sections from rats at various ages and of cultured optic nerve cells was performed using polyclonal antibodies generated against a synthetic peptide (p30 N-terminal 1-13, Rauvala et al., 1988, J. Cell Biol. 107:2293-2305) and against intact p30.

P30 is expressed in the developing CNS in cells that extend processes which end in myelin sheaths, suggesting that they are oligodendrocytes. Galactocerebroside-positive oligodendrocytes in cultures of rat optic nerve are also labelled by anti-p30. Oligodendrocytes labelled by anti-p30 are first apparent in the spinal cord at P1 and are most numerous at P8. In the adult spinal cord anti-p30 labelled oligodendrocytes are infrequent. P30 staining is also present, at much lower levels, in most or all developing CNS neurons. Neuronal staining persists into adulthood. The expression of p30 in numerous oligodendrocytes in early postnatal development and a sub-population of oligodendrocytes in the adult is consistent with the hypothesis that p30 expression is limited to actively myelinating oligodendrocytes.

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153.18

PHOSPHORYLATION OF GFAP AND VIMENTIN BY A CYTOSKELETAL-ASSOCIATED INTERMEDIATE FILAMENT PROTEIN KINASE ACTIVITY IN CULTURED ASTROCYTES. B.C. Harrison and P.L. Mobley. *Univ. Texas Health Science Center, San Antonio, TX, 78284.*

A cytoskeletal-associated protein kinase is present in cultured astrocytes which is capable of phosphorylating the cytoskeletal proteins glial fibrillary acidic protein (GFAP) and vimentin. Studies were conducted to determine if the intermediate filament protein kinase (IFPK) activity was distinct from that of protein kinase C (PK-C) and the cyclic AMP-dependent protein kinase (PK-A). All three kinases phosphorylated GFAP and vimentin, and the sites of phosphorylation of these proteins were compared by 2-dimensional tryptic peptide mapping. Although there were sites of phosphorylation common to all the kinases, the IFPK produced unique phosphopeptide maps of both GFAP and vimentin. Phosphoamino acid analysis was also conducted to determine which amino acids were phosphorylated by the IFPK, PK-C and PK-A. The IFPK increased the ^{32}P-incorporation into both serine and threonine residues of GFAP and vimentin. PK-A increased the ^{32}P-incorporation into only serine residues of the proteins, while PK-C increased the ^{32}P-incorporation into serine and threonine residues of GFAP, and serine residues of vimentin. Vimentin and GFAP have been shown to be phosphorylated by PK-C and PK-A in the amino-terminal domain. The IFPK also phosphorylated GFAP in the amino-terminal domain; however, the IFPK was capable of phosphorylating both the amino- and carboxy -terminal domains of vimentin. Therefore, these studies suggest that the IFPK activity observed in cultured astrocytes is distinct from that of PK-C and PK-A, and is capable of phosphorylating unique sites on GFAP and vimentin.

153.19

ADULT AND FETAL ASTROCYTES IN CULTURE DIFFER IN MORPHOLOGY AND GFAP EXPRESSION. M.S. Bull, J. Qian, A. Tessler and P.Levitt, Dept. Anatomy, Medical College of Pa., Phila., PA 19129.

Astrocyte differentiation and growth in the developing brain are regulated by a complex array of environmental factors. The regulation of growth of adult astroglia, however, is still poorly understood. We have begun culturing astrocytes from the spinal cord of one year old rats and comparing their growth, morphological and immunohistochemical properties with those of embryonic day (E) 18 spinal cord astrocytes in order to study the cellular processes regulating adult astrocyte growth. The results of these comparisons show marked differences in the complexity of astroglial morphologies and level of GFAP immunoreactivity between adult and fetal astrocytes prepared and cultured under similar conditions, even when the fetal glia are maintained for several months. Whereas adult astrocytes grow slowly, tend to be of similar morphology (Type I) and have low overall GFAP expression, fetal astrocytes grow quickly and are separable into different morphological types (both I and II) with high GFAP expression. In addition, pronounced cytoplasmic tangles, which are GFAP-immunoreactive, are often visible in the somas of adult astrocytes but never in embryonic astrocytes. These findings indicate that in culture, the capacity for morphological differentiation of adult astrocytes is different from fetal-derived astroglia. In addition, the results raise the possibility of substantial age-related changes in adult astrocytes that are never expressed by fetal astroglia, even when maintained for long periods of time *in vitro*. Supported by VA Medical Research Service, USAMRDC grant 5193002, and NIH-NS24707.

153.20

EXPRESSION OF AN INTERMEDIATE FILAMENT-ASSOCIATED PROTEIN, IFAP-70/280kD, IN RADIAL GLIA AND IN REACTIVE ASTROCYTES OF RATS. H.-Y. Yang, V. Krihо*, D. Johnson-Seaton*, and G. D. Pappas. Dept. of Anatomy & Cell Biology, University of Illinois at Chicago, IL 60612.

Injury to CNS results in gliosis. The reactive astrocytes are characterized by their augmented intermediate filament (IF) cytoskeleton, in which two IF structural proteins, glial fibrillary acidic protein (GFAP) and vimentin, have been found. Because vimentin is a major IF constituent of immature astrocytes, the expression of this protein may suggest an immature cell state. However, vimentin is found to be present in many mature astrocytes in the white matter. Our research is currently concentrated on expression of intermediate filament-associated proteins (IFAP) in cells of astrocytic lineage. Recently, we have identified an IFAP, IFAP-70/280kD, in a subgroup of non-stellate (type-1, based on morphology) neonatal astrocytes in primary cultures. The ontogeny of this IFAP has also been studied by immunofluorescence in rat spinal cord from E12 through postnatal day 28, as well as in adult rats. The IFAP-70/280kD immunoreactivity was detected in the radial glial cells from the earliest day examined, diminished following the expression of GFAP in these cells, and disappeared totally in spinal cord astrocytes after postnatal day 5. The immunoreactivity of this IFAP was not found in mature astrocytes in rat CNS. However, the immunoreactivity of this IFAP was detected in reactive astrocytes of rat brain induced by stab injury with a needle. We have used double-labeling immunofluorescence microscopy with antibodies to GFAP and IFAP-70/280kD (or vimentin) to study the gliotic reaction as it occurred from 4 hrs to at least 20 days post-lesion in the area of the wound. The results show that the vimentin/GFAP-containing astrocytes appear first in the wound area at 8 hrs post-lesion, and the IFAP-70/280kD/GFAP-containing astrocytes appear at 2 days post-lesion. The results indicate that the reactive astrocytes are different from the mature astrocytes by their expression of IFAP-70/280kD which is expressed in radial glia-related cells, and that these astrocytes may be derived from type-1 astrocytes. (Supported by NIH grant NS26395)

153.21

BRAIN GANGLIO-N-TETRAOSYLCERAMIDE (GA1): PRESENCE IN ADULT BOVINE AND HUMAN MYELIN. E.L. Hogan and S. Dasgupta*. Dept Neurology, Med Univ of SC, 171 Ashley Avenue, Charleston, SC 29425-2232.

Ganglio-N-tetraosylceramide ($GgOse_4Cer$) is also designated GA1 or asialo-GM1. Its presence in mouse CNS myelin has been indicated by immunohistology (Kusonoki et al., Brain Res. 334, 117, 1985). We have purified a neutral glycosphingolipid (GSL) from bovine brain by column chromatography. The purified homogenous compound has an Rf near pentaglycosylceramide of bovine RBC. The gal:galNAc:glc molar ratio is 2:1:1. Permethylation analysis and stepwise hydrolysis with β-galactosidase and β-hexosaminidase indicated its structure as Galβ(1-->3) GalNAcβ(1-->4) Galβ(1-->4) GlcCer (GA1). The ceramide contains sphingosine and C18:0, C18:1 and C16:0 fatty acids. GA1 has been detected in bovine and in human myelin by tlc. Another neutral GSL has also been purified and tentatively characterized as IV^3Gal $III^3GalNAc$ $GbOse_3Cer$ (Gal $GbOse_4Cer$) by GC. This GSL has been reported in a human teratocarcinoma cell line (Kannagi et al. The EMBO J. 2, 2355, 1983). This is, to our knowledge its first identification in the nervous system. Purification for complete characterization and NMR characterization of the purified GA1 and determination in brain cellular and subcellular compartments is presently being done.

153.22

ISOLATION AND CHARACTERIZATION OF SCHWANN CELLS DERIVED FROM ADULT NERVE. T.K. Morrissey, N.Kleitman and R.P. Bunge, The Miami Project to Cure Paralysis, University of Miami School of Medicine, 1600 NW 10 Ave., R48, Miami, Fl 33136.

Should implants of Schwann cells (ScCs), or prosthetic devices containing ScCs, prove to be a practical approach to treating spinal cord injury it would be advantageous to be able to harvest and grow a patient's own ScCs for autologous grafting. Toward this end we have been studying methods of purifying and amplifying ScCs obtained from adult peripheral nerve. Adult rat sciatic nerves were removed and enzymatically dissociated either immediately or following a period of "in vitro Wallerian degeneration" as 1x1mm explants in tissue culture. Immediate dissociation yielded populations of cells containing less than 20% ScCs. Contaminating cells were mostly endoneurial fibroblasts. Conversely, serial explantation of explants over 4-5 weeks decreased fibroblast contamination as these grow out from the explants. After these explantations, dissociation yielded up to 98% pure ScCs. ScCs from this source retained functional capacity in that they associated with sensory neurons in culture and form myelin under appropriate conditions. These cells proliferated in response to axonal mitogens as well as in the presence of GGF and forskolin. Studies are underway to determine the most efficient way to acquire large numbers of these cells. Concurrently we are applying these techniques to adult human peripheral nerve. Preliminary results indicate that serial explantation yields a purer population of ScCs than primary dissociation. It thus appears to be possible to obtain quite pure populations of Schwann cells by simple culture methods; it remains to be determined, however, how extensively this population can be expanded while retaining growth control and full functional capacity.

Supported by NS09923 and the Miami Project to Cure Paralysis.

153.23

OXYGEN RADICAL INDUCED CELL DEATH OF OLIGODENDROCYTES IN CULTURE. Y.S. Kim* and S.U. Kim. Division of Neurology, Univ. British Columbia, Vancouver, B.C. V6T 1W5, Canada.

Previous studies have suggested that oxygen radicals and lipid peroxidation may be implicated in the irreversible loss of neurons and glial cells following brain or spinal cord injury, degenerative neurological diseases such as Parkinson disease and Huntington disease and in multiple sclerosis. The cytotoxic effects of oxygen radicals have been studied in enriched population of mature bovine oligodendrocytes in culture. Oxygen radicals were generated enzymatically by glucose and glucose oxidase, and hypoxanthine and xanthine oxidase. Cytotoxicity was assessed by trypan blue exclusion and percent lactate dehydrogenase release into the media. Incubation of oligodendrocytes with these oxygen radical generating systems for 4 h resulted in significant cell death, especially in the glucose oxidase system. The cytotoxic effects were completely protected by catalase in both oxygen radical generating systems. However, superoxide dismutase, dimethylsulfoxide and antioxidants such as vitamin E and glututhione did not protect the oxidant-mediated cytotoxicity of oligodendrocytes. It appears that hydrogen peroxide is a major toxic oxidant in these oxygen radical generating systems to induce the cell death of oligodendrocytes.

GENE STRUCTURE AND FUNCTION II

154.1

IMMUNOCYTOCHEMICAL LOCALIZATION OF CHLORAMPHENICOL ACETYL TRANSFERASE (CAT) IN BERGMANN GLIA OF THE CEREBELLUM IN TRANSGENIC MICE EXPRESSING HUMAN IMMUNODEFICIENCY VIRUS-LONG TERMINAL REPEAT-CAT (HIV-LTR-CAT) CONSTRUCTS. RICHARD E. HARLAN AND OM PRAKASH.* Department of Anatomy, Tulane Medical School (REH) and Dept. Mol. Oncology, Alton Ochsner Medical Foundation (OP), New Orleans, LA 70112 and 70121.

Involvement of the nervous system in HIV infection has been demonstrated in 70-80% of AIDS cases at autopsy. The nature of this neurotropism is not well understood. Glial cells are a primary target for HIV infection and replication.

Transgenic mice bearing the LTR of HIV fused to the bacterial reporter gene CAT were constructed. One line of mice showed high, constitutive levels of CAT activity in the cerebellum, compared to other brain regions. Immunocytochemical analysis using a polyclonal antibody to CAT (5-Prime-3-Prime, Inc.) revealed intense immunostaining of Bergmann glia. Immunostaining was found in the cell body and throughout the processes extending through the molecular layer. Subpopulations of glia (presumably astrocytes) in the cerebral cortex, caudate-putamen and olfactory bulb were also immunostained. No staining was seen in non-transgenic mice. It is likely that certain glial cells express proteins that transactivate the HIV-LTR.

154.2

NEURONAL SPECIFIC EXPRESSION OF THE HUMAN NEUROFILAMENT L PROMOTER IN A HSV-1 VECTOR. H.J. Federoff, A. Geller, and B. Lu. The Departments of Medicine and Neuroscience, The Albert Einstein Coll. of Med., Bronx, N.Y. 10461, The Dana Farber Cancer Institute, Boston, MA 02115.

The study of gene function and regulation in neurons has been hampered because of the difficulty of introducing genes into neurons. Recently, a Herpes Simplex Virus (HSV-1) vector has been developed that can efficiently transfer genes into cultured peripheral and central neurons (A. I. Geller and Breakefield, X.O. Science 241:1667, 1988, A.I. Geller and A. Freese, PNAS 87: 1149,1990). The prototype vector, pHSVlac, places the E. coli lac Z gene under the control of the HSV immediate early (IE) 4/5 promoter. The IE 4/5 promoter and therefore pHSVlac virus, expresses beta-galactosidase in many cell types including fibroblasts, glia and neurons. To determine if a neuronal specific promoter could function properly in a HSV-1 vector we replaced the IE 4/5 promoter with the human neurofilament L promoter (pNFLlac). Initial experiments performed by lipofection into three neuronal cell lines (PC12, NB2a, SKN-SH) and three non-neuronal cell lines (COS 7, NIH 3T3, and a glioblastoma) demonstrated marked neuronal specificity. The neurofilament promoter was 25-75 fold more active in neuronal than in non-neuronal cell lines. Neuronal specificity of expression of beta-galactosidase form pNFLlac virus is currently under study. The packaging of pNFLlac into viral particles will be performed using new HSV-1 deletion mutant packaging system.

154.3

STRUCTURE AND EXPRESSION OF THE HUMAN GENE FOR GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP). F. Besnard*, E. T. Browning, M. Brenner*, R. Chao*, Y. Nakatani* and E.Freese. Lab. of Molec. Biol., NINDS-NIH, Bethesda, MD 20892, and Dept. Pharmacol., Robt. Wood Johnson Med. School, Piscataway, NJ 08854.

GFAP is an intermediate filament protein expressed almost exclusively in astrocytes. We have sequenced a 12 kb segment of a human genomic GFA clone; the sequence obtained contains the entire GFA coding region, 2 kb of the 5'-flanking region, and 0.5 kb of the 3'-flanking region. Comparison of this sequence to that of the mouse shows strong conservation of the size and sequence of the nine exons, but not of the introns. In particular, many of the sequence repeats present in the mouse introns were absent in the human sequence. For example, the $(TG)_6(TC)_{19}(AC)_8$ repeats in mouse intron 3 and the $(TC)_{25}$, 28 bp (R1) and 34 bp (R2) repeats in mouse intron 7 were not conserved in the human sequence. These results suggest that these repeats are not important for expression of the GFA gene.

To identify cis-acting sequence elements responsible for glial-specific GFA transcription, transient transfections were conducted with chloramphenicol acetyl transferase reporter gene constructs. A 2 kb 5'-flanking segment was active in the glial cell lines C6 and U251, but not in the non-glial cell lines HepG2 and HeLa, indicating that it contains cell-specific regulatory sites. Deletion analysis of this fragment identified two regions required for efficient expression; one located close to the RNA start point, the other about 1.7 kb upstream. These two regions apparently act synergistically, as removal of either one drastically reduced expression. Interestingly, both regions contain similar sequences which display strong DNA footprints. (Supported in part by NSF Grant BNS-8708539).

154.4

ISOLATION AND CHARACTERIZATION OF cDNA CLONES ENCODING β-TUBULIN AND β-ACTIN FROM SQUID GIANT FIBRE LOBE. A.E. Gioio, L. Barbieri*, L.M. Turzai*, J.S. Stiffler*, C. Perrone*, A. Giuditta and B.B. Kaplan. Dept. of Psychiatry, Univ. of Pittsburgh Sch. of Med., Pittsburgh, PA 15213.

A cDNA library from squid giant fibre lobe total RNA was constructed in a λ-zap vector using a modification of the Gubler-Hoffman method. The library contained approx. 6.0 X 10^7 pfu with insert sizes ranging from 600-4000 nucleotides in length. Library screening with rodent cDNA probes for β-tubulin and β-actin mRNAs yielded multiple positive plaques. One β-tubulin clone (pGFL-6) was 1874 bp in length and contained 118 and 424 bp of 5' and 3' untranslated sequence, respectively. Northern analysis using pGFL-6 and rat β-tubulin cDNAs as probes yielded a single major RNA species approx. 1.9 kb in length. Remarkably, squid β-tubulin manifests approx. 81% and 94% sequence similarity to the mammalian homologues at the nucleic acid and amino acid levels, respectively. Most of the variance represented conservative amino acid substitutions at the N- and C-terminal regions of the molecule.

The β-actin clone isolated is approx. 2.0 kb in length and contains the entire coding region of the protein (1.1 kb) and 128 bp of 5' noncoding sequence. Sequence comparison to the murine form of the protein revealed >95% sequence identity at the amino acid level. Taken together, these findings demonstrate the marked evolutionary conservation of sequence manifest by these cytoskeletal proteins.

154.5

PROMOTERS OF RAT BRAIN TYPE I AND II SODIUM CHANNEL GENES SHARE A COMMON MECHANISM OF REGULATION. S.D.Kraner, K.M.Dains, M.Azoulay, and G.Mandel, Dept. of Neurobiology & Behavior, SUNY at Stony Brook, Stony Brook, N.Y. 11794.

The sodium channel is encoded by a multi-gene family whose members are expressed in a tissue-specific manner. The excitable rat pheochromocytoma cell line, PC12, expresses the type II sodium channel gene, but not the type I gene. We have previously reported that multiple negatively-acting elements within the 5'flanking region of the type II sodium channel gene confer cell-specificity *in vitro*. Reporter fusion genes containing this region are expressed to high levels in PC12 cells, but are silent in the skeletal muscle cell line, L6. Using a transient expression assay we have further characterized the strongest of these negatively-acting elements. Removal of sequences between -1051 and -938 nucleotides upstream of the type II promoter results in a ten-fold increase in fusion gene activity in L6 cells, and no change in expression in PC12 cells. We have now isolated a 4 kb piece of the 5'flanking region of the type I gene. Fusion genes containing this entire region, or 5' deletion mutants of this region, have been analyzed in a variety of cell types including PC12, L6, epithelial cells, and a human neuroblastoma cell line, SY5Y. The 4kb type I fusion gene is active in the SY5Y cells but inactive in PC12 cells. Sequential 5' deletions of the type I 5'flanking region result in inappropriate expression of the reporter gene in PC12 and L6 cells. Further analysis of the sequences and promoter activities of these two neuronal ion channel genes will facilitate identification of the elements involved in cell-specific regulation.

154.6

GENERATION OF NCAM-TRANSCRIPT DIVERSITY. D.Barthels[1], G.Vopper[1]*, C.Goridis[2]*, and W.Wille[1]. [1]Inst. f. Genetik, University of Cologne, Köln, FRG, [2]Centre d'Immunologie, INSERM-CNRS, Marseille, France.

Neural cell adhesion molecules (NCAMs) are cell surface glycoproteins known to be involved in several important events during vertebrate nervous system development. There exist three classes of NCAM-polypeptides (120, 140 and 180 kD) which differ mainly in size of their C-terminal extensions.

Alternative processing of primary RNA-transcripts has been shown as a major strategy to create diversity of the gene products. In the case of murine NCAM the various mRNAs are generated by alternative splicing and differential polyadenylation of transcripts of a single gene which contains at least 24 exons. Since exons 0-14 are common to all NCAM-mRNAs, the utilization of exons 15-19 determines whether the resulting protein is membrane-associated or has transmembrane and cytoplasmic domains.

With PCR and S1 nuclease protection analyses we identified three additional alternative splice sites. The most complex pattern was detected at splice site a (exon borders 12/13), where at least five different combinations of sequences can be observed. The shortest extra segment consisted only of the trinucleotide AAG which was found to be inserted between exons 12 and 13 or 13 and 14 in several independent cDNA-clones, suggesting the existence of an unusual splicing mechanism in this region of the NCAM gene or a novel RNA editing system.

154.7

Analysis of Molecular and Cellular Variation in NCAM Expression Aoshuang Chen*, Brian Key*, Sue Haines*, Antonio Reyes*, Stephen Small*, and Richard Akeson Childrens Hospital Research Foundation, Cincinnati, Ohio 45229

Expression the the neural cell adhesion molecule (NCAM) is regulated at several levels. NCAM expression is restricted to discrete tissues and times of development. NCAM transcripts are initiated from identical genomic positions is several tissues and cell types. Initial studies suggest that function promoter elements are found within 800 bp of DNA immediately 5' to the start of transcription. This DNA segment contains additional putative regulatory elements which are being evaluated by transient transfection assays into neuronal and non-neuronal cells. Alternative splicing of the single NCAM gene generates a number of different transcripts. We have analyzed regulation of expression of an alternative exon, VASE, found in the fourth immunogluobulin-like domain. VASE appears primarily postnatally in rat brain and somewhat earlier in heart. Within the nervous system, PCR analysis of VASE levels indicates significant variation among brain regions with very low levels observed in the olfactory bulb and also in the olfactory epithelium. The cellular basis of the regulation of alternative splicing of NCAM is being analyzed by in situ hybridization. An additional level of NCAM regulation is differential glycosylation of the NCAM polypeptides within distinct brain regions. Unique N-linked carbohydrate groups are found on NCAM from olfactory tissues but not other brain regions. These multiple levels of regulation of NCAM expression and NCAM form suggest that distinct NCAM forms may have varying biologic roles during nervous system development and function. Supported by grants HD21065 and DS00347.

154.8

NK-10, A ZINC-FINGER ENCODING GENE DEVELOPMENTALLY EXPRESSED IN THE MOUSE BRAIN. R.Lange-Sablitzky*, D.Barthels, and W. Wille. Institute of Genetics, University of Cologne, Zülpicher Str. 47, D-5000 Köln 1, Fed. Rep. Germany

NK-10 has been cloned as cDNA from neonatal mouse cerebellar poly(A)$^+$RNA in λgt11. The NK-10 transcript is 1.85 kb in length containing a 0.4 kb 5'-non-coding region, an open reading frame of 1296 nt (coding for 432 AA), and a 3'-non-translated region of 0.16 kb carrying a noncanonic polyadenylation signal ATTAAA. The encoded NK-10 protein is composed of repetitive zinc-finger motifs exclusively, some of which are cryptic and non-functional (written below in square brackets). The overall organization of the fingers and pseudo-fingers is A-[B1]-[B2]-C1-C2-C3-C4-C5-[D]-E-[F]-G1-G2-G3-G4-G5.

The upstream region of the NK-10 gene contains a fairly good TATA-box consensus sequence followed by several major and minor cap-sites. An unusual feature of the genomic organization of NK-10 (the locus is unique and not a pseudogene) is the total lack of introns. NK-10 is expressed during the entire late embryonic and postnatal development of the brain with a peak relative level at approx. 3-7 days after birth. No expression was found in the liver as determined by S1 nuclease protection assay. Financed through DFG-SFB 243 grant to WW.

154.9

CONSTRUCTION OF A HUMAN SPINAL CORD cDNA LIBRARY AND SUBTRACTIVE CLONING OF SPINAL CORD SPECIFIC cDNAs. H. Kobayashi[1)], H. Takahashi*[2)], K. Oyanagi*[2)], T. Miyatake*[1)] and S. Tsuji[1)]. 1)Dept. of Neurology, 2)Dept. of Pathology Brain Res. Inst. Niigata University., Niigata 951, Japan.

Neurodegenerative diseases are characterized by neuronal degeneration of specific neurons, e.g. degeneration of motoneurons in amyotrophic lateral sclerosis. As the first step to understand molecular mechanisms of neuronal degeneration of spinal cord motoneurons, we have constructed a human spinal cord cDNA library and developed a strategy for isolating spinal cord specific genes by subtractive cloning. To isolate human spinal cord specific cDNAs, spinal cord specific [^{32}P]-cDNA probes were generated by phenol emulsion-enhanced reaction (G. H. Travis and J. G. Sutcliffe., 1988). Approximately 0.5% of ten thousand colonies gave strong signals with the subtracted probe and individual spinal cord specific cDNAs were isolated. The results demonstrate that the subtractive cDNA cloning strategy is quite feasible for isolating genes which are differentially expressed in specific neurons.

154.10

MOLECULAR HOMOLOGY OF A NEUROGLIAL GENE EXPRESSED IN DROSOPHILA AND HUMAN NERVOUS SYSTEMS. J. Martin*, M. Rudnicka*, J. Thomas*, and C. Miller. Department of Pathology, University of Southern California, School of Medicine, Los Angeles, CA 90033.

Monoclonal antibodies have identified immunocytochemical cross-reactions among cells in the human and Drosophila nervous systems (Miller and Benzer, PNAS 80:7641-7648, 1983). We are now exploring which of these represent homologies at the primary sequence level. MAb 23E9 identifies cells in Drosophila retina and optic lobes as well as human oligodendroglia and neurons (Rudnicka et al., J. Neuropath. Exp. Neurol. 48:363, 1989). Using the MAb, cDNA clones have now been isolated from both Drosophila and human brain cDNA λ gt11 expression libraries. A Drosophila cDNA clone has a 1600bp insert, including 1500bp of open reading frame. The nucleotide sequence and deduced amino acid sequence show no identity with any protein in the NBRF Protein Sequence Data Base. In situ hybridization with this clone shows localization to the Drosophila retina in a pattern closely similar to the immunocytochemical staining. Western blots with MAb 23E9 show immunoreactivity with a 220kDa protein on fly head homogenates and two bands of 130 and 80kDa on human brain homogenates. The human cDNA clone of 1600bp cross-hybridizes strongly with the Drosophila cDNA clone on Southern blots. These results suggest possible evolutionary conservation of this gene.

154.11

ORGANIZATION OF THE RAT AND THE HUMAN B-50 (GAP-43) GENE. L.H. Schrama, H.B. Nielander*, A.J. van Rozen*, P. Schotman*, and W.H. Gispen*. Div. Mol. Neurobiol., Rudolf Magnus Inst., Lab. Physiol. Chem., Inst. Molec. Biol. & Med. Biotechnol., Univ. of Utrecht, Padualaan 8, 3584 CH Utrecht, NL.

The neuron-specific phosphoprotein B-50 (GAP-43) has been shown to be associated with axon development and regeneration. How B-50 transcription is regulated is currently not understood. B-50 is encoded by a single copy gene. The increase in B-50 protein expression during regeneration of the n. ischiadicus is preceded by an increase of B-50 mRNA (Van der Zee et al., *J. Neurosci.*, 9:3505, 1989).

In order to elucidate the regulation of B-50 transcription, we have isolated coding and non-coding portions of the human and the rat B-50 gene, and determined the exon/intron structure of the human B-50 gene. The human exon 2 encodes for amino acids 11-209 and contains the first nucleotide of amino acid 210, and exon 3 encodes for the remainder of the protein. The 10 amino acid insert present in the human B-50 protein as compared to the rat B-50 protein is encoded by exon 2. Currently we are subcloning the human exon 1 using inversed PCR of a 1.8 kb genomic fragment containing exon 1. We have sequenced a 0.8 kb HindIII - NheI fragment upstream of the translation start of the rat B-50 gene. This presumed promoter region of the rat B-50 gene contains repetitive sequences capable of forming H-DNA and Z-DNA. In contrast to recent results published by others (Grabczyk et al., *Soc. Neurosci. Abstr.*, 15:381.3, 1989, Nedivi et al., *Soc. Neurosci. Abstr.*, 15:503.15, 1989), we found that the 0.8 kb fragment also contains consensus sequences for binding of regulatory and transcription factors, including those for AP-1 and the TATA box. Currently the promoter activity of the elements upstream of the proposed various transcription starts is tested in embryonal carcinoma (EC) and PC12 cells after neuronal differentiation using luciferase as the reporter gene.

154.12

ISOLATION AND CHARACTERIZATION OF THE CHICKEN GENE FOR SNAP-25. Christina Bark, Dan Larhammar#, and Michael C. Wilson##. Department of Developmental Biology, #Department of Medical Genetics, Uppsala University, S-751 23 Uppsala, Sweden. ##Scripps Clinic and Research Foundation, La Jolla, CA 92037, USA.

SNAP-25 (synaptosome-associated protein-25) has been shown to localize to presynaptic terminals in certain subpopulations of neurons in mouse and chicken (Oyler et al., 1989; Catsicas et al., submitted). The exact biological role of the SNAP-25 protein has yet to be resolved. Potential functional protein domains include a cluster of four cysteine residues that could mediate binding of metal ions as well as an amphipathic alpha helix that may interact with the synaptosomal membrane. The SNAP-25 protein shows a remarkable degree of evolutionary conservation in that the chick and mouse proteins are identical throughout the 206 amino acids and show 60% sequence identity to the *Drosophila* protein.

The precise onset of gene expression at the time of synaptogenesis and also the localization of SNAP-25 to only a subset of neurons suggests a stringent regulatory control of the gene at the molecular level. In order to analyze in more detail the *cis*- and *trans*-acting elements influencing gene activation, the chicken gene for SNAP-25 has been isolated. Preliminary characterization indicates that the gene contains at least eight exons that may span as much as 50 kilobasepairs. Exon organization as well as promoter structure will be discussed. (Supported by the Swedish Natural Science Research Council, B-BU9714-300, C.B.)

154.13

CHROMOSOME MAPPING AND MUTATIONAL ANALYSIS OF THE NEURON-SPECIFIC PROTEIN, SNAP-25. M.C. Wilson, C.A. Kozak*, T.N. Sato and E.J. Hess, Dept. of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037 and NIH, Bethesda, MD 20892.

SNAP-25 is a neuron-specific synaptosomal associated protein which appears to contribute to synapse formation. In order to better characterize SNAP-25 expression and function, we have mapped the SNAP-25 gene. The SNAP-25 restriction fragment length polymorphisms (RFLPs) observed in the southern blot analysis of a panel consisting of 24 hamster-mouse somatic cell hybrids demonstrated that SNAP-25 is located on mouse chromosome 2. Further analysis of 72 recombinant inbred mouse strains using a Sca I RFLP situated the SNAP-25 locus approximately equidistant between marker genes *Hdc* and *Psp* in the medial region of mouse chromosome 2, 49 to 57 centimorgans from the centromere. Several mouse mutants map in this region of chromosome 2, including the recessive mutants blind sterile (*bs*), undulated (*un*), lethal milk (*lm*) and the semi-dominant mutation coloboma (*Cm*). Brain RNA and protein was prepared from each mouse mutant and a control littermate; no difference in the mobility of SNAP-25 mRNA or protein was observed among the mutants. However, a ~50% reduction in SNAP-25 mRNA expression and a ~50% reduction in SNAP-25 protein expression was observed on Northern and Western blots, respectively, of the heterozygous mouse mutant, coloboma (*Cm*/+); the coloboma mouse mutant is a neurological mutant whose phenotype includes opthalmic deformation and stereotypic head shaking, a phenotype which may be consistent with a reduction in SNAP-25 expression. Further experiments are underway including extensive Southern blot analyses of coloboma genomic DNA to determine if the coloboma phenotype is a result of a mutation in the SNAP-25 gene. Supported by PHS CA33730, NS23038 and an American Epilepsy Society Research Fellowship.

154.14

STRONG EVOLUTIONARY CONSERVATION OF SNAP-25 BETWEEN DROSOPHILA, GOLDFISH, CHICKEN, AND MOUSE. C. Risinger*, A. G. Blomqvist*, I. Lundell*, S. Catsicas#, M. C. Wilson##, and D. Larhammar*. Dept of Medical Genetics, Uppsala University, Box 589, S-751 23 Uppsala, Sweden. #Institute de Biologie Animale, Lausanne, Switzerland. ##Scripps Clinic, La Jolla, CA, U.S.A.

SNAP-25 is a 25-kDa protein which is expressed exclusively by some neuronal subpopulations. It is localized to presynaptic nerve terminals and is associated with synaptosomal membranes (Oyler et al., 1989). Its onset of expression correlates precisely with the time of synaptogenesis and indicates a role in this process (Catsicas et al., submitted).

We have previously shown that the chick and mouse SNAP proteins are identical throughout the 206 amino acids (S. Catsicas et al., submitted). To investigate further the evolutionary conservation of SNAP we have isolated clones from goldfish *(Carassius auratus)* and *Drosphila melanogaster* libraries. Drosophila SNAP displays 60% sequence identity to the mouse protein. A segment of 60 amino acids shows 85% sequence identity.

Several SNAP clones were isolated from a goldfish retina cDNA library. Six of these have been partially sequenced. Surprisingly, all six clones are distinct. Overall, they show approximately 90% amino acid sequence identity to the mouse protein and somewhat higher identities to each other. Thus, the strong evolutionary conservation of SNAP contrasts with the polymorphism in goldfish. However, goldfish may have been artificially inbred by man for 2,000 years.

The sequences of SNAP-25 from Drosophila, goldfish, chicken, and mouse reveal an extremely well-conserved protein.

ION CHANNELS: MODULATION AND REGULATION II

155.1

ANOMALOUS EFFECTS OF INTRACELLULAR Ca^{2+} CHELATION IN CAT NEOCORTICAL NEURONS. P.C. Schwindt, W.J. Spain & W.E. Crill. Dept. of Physiology & Biophysics, Univ. Washington Sch. of Med., Seattle, WA 98195.

Excitability and ionic currents were examined in layer V neurons from cat sensorimotor cortex after impalement with microelectrodes containing KCl plus 200mM or 2mM of the Ca^{2+} chelator BAPTA. Cells impaled with microelectrodes containing KCl alone served as controls. Impalement with electrodes containing 200mM BAPTA resulted in rapid abolition of Ca^{2+}-mediated afterhyperpolarizations (AHPs). Spike duration was normal, but rheobase was greatly elevated and subthreshold rectification was absent. Both the persistent Na^+ current and the anomalous rectifier current were absent during voltage clamp of these cells, whereas both currents were prominent in controls. Cells impaled with 2mM BAPTA displayed a different abnormality. The Ca^{2+}-mediated AHP following a spike train was markedly enhanced in both duration and amplitude. Injection of 1 second current pulses evoked an initial burst of spikes followed by a prolonged cessation of firing instead of the tonic firing seen in controls. Ca^{2+}-mediated K^+ currents were evoked by smaller depolarizations than in control cells, and they were markedly larger and slower than seen in controls when evoked at equivalent potentials. These currents were unaffected by apamin or 1mM TEA, but were abolished by muscarinic and adrenergic agonists. We conclude that small changes of $[Ca^{2+}]_i$ can markedly alter both neuronal firing properties and the apparent voltage dependence and kinetics of Ca^{2+}-mediated K^+ currents. A large reduction of $[Ca^{2+}]_i$ results in the disappearance of voltage-gated currents that are normally present. Supported by NINCDS grants NS001166, NS620322 & NS620486.

155.2

MODULATORY EFFECTS OF SEROTONIN ON CAT NEOCORTICAL NEURONS. W.J. Spain, P.C. Schwindt & W.E. Crill. Depts. of Medicine and Physiology & Biophysics, Univ. Washington Sch. of Med., Seattle, WA 98195.

We are interested in the mechanisms for the transduction of synaptic currents into spike trains by neocortical neurons that project to lower brain centers. These layer V pyramidal neurons respond to steady injected current with an initial period of phasic firing followed by adaptation to steady tonic firing rates. The largest neurons of area 4γ (termed PHE cells) are recognized by short duration action potentials (mean=0.4 ms). Deactivation of a large inwardly rectifying cation current (I_h) increases their initial firing rate to steady injected current following a period of hyperpolarization. The smaller neurons (termed PHI cells) have wider action potentials (mean=0.8ms), decreased initial firing rate following a period of hyperpolarization and a smaller I_h. The effect of serotonin (5-HT) 10-100μM on these cells in slices was investigated in current and voltage clamp. In PHE cells, 5-HT caused a depolarization of resting potential (RP) and an increase in initial phasic firing rate with a decrease in the tonic firing rate to steady depolarizing current pulses. In addition, the increase in initial firing rate following a period of hyperpolarization was enhanced by 5-HT. In PHI cells, 5-HT caused either a small depolarization, hyperpolarization or no change in RP. 5-HT increased the tonic firing rate and reduced slow adaptation during steady depolarizing current pulses. In both types of pyramidal cells, action potential duration did not change, however, the medium duration afterhyperpolarization was reduced. Following repetitive firing, a slow duration afterhyperpolarization (sAHP), caused predominantly by a Na+ dependent K+ conductance, was markedly reduced in all neurons. In voltage clamp experiments on both cell types 5-HT caused and an increase in I_h. The increase in I_h was accompanied by a positive shift of its voltage-dependent activation, decrease of activation time constants and increase of deactivation time constants. It is concluded that in PHE cells, the dominant role of I_h contributes to the depolarization, and increase in phasic firing in the presence of 5-HT; and in PHI cells, the mechanism underlying the reduction of the sAHP by 5-HT results in the increase in tonic firing. Supported by grants NS001166, NS620322 & NS620486.

155.3

A PATCH-CLAMP ANALYSIS OF THE EFFECTS OF MUSCARINE ON DOPAMINERGIC NEURONS OF THE SUBSTANTIA NIGRA.

S.V.P. Jones, N.L. Silva and M.R. Brann. Laboratories of Neurophysiology and Molecular Biology, NINDS, National Institutes of Health, Bethesda MD 20892.

Of the 5 muscarinic receptors that have been cloned, *in situ* hybridization experiments have revealed that the dopamine (DA) neurons within the substantia nigra zona compacta (SNZC) contain only m5 muscarinic receptor mRNA (Weiner *et al.* 1990). Therefore, electrophysiological analysis of the effects of muscarinic agonists on DA neurons should reveal the actions of the m5 muscarinic receptor subtype. The striatum of 6 day old rats were injected with fluorescent beads. These beads retrogradely label the DA neurons of the SNZC (Silva *et al. J. Neurophysiol* in press 1990). The cells of the SNZC were acutely dissociated from 9-12 day old rats. Cells containing the fluorescent beads were recorded from using the whole-cell patch-clamp technique. Application of 50 μM muscarine resulted in several different responses. These included an outward current response in cells voltage-clamped at -60 mV and inhibition of a tail current observed on return to -60 mV from positive potentials. A reduction in outward current was also noted on application of muscarine in cells held at positive (0 to +40mV) potentials. The identity of the conductances underlying these responses is being further characterized.

155.4

ANTIBODIES AGAINST THE G-PROTEIN G_i ATTENUATE COUPLING OF OPIATE (μ) RECEPTOR-EFFECTOR MECHANISMS - A WHOLE-CELL PATCH CLAMP STUDY IN RAT LOCUS COERULEUS (LC) SLICES. M.Alreja*, E.J. Nestler and G.K. Aghajanian. Depts. of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT 06508.

Previous intracellular studies have demonstrated that the opiate-induced outward K^+ current in noradrenergic LC neurons is mediated by a pertussis toxin (PTX)-sensitive G-protein. We used the whole-cell mode of patch clamping in LC slices (400 μm) to study the effect of loading these neurons with antibodies to $G_{i\alpha}$ and $G_{o\alpha}$ (the principal PTX substrates in LC) on the opiate-induced outward K^+ current We also tested the effect of pretreatment of LC membranes with the antibodies on opiate-induced inhibition of adenylate cyclase activity.

The antibodies used (Spiegel/NEN, USA) had been raised against C-terminal decapeptides of the α-subunits of G_i (recognizing $G_{i\alpha\text{-}1}$ and $G_{i\alpha\text{-}2}$) or G_o (recognizing $G_{o\alpha}$ and $G_{i\alpha\text{-}3}$). To test the specificity of the antisera, immunoblots were performed on SDS-PAGE separated G-proteins from rat LC homogenates. G_i and G_o antisera did not cross-react up to the tested dilutions (G_o, 1:10; or G_i, 1:50); the G_o antiserum had a lower titer than the G_i antiserum.

Whole-cell patch clamp recordings from identified LC neurons were made using low resistance (≈3 MΩ) electrodes filled with typical "intracellular" solution containing GTP with or without antiserum. D-ala^2,D-leu^5 enkephalin (DADLE) was used to evoke the outward K^+ current. At 1:50 dilution, antibodies against G_i but not G_o caused over 50% attenuation of the DADLE-evoked response. In preliminary experiments, G_i antiserum also attenuated DADLE-induced inhibition of forskolin-activated adenylate cyclase activity in LC membranes.

Thus the G-protein G_i ($G_{i\alpha\text{-}1}$ and/or $G_{i\alpha\text{-}2}$) appears to mediate, at least in part, the opiate-induced opening of K^+ channels in rat LC neurons; however, because of the relatively low G_o antibody titer, G_o involvement could not be ruled out.

155.5

LACK OF EFFECT OF T-CHANNEL BLOCKERS ON PACEMAKER-LIKE FIRING OF DORSAL RAPHE NEURONS RECORDED *IN VITRO*. A. L. Mueller. Natural Product Sciences, Salt Lake City, UT 84108.

Dorsal raphe neurons fire action potentials in a rhythmic, pacemaker-like fashion. It has been hypothesized that T-type calcium channels may underlie the generation of such activity in these and other central neurons (Synapse 1:582, 1987; TINS 11:431, 1988; Science 242:1654, 1988). The aim of the present study was to determine the effect of known T-channel blockers on the spontaneous pacemaker-like activity of 5HT-containing dorsal raphe neurons in an *in vitro* brain slice preparation. Coronal slices (400 μm) of rat brainstem containing dorsal raphe were submerged in and superfused with warm (35 °C), oxygenated artificial CSF (aCSF) containing (in mM) 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 1.3 $MgSO_4$, 2 $CaCl_2$, 25 $NaHCO_3$, 11 glucose. Methoxamine (10 μM) was included to increase spontaneous neuronal activity (Brain Res. 289:109, 1983). In experiments involving Cd^{+2} or Co^{+2}, 1.25 mM HEPES replaced the NaH_2PO_4. Putative 5HT-containing neurons, identified on the basis of spontaneous firing rate, action potential duration and waveform, and sensitivity to 5HT, were recorded with standard extracellular recording techniques. Cd^{+2} (10-100 μM) or Co^{+2} (1-3 mM) totally suppressed the spontaneous firing of all neurons tested; with Cd^{+2}, a transient excitation preceded the inhibition. Ni^{+2} had no effect at concentrations up to 300 μM; at 1 mM, Ni^{+2} elicited a slow biphasic increase-decrease in firing rate. Neither amiloride (300 μM) nor 1-octanol (300 μM) produced a marked suppression of spontaneous firing. Taken together, these findings, and especially the rank order of potency of $Cd^{+2} > Ni^{+2}$, do not support the hypothesis that conventional T-type calcium channels (where $Ni^{+2} > Cd^{+2}$) are involved in the generation of pacemaker-like neuronal activity in 5HT-containing neurons in the dorsal raphe.

155.6

MODULATION OF GLYCINE RECEPTOR Cl- CHANNELS BY PROTEIN KINASE A (PKA) IN SPINAL TRIGEMINAL NEURONS. Y. Song and L.-Y.M. Huang, Marine Biomedical Institute, Univ. TX Med. Br., Galveston, TX 77550.

Glycine is an important inhibitory transmitter in the brainstem and spinal cord. The electrophysiological and molecular properties of glycine receptor Cl- channels have been studied extensively. But very little is known about the modulation of this channel. We have examined the regulation of strychnine-sensitive glycine receptor channels in isolated neurons acutely dissociated from spinal trigeminal nucleus of rat using the patch clamp and intracellular perfusion technique. Under whole cell recording conditions, internally perfused 3′5′-monophosphate (cAMP)-dependent protein kinase (PKA) or cAMP or GTP-γ-s dramatically increased the glycine-induced Cl- currents in these cells. To determine which G protein was involved in the modulation, we studied the effects of cholera toxin (CTX) and pertussis toxin (PTX) on the glycine-activated Cl current. PTX had no effect on glycine response. CTX, on the other hand, increased the glycine response substantially. This increase was blocked when protein kinase inhibitor and CTX were simultaneously introduced into the cells. This result suggested that G_s protein in the cAMP pathway is involved in the modulation of glycine responses. Supported by NS01050 and NS23061.

155.7

MODULATION OF THE GLYCINE RECEPTOR BY CHLORMETHIAZOLE (CLM) AND 2,6-DIISOPROPYLPHENOL (PROPOFOL). T.G.Hales[1]*, J.A.Peters[2]*, J.J.Lambert[2]* & R.L.Katz[1]. [1]Dept.Anesthesiology, UCLA, Sch. of Med. L.A., CA. 90024. [2]Dept. Pharmac. & Clin. Pharmac. Dundee Univ. Sch. of Med. Ninewells Hosp. Dundee. DD19SY. Scotland.

A number of compounds with anticonvulsant and anesthetic properties potentiate the actions of GABA on the $GABA_A$ receptor. These compounds include pentobarbitone, alphaxalone, CLM (Simmonds & Turner, Neuropharmac. 26:923, 1987) and propofol (Hales & Lambert, Br.J.Pharmac. 94: 393P, 1988). CLM has also been reported to modulate the glycine receptor and this property of the drug may underlie its use in the treatment of *status epilepticus* in patients failing to respond to benzodiazepines and anticonvulsant barbiturates (Harrison & Simmonds, Br.J.Pharmac. 80:387, 1983).

The whole-cell configuration of the patch-clamp technique was used to record, from tissue cultured murine spinal neurones and bovine chromaffin cells, currents evoked by the local application of glycine (100 μM) and GABA (100 μM), respectively. Glycine activated currents were blocked by the bath application of strychnine (100 nM), but were unaffected by bicuculline (1 μM). Bath application of alphaxalone (1 & 10 μM) and pentobarbitone (10 & 100 μM), at doses found to cause marked potentiation of GABA-evoked currents, had no effect on currents activated by glycine. In contrast, CLM (10 - 100 μM) and propofol (0.84 - 16.8 μM) potentiated currents activated by glycine in a dose dependent manner. Within this range of concentrations CLM and propofol were found to cause a much greater potentiation of GABA-evoked currents.

Unlike the other modulators of the $GABA_A$ receptor investigated, CLM and propofol potentiated strychnine sensitive currents, evoked by glycine.

155.8

Extracellular ATP Modulates Currents in Chromaffin Cells Through a G-protein Mediated Pathway. Diverse-Pierluissi,M., Dunlap,K.*,and Westhead,E.W.Molecular and Cellular Biology Program, Univ. of Massachusetts, Amherst, MA 01003 and *Dept. of Physiology, Tufts Med. Sch., Boston, MA 02111.

Chromaffin cells cosecrete 150mM ATP with other nucleotides,catecholamines and neuropeptides.We have shown that extracellular ATP can both enhance and inhibit catecholamine secretion,suggesting that it plays a transmitter role.Enhancement is blocked by pretreatment with cholera toxin (CTX) and inhibition by pretreatment with pertussis toxin (PTX).We are employing patch clamp techniques to determine whether ATP exerts this action on chromaffin cells via alterations in calcium entry mechanisms. Both low threshold,transient(T)and high threshold,long-lasting(L) voltage-dependent calcium currents can be recorded.ATP(10-100μM)has complex effects:in virtually all cells it inhibits the L current(but not T)by an average of 30%.This action is mimicked by ADP and blocked by PTX pretreatment.In 20% of these cells,ATP-induced inhibition is eclipsed by a rapid enhancement of inward holding current and a concomitant increase in the L component.These two enhancing effects desensitize during steady ATP application,uncovering the non-desensitizing inhibition of L current.Neither the increase in holding current nor the potentiation of calcium current is blocked by CTX pretreatment.Thus, the actions of ATP on secretion can,only in part,can be explained by a G-protein mediated modulation of calcium influx. (Work supported by PHS grants NS26606(EW) and NS16483(KD).

155.9

MODULATION OF Ca^{++} CHANNELS IN BOVINE ADRENAL CHROMAFFIN CELLS (BCCs). Y. Ohya and R.Y.K. Pun. Dept. of Physiology and Biophysics, University of Cincinnati, Cincinnati, OH 45267, and Department Internal Medicine, Kyushu University, Japan.

Biochemical and electrophysiological evidence indicate that L-type Ca^{++} channels are phosphorylated and modulated by protein kinases. We have studied the effects of the phorbol ester, 12-O-tetradecanoly phorbol-13-acetate (TPA), which activates protein kinase C, on the Ca^{++} channels in BCCs using both whole cell voltage clamp recording and single channel measurement. TPA (80-160 nM) increased the peak amplitude of whole cell Ca^{++} current and revealed a fast decaying component. Tail-current measurements with short voltage pulses showed that the conductance was enhanced. The inactive phorbol ester 4-α phorbol (1 μM) did not enhance the amplitude nor alter the decay. TPA had similar effects on Ba^{++} currents. Single channel measurements were performed with 100 mM Ba^{++} inside the recording pipette under cell attached mode. Inward currents with slope conductances of about 22 pS and 8 pS were recorded. Both channels had similar voltage dependency in activation and inactivation, and open time. Channel opening was prolonged by the agonist BAY K-8644 (1 μM) and was reduced by the antagonist nitrendipine (1 μM). TPA increased the probability of channel opening, prolonged the open time, and prolonged the channel burst duration. TPA also shifted the steady-state inactivation relationship to a more negative potential. The shift in inactivation relationship was similar to that observed for whole cell Ca^{++} current. Our results indicate that protein kinase C has a profound effect on Ca^{++} channels in BCCs. (This work is supported by NSF Grant DCB-8812562)

155.10

CALCIUM FLUXES IN ISOLATED BOVINE RETINAL CELLS. T.G.Ma*, M.Zhou*, R.D.Radtke* and M.T.Tseng. Departments of Anatomical Sciences and Neurobiology and of Ophthalmology and Vision Sciences, School of Medicine, University of Louisville, Louisville, KY 40292

Transmembrane movement of calcium is tightly regulated and failure of this regulation contributes to the calcium overload phenomenon observed in the ischemic tissues. We have previously examined the calcium flux in retinal P2 membrane fractions (Ma et al., J Cell Biol 109:334a, 1989) and now report investigations of whole cell preparations. Bovine retinas were digested with papain for 40 min at 37°C to yield >90% viable cells. Cells were incubated with calcium uptake modulators at 37°C for 20 min in the presence of [$^{45}Ca^{++}$]. Results demonstrated that Ca^{++} uptake was ATP dependent and inhibited by Ca^{++} channel blockers. Without exogenous ATP, Ca^{++} uptake was directly proportional to $[Ca^{++}]_o$. In the presence of 1 mM ATP, Ca^{++} uptake was affected by the varying $[Ca^{++}]_o$ in the following manner; a gradual decline between 0-1.25 mM $[Ca]_o$ and a mirrored increase at higher $[Ca^{++}]_o$ (2.5- 5.0 mM). Also, sodium orthovanadate inhibited and ouabain enhanced calcium influx. Verapamil and ketamine inhibited Ca^{++} uptake in a dose dependent manner. The result indicate that Ca^{++}-ATPase and calcium channels are functional and that isolated bovine retinal cells are useful for examining calcium flux in excitable tissues.

155.11

ADENOSINE ANTAGONISTS FAIL TO BLOCK THE ADENOSINE-INDUCED REDUCTION OF CALCIUM CURRENT IN CHICK SENSORY NEURONS. S.C. Nam, L. Yousif and P. E. Hockberger, Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

Adenosine analogues reduce calcium and potassium currents in several types of neurons presumably by acting through adenosine receptors (cf. Fredholm & Dunwiddie, Trends in Pharm. Sci., 9:130, 1988). We have investigated this possibility by testing the effects of several adenosine agonists and antagonists on the calcium currents recorded from acutely isolated chick DRG cells, as described elsewhere (Hockberger et al. Nature 338: 340, 1989). High-threshold (HT) currents were monitored during local application of agents via micropipet. The following agonists decreased the HT current in a dose-dependent and reversible manner: 2-chloro-adenosine (CA; 0.1μM reduced the current by 68% on average, n=8); N^6-cyclohexyl-adenosine (CHA; 0.1μM a 35% reduction, n=3); and SC-32796 (1 nM a 29% reduction, n=3). Likewise local application of adenosine antagonists also decreased the HT current in a dose-dependent and reversible manner: 8-phenyl-theophylline (PT; 1μM a 44% reduction, n=12); 8-cyclopentyl-theophylline (CPT; 0.5 μM a 43% reduction n=4), and 1,3-dipropyl-8-p-sulfophenyl-xanthine (DSPX; 1μM a 20% reduction, n=3). However, bath application of the antagonists, even at 10μM , did not affect the responses to the micropipet-applied agonists. These results suggest that the inhibitory effect of adenosine on calcium current in chick DRG cells is not mediated by adenosine (A1 or A2) receptors. This research was supported by NIH grant #NS-26915.

155.12

DOPAMINE INHIBITS VOLTAGE-ACTIVATED CALCIUM CURRENTS IN RAT NEUROINTERMEDIATE PITUITARY CELLS. I. Nussinovitch & A.L. Kleinhaus. Dept. of Anat. Heb.U.Hadassah Med. Sch., Jerusalem, Israel.

Dopamine, a potent inhibitor of MSH-secretion and of spontaneous action potentials in neurointermediate pituitary cells (melanotrophs), is thought to act in a way similar to that of calcium channel blockers (Douglas & Taraskevich 1982 J.Physiol.326). Therefore, we examined the effects of dopamine on voltage-gated calcium currents in dissociated rat melanotrophs. Calcium currents were recorded using the whole-cell mode of the patch-clamp technique with TTX in the bath solution and with N-methylglucamine as the main intracellular cation. We observed two types of calcium currents similar to the T and L types described elsewhere. The T-type current activated at membrane potentials between -50 to -40 mV and the L-type activated at membrane potentials between -30mV to -20mV. Dopamine (1-20μM), applied from a pipette, reversibly reduced the size of both T and L types of calcium currents by 20%-100%. These findings suggest that dopamine receptors are coupled to calcium channels in the melanotroph and that dopamine inhibits secretion in melanotrophs by decreasing calcium currents. Supported by the U.S-Israel BSF grant # 88-00347 to I.N. and the Berman Visiting fund to A.L.K.

155.13

TROPHIC EFFECTS OF NERVE GROWTH FACTOR (NGF) ON TETRODOTOXIN (TTX) AND CAPSAICIN SENSITIVITY IN ADULT SENSORY NEURONS. Luis G. Aguayo, Geoffrey White and Forrest F. Weight. Section of Electrophysiology, LPPS, NIAAA, Rockville, Md 20852.

Although NGF is critical for the survival of developing dorsal root ganglion (DRG) neurons, adult neurons can survive in the absence of NGF. We have investigated the effects of NGF (2.5 ng/ml for 1-2 weeks) on adult rat DRG neurons maintained in cell culture in defined-media without background cells. Whole-cell recordings revealed no significant difference in resting membrane potential and input resistance between cells cultured in the absence (NGF^-) and presence (NGF^+) of NGF. The sensitivity of the Na^+ spike to TTX (1 μM) was different in cells cultured in the absence or presence of NGF. Spikes were abolished by TTX in 100% of NGF^- cells (n=15), while in NGF^+ cells the spike was abolished in only 41% of the neurons (n=29) ($p<0.001$, chi-square, x^2). The threshold for spike generation was significantly lower in NGF^- cells than in NGF^+ cells, -25 ± 1.1 mV vs -18.9± 2.2 mV ($p<0.05$, ANOVA), respectively. Voltage clamp experiments revealed that the action potentials were predominantly carried by TTX-sensitive and TTX-resistant Na^+ currents in the absence and presence of NGF, respectively. Chemosensitivity of DRG neurons was also different in the absence and presence of NGF. For example, the percent of neurons in which capsaicin (500 μM) current was detected increased from 19% (n=16) in NGF^- cells to 55% (n=29) in NGF^+ cells ($p<0.05$, x^2).

The results suggest that NGF has trophic effects on voltage-dependent and chemosensitive properties in adult mammalian neurons.

155.14

UNSATURATED FATTY ACIDS MODULATE SODIUM CURRENT IN CULTURED SKELETAL MUSCLE. S.J.Wieland, J.E.Fletcher, Q.H.Gong* and H.Rosenberg*. Hahnemann Univ., Philadelphia, PA 19102

Fatty acids may act as second messengers in signalling pathways, or may interact directly with membrane-associated structures, including ion channels, altering their functional properties. Using the tight-seal whole-cell recording method, we monitored the differential effects of intracellular and extracellular application of 1-20 μM free fatty acids on cultured human skeletal muscle myocytes. Internal exposure to 5-15 μM arachidonic acid caused an increase (16-72%, mean= 41%, n=7, $p<.03$) in peak inward sodium current during voltage clamp steps from a holding potential of -100 mV. Intracellular oleic acid at 15 μM also increased the magnitude of inward currents. In contrast, external arachidonic acid (5-15 μM) reversibly inhibited inward currents 40-80%, while internally or externally applied stearic acid had no effect at up to 20 μM. Preliminary data indicates that cells from malignant-hyperthermia susceptible individuals show weaker sensitivity to internally applied arachidonic acid. Supported in part by MDA.

155.15

Ca^{2+} CHANNEL ANTAGONISTS REDUCE THE MAGNITUDE AND ALTER THE KINETICS OF M-CURRENT IN BULLFROG SYMPATHETIC B NEURONS. Y.-Y. Peng and J. P. Horn Department of Physiology, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

In bullfrog sympathetic B neurons Ach and LHRH each produces a slow epsp that is mediated primarily through suppression of M-current (I_M), a voltage- and time-dependent K^+ current. In preliminary experiments, we observed that nifedipine and ω-Conotoxin GVIA (ω-CgTx GVIA), antagonists of high-threshold Ca^{2+} channels, partially inhibited both the muscarinic and LHRH epsps, and also the responses to exogenous teleost and chicken II LHRH. We then investigated the effects of Ca^{2+} channel blockers by measuring I_M in B cells of isolated sympathetic ganglia using a single-electrode voltage clamp.

I_M was measured with 750 ms voltage steps to potentials between -30 and -90 mV from holding potentials of -30 to -50 mV. The slow current relaxations characteristic of I_M were reduced in amplitude, but never fully blocked, by bath application of nifedipine (1-10 μM) and ω-CgTx GVIA (3, 10 μM), and by switching to Ringer containing 0 Ca^{2+}, 10 mM Mg^{2+} and 0.1 mM EGTA. The effects of nifedipine were more pronounced at depolarized potentials whereas those of ω-CgTx GVIA and 0 Ca^{2+} were larger at hyperpolarized potentials. The effects of nifedipine and 0 Ca^{2+} were rapidly reversible while those of ω-CgTx GVIA were only partially reversed by several hours of washing. In addition to reducing its magnitude, Ringer containing either ω-CgTx GVIA or 0 Ca^{2+} altered the kinetics of I_M. Normally the time constant for deactivation of I_M decreases at hyperpolarized potentials. ω-CgTx GVIA and 0 Ca^{2+} reduced the voltage dependence of the time constant such that it resembled that seen at depolarized potentials.

These results demonstrate an interaction between voltage gated Ca^{2+} channels and the gating of M-current. Supported by NIMH training grant MH18273 (YYP) and NIH grants NS21065 and NS01427 (JPH).

155.16

TRH INHIBITS AN INWARD-RECTIFYING POTASSIUM CURRENT IN GH_3 CELLS. J.R. Schwarz* and C.K. Bauer* (SPON: European Neuroscience Association), Physiologisches Institut, Universitätskrankenhaus Eppendorf, D-2000 Hamburg 20, F.R.G.

Thyrotropin-releasing hormone (TRH) induces a biphasic prolactin secretion in clonal rat anterior pituitary cells (GH_3). The hyperpolarization accompanying the first phase of secretion is induced by an opening of Ca^{++}- dependent K^+ channels, whereas the depolarization and increase in repetitive firing during the second phase are assumed to be caused by a closing of K^+ channels. We now describe a K^+ current which is a likely candidate for mediating the second phase of secretion.

Whole-cell inward K^+ currents were elicited by hyperpolarizing pulses from a holding potential of -40 mV in isotonic KCl using the patch clamp technique. These K^+ currents showed time- and voltage-dependent inactivation at potentials more negative than -60 mV. Inactivation was faster and more complete at larger hyperpolarizations. The amplitude of inward K^+ current increased by 10-20% after depolarizing pre-pulses of 5 s indicating the presence of a steady inward K^+ current at -40 mV. The inward K^+ current was drastically reduced by Cs^+, Ba^{2+}, quinidine, 4-aminopyridine and TEA. TRH consistently reduced the inward K^+ current in the presence of internal Ca^{2+}. This reduction was abolished if the pipette solution contained GDPβS (400 μM), confirming the involvement of G-proteins in the signal transduction pathway.

In intact cells, closing of K^+ channels would result in a depolarization which could readily explain the TRH-induced increase in action potential firing underlying the sustained second phase of secretion.

155.17

COMPARISON OF GALANIN AND BETHANECHOL ACTIVATED POTASSIUM CURRENTS IN DISSOCIATED ATRIAL MYOCYTES OF THE MUDPUPPY. L.A. Merriam and R.L. Parsons. Dept. of Anatomy and Neurobiology, University of Vermont, Burlington, VT 05405.

Previously we demonstrated that a galanin-like neuropeptide is co-localized with acetylcholine in cardiac parasympathetic postganglionic neurons of the mudpuppy, Necturus maculosus (Parsons, et al., Neuroscience 29:749, 1989). Galanin and muscarinic agonists both initiate hyperpolarization of atrial myocytes (Merriam et al, Neurosci. Abstr. 15:219, 1989). The present studies utilized the whole cell patch clamp recording technique to analyze the currents underlying these hyperpolarizations. In myocytes clamped to -50 mV, galanin and the muscarinic agonist bethanechol produced outward currents that differed markedly in their time course characteristics. Bethanechol currents rose to peak more quickly and decayed much faster than galanin-induced currents. The responses to both agonists exhibited voltage-dependence and reversed at the same membrane potential, which was dependent upon external [K+] in accordance with the Nernst equation for a K+ conductance. Both currents exhibited rectification at membrane potentials more positive than the reversal potential. The bethanechol-induced current could be typically reproduced throughout recordings with little or no decrement, while the amplitude and duration of the galanin response often decayed with repetitive agonist application. This decay may suggest sensitivity to dialysis of some intracellular component. Results of initial experiments with the GTP binding protein activator GTP-γS and the inhibitor GDP-βS suggest a G protein is involved in the mediation of both responses.

This work is supported by PHS grants NS 25973 and NS 23798.

155.18

DUAL REGULATION OF A POTASSIUM CHANNEL BY G PROTEIN AND INOSITOL POLYPHOSPHATES. J.T. WU*, S.J. Wieland, O.H. Gong* and R.H. Chou*, Department of Anatomy, Hahnemann University, Philadelphia, PA.

Second messenger systems may modulate ionic channels through GTP-binding proteins, polyphosphoinositide metabolites and protein phosphorylation. The effects of various second messengers on the voltage-activated inward-rectifying K^+ current (I_{ki}) were examined in macrophages differentiated from the HL-60 promyelocytic leukemia cell line. The amplitude of I_{ki} in untreated cells remained stable during whole-cell voltage-clamp recording for up to 20 minutes. The presence of 100 uM guanosine 5'-[γ-thio] triphosphate (GTP[γS]) in the patch pipette solution inhibited I_{ki} beginning within 3 minutes and lasting as long as 20 minutes post-intracellular application. The effect of GTP[γS] on pertussis toxin-treated cells suggests that a pertussis toxin substrate is not involved in the suppression of I_{ki} in HL-60 macrophages. In contrast, 1uM of inositol-1,4,5-trisphosphate or inositol-1,3,4,5-tetrakisphosphate caused an increase in the same voltage-dependent K^+ conductance which was independent of a rise in intracellular Ca^{+2}. Hence the present findings demonstrate for the first time a dual modulation of a K^+ conductance by G protein and inositol polyphosphates.

Supported in part by MDA and by a Biomedical Research Support Grant.

155.19

CYCLIC AMP MODULATES A SLOWLY ACTIVATING K+ CURRENT EXPRESSED IN XENOPUS OOCYTES.
E. M. Blumenthal and L. K. Kaczmarek Dept. of Pharmacology, Yale University School of Medicine, New Haven CT 06510.

A cDNA cloned from rat kidney and uterus, termed I_{sk}, encodes a 15kDa protein with one transmembrane segment and induces a very slowly activating K+ current when expressed in *Xenopus laevis* oocytes (Takumi et al, Science, 1988; Pragnell et al, Neuron, 1990). Incubation of I_{sk}-expressing oocytes in 1 μM progesterone, which lowers intracellular cAMP, caused a 37% decrease in the slow K+ current along with a 7% decrease in membrane capacitance (Cm). Conversely, 1 mM 8-Bromo-cAMP increased both the current and Cm. The current increased up to 300% within 60 minutes of drug treatment while Cm increased up to 15%. Similar experiments were carried out with oocytes expressing both I_{sk} and Kv1, a delayed rectifier type K+ channel cloned from rat brain (Swanson et al, Neuron, 1990). The Kv1 current was unaffected by both drugs while I_{sk} and Cm responded as described above. Additional studies are being conducted to determine if these effects are the result of 1) a change in the number of I_{sk} channels present in the plasma membrane through selective endocytosis and exocytosis, or 2) modification of existing channels.

156.1

PHYSIOLOGICALLY LOW LEVELS OF ADP REVERSE THE INHIBITION OF ATP-SENSITIVE K CHANNELS BY ATP.
S. Fatherazi, D.L. Cook*, K. Tornheim*^ and B.E. Corkey*^ Seattle VA Med Ctr and Dept of Physiol/Biophys, U. of Washington, Seattle, WA 98108 and ^Diabetes/Metab. Div., Boston U., Boston MA 02118.

Both ATP (Kd≈15 μM) and ten-fold higher doses of ADP (Kd≈150 μM) block ATP-sensitive K channels (KATP) in pancreatic B-cells. Paradoxically, a high level (2 mM) of ADP shifts the ATP-dose response to the right suggesting ATP-ADP competition and implicating ADP in the regulation of KATP activity (and electrical activity) in B-cells. The physiological interpretation of this ADP effect is complicated because steady-state free cytosolic ADP levels are generally only 20-50 μM and thus may not compete effectively with the mM ATP levels in cells. To test this, we exposed KATP channels in inside/out patches from cultured mouse B-cells to a control (0 ATP, 0 ADP) buffer and to ≈3.7 mM ATP with ADP levels fixed using creatine phosphokinase with different ratios of creatine/creatine phosphate (total = 12 mM). KATP activity in control buffer was maximal (NPo = 100%). Low ADP (≈ 18 μM; ATP/ADP ≈ 220) completely suppressed channel activity (NPo = 0%), as expected with ATP alone. In high ADP (≈ 500 μM; ATP/ADP ≈ 7) the ATP block was significantly relieved (NPo = 15% and 25% in 5 trials in 2 patches).

Quantitatively, this increase of KATP activity is significant because KATP channels completely determine the resting membrane potential of B-cells and, in physiological conditions, are only 1-2% active. Changes of intracellular free ADP may contribute to the regulation of B-cell electrical activity. The mechanism of interaction of ATP and ADP remains to be determined.

156.2

ATP SENSITIVE K+ CHANNELS MODULATE SYNAPTIC TRANSMISSION IN THE HIPPOCAMPAL SLICE. Y. Ben-Ari, V. Crépel, S. Zini and D. Hazboun. INSERM U29, 123 Bd Port-Royal, 75014 Paris, France.

We have recently shown that peripheral activators of ATP sensitive K+ channels (K+ATP) such as the vasodilator drug diazoxide (Ben-Ari et al. 1990, Neurosci. in press) reduce the enhanced glutamate release observed in the CA3 region during anoxic episodes. The selective K+ ATP blocker glibenclamide has an opposite effect (Ben-Ari 1990, Eur. J. Neurosci. 2,62). We have now examined the effects of these drugs on synaptic responses.

In normoxic conditions bath applications of diazoxide (650 uM) increased the amplitude and duration of the field EPSP in CA1. In CA3 the EPSPs were increased in duration and associated with interictal bursts similar to those evoked by convulsive agents. Glibenclamide (10 uM) usually had no effect on synaptic responses in CA1 and CA3 ; it did however occasionally generate paroxysmal activity, in particular in slices subjected to repeated brief hypoxic episodes. K+ ATP activators or antagonists had little effect on resting membrane potential or input resistance of CA1 and CA3 neurons, particularly in presence of TTX or the broad spectrum excitatory amino acid antagonist kynurenate (1 mM). In preliminary experiments using hippocampal synaptosomal preparations, glibenclamide enhanced the ongoing release of endogenous glutamate. We propose that transmitter release is modulated by K+ ATP channels, with consequences that depend on the metabolic status of the circuit. The enhanced synaptic responses caused by diazoxide may be in part due to a reduction of GABA release (Abst. Crépel).

156.3

EFFECTS OF POTASSIUM CHANNEL OPENERS ON TRANSMISSION AT THE FROG MUSCLE ENDPLATE. Eugene W. Shek, Lian-Ming Tian and Karim A. Alkadhi, Department of Pharmacology, College of Pharmacy, University of Houston, Houston, TX 77204-5515.

The ATP-regulated K^+ channels are known to exist in high density in skeletal muscle fibers. These channels which also exist in cardiac and smooth muscles as well as in ß-cells of islets of Langerhans, are shut under normal circumstances but open when ATP in the cell falls below the normal level. However, the function of these ATP-regulated K^+ channels is not clear especially in skeletal muscle where the ATP level is high and well maintained. In this preliminary study we investigated the effects of a new class of "K^+ channel openers" on the parameters of release at the frog endplate. Conventional intracellular recording was used to study the effects of cromakalim (CKM) and RP52891 on evoked and spontaneous transmitter release. Neither cromakalim (0.1 - 0.5 mM) nor RP 52891 (0.1 mM) caused any significant change in the resting membrane potential when superfused for 1 hr (control: -92±2; CKM 0.3 mM; 1 hr: -91± 6, n=6). No significant effect on MEPP frequency was observed. However, the drugs caused a marked decrease in EPP amplitude (control 7.6±1 mV; CKM 0.3 mM, 1 hr: 3.3±1 mV, n=6). The MEPP amplitude was also decreased but to a lesser extent (control quantal content, m=13±3; after CKM, m=8±3). Pretreatment with the ATP-regulated K^+ channel blocker glibenclamide (0.3 mM) did not antagonize these effects. It is concluded that these agents have postjunctional effects at the endplate unrelated to K^+ channel opening.

156.4

CHARACTERIZATION OF POTASSIUM CURRENTS ACTIVATED BY CROMAKALIM IN CULTURED RAT HIPPOCAMPAL NEURONS. Dora M. Politi, Susan M. Jones and Michael A. Rogawski. Neuronal Excitability Section, Medical Neurology Branch, NINDS, NIH, Bethesda, MD 20892.

Previous studies in our laboratory have shown that cromakalim activates a tetraethylammonium (TEA)-sensitive, voltage-dependent K^+ current in cultured embryonic rat hippocampal neurons (*Eur. J. Pharmacol.* **168**: 7, 1989). This phenomenon was further characterized using whole-cell voltage-clamp and single-channel recording techniques. Glyburide (1-25 μM), an antagonist of ATP-dependent K^+ channels, produced a slow concentration-dependent depression of the cromakalim-activated current, whereas charybdotoxin (100 nM; CTX), a blocker of some Ca^{2+}-dependent and Ca^{2+}-independent K^+ channels was inactive. Neither glyburide nor CTX affected resting or voltage-activated K^+ currents in the absence of cromakalim. Single channel recordings in the cell attached configuration showed that cromakalim (100 μM) activated flickery channels that have a single channel conductance of 15-30 pS. In the presence of cromakalim, the open state probability of the channels was typically >0.8-0.9 at depolarized potentials; channel openings were not observed in the absence of cromakalim. These data are compatible with an interaction of cromakalim with ATP-dependent K^+ channels.

156.5

IS THE ENHANCEMENT OF HIPPOCAMPAL EPSPs BY DIAZOXIDE CAUSED BY DISINHIBITION? V. Crépel, K. Krnjevic*, Y. Ben-Ari and A.T. Tan*. INSERM U29, Hôpital Port-Royal, 75014, Paris, France and *Anaesthesia Research Dept., McGill University, Montréal, H3G 1Y6, Canada.

Selective activation of ATP-sensitive K-channels suppresses glutamate release in the CA3 region of hippocampal slices (Ben-Ari, 1990, Eur. J. Neurosci. 2: 62). However, as described in another abstract (Ben-Ari et al.), diazoxide potentiates EPSPs in both CA1 and CA3; in the latter, it can generate seizure activity. A simple explanation is that diazoxide blocks synaptic inhibition. To test this possibility, we examined the effects of diazoxide after blocking GABA receptors.

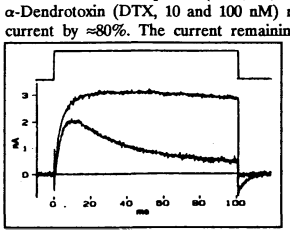
EPSP fields were recorded simultaneously from the apical dendritic regions of CA1 and CA3 - separated by cutting the slice. In ACSF with 6 mM Mg^{2+} and 4 mM Ca^{2+} (to avoid seizure activity), diazoxide (0.65 mM) prolonged EPSPs equally in CA1 and CA3 (~ 30%) and increased their height by 9-12%. After treatment with bicuculline (10 μM, n=7), the increase in height was abolished in CA3, but unchanged in CA1. Conversely, the change in EPSP duration was reduced in CA1, but not CA3. The addition of phaclofen (0.5 mM, n=3) caused no further suppression of diazoxide effects.

We conclude that disinhibition can only partly explain the effects of diazoxide, and that EPSPs are facilitated by another mechanism, perhaps as a result of depression of on-going glutamate release or a block of K channels (cf. Kozlowski et al. 1989, Br. J. Pharmacol. 97, 1039).

156.6

BLOCKING POTASSIUM CURRENTS IN CHICK DORSAL ROOT GANGLION (DRG) NEURONS. J. L. Kenyon, C. D. Westbrook, and M. L. Hall*. Department of Physiology, Univ. of Nevada Sch. of Med., Reno, NV 89557.

We are investigating the development of neurons with differing K currents in chick DRGs and we tested low concentrations of K channel blockers in order to classify voltage-gated K currents. Neurons were isolated from 10 day old chick embryos and voltage-clamped within 12 hours. They were bathed in Ca-free physiological saline with TTX to inhibit inward and Ca-activated currents. K channel blockers were applied via a puffer pipette. K currents were activated by 100 ms depolarizations positive to -40 mV. Control currents were dominated by sustained current components. TEA (20 mM) rapidly and reversibly reduced K current amplitude by ≈80%. 3,4-diaminopyridine (50 μM) reversibly reduced K current amplitude by ≈50% over several minutes. For both agents, the insensitive current looked like a small version of control. β-Bungarotoxin (100 μM) did not reduce K currents. Capsaicin (CAP, 50 μM) reduced late K current by 20 - 50%. α-Dendrotoxin (DTX, 10 and 100 nM) rapidly and reversibly reduced late K current by ≈80%. The current remaining in CAP and DTX increased then decreased during the step (figure) while the toxin-sensitive current was sustained. The figure shows currents elicited by steps from -95 to +30 mV in control (larger current) and in 10 nM DTX. Thus, all effective blockers reduced sustained currents while only CAP and DTX revealed transient current components. Supported by AHA 87-648.



156.7

Effects of various potassium channel blockers on inositol phosphate accumulation in rat striatal slices. R.Levent Buyukuysal and Richard J.Wurtman. Massachusetts Institute of Technology, Department of Brain and Cognitive Sciences, Cambridge MA, USA.

The effects of various potassium channel blockers on inositol phosphate (IP) turnover were tested using cross-chopped striatal slices. The following blockers were tested: 3,4-diaminopyridine (DAP), phalloidine (Phl), dendrodotoxin (Ddx), charybdotoxin (Chx), apamin (Apm), tetrahydroaminoacridine (THA), quinine (Quin) and tetraethylammonium (TEA). All blockers except Phl, Apm and TEA increased IP accumulation in a dose dependent manner. DAP, Chx and Ddx were effective in a Ca^{+2}-free medium. In contrast, THA and Quin decreased IP accumulation in Ca^{+2}-free medium. The DAP and Chx-induced increases were sensitive to the sodium channel blocker tetrodotoxin, while the THA, Quin and Ddx-induced increases were not. The effects of DAP and of low concentrations of THA were partially blocked, and that of Ddx abolished, by atropine. In eserine-containing medium, DAP and Chx further increased IP accumulation, but THA, Quin, Ddx and TEA decreased it. Moreover THA, Quin and Ddx (but not DAP and Chx) decreased carbachol-induced IP accumulation. These results indicate that although blockade of certain potassium channels can enhance the production of second messengers and potentiate postsynaptic events, most of the above blockers appear to have additional effects (e.g., on muscarinic receptors or on calcium channels).

Supported in part by a grant from NIMH.

156.8

INHIBITION OF OUTWARD CURRENTS IN DORSAL ROOT GANGLION CELLS BY DENDROTOXIN HOMOLOGUES. [Ω]D. G. Owen, [Θ]A. Hall*, [♣]R. G. Sorensen & [Ω]J. Stow*. [Ω]Wyeth Research (UK), Taplow, UK; [Θ]Dept. Biochemistry, Imperial College London, UK; [♣]Dept. Medicine, Jefferson Medical College, Philadelphia, USA.

α-dendrotoxin (α-dtx), a polypeptide fraction of Green Mamba venom, and Toxin I, a structurally very similar toxin obtained from Black Mamba venom, are known to facilitate transmitter release, most likely through inhibition of a voltage-activated transient outward (potassium) current (TOC) (see Harvey & Anderson, 1986). We have compared the effects of the peptide homologues (*ex* green mamba), β_1-dtx (1μM), γ-dtx (1μM) and δ-dtx (1μM), with α-dtx (100nM) and Toxin I (100nM) on outward currents in rat dorsal root ganglion (DRG) neurones.

DRGs were obtained from rat pups (neonates to *ca.* 4 days) and cultured together with glia and glial conditioned medium (*ex* C_6 glioma cells). Voltage clamp recordings were made 4-8 days post-plating using a switching voltage clamp amplifier and patch electrodes (5-7MΩ) filled with (mM): 140 KGluconate; 1 $MgCl_2$; 1.1 EGTA/KOH; 5 HEPES; pH 7.2. Bathing solution consisted of 140 TrisCl; 4$MgCl_2$; 5 HEPES; 10 glucose; 20 sucrose; pH 7.4. All the homologues reduced voltage-activated outward current in a sub-population of DRG neurones. Inhibition by Toxin I, β_1-dtx and γ-dtx was indistinguishable in a given neurone, blocking both sustained outward current (I_K) and slowly-inactivating TOC. δ-dtx blocked I_K preferentially, in contrast to α-dtx which was more selective for TOC whereas, in the same cells, Toxin I blocked TOC and I_K with little discrimination.

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156.9

REGULATION OF THE CA^{++} SENSITIVITY OF K(CA) CHANNELS BY PROTEIN PHOSPHORYLATION IN A DRG NEURONAL CELL LINE. K. Naruse* and G.S. Oxford. Department of Physiology, University of North Carolina, Chapel Hill, NC 27599.

F-11 cells, a dorsal root ganglion × neuroblastoma hybrid cell line can be cultured under conditions that promote expression of neuronal morphology. Single Ca^{++}-activated K(Ca) channels (≈300pS) in inside-out membrane patches excised from such "differentiated" cells exhibit a higher Ca^{++} sensitivity than in excised patches from cells grown in normal growth media or from cultured rat embryonic DRG neurons (Naruse et al., 1990). As the differentiation of F-11 cells is, in part, triggered by agents that promote intracellular cAMP levels (e.g. dibutryl cAMP, IBMX), we examined the possibility that Ca^{++} sensitivity of K(Ca) channels is regulated by the activity of cAMP-dependent protein kinase (PK-A).

To test this hypothesis, we (1) acutely incubated undifferentiated F-11 cells with forskolin or dibutyrl cAMP to increase the activity of endogenous PK-A, or (2) exposed excised inside-out membrane patches to the purified catalytic subunit of PK-A from bovine heart. Using only patches with single K(Ca) channels, open probabilities were computed at several membrane voltages and buffered [Ca^{++}]. Both endogenous and exogenous PK-A increased the activity of K(Ca) channels from the undifferentiated cells. The effects were most pronounced at [Ca^{++}] of 0.5 - 1.0μM.

Our results suggest that the Ca^{++} sensitivity of neuronal K(Ca) channels is regulated by PK-A activity with possible consequences for excitability during transmitter induced changes of cellular cAMP levels. Supported by NIH grant NS23804

156.10

SUBCONDUCTANCE BEHAVIOR IN LARGE CONDUCTANCE CA^{2+}-ACTIVATED K^+ CHANNELS INDUCED BY BOVINE PANCREATIC TRYPSIN INHIBITOR, BPTI. K. J. Lucchesi* and E. G. Moczydlowski, Dept. Pharmacology, Yale Univ. Sch. Med., New Haven, CT, 06510.

Kunitz protease inhibitors are ≈ 60-residue peptides homologous to mamba snake dendrotoxins (DTX) that block certain voltage-dependent K^+ channels. We previously observed that DTX-I induces long-lived subconductance events in maxi-K(Ca) channels (Neuron, 2: 141-148, 1990). To study structure-activity relationships for this inhibition, we tested BPTI, a well known trypsin inhibitor and model peptide for investigations of protein folding. Like DTX-I, BPTI produced subconductance events by a one-site binding reaction at the inside surface of single K(Ca) channels from rat skeletal muscle incorporated into planar lipid bilayers (Kd = 4.9 μM in 50 mM symmetrical KCl). Several differences between the action of BPTI and DTX-I are noteworthy. The current amplitude of the BPTI-induced substate is 1.2 ± 0.1 pA at + 20 mV, significantly below the 2.8 pA substate seen in the presence of DTX-I. BPTI substates also undergo stronger inward rectification at positive voltages than those induced by DTX-I. Substate lifetimes in the presence of BPTI are much shorter than those produced by DTX-I, yielding respective koff's of 3.7 sec^{-1} and 0.027 sec^{-1}. Apparent association rate constants of both BPTI and DTX-I are increased by depolarizing voltage and are dramatically reduced at high ionic strength. To probe the mechanism of peptide induced substates, we tested the possibility of binding competition with TEA and Ba^{2+}, two internal K(Ca) channel blockers. We found that internal TEA blocks substates and open states with equal affinity (Kd = 37 mM); blocking/unblocking rates of internal Ba^{2+} are virtually unaffected by the presence of up to 100 μM BPTI. These results suggest that the site of peptide-inhibitor binding does not lie within the internal channel vestibule. BPTI may affect K^+ permeation by an allosteric mechanism. Supported by NIH AR38796 and HL38156.

156.11

OPEN CHANNEL NOISE OF SUBCONDUCTANCE STATES. M.I. Glavinovic, Dept. Anaesthesia Research, Physiology and Biomedical Engineering, McGill University, Montreal, Quebec, Canada.

Subconductance states within the main open state have been reported for a variety of channels (Barrett et al., J. Physiol., 331, 211-230, 1982). They are usually explained by the partial occlusion of the open channel. Alternatively they may represent a presence of parallel conductive subunits (Hunter and Giebisch, Nature, 327, 522-524,1987). Resolution of this problem is of considerable importance for understanding of the molecular structure and permeation of ion channels. To resolve it the open channel current noise of different current sublevels was examined.

In Ca^{++} activated K^+ 'maxi' channels of bovine chromaffin cells the variances of current fluctuations through the subconductance states were as a rule greater and more variable than the variances of the main open state. No simple relationship between the variances and the current amplitudes of sublevels was obvious. In contrast, when several channels were simultaneously open the variances of the current fluctuations increased in an apparently linear manner as more channels were open. This argues against the idea that the main open state is a result of simultaneous opening of several conductive subunits, although it does not eliminate it completely. Opening of all subunits may somehow 'stabilize' the channel segments that are the noise sources of the current sublevels. Supported by MRC (Canada).

156.12

A NOVEL K^+ CONDUCTANCE OF RAT MOTONEURONES IS BLOCKED BY TRH. A. Nistri, N.D. Fisher* and M. Gurnell*, Pharmacology Dept., St. Bartholomew's Hospital Medical College, London EC1M 6BQ, U.K.

The excitatory action of the neuropeptide TRH was studied on motoneurones of the neonatal rat spinal cord *in vitro*. Cells were voltage clamped with a single KCl or CsCl electrode in the presence of TTX at room temperature. Fast superfusion with TRH (1 μM) elicited, with a 2-4 min latency, an inward current (I_{TRH}) with a slow offset (> 10 min). At -70 mV I_{TRH} was -0.59 ± 0.06 nA with a 28 ± 5% fall in slope conductance (n=12). I_{TRH} reversed near -100 mV and peaked at -55 mV. In 13.5 mM K^+ I_{TRH} reversal and peak underwent a positive shift. In the presence of TRH the threshold for voltage sensitive Ca^{2+} currents was reduced and slow oscillatory currents appeared, while -10 to -30 mV hyperpolarizing steps induced an instantaneous current (smaller than control) which relaxed outwardly to steady state level within 150 ms (no such relaxation was found in control solution). I_{TRH} was not blocked by TEA (20 mM), 4AP (1 mM), Cs^+ (2 mM or injected intracellulary) or Cd^{2+} (0.2 mM) but was depressed by Ba^{2+} (0.2 - 1.5 mM). It is suggested that TRH blocks (perhaps via internal messengers) a Ba^{2+} sensitive K^+ conductance active at resting potential and apparently distinct from previously reported K^+ currents of mammalian central neurones. Supported by Cyanamid-Takeda, NATO and an Alliance grant.

156.13

EFFECTS OF INTRACELLULAR GDPβS ON THE RESPONSES TO LHRH, MUSCARINE AND SUBSTANCE P IN BULLFROG SYMPATHETIC NEURONS Mark A. Simmons & Robert J. Mather* Neuropharmacology Lab Dept. Pharmacol., Marshall Univ., Huntington, WV 25755

In sympathetic neurons, LHRH, muscarine, and substance P (SP) all inhibit the M current (I_M). GDPβS was applied intracellularly to determine the interactions between the three types of receptors, G proteins and I_M. Whole cell recordings were made from enzymatically dissociated neurons. The inhibition of I_M by agonists was dependent upon the ratio of GDPβS:GTP in the pipette solution. At 10:1 GDPβS:GTP, agonist inhibition of I_M was either small or absent. At 2:1 GDPβS:GTP the responses to agonists appeared normal. When the GDPβS:GTP ratio was 4:1, the responses to agonists decreased with consecutive agonist applications. The response to SP was almost completely blocked after three SP applications, but LHRH or muscarine was still capable of inhibiting I_M. When LHRH was applied first, successive responses diminished; however, SP or muscarine was still effective in inhibiting I_M. Successive responses to muscarine were less sensitive than the peptide responses to inhibition by GDPβS.

These results suggest that each type of receptor is associated with a distinct population of G proteins. It is not clear whether this represents a diversity in the molecular structure of the separate G proteins or reflects a physical compartmentation of the G protein populations activated by the different receptors.

156.14

SOMATOSTATIN, OPIOIDS AND α_2 RECEPTOR AGONISTS INCREASE ACTIVITY OF POTASSIUM CHANNELS AND DECREASE ACTIVITY OF CALCIUM CHANNELS IN EXCISED MEMBRANE PATCHES. K-Z. Shen, A. Surprenant and R.A. North. Vollum Institute, Oregon Hlth. Sci. Uni., Portland, OR 97201.

Outside-out recordings were made from neurons enzymatically dissociated from guinea pig submucous plexus. Several potassium channels of different conductances were active, and the unitary currents reversed polarity at potentials appropriate to the Nernst equation when external potassium was 10, 25, 55, 105 and 160 mM. Noradrenaline (0.1-10 μM), the selective α_2-adrenoceptor agonist UK14304 (60-400 nM), somatostatin (40-200 nM) and [Met^5]enkephalin (2-10 μM) increased potassium channel activities, and these effects were blocked by appropriate antagonists (idazoxan and naloxone). All agonists were effective when applied sequentially to the same patch. Quantitative analysis was difficult in patches with channels of several conductances; however, for the largest conductance channel (about 200 pS in equal 160 mM potassium) agonists prolonged burst duration. Agonist actions were mimicked by including GTP-γ-S (500 uM) in the electrode, and prevented by GDP-β-S (500 uM). With external $BaCl_2$ (110 mM) depolarization of the patch opened unitary N-like calcium channels: openings were much reduced by cadmium (100 μM) and significantly decreased by the three agonists. The same receptors couple to several potassium channels as well as a calcium channel in isolated membrane patches.

156.15

SPONTANEOUS FIRING IN SQUID AXONS INDUCED BY CHANGES IN INTRACELLULAR pH. John R. Clay*, Vijay Kowtha*, and Keith E. Krebs, Lab of Biophysics, NINDS, NIH, Bethesda, MD 20892, and Marine Biology Lab, Woods Hole, MA 02543.

We have observed automaticity (firing frequency ~ 20 Hz) from squid giant axons internally perfused with solutions having a pH > 8.5. T = 12° C. The effect is robust and reproducible, lasting in some preparations for as long as 45 min. It is associated with a hyperpolarizing influence on the membrane potential, inasmuch as the recovery of the membrane potential from the foot of the action potential (AP) is slower during automaticity compared to the recovery of the membrane potential toward rest in control (pH_i = 7) following an elicited AP. Moreover, the resting potential is hyperpolarized by ~ 5 mV with a change of pH_i from 7 to 9 with TTX seawater. Voltage clamp measurements revealed a lack of effect of a change in pH_i ($7<pH_i<9$) on I_{Na}. The delayed rectifier is modified in this pH range by a shift of its inactivation curve along the voltage axis in the positive direction as the pH is made basic. This effect produces a hyperpolarizing influence, theoretically, but it is insufficient to explain the excitability and resting potential results. A change in pH_i from 7 to 9 appears to reduce the leak conductance, an effect which may be sufficient to explain these observations. The proposed mechanism underlying automaticity is a reduction of I_{Na} inactivation by this hyperpolarization, thereby increasing the amount of inward current available to take the membrane potential beyond threshold following recovery from the foot of the previous AP.

PEPTIDES: ANATOMICAL LOCALIZATION I

157.1

DISTRIBUTION OF PREPRO-MELANIN-CONCENTRATING HORMONE-DERIVED PEPTIDES AND mRNA IN THE RAT BRAIN. J.C. Bittencourt, F. Presse*, J.L. Nahon*, C.A. Peto*, W. Vale and P.E. Sawchenko. The Salk Institute, La Jolla, CA 92037.

In addition to a nonadecapeptide homologous to the teleost melanin-concentrating hormone (MCH), the amino acid sequence predicted from a rat prepro-MCH cDNA (Endocrinology 125:2056, 1989) suggested that at least one (neuropeptide EI, or NEI), and possibly a second (NGE), neuropeptide may be encoded by this precursor. Cross-reactivity with epitopes of NEI or NGE can account for reported localization of α-MSH, rat CRF and human GRF in rat dorsolateral hypothalamic neurons. We have used antisera raised against rat MCH and NEI in immunohistochemical studies at the light and EM levels, along with hybridization histochemical localization of prepro-MCH mRNA, to define the organization of this system. As expected, prepro-MCH mRNA is prominently expressed in cells in the lateral hypothalamic area and zona incerta. In addition, smaller cell groups in the olfactory tubercle and pontine tegmentum were also positively hybridized using cRNA probes. The MCH and NEI peptides were extensively colocalized in neurons in each of these areas. Fibers stained for MCH and NEI were similarly and broadly distributed throughout the CNS in patterns that generally conformed with known projection fields of the lateral hypothalamic area and zona incerta. A differential distribution was seen in at least one region, the interanterodorsal nucleus of the thalamus, which contained a prominent terminal field stained for MCH but not NEI. At the EM level, a subset of MCH stained perikarya displayed prominent staining associated with the Golgi apparatus; this was not encountered in NEI stained cells. Both peptides were localized in terminals in the septal nuclei, lateral hypothalamic area and median eminence, with staining associated principally with dense-cored vesicles.

157.2

LOCALIZATION OF ATRIAL NATRIURETIC FACTOR-LIKE mRNA IN THE RAT BRAIN BY IN SITU HYBRIDIZATION. F. Riftina and B.S. McEwen. Lab of Neuroendocrinology, The Rockefeller University, New York, NY 10021

Atrial natriuretic factor (ANF) is a 28 amino acid peptide that is proteolytically released from the carboxy-terminal end of a larger (126 aa) precursor. ANF released from the atrium of the heart exerts effects on the regulation of water and salt balance. ANF-like immunoreactivity has been shown to be present in the brain where it is implicated in the central regulation of blood-pressure homeostasis, and control of salt appetite. In our experiments, a synthetic oligonucleotide probe complementary to the part of the carboxy-terminal region of the rat ANF (amino acids 107-116) was used to study the distribution of ANF-like mRNA in the rat brain. Perikarya containing ANF-like mRNA were detected in the cortex, septum, hippocampus, preoptic area, thalamus and hypothalamus.

This work was supported by the NIH Grant 5T32 DK 07313-12 and NIMH Grant MH 43787.

157.3

FLFQPQRF-AMIDE-LIKE PEPTIDES IN RAT CNS: ONTOGENY, ORGANIZATION OF NEURONAL SYSTEMS, AND RELATIONS TO OTHER NEUROPEPTIDES. L. Kivipelto and P. Panula. Dept. Anatomy, Univ. of Helsinki, 00170 Helsinki, Finland.

FLFQPQRFamide (F8Fa), isolated from bovine brain, shares the carboxy terminus with the molluscan cardioexcitatory peptide FMRFamide. We used immunohistochemistry and retrograde tract tracing method to study the neuroanatomy of the F8Fa-immunoreactive (ir) system in rat CNS.

Intraventricular and intraspinal colchicine treatment was applied to visualize F8Fa-ir perikarya. To trace F8Fa-ir fiber connections retrograde tracer True Blue® was injected into F8Fa-ir terminal areas. Rats of different ages were studied to establish the ontogeny of F8Fa-like immunoreactivity (LI) containing system. Light microscopic mirror method was applied in a comparative study with other peptides. Tissues were fixed with 4% paraformaldehyde.

F8Fa-ir cells of periventricular hypothalamus projected to the nucleus of the solitary tract (Sol). Neurons of Sol projected within the same nucleus, to periambigual area and lateral parabrachial nucleus. The spinal F8Fa-LI was mainly of intrinsic origin. The spinal F8Fa-ir cells were located in laminae I, II, X and in the dorsal gray commissure of sacral cord. Fibers and terminals were found in laminae I, II, X, intermediolateral cell column and sacral parasympathetic nucleus. No F8Fa-LI was detected before embryonal day 20 when terminals were detected in median eminence. After that the F8Fa-ir system developed very rapidly and reached its adultlike form by the end of fourth postnatal week. F8Fa-ir system appeared to be anatomically distinct from enkephalin and neuropeptide Y-ir systems, although some overlapping of terminal fields was observed, and coexistence of F8Fa and NPY-LI was detected in a small group of hypothalamic neurons.

The F8Fa-ir system appears to be distinct from any previously described peptide system. Our results show a perinatal development of the F8Fa-ir structures in rat CNS suggesting a neurotransmitter role of these peptides. These anatomical studies further support the concept that F8Fa-like peptides are involved in the regulation of nociception and autonomic and endocrine functions.

157.4

LOCALIZATION OF DIAZEPAM BINDING INHIBITOR (DBI) IN THE RAT BRAIN AND PITUITARY BY HIGH RESOLUTION IN SITU HYBRIDIZATION. Y. Tong*, E. Rhéaume*, J. Simard*, A. Dupont and G. Pelletier. MRC Group in Molecular Endocrinology, CHUL Research Centre, Quebec, Canada.

An endogenous polypeptide which exhibits high affinity for brain benzodiazepine (BDZ) receptors has been recently been isolated and characterized. It has been named diazepam binding inhibitor (DBI). Using antibodies against an octadecaneuropeptide (ODN) derived from DBI, we have very recently localized this peptide in glial cells of the rat brain and pituicytes of the rat posterior pituitary. In order to determine whether or not these non-neuronal cells are involved in DBI biosynthesis, high resolution in situ hybridization was performed using [^{35}S]labeled antisense RNA as well as control sense RNA (plasmid supplied by Dr. H. Tiedge). Hybridization signal was observed in both semithin (1 µm) and ultrathin sections (0.1 µM) in the hypothalamus, cerebral cortex, cerebellum and posterior pituitary. In the different brain areas, specific labeling was exclusively observed in astrocytes and ependymal cells. In the posterior pituitary, only pituicytes were labeled. These results clearly indicate that DBI is synthesized in non-neuronal cells in the rat brain and posterior pituitary. The present findings together with previous results indicating the presence of BZD receptors in glial cells suggest that DBI may play an important role in glial cell function.

157.5

DISTRIBUTION OF SOMATOSTATIN-IMMUNOREACTIVE CELL BODIES AND PROCESSES IN THE TELENCEPHALON OF *MACACA FUSCATA*. S. Iritani* and K. Satoh. Department of Psychiatry, Shiga University of Medical Sciences, Otsu 520-21, Japan.

Distribution of somatostatin-immunoreactive cell bodies and processes was studied in the telencephalon of the macaque monkey (*Macaca fuscata*), by applying an immunohistochemical technique using a monoclonal antibody raised against somatostatin tetradecapeptide. Many somatostatin-immunoreactive cell bodies and processes were observed in all regions of the cerebral cortex. The cell bodies were distributed in layers II to VI of the neocortex, and also in the underlying white matter. Three types of somatostatin-containing cell bodies were distinguished in the cerebral cortex. A large number of somatostain-containing cell bodies and processes were observed throughout the hippocampal formation, including the subiculum. The regional distribution of somatostatin-immunoreactive puncta was most dense in the molecular layer of the dentate gyrus. Many somatostatin-containing cell bodies were observed in the stratum oriens of CA1 and CA2 and in the field of CA4. In the entorhinal cortex, the distribution pattern of somatostatin-containing cell bodies and fibers was similar to that of the neocortex. Within the amygdaloid nuclei, a dense distribution of immunoreactive cell bodies and fibers were observed in the central nucleus. The present observations demonstrate that somatostatin-containing neuronal systems are highly developed in the forebrain limbic structures of the primate.

157.6

SOMATOSTATIN IN PRIMATE DIENCEPHALON. C. Desjardins* and A. Parent. Lab. of Neurobiol., Laval Univ., Québec, Canada.

As part of a research program designed to investigate the anatomical and functional organization of somatostatin (SS) neuronal systems in primate forebrain, we studied the distribution of SS immunoreactivity in the diencephalon of squirrel monkeys (*Saimiri sciureus*) with antibodies SS 309 and SS 320 (R. Benoit). In **thalamus** dense plexuses of SS fibers were noted in medial pulvinar, medial habenula, and causal portions of mediodorsal nucleus. Other fibers and terminals were scattered in midline nuclei, centralis medialis, subparafascicularis, centralis superior lateralis and lateral habenular nuclei. Weakly stained cells occurred in rostral and caudal poles of reticular nucleus and in centralis medialis, mediodorsal, centralis superior lateralis and laterodorsal nuclei. In **subthalamic region** a dense fiber plexus capped the dorsolateral pole of subthalamic nucleus (STH), whereas few fibers occurred in ventral part of zona incerta and in ventromedial portion of STH. In **hypothalamus** very dense fiber plexuses were noted in medial preoptic area, suprachiasmatic nucleus, around central core of ventromedial nucleus, in arcuate nucleus and median eminence. Other fibers were scattered in lateral area, periventricular gray, and supramammillary area. Multipolar SS cells were noted in median anterior area, paraventricular nucleus, at the periphery of ventromedial nucleus and in lateral area. Large SS fibers of the "wooly" type coursed along the dorsal surface of the optic tract in lateral hypothalamus. These fibers were morphologically similar to and continuous with the abundant wooly fibers in substantia innominata and amygdala. These results suggest that SS mediates some of the complex interactions that occur between hypothalamus and amygdala.

157.7

CAJAL-RETZIUS CELLS IN LAMINA I OF THE FELINE CEREBRAL CORTEX EXHIBIT CORTICOTROPIN RELEASING HORMONE-LIKE (CRH) IMMUNOREACTIVITY. V. John Massari, Y. Tizabi, and P.J. Gatti. Howard Univ. Coll. Med., Wash., D.C. 20059.

We have examined the distribution of immunoreactivity for CRH, Neuropeptide Y (NPY), and choline acetyltransferase (ChAT) in lamina I of the cerebral cortex of colchicine treated cats utilizing an avidin-biotin immunoperoxidase method. In all cortical areas examined, CRH immunoreactive perikarya were found in lamina I. Some of these cells were large and had long horizontal processes, which are the characteristics of Cajal-Retzius cells. CRH immunoreactive perikarya were also found in lamina II. No NPY-like immunoreactive cells were found in lamina I. NPY cells however, were numerous in the deepest layers of the cerebral cortex and particularly in the subcortical white matter . ChAT-like immunoreactive perikarya were not detected in lamina I or in any other lamina of the cerebral cortex examined, despite colchicine pretreatment. However, numerous well stained ChAT cells were seen in other nuclei throughout the brain. Since Cajal-Retzius cells receive input from noradrenergic terminals from the locus ceruleus and synapse upon dendritic bouquets of all cortical pyramidal cells, it appears that CRH-neurons in lamina I are ideally situated to modulate cortical neuronal functions. Supported by A.H.A./N.C.A.

157.8

CRF IMMUNOSTAINING IN THE SYRIAN HAMSTER FOREBRAIN. R. L. Meisel and S. N. Del Paine. Dept. of Psychological Sciences, Purdue University, West Lafayette, IN 47907.

Syrian hamsters differ from many species in the regulation of adrenocortical secretions. For example, following unilateral adrenalectomy, Syrian hamsters do not show compensatory adrenal growth characteristic of other species. This lack of compensation for diminished adrenal output indicates that there may be differences in negative feedback sensitivity to adrenal corticoids. In this study we examined the distribution of CRF immunoreactivity in the forebrain of the hamster as part of our investigations into the regulation of adrenal function in this species. Immunocytochemistry for CRF was conducted using a primary antibody to oCRF and a peroxidase-based Vector ABC kit. A consistent pattern of neuronal labelling was observed among hamsters treated with colchicine. Large numbers of labelled cells were located in the bed nucleus of the stria terminalis, the preoptic area, the supraoptic nucleus, and the paraventricular nucleus of the hypothalamus. A prominent cell group was also found in the caudal retrochiasmatic area. Fewer cells were seen in several thalamic nuclei, with a sparse distribution of neurons in the lateral septum, and the medial and central amygdaloid nuclei. Some labelled cells were also found in the caudal arcuate nucleus. In the cerebral cortex, labelled cells were evident in the superficial layers of the parietal cortex. None of these cellular groups were labelled in noncolchicine-treated hamsters, even after unilateral adrenalectomy. The pattern of CRF staining reported here for the Syrian hamster is very similar to that reported for the rat, though the central nucleus of the amygdala was only weakly stained in the hamster. These results suggest that CRF in the hamster, as in the rat, may regulate extracortocotrophic functions.

157.9

DISTRIBUTION OF PRODYNORPHIN PEPTIDES IN PRIMATE CORTEX D.J. Healy, J.H. Meador-Woodruff, and H. Akil. Mental Health Research Institute and Department of Psychiatry, University of Michigan, Ann Arbor, MI 48109.
Prodynorphin (prodyn) is one of the three endogenous opioid peptide precursors, and can be processed into several functional domains with affinities for the opiate receptors. Prodyn is of particular interest, as it is the only opioid peptide precursor that produces peptides with significant affinities for the kappa opiate receptor. Prodyn peptides have been previously studied in various brain regions such as the hippocampus and the basal ganglia; in primates, these peptides also exist in fairly high concentrations in the cortex, but a detailed investigation of the distribution of these peptides in cortex has not yet been performed. In an attempt to understand the nature of cortical prodyn-derived peptides, we studied the distributions of Dyn A (1-17), Dyn A (1-8), Dyn B, and α-neo-endorphin in twelve cortical areas in six monkeys (*macaca nemestrina*) using specific radioimmunoassays. There were between-region differences in amount of each peptide in the twelve cortical areas studied, suggesting regional heterogeneity in the distribution of prodyn in primate cortex. [Supported by Grants DA02265, MH00818, MH422251, and the Theophile Raphael Fund].

157.10

NEUROPEPTIDE-Y IMMUNOREACTIVITY IN THE HUMAN STRIATE CORTEX. Nancy E.J. Berman. Dept. of Anatomy and Cell Biology, Univ. of Kansas Med. Ctr., Kansas City, KS 66103

Neuropeptide-Y (NPY) is a potent vasoconstrictive agent found in perivascular axons of peripheral vessels. Little is known about its function in the cerebral cortex. We examined the morphology and distribution of NPY-immunoreactive (NPY-ir) neuronal cell bodies and axon plexuses in human striate cortex using a polyclonal antibody (Cambridge Biochemicals). The stability of NPY in postmortem tissue (Norvell and Macbride, '90), makes this approach feasible.

A dense plexus of NPY-ir axons was seen in the upper part of layer I, just beneath the pia. This plexus was especially pronounced in regions where the pia is penetrated by small capillaries, and NPY-ir beaded axons often surrounded these capillaries. In the lower half of layer I, small multipolar NPY-ir neurons were seen. Many of these had beaded axons with tortuous trajectories, including "hairpin" loops. These axons may contribute to the plexus in the upper part of layer I. In layers II-IIIb neurons with a variety of multipolar, nonpyramidal morphologies including neurons with axon loops were found. In layers IVb,c, V, and VI, NPY-ir neurons were relatively less numerous than in the supragranular layers. NPY-ir neurons were relatively more numerous in the white matter. Many of these cells had perikarya elongated in the dimension parallel to the axon bundles.

In addition to the NPY-ir axons surrounding pial capillaries, NPY-ir neurons were found in close approximation to small blood vessels throughout the cortex. Control of cerebral blood flow is thought to be achieved primarily by autoregulation. These results suggest an additional mechanism. As in some peripheral vascular beds, cortical blood flow may be influenced by neuropeptide-Y innervation of arteries. This innervation may originate from intrinsic cortical NPY containing neurons. Supported by MH38399 and BNS 8819971.

157.11

DISTRIBUTION OF VASOACTIVE INTESTINAL POLYPEPTIDE IMMUNOREACTIVE(VIP-IR) NEURONS IN CANINE FOREBRAINS
D.S.Lee and C.I.Cha, Department of Anatomy, College of Medicine, Seoul National University

To compare the morphological differences in the distribution of the vasoactive intestinal polypeptide immunoreactive (VIP-IR) neurons in the brains of higher animals with those of rodents, an immunohistochemical study of canine forebrains was conducted using the ABC method. Unlike the previous report on rats and mice, we found some interesting characteristics of canine VIP-IR neurons in the cerebral cortex and hypothalamus.

In the cerebral cortex, VIP-IR neurons were present in all layers of the cortex and were usually bipolar. In contrast to rodents, 1) the proportion of multipolar neurons was much higher, 2) the staining density of multipolar neurons was higher than that of bipolar cells, 3) two band-like distributions of cells were observed one in layers II and III and another in layers V and VI, and 4) among bipolar cells, obliquely lying cells were frequently found even in the middle layers of the cortex.

In the hypothalamus, densely stained cell bodies and fibers were found in the suprachiasmatic nucleus largely in accordance with the previous descriptions in rodents. In addition, there were strong VIP-IR cell bodies and fibers in the magnocellular portion of the supraoptic and paraventricular nucleus and also many immunoreactive fibers in the internal layer of the median eminence. Some scattered fibers were seen in the external layer of the median eminence and a few fibers around the blood vessels. VIP-IR cells were also seen in the magnocellular portion of the medial preoptic area.

The above findings in the hypothalamus were largely consistent with previous biochemical studies and presented morphological evidence that VIP had some neuroendocrinological functions, especially in the hypo-thalamo-neurohypophysial system.

157.12

VASOACTIVE INTESTINAL PEPTIDE mRNA EXPRESSION IN PRIMARY SOMATIC SENSORY AND VISUAL CORTEX OF MONKEY AND RAT
D.L.Benson, P.J.Isackson and E.G.Jones. Department of Anatomy and Neurobiology, Univ. of California, Irvine, CA 92717.

Previous immunocytochemical studies have failed to localize vasoactive intestinal peptide (VIP) containing neurons in monkey neocortex. In this study, using in situ hybridization techniques, the areal and laminar distribution of VIP cRNA was examined in primary somatic sensory and visual cortex in rats and monkeys. The cDNA corresponding to the mRNA which encodes VIP and PHM, a related peptide, in monkey neocortex was isolated using polymerase chain reaction amplification. The monkey VIP cDNA was 488 base pairs long and had a 98% sequence identity with the published human cDNA in the comparable region (Itoh, et al, Nature 304:547,1983). Using the monkey VIP clone, we synthesized 35-S labeled antisense RNA probes for in situ hybridization studies. In primary somatic sensory cortex and primary visual cortex, the distribution of VIP cRNA hybridization was very similar. In both rat and monkey, labeled cells were evident in all layers, including layer I. Occasional, labeled cells were also found in the underlying white matter. The greatest density of antisense VIP cRNA labeled cells was in layers II and III. The present results indicate that both rat and monkey neocortex express VIP mRNA. Supported by N.I.H. grant number NS 21377.

157.13

IMMUNOCYTOCHEMISTRY OF THE GNRH SYSTEM IN DEVELOPING XENOPUS. J.W. Witkin. Dept. Anatomy and Cell Biology, Columbia Univ., Coll. of P&S, New York, NY 10032

The anatomy of the gonadotrophic releasing hormone (GnRH) system has been described in the adult African clawed toad (Xenopus laevis) (Doerr-Schott, J. and M.P. Dubois, Cell Tiss. Res., 172:477, 1976); however it is not known when the system differentiates or whether it is fully (or differently) developed in premetamorphic stages.

Embryonic, larval and young metamorphic toads (Nieuwkoop and Faber stages 23 through 66) were anesthetized with MS222 and fixed by immersion in 4% paraformaldehyde, 7% sat. picric acid in 0.1M phos. buffer, pH 7.3 for 1/2 hr. to 24 hr., depending upon the age of the animal. Tissues were either: 1) embedded in OCT and cut at 12 um on the cryostat, 2) embedded in 12% gelatin and cut at 40 um on the vibratome or 3) embedded in egg yolk/gelatin and cut at 40 um on the freezing microtome. Sections (either on slides or free floating) were incubated in an antibody to GnRH (LR1 of Benoit or Rb4468 of our laboratory) followed by the standard Vectastain avidin biotin HRP (ABC) procedure with DAB as the chromogen. Controls in which the primary antibody was omitted or preabsorbed with GnRH peptide had no specific staining.

By larval stage 47, there were GnRH fibers in the anterior telencephalon and the lateral acellular region of the diencephalon. In addition, there was a dense fiber projection in the basolateral region of the entire spinal cord. In young metamorphic toads, there were GnRH neurons in the following locations: the septal/preoptic area, continuing ventrally and posteriorly, with projections towards the infundibulum; ventrolaterally in the diencephalon with projections to the lateral geniculate, habenula and optic tectum; along the pia at the medial surface of the telencephalon, probably in association with the nervus terminalis. Some GnRH neurons were also found along the pial surface of the olfactory bulb and in the region of the entrance of the optic nerves. There were traceries of fibers in the amygdala, olfactory bulb, pallial regions, midbrain and hindbrain. Innervation of the spinal cord was notably reduced after metamorphosis, with only an occasional fiber visible in the mid ventral region. USPHS HD10665

157.14

LOCALIZATION OF A NEUROKININ B PRECURSOR PROTEIN FRAGMENT IN GUSTATORY AND OLFACTORY PATHWAYS IN RAT BRAIN BY IMMUNOCYTOCHEMISTRY. L.R. Lucas, J.E. Krause and R.E. Harlan. Neuroscience Training Program and Dept. of Anatomy, Tulane Univ. Sch. Med., New Orleans, LA 70112 and Dept. of Anatomy and Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Neurokinin B (NKB), a bioactive tachykinin, is derived from the preprotachykinin (PPT) B precursor protein. Raising antisera that do not cross react among the tachykinins has proven difficult in the past given the homology in sequence of the C-terminus region of these peptides. We have raised antisera in rabbit specific for a 30-amino acid fragment (Peptide 2) found in PPT B protein, upstream of the NKB peptide. Immunohistochemical localization of the peptide in rats perfused with Zamboni's fixative revealed a widespread distribution in gustatory and olfactory pathways in the brain. Our initial mapping studies have shown immunoreactive fibers and cell bodies in non-colchicine treated animals at dilutions of 1:1000 to 1:10,000. The parabrachial nucleus, central nucleus of the amygdala, and the olfactory tubercle showed very dense immunoreactivity. More moderate staining was observed in the nucleus of the stria terminalis, anterior olfactory nucleus, and olfactory bulb. Immunoreactivity was completely abolished by preabsorption of the antiserum with a 10 μmolar concentration of the 30-residue peptide. Our results agree favorably with previously published reports of NKB mRNA distribution.

157.15

ACTH AND ENKEPHALIN (ENK) AXONAL INPUT TO C-FOS CONTAINING PARAVENTRICULAR (PVN) NEURONS. S. Preiel and D.T. Piekut, Neuroendocrine Unit, University of Rochester, Rochester, N.Y. 14642

Recent immunocytochemical studies suggest that the appearance of c-fos in neuronal nuclei of adult rats is dependent on neuronal activity and occurs in particular following stressful stimulation. It is of interest to determine which neurotransmitters modulate specifically those neurons, activated by a certain stress stimulus. We have focused on the afferent input of two peptides, known to be involved in processing of stress information, to c-fos containing neurons in the PVN.

Under ether anesthesia, mustard oil (MO) was gently applied to the left hindfeet of experimental rats, control rats received applications of saline. One to three hours later, the animals were transcardially perfused with phosphate buffer followed by 4% paraformaldehyde. Hypothalamic tissue sections were processed for c-fos (gift of Dr. D. Slamon) immunocytochemistry followed by ACTH or ENK immunocytochemistry using the ABC Vectastain Kit. The c-fos antibodies were visualized with the 3,3'- diaminobenzidine (DAB) procedure, the ACTH or ENK antibodies with the DAB/Nickel procedure. Some sections were plastic embedded for semi-thin sectioning.

The c-fos antiserum labeled only neuronal nuclei; the ACTH and ENK antisera labeled peptide fibers. Mustard oil is known to activate small diameter, nociceptive nerve fibers. Unilateral application of MO increased the number of PVN neurons with c-fos labeled nuclei bilaterally. These c-fos labeled neurons were present in the parvocellular components of the PVN. The data showed that following a stressful, e.g. noxious stimulus, subpopulations of PVN neurons were activated. Double-labled tissue sections demonstrated ACTH and ENK immunoreactive axons in close proximity to these c-fos containing neurons. Two micron sections substantiated that ACTH and ENK varicosities were positioned such, as to be able to modulate the activity of neurons and thus the stress induced expression of c-fos in these neurons. (Supported by NS 18626.)

157.16

INTERACTIONS OF PROPIOMELANOCORTIN (POMC)-RELATED PEPTIDES, NEUROPEPTIDE Y (NPY) AND DOPAMINE β-HYDROXYLASE (DβH) FIBERS AND THYROTROPIN-RreleasING HORMONE (TRH) NEURONS IN THE PARAVENTRICULAR NUCLEUS OF RAT HYPOTHALAMUS. N. Liao[1]*, M. Bulant[2]*, P. Nicolas[2]*, H. Vaudry[2]* and G. Pelletier[1]. [1]MRC Group in Molecular Endocrinology, CHUL Reserach Centre, Quebec, Canada and [2]GREM, Faculté des Sciences, Université de Rouen, France.

In order to determine the nature of afferent fibers contacting TRH neuronal cells bodies in the rat hypothalamic paraventricular nucleus, we used dual immunostaining procedures which employed antibodies to ACTH (to label POMC neurons), NPY and DβH and peroxidase-labeled goat anti-rabbit lgG as a first sequence and antibodies to a cryptic fragment (Ps5) of pro-TRH (to label TRH neurons) and alkaline phosphatase-labeled goat anti-rabbit IgG as the second sequence. A rich innervation of the paraventricular nucleus by immunoreactive POMC, NPY and DβH fibers was observed. Numerous NPY and POMC fibers were in intimate anatomic proximity and often appeared to surround in remarkable density TRH-containing cell bodies. Less frequent oppositions between DβH fiber and TRH cell bodies were detected. These results strongly suggest that TRH neurons might be regulated by POMC, NPY as well as adrenergic and/or noradrenergic systems. These interactions might be the neuroanatomical basis for the already observed effects of opiate peptides, NPY and catecholamines on TSH secretion.

157.17

INTRAVESICULAR ORGANIZATION OF THE NEUROPHYSINS AND THE C-TERMINAL GLYCOPEPTIDE OF PROPRESSOPHYSIN (CPP) IN RAT NEURAL LOBE. M. Tian and W.E. Armstrong. Dept. of Anat. Neurobiol., Univ. Tenn., Memphis, The Health Science Center, Memphis, TN 38163.

Prior to characterization of the vasopressin precursor as a glycopeptide, Tasso et al (*Cell Tiss. Res.* 180: 11, 1977) reported a probable glycopeptide in the perimeter of vasopressin-containing dense core vesicles of the neural lobe using silver protein stains. By quantifying the distribution of gold particles over dense core vesicles from highly magnified electron micrographs, we find that CPP indeed occupies a distinctly more peripheral part of vasopressin vesicles than does vasopressin-associated neurophysin (VP-NP).

Oxytocin and vasopressin granules were immuno-labeled on thin sections from rapidly frozen, freeze substituted rat neural lobes. In vasopressin vesicles, an antibody to CPP preferentially labels the perimeter of the dense core (~70% of gold particles), compared to a circular core region of equal area (~30%), whereas an antibody cross reacting with both VP-NP and OT-NP (PS45) labels the central region preferentially (~65% central, ~35% perimeter). Thus, in vasopressin vesicles, NP and CPP distributions are complementary. In oxytocin vesicles, an antibody specific to OT-NP (PS38) and PS45 both preferentially label the central region (~60%) of the vesicle compared with the perimeter (~40%).

Thus, within neurohypophysial dense core vesicles, the different products derived from the vasopressin and oxytocin precursor are not homogeneously distributed. Supported by NIH grant #NS23941.

157.18

THE PATTERN OF HYPOTHALAMIC VASOPRESSIN MAGNOCELLULAR NEURONS DIFFERS ACROSS HAMSTER SPECIES. T.P. Goodness and G.J. DeVries. Prog. in Neurosci. and Behav., Univ. of Mass., Amherst, MA. 01003.

Magnocellular vasopressin (VP) neurons respond to osmotic challenge and temperature changes. Several differences in these responses have been observed across hamster species. We compared the pattern of VP-immunoreactive (VP-IR) cells in *Cricetellus migratorius* (CM) and *griseus* (CG), *Phodopus campbelli* (PC) and *sungorus* (PS), and *Mesocricetus auratus* (MA) to generate hypotheses about the role of these neurons. In addition to the paraventricular and supraoptic nucleus, MA had all the accessory VP-IR cell groups previously reported in rats, i.e., nucleus circularis, perifornical nucleus and nucleus of the medial forebrain bundle and scattered VP-IR cells in the anterior and lateral hypothalamic area. CM and CG lacked VP-IR neurons in the nucleus circularis and had few in the anterior hypothalamic nucleus. PS also lacked a nucleus circularis, but had more cells scattered rostrally in the anterior hypothalamic nucleus. PC had fewer VP-IR cells in the anterior hypothalamic nucleus, but had a prominent nucleus circularis. CM, CG, and MA had a discrete group of perifornical VP-IR cells, while PC and PS had VP-IR cells spread out throughout the lateral hypothalamus. Finally, CM lacked cells in the nucleus of the medial forebrain bundle. Since some of these accessory nuclei have been linked to temperature and osmoregulation, the species differences may be related to the different environmental demands.

157.19

ULTRASTRUCTURAL CHARACTERIZATION OF GALANIN SYNTHESIZING NEURONS IN HYPOPHYSIOTROPHIC REGIONS OF THE RAT DIENCEPHALON. Z. LIPOSITS[1,2], I. MERCHENTHALER[2] and A. NEGRO-VILAR[3]. [1]Dept. of Anatomy, Univ. Med. School, Pecs 7643, Hungary and [2]Funct. Morphol. and [3]Reprod. Neuroendocrinol. Sections, LMIN, NIEHS, NIH, Research Triangle Park, NC 27709, USA.

Galanin has been shown to be synthesized in the brain (Skofitsch and Jacobowitz, Peptides, 6: 509-546, 1985) and to act upon the pituitary gland (Ottlecz et al., Proc. Natl. Acad. Sci. USA, 85: 9861-9865, 1988). In order to characterize the hypophysiotrophic galanin immunoreactive (IR) system morphologically, parvocellular neurons expressing the peptide were studied, by means of ultrastructural immunocytochemistry, in the medial preoptic area, the paraventricular and arcuate nuclei of colchicine treated (30 μg/100 g b.w.), adult male rats. The neurons contained immunolabeled ribosomes, neurosecretory granules of 80-120 nm and received synapsing axon terminals on their cell bodies and dendrites. Galanin-containing axons established neuro-hemal junctions with portal vessels in the median eminence and also formed synaptic connections with neurons residing in the preoptic, paraventricular and arcuate regions. Light microscopic double labelling performed in the paraventricular nucleus revealed that the parvocellular galanin-IR cells were densely innervated by adrenergic and neuropeptide-Y-IR axons and received a modest serotonergic input. Galanin-IR axons were in juxtaposition to somatostatin-IR neurons. These data indicate, that both hypophysiotrophic and neuromodulatory functions can be attributed to galanin synthesizing neurons.

157.20

THE HYPOPHYSIOTROPIC GALANIN SYSTEM OF THE RAT BRAIN AS REVEALED BY COMBINATION OF RETROGRADE TRACING AND IMMUNOCYTOCHEMISTRY. I. Merchenthaler and A. Negro-Vilar*. Functional Morphol. and Reprod. Neuroendoc. Sections, NIEHS, NIH, Res. Triang. Park, NC 27709

Fluoro-Gold is a retrograde tracer which does not penetrate the blood-brain-barrier (BBB) but is taken up by nerve terminals from areas which are outside of the BBB. The median eminence (ME) is a circumventricular organ lacking a BBB and it contains large number of galanin-immunoreactive (GALi) nerve terminals. In order to identify those GALi neurons which have access to capillaries of the ME (the hypophysiotropic GALi neurons), Fluoro-Gold was injected peripherally (15 mg/kg). Five days later colchicine was injected into the lateral ventricle. The animals were perfused with fixative one day later. Thin paraffin sections were immunostained for GAL by the indirect immunofluorescence technique. The retrogradely transported Fluoro-Gold and the endogenous GAL immunoreactivity were examined with an Axiophot photomicroscope equipped with the necessary filter combination. Our studies indicate that the majority (approx. 70%) of the hypophysiotropic GALi neurons are located in the arcuate nucleus. The paraventricular nucleus, and to a lesser extent, the preoptic area also contribute to this system. Since the organum vasculosum of the lamina terminalis, another circumventricular organ, also contains GALi nerve terminals, the proper number of GALi neurons projecting to the median eminence from the preoptic region cannot be determined by this retrograde technique. The hypophysiotropic GALi neurons are intermixed with other non-hypophysiotropic GALi neurons which project to other brain areas, where GAL may function as a neurotrasmitter or neuromodulator.

157.21

CONNECTIVITY BETWEEN AV3V AND BRAINSTEM NUCLEI. S.A. Joseph, C. Bulson*, W.H. Pilcher and L.J. Sim. Neuroendocrine Unit, University of Rochester, Rochester, N.Y. 14642.

The AV3V nuclei encompassing the rostral part of the third ventricle have been shown to exert a significant influence in the regulation of cardiovascular function. Numerous electrophysiological studies have documented the reciprocity and potential interactions between brainstem and forebrain cardiovascular centers. These neuroanatomical studies demonstrate the intrinsic organization of the AV3V region and its afferent and efferent connections with brainstem nuclei.

Horseradish peroxidase conjugated wheat germ agglutinin (HRP-WGA) was injected into central sites of the AV3V region in the rat to elucidate cells of origin projecting to this region. Retrograde transport was identified most densely in the dorsal raphe, lateral parabrachial nucleus, locus coeruleus and the arcuate hypothalamic nucleus. Immunohistochemical analysis of these cell groups demonstrates that noradrenergic and serotonergic neurons contribute to these afferent projections.

Phaseolus vulgaris leucoagglutinin (PHA-L) anterograde marker was iontophoretically instilled into central sites of the AV3V region to determine projections to brainstem nuclei. Efferent projections in the form of terminals and fibers were found in the arcuate nucleus of the hypothalamus, dorsomedial nucleus, locus coeruleus, dorsal raphe and periaqueductal grey. These studies demonstrate neuroanatomical connectivity between forebrain and brainstem cardiovascular centers. (Supported by American Heart Association #871011 and NIH NS21323 grants.)

OPIOIDS: RECEPTORS I

158.1

MOLECULAR CHARACTERIZATION OF OPIOID RECEPTORS IN SUBCELLULAR FRACTIONS OF RAT BRAIN.
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Previously we have reported the binding characteristics of opioid receptors in synaptic plasma (SPM) and light (LM) membrane fractions of rat brain. In the present work the subunit structure of opioid binding sites in these fractions was investigated with the mu receptor specific affinity reagent (^{3}H)Tyr-D-Ala-Gly-N(Me)Phe-CH_2Cl, (^{3}H)DAMCK spec. activity 56.8 Ci/mmol, prepared by Varga et al. (Neuropeptides 12, 135, 1988). Labelled proteins were separated by SDS-PAGE and evaluated by gel slicing and fluorography. Predominant labelling was seen at about 58 kDa in SPMs as well as LMs. This polypeptide was also detected in subcellular fractions of 1-day-old rats (P-1), but additionally a heavily labelled band at Mr 48 kDa was apparent. This protein might be characteristic for the neonates, since only very weak incorporation into the 48 kDa protein was seen in adults. Incubation of rat brain SPMs under phosphorylating conditions resulted in specific incorporation of ^{32}P from (γ-^{32}P)ATP into the 58 kDa protein. Results suggest that the 58 kDa protein might be the integral binding site of the opioid receptors.

158.2

AFFINITY LABELING OF THE μ OPIOID RECEPTOR IN BRAIN.
J.M. Bidlack[1], R.A. Kaplan[1*], A. Seyed-Mozaffari[2*] and S. Archer[2*]. [1]Dept. of Pharmacology, University of Rochester, School of Medicine and Dentistry, Rochester, NY 14642 and [2]Dept. of Chemistry, Cogswell Laboratory, Rensselaer Polytechnic Institute, Troy, NY 12181.

Previously, we have shown that after reduction of a disulfide bond at or near the μ opioid binding site in rat brain membranes, 14ß-(bromoacetamido)-7,8-dihydromorphine (H_2BAM) alkylated this site, resulting in the irreversible inhibition of μ opioid binding, without altering binding to δ and κ opioid sites. [^{3}H]H_2BAM was synthesized and tested for its ability to bind reversibly and irreversibly to the μ opioid receptor. In the absence of a reducing reagent, [^{3}H]H_2BAM bound reversibly to rat brain membranes with a K_d value of 0.51 ± 0.08 nM and a B_{max} value of 185 fmol/mg membrane protein. The μ-selective peptide DAGO had a 700-fold lower IC_{50} value for the inhibition of 0.4 nM [^{3}H]H_2BAM binding than either the δ-selective peptide DPDPE or the κ-selective alkaloid U50,488H. When membranes were treated with dithiothreitol (DTT), followed by the addition of [^{3}H]H_2BAM, the μ opioid binding site was alkylated with the affinity ligand. The alkylation was dependent on pH, temperature, time, and on the concentrations of DTT and [^{3}H]H_2BAM. Only opioids known to bind to the μ opioid binding site were able to block the affinity labeling. When membranes were separated on SDS polyacrylamide gels, proteins with molecular weights of 54,000 and 31,000 were specifically alkylated with [^{3}H]H_2BAM. (Supported by grants DA03742 and DA01674).

158.3

MONOCLONAL ANTIBODIES TO MORPHINE AND HALOPERIDOL AS MODELS OF RECEPTOR-LIGAND INTERACTIONS J.M. Anchin, P.H. Kussie, C. Mandal, D.S. Linthicum, Dept. Vet. Microbiol., College of Vet. Medicine, Texas A&M University, College Station, TX 77843

Knowledge-based computer-assisted molecular modelling of monoclonal antibodies is now feasible using a modelling protocol involving "canonical structures" from known crystallographic coordinates of emperically solved myeloma proteins and monoclonal antibodies. In our study we examined the binding sites of monoclonal antibodies directed against morphine and haloperidol. Both of these drugs have piperidinyl nitrogens with similar pKa's and the protonated form of this moiety is thought to be important in drug-receptor interactions for both drugs. Molecular modelling of the antibody binding sites reveals structural features and chemical interactions which may be similar to those found in the neurogenic receptor. For the anti-morphine antibody we have discovered that a hydrophobic pocket is formed by key residues in the hypervariable loops and H:50 glutamate forms a salt-bridge with the piperidinyl nitrogen of morphine. In the anti-haloperidol antibody, the flurophenyl ring of haloperidol "stacks" with an isolated tryptophan (H:50) thereby quenching it's natural fluorescence and also forms a salt-bridge with H:107 aspartate. The use of antibody binding sites as a paradigm for neurogenic receptor binding sites can be an important concept in our understanding of drug-receptor interactions. Detailed structure-activity relationships and molecular modelling studies are important tools if valid comparisons are to be made. (Supported by Am. Heart Assoc. Grant-in-Aid 89-1066)

158.4

NALOXONAZINE AND ß-FUNALTREXAMINE ANTAGONISM OF μ ACTIONS: FURTHER EVIDENCE FOR DISTINCT μ_1 AND μ_2 RECEPTORS.
C.G. Pick, D. Paul and G.W. Pasternak. The Cotzias Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Depts. of Neurology and Neuroscience and Pharmacology Cornell Univ. Medical College, New York NY 10021.

In binding studies, naloxonazine (NAZ) blocks μ_1 receptors whereas ß-funaltrexamine (ß-FNA) alkylates both μ_1 and μ_2 receptors. Because NAZ antagonized analgesia produced by i.c.v. DAMGO but not i.t. DAMGO, we proposed that μ_1 receptors mediate analgesia supraspinally whereas μ_2 receptors are active spinally. However, these results may reflect differing efficacies and levels of spare receptors with a single μ receptor type. If the differences between i.c.v. and i.t. analgesic sensitivity of DAMGO to NAZ were due to spare receptors, the μ-selective antagonist, ß-FNA should show similar results. NAZ antagonized i.c.v. DAMGO analgsia (ID_{50}=6.1 mg/kg), 6-fold more potently than i.t. DAMGO analgesia (ID_{50}=38.8 mg/kg). In contrast, ß-FNA blocked both i.c.v. (ID_{50}=6.1 mg/kg), and i.t. (ID_{50}=7.7 mg/kg) DAMGO analgesia equally well. ß-FNA also blocked morphine's inhibition of gastrointestinal transit, a NAZ-insensitive (μ_2) action, as effectively as i.c.v. and i.t. DAMGO analgesia. These differences between NAZ and ß-FNA strongly argue against the efficacy/spare receptor hypothesis and support the concept of distinct μ_1 and μ_2 receptors.

158.5

POSSIBLE CONTRIBUTION OF A GLUTATHIONE CONJUGATE TO THE LONG DURATION OF OPIOID ANTAGONISM BY ß-FUNALTREXAMINE (ß-FNA). D. L. Larson,*† A. E. Takemori,*‡ and P. S. Portoghese,† Departments of Medicinal Chemistry† and Pharmacology,‡ Univ. of Minnesota, Minneapolis, MN 55455.

The nonequilibrium opioid antagonist, ß-FNA, is an important pharmacological tool in opioid research. In addition to being highly μ-selective in smooth muscle preparations, it possesses an ultra-long duration blockage of μ-opioid receptor sites in vivo. As it is known that ß-FNA readily reacts with sulfhydryl groups, and the fact that glutathione is found in abundant levels in the brain, the possibility exists that a ß-FNA-glutathione adduct (ß-FNAG) may contribute to its long duration of action in vivo. Here we report on the activity of this adduct and related conjugates. In guinea-pig ileum and mouse vas deferens preparations, ß-FNAG showed no irreversible antagonism of opioid agonists. Administration i.c.v. (4.8 nmole/mouse) elevated the ED_{50} of morphine by sixfold after 24 hours. A threefold increase occurred 24 hours following 5 mg/kg s.c. This action in vivo closely paralleled that of ß-FNA itself. The ß-FNA-cysteine adduct (ß-FNAC) also showed reversible activity on the GPI, but in vivo (4.8 nmole/mouse i.c.v.) it exhibited greater (46-fold) antagonism of morphine at the 24 hour interval. The possibility exists that both ß-FNAG and ß-FNAC are either trapped in the brain due to their polarity, or they are enzymatically reconverted to their precursor, ß-FNA.

158.6

COMPARATIVE ACTIVITIES OF OPIOIDS AT μ-RECEPTORS IN GUINEA PIG ILEUM AND IN A NEUROBLASTOMA CELL LINE. L. Toll and I. Berzetei-Gurske. Neuroscience Department, SRI International, Menlo Park, CA 93025.

The μ-opioid receptor is the site mediating the analgesic activity of morphine and most fused ring and peptide opiates. In order to better understand the μ-activities of opioid ligands, experiments were designed to determine the activities of various families of opioid compounds at μ-opioid receptors without interference by other receptor subtypes. Experiments were conducted in guinea pig ileum in the presence of the selective κ-antagonist nor-BNI. The IC_{50} values obtained for the inhibition of contractions were compared with activities found for the inhibition of cAMP accumulation in the μ-containing neuroblastoma cell line SH-SY5Y. In the guinea pig ileum, nor-BNI caused about a 2-fold shift in the IC_{50} value of μ-selective ligands normorphine and DAGO, and a 360-fold shift in the IC_{50} value of the κ-selective ligand U-69593. For the κ-agonist EKC, the shift was about 60-fold, resulting in an IC_{50} of approximately 12 nM at μ-receptors, compared to 8 nM for DAGO. In SY5Y cells, the κ-agonist EKC appears to be 10 times more effective than DAGO, with IC_{50} values for inhibition of cAMP accumulation of 3 nM and 30 nM, respectively. The results indicate that EKC is an effective μ- as well as κ-agonist. Differences between guinea pig ileum and SY5Y cells are being investigated.

158.7

EVIDENCE FOR A ROLE OF KAPPA OPIOIDS IN THE TONIC REGULATION OF TUBEROHYPOPHYSIAL DOPAMINE (THDA) NEURONS IN THE MALE RAT J. Manzanares, K.J.Lookingland, S.D. LaVigne and K.E. Moore. Department of Pharmacology and Toxicology. Michigan State University. East Lansing, MI 48824.

The purpose of the present study was to characterize the effects of the kappa agonist U-50488 and the antagonist nor-binaltorphimine (NOR-BNI) on the activity of THDA neurons in the male rat. The activity of THDA neurons was estimated by measuring the concentration of 3,4-dihydroxyphenylacetic acid and the accumulation of 3,4-dihydroxyphenylalanine following administration of a decarboxylase inhibitor (NSD 1015) in the intermediate lobe (IL) and neural lobe (NL) of the pituitary. U-50488 (2.5-20 mg/kg; sc; 60 min) caused a dose-related decrease in the activity of THDA neurons projecting to both the IL and NL. NOR-BNI (25-50 μg/rat; icv; 120 min) increased THDA activity and this effect was reversed by U-50488. These neuronal results suggest that endogenous kappa opioids regulate the basal activity of THDA neurons projecting to the IL and NL of the male rat. (Supported by NIH grant NS 15911.)

158.8

THE β-ENDORPHIN INDUCED PROLACTIN INCREASE INVOLVES THE KAPPA OPIATE RECEPTOR SUBTYPE IN FEMALE RATS. L. Kehoe and P. Callahan. Dept. of Zoology, Miami University, Oxford, OH 45056.

The Prolactin (PRL) secretory response to β-endorphin (β-end) was determined in female Sprague Dawley rats following pretreatment with the specific κ opiate receptor antagonist, nor-Binaltorphimine Hydrochloride (norBNI).

Intraventricular (IVT) cannulae were chronically implanted in the lateral ventricle of cycling and lactating rats (day 2 post partum). Following a 5-7 day recovery period, and one day prior to the experiment, animals received chronic jugular cannulae. On the day of the experiment, pups were removed from the post partum females for two hours prior to blood sampling. β-end was injected alone (0.025, 0.05, 0.1 μg in 5 μL saline, IVT), or 45 min after pretreatment with norBNI (10 nm, IVT).

All doses of β-end produced a large, significant increase in circulating levels of PRL. PRL concentrations exceeded 200 ng/ml after 15 min. The 0.1 μg dose produced significant elevations in circulating PRL levels during the entire 60 min sampling period, while the other doses produced significant increases which persisted for 45 min. Cycling females also exhibited a significant and dramatic increase in circulating levels of PRL following the administration of β-end. However, the levels were less than those observed in lactating females. NorBNI completely blocked the increase in PRL levels at all doses of β-end tested. Furthermore, PRL levels were significantly lower than basal levels by 15 min, and remained depressed throughout the 60 min period. These results indicate that β-end elicits an increase in PRL secretion in both lactating and cycling female rats through its action at the κ opiate receptor subtype.

158.9

THE PROLACTIN SECRETORY RESPONSE TO ß-ENDORPHIN DOES NOT INVOLVE THE μ_1 OPIATE RECEPTOR SUBTYPE IN THE DIESTROUS FEMALE RAT. J. Janik, P. Callahan and J. Rabii. Dept. of Biological Sciences, Rutgers University, Piscataway, NJ 08854.

It has been proposed that morphine elicits a prolactin secretory response through its activity at the μ opiate receptor subtype. Pasternak and colleagues have reported that the μ_1 site is the key receptor subtype involved in the prolactin release response to morphine. However, the role of this subtype in the action of the endogenous opioid peptides is not firmly established. The purpose of this study was to determine whether or not the μ_1 receptor subtype is involved in the ß-endorphin induced increase in circulating levels of prolactin.

Female Sprague-Dawley rats in diestrus were chronically implanted with intraventricular (IVT, lateral ventricle) cannulae and allowed a minimum of 5 days to recover. They were implanted with jugular cannulae 24 hours prior to their use in an experiment. One group of animals received saline or WIN 44,441-3 (WIN, 4 mg/kg, ip), an antagonist of the μ, δ and κ sites. A second group received saline or Naloxonazine (NAZ, 20 mg/kg, iv) at the time of the jugular implantation. All animals received saline or ß-endorphin (0.5, 2.5 or 5 mg) in a 5 μl volume.

All doses of ß-endorphin produced a large, significant increase in circulating levels of prolactin which was significantly attenuated by WIN. In order to determine the involvement of the μ_1 site, animals were pretreated with NAZ. In contrast to NAZ's ability to antagonize a morphine induced prolactin increase, NAZ had no effect on the increased circulating levels of prolactin produced by ß-endorphin administration, indicating that the μ_1 site is not involved in this response.

158.10

MULTIPHASIC EFFECTS OF MORPHINE ON SUBSTANCE P RELEASE FROM TRIGEMINAL NUCLEUS CAUDALIS SLICES. W. Maixner, H. Suarez-Roca, J.R. Zuniga*, and S. Madison*. Dental Research Center and Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, N.C. 27514.

It is generally accepted that morphine acts presynaptically to inhibit substance P (SP) release from afferent terminals in the trigeminal nucleus caudalis (TNC; Nature 268:549, 1977). Recent studies, however, provide evidence that opioids produce both inhibitory and excitatory effects on SP release which are concentration and receptor subtype dependent (J. Neurochem. 48:529, 1987). In the present study, we have examined a wide range of morphine concentrations on K^+-evoked SP release from rat TNC slices. Immunoreactive SP was measured in perfusates. Morphine produced multiphasic effects on K^+-evoked SP release without affecting basal release. Very low concentrations (1.0 and 3.0 nM) suppressed release, intermediate concentrations (100-300 nM) facilitated release, high concentrations (1-3 uM) suppressed release, and a very high concentration (30 uM) facilitated release. These effects were abolished by opiate-receptor blockade with naloxone (30 nM). Thus, morphine produces complex effects on SP release from TNC which are concentration and possibly receptor subtype dependent. Supported by DE08013 & RR05333.

158.11

MORPHINE PRODUCES MULTIPHASIC EFFECTS ON SUBSTANCE P RELEASE FROM TRIGEMINAL NUCLEUS CAUDALIS SLICES BY STIMULATING DIFFERENT OPIOID RECEPTOR SUBTYPES. H. Suarez-Roca, J.R. Zuniga*, S. Madison* and W. Maixner. Dept. of Pharmacology, University of North Carolina, Chapel Hill, N.C. 27514.

Morphine (MOR) produces concentration dependent multiphasic effects on K^+-evoked substance P (SP) release from rat trigeminal n. caudalis (TNC; Maixner et al., this volume). In this study, we tested the action of selective opioid receptor antagonists on these multiphasic effects of MOR. The mu opiod receptor antagonist, β-funaltrexamine (FNA; 20nM), selectively abolished the increase in SP release elicited by 100 nM MOR, whereas n-binaltorphimine, a kappa opioid receptor antagonist (3nM), selectively reversed the facilitation produced by 30uM MOR. The delta opioid receptor antagonist, ICI173,864 (0.3uM), reversed the inhibition of SP release induced by 3uM MOR and attenuated the facilitation with 100 nM MOR. The initial inhibition of SP release with 1nM MOR was not affected by the antagonists. In conclusion, the increase in SP release produced by 100 nM and 30uM MOR may be mediated by FNA-sensitive mu and kappa opioid receptors respectively, whereas 1nM and 3uM MOR may inhibit release by stimulating FNA-insensitive mu and delta opioid receptors respectively. Supported by DE08013, RR05333 & OAS Fellowship.

158.12

EFFECTS OF OPIATE RECEPTOR ANTAGONISTS ON β-CASOMORPHIN-INDUCED ANALGESIA IN NEONATAL RATS. J. Giordano. Coll. Pharm. Health Sci.,Drake Univ., Des Moines, IA 50311

β-casein, and derived peptides, produce analgesia in neonatal rats. While the opioid activity of these ligands has been linked to the μ-receptor, developmental, and pharmacologic patterns of analgesia were inconsistent with the putative profile of μ mediation. The present study directly investigated whether β-casomorphin (BC)-induced analgesia is mediated by multiple opioid receptors during ontogeny. Rat pups, at post-natal day (PND) 7 or 14 were pretreated with the μ-antagonist β-funaltrexamine (β-FNA: 10-50 nmol; 5ul, icv), δ-antagonist naltrindole (NTI: 25-100 nmol; 5ul, icv) or κ-antagonist nor-binaltorphimine (BNI: 25-100 nmol; 5ul, icv) and patterns of analgesia in forepaw (FP) and tail (T) produced by BC (20 mg/kg,ip) were examined. At PND 7, reduction of BC-analgesia by β-FNA was greater in FP than T. At PND 14, β-FNA equipotently decreased BC effects in both FP and T. At PND 7, NTI most effectively reduced BC-analgesia in T. By PND 14, NTI effects in FP and T were less potent. BNI did not affect BC-analgesia at either age. These data suggest that BC-analgesia may be differentially subserved by μ and δ, but not κ receptor systems during post-natal ontogeny.

158.13

CHARACTERIZATION OF [D-SER^2,LEU^5,THR^6]ENKEPHALIN (DSLET) ANTINOCICEPTION: DIRECT AND MODULATORY ACTIONS. Qi Jiang and Frank Porreca, Department of Pharmacology, University of Arizona Health Sciences Center, Tucson, AZ 85724

It has been suggested that some supraspinal opioid δ receptors may be functionally associated with opioid μ receptors. Agonists at these $\delta_{complexed}$ (δ_{cx}) sites produce modulation of μ-mediated effects such as antinociception, while compounds acting at the $\delta_{non\text{-}complexed}$ (δ_{ncx}) receptor do not. Our previous studies have shown that the δ agonist, [D-Pen^2,D-Pen^5]enkephalin (DPDPE), and the δ antagonist, ICI 174,864, act on both δ sites; while [D-Ala^2,Leu^5,Cys^6]enkephalin (DALCE) has agonist and antagonist actions only at the δ_{ncx} site. The present study investigated the direct agonist and modulatory actions of the synthetic opioid peptide, DSLET in the mouse. Antinociceptive effects of DSLET, DPDPE and morphine were evaluated in the 55° C warm-water tail-flick test following intracerebroventricular *(i.c.v.)* administration. *I.c.v.* DSLET (0.044-1.46 *nmol*) produced dose- and time-related antinociception (10-60 min) with a maximal effect at + 10 min; the A_{50} value (and 95% C.L.) was 0.22 (0.15 - 0.31) *nmol*. Similarly, *i.c.v.* DPDPE and morphine also produced dose-related antinociception, but were approximately 130 and 4-fold less potent than DSLET. The effects of DSLET and DPDPE, but not morphine, were antagonized by co-administration of ICI 174,864 (4.4 *nmol*). Administration of DALCE (4.57 *nmol* at -24 hr) blocked only the antinociception produced by DPDPE, but not that of DSLET or morphine. Sub-antinociceptive doses of DPDPE (1.55 *nmol*) and DSLET (0.0015 *nmol*) displaced the morphine dose-response line to the left by approximately 4.5 and 10.6 fold, respectively. The modulation of morphine antinociception by DPDPE and DSLET, but not the direct morphine effect, was blocked by ICI 174,864 (4.4 *nmol*), but not by DALCE pretreatment. These results suggest that both the direct antinociception and μ-modulatory actions of DSLET may occur at the δ_{cx} receptor and support the hypothesis of δ sites which can be differentiated on the basis of interactions with μ receptors.

158.14

REGULATION OF ^{3}H-THYMIDINE INCORPORATION BY OPIOIDS IN RAT BRAIN AGGREGATING CULTURES J. BARG*, M. BELCHEVA*, AND C. J. COSCIA. Dept. of Biochem. and Mol. Biol., St. Louis Univ. Sch. Med., St.Louis, MO 63104.

Studies performed to date have not demonstrated definitively whether opioids directly affect DNA synthesis during brain ontogeny. Herein we present evidence of an opioid receptor-mediated effect of D-ala^2,mephe^4,gly-ol^5 enkephalin (DAMGE) on ^{3}H-thymidine incorporation that depends on the developmental state of the culture. Rat brain aggregates (embryonal day 15) were grown for various time intervals and exposed to different concentrations of opioids 48 h prior to harvesting and to ^{3}H-thymidine for the final 23 h. A decrease of ^{3}H-thymidine incorporation was observed, with the greatest effect in 7-day cultures whereas DAMGE had no influence in 20-day cultures. Dose dependency studies demonstrated that at levels $\geq$ 0.5 μM DAMGE a substantial proportion (40%) of the cell's DNA synthesis is inhibited and the decrease was naltrexone-reversible but not influenced by (+)naloxone or (+)morphine. Both κ-selctive agonists, U50488 and U69593, also decreased ^{3}H-thymidine incorporation, whereas DADL did not alter the incorporation as compared to control. Although the normal developmental role of opioids remains to be determined, these findings may have immediate clinical relevance to fetuses at risk as a result of maternal drug abuse.

158.15

PRESYNAPTIC INHIBITION OF HIPPOCAMPAL MOSSY FIBER SYNAPTIC TRANSMISSION BY KAPPA OPIOIDS. R.L. Gannon and D.M. Terrian. Dept. of Anatomy, East Carolina Univ. Sch. Med.,Greenville, NC 27858.

Recently, this laboratory demonstrated the concomitant release of L-glutamate (Glu) and prodynorphin-derived peptides from hippocampal mossy fiber (MF) synaptosomes. Dynorphins are believed to be endogenous ligands for the kappa opiate receptor subtype. However, presynaptic opioid autoregulation at the MF-CA3 synapse has not been reported. In this study, we describe the effects of the kappa agonist U50488H on calcium availability and transmitter release from guinea pig hippocampal MF synaptosomes. MF synaptosomes were loaded with the calcium indicator FURA-2. Application (30 s) of U50488H dose dependently inhibited KCl-evoked increases in MF cytosolic calcium availability. The inhibitory effect of U50488H was reversed by the selective kappa antagonist Nor-Binaltorphimine. In other experiments, superfused MF synaptosomes were depolarized and the superfusates assayed for Glu and dynorphin B. U50488H significantly inhibited transmitter release with an approximate IC_{50} of 30 uM U50488H. These results suggest the existence of a kappa opioid autoreceptor capable of modulating MF synaptic transmission. Supported by AFOSR 89-0531, NC United Way

158.16

OPIOID RECEPTOR MEDIATED POTASSIUM CONDUCTANCES RECORDED FROM ACUTELY DISSOCIATED HIPPOCAMPAL NEURONS T.L.Wimpey and C. Chavkin. Dept. of Pharmacology, University of Washington, Seattle, WA 98195

The potassium channels controlled by opioids in neurons from the hippocampal CA1 region or the presubiculum/subiculum have different voltage dependencies. Hippocampal neurons acutely dissociated from adult rats were voltage-clamped using the whole cell patch clamp technique. The neurons were continuously superfused with oxygenated buffer (extracellular $[K^+]$ = 5 mM, pipette $[K^+]$ = 140 mM) containing TTX; drug responses were determined by pressure application through drug pipettes. CA1 neurons were found that responded to application of the mu selective opioid agonist PL017 with an outward current averaging 500 pA at -50 mV holding potential (n=4). The properties of this ligand-gated potassium current were similar to those described previously by Williams and coworkers. In recordings from neurons dissociated from the presubiculum/subiculum (a region rich in mu opioid receptor binding) a population of cells was found that responded to application of PL017 or DAMGO. However, unlike the responses seen in the CA1 little or no current resulted from application of the opioid to the cell at a holding potential of -50 mV; rather, the opioid effect was only seen when the cells were step depolarized. The opioids significantly increased the step depolarized (-40 to 0 mV) outward current (n=8) and this effect was blocked by simultaneous application of the antagonist naloxone. The outward currents elicited by step depolarization and the opioid effect were abolished by cesium but unaltered by cadmium indicating that these currents were primarily carried by potassium. The heterogeneity of responses seen suggests that opioid receptors can control more than one type of potassium current. Supported by DA 04123.

158.17

CELLULAR LOCALIZATION OF ∂-OPIOID RECEPTORS USING FLUORESCENT DERIVATIVES OF NALTRINDOLE. R. ELDE, M. SULTANA*†, V. BOYAPATI*†, J. ELLS*, J. WANG AND P.S. PORTOGHESE*†, Departments of Cell Biology and Neuroanatomy and Medicinal Chemistry†, University of Minnesota, Minneapolis, MN 55455

The present understanding of the expression and distribution of opioid binding sites has been established using radiolabeled ligands selective for the three subtypes of opioid receptors. Although much is known concerning the regional occurrence of these binding sites, little is known about their cellular and subcellular localization.

As a step toward understanding the cellular mechanisms that regulate the expression of functional opioid receptors, we have initiated the development of subtype-selective fluorescent ligands which can be visualized using high resolution imaging methods. Naltrindole is a recently described, selective antagonist of the ∂-opioid receptor (Portoghese et al., *J. Med. Chem.* 31:281, 1988). Syntheses and purifications of three fluorescent derivatives of this compound were achieved, each of which retained a significant degree of potency and selectivity as determined in the mouse vas deferens bioassay. NG108-15 cells, a clonal line known to synthesize ∂-opioid receptors, were cultured on cover slips using standard conditions, and differentiated by withdrawal of serum from the media. Three days after serum withdrawal, each of the ligands was incubated with the cells at concentrations ranging from 1 - 100 nM with or without DADL (5 mM) and observed using laser scanning confocal microscopy. In the absence of DADL, patches of fluorescence were observed associated with the membranes of cells and their processes. The intensity of the fluorescent patches was concentration dependent. Patches were frequently associated with varicosities found along the processes. These results suggest that the mechanisms regulating the expression of ∂-opioid receptors and their association with other membrane proteins can be visualized at the cellular level. Supported by DA 02148 and DA 07234.

158.18

UP-REGULATION OF δ-OPIOID BINDING IN SUBCELLULAR FRACTIONS FROM NEURONAL CELL CULTURES. M. M. Belcheva*, J. Barg*, R. J. McHale*, X-M. Gao, D-M. Chuang and C. J. Coscia. St. Louis Univ. Sch. Med., St.Louis, MO 63104, Biological Psychiatry Branch, NIMH, Bethesda, MD 20892

In contrast to butyrate-induced up-regulation of receptors, antagonists elicit an increase in opioid receptors which may not be inhibited by cycloheximide. This was interpreted as possibly due to changes in G protein coupling. To assess this hypothesis, the modulation of agonist δ-binding by Gpp(NH)p and receptor distribution in light (LMs) and heavy (HMs) membrane fractions were examined after either naltrexone (antagonist) or butyrate induced up-regulation. Treatment of NG108-15 cells with naltrexone (1 μM, 48 h) and NCB-20 cells with Na butyrate (1 mM, 3 days) increased Bmax values of ^{3}H-DADLE and ^{3}H-diprenorphine in LMs and HMs 2-3 fold, without changing Kd. Insignificant differences were observed in HM and LM Bmax values for the agonist and antagonist under both up-regulation conditions. Upon cycloheximide treatment (1 μg/ml, 48 h) of NG108-15 cells Bmax values of DADLE and diprenorphine binding in HMs were comparable and increased upon up-regulation in agreement with previous findings. Although LM Bmax values for the agonist were elevated 2-fold in the presence of cycloheximide, no change occurred for antagonist Bmax which was twice that of DADLE. Since up-regulation also increases LM Gpp(NH)p sensitivity, the results indicate that antagonist-induced up-regulation involves G-protein coupling alterations.

OPIOIDS: RECEPTORS II

159.1

AGE-RELATED CHANGES IN THE EXPRESSION OF BRAIN OPIOID RECEPTORS. *E.M. Unterwald and M.J. Kreek**, The Rockefeller University, New York, N.Y. 10021

Possible alterations in the endogenous opioid system have been suggested for the mediation of age-related differences in basal pain threshold, response to morphine administration, and temperature control. The present study investigates the expression of opioid receptors in the brains of adolescent, young adult, and naturally aged guinea pigs using quantitative *in vitro* receptor autoradiography with highly selective radioligands. *Mu* receptors were labelled with [^{3}H]D-ala^2,N-me-phe^4,gly-ol^5-enkephalin, *delta* receptors with [^{3}H]D-pen^2,D-pen^5-enkephalin, and *kappa* receptors with [^{3}H]U-69,593. Results demonstrate that throughout most of the brain the density of *mu* opioid receptors was lower in the aged animals than in either the adolescent or young adult groups. The largest reductions in *mu* receptor number were found in the posterior region of the nucleus accumbens, frontal cortex, hypothalamus, anterior portion of the caudate putamen, septum, and substantia nigra reticular. The density of *delta* opioid receptors was reduced in some brain regions of the old animals and was increased in others. Areas that showed the largest reductions in *delta* receptor number were the caudate putamen, hippocampus, and nucleus accumbens whereas the largest increases were found in the amygdala and hypothalamus. These alterations in the expression of opioid receptors may in part underlie the age-related changes in nociceptive thresholds observed in elderly animals and humans.

159.2

THE PRENATAL DEVELOPMENTAL PROFILE OF OPIOID PEPTIDES AND RECEPTORS OF THE MOUSE BRAIN. R.A. Rius*+, J. Barg*++, C.J. Coscia++, and Y.P. Loh+. Laboratory of Developmental Neurobiology, NICHD, Bethesda, MD 20892+ and Department of Biochemistry, St. Louis University, School of Medicine, St. Louis, MO 63104++.

Although the opioid system of mouse brain has been studied postnatally, little is known about the development and relationship between embryonic opioid peptides and their receptors. The ontogeny of opioid peptides and receptors was studied during the embryonic period (E) 11.5 to postnatal day 1 (P1). Met-enkephalin, dynorphin and β-endorphin were detected as early as E11.5 and significantly increased from E14.5 to E18.5. μ and κ receptors were first detected at E12.5 and at E14.5 respectively. Differences in immunoreactivity levels of the three peptides occurs with dynorphin being much lower than met-enkephalin and β-endorphin. Expression of opioid peptides and receptors from E14.5 to E18.5 reveals an apparent association between the increasing amount of the three peptides and the rise in their corresponding opioid receptors. Interestingly, levels of β-endorphin but not met-enkephalin or dynorphin diminish by P1, the stage at which a sharp increase of μ receptors occur. In a comparative study of the binding of β-endorphin, its truncated form (1-27) and their N-acetyl derivatives, to E14.5 brain membranes, β-endorphin exhibited the highest affinity.

159.3

CHANGES IN MU, DELTA, AND KAPPA OPIOID BINDING SITES IN RAT SPINAL CORD AFTER DORSAL RHIZOTOMY. C.W. Stevens and V. S. Seybold, Department of Cell Biology and Neuroanatomy, University of Minnesota, Minneapolis, MN 55455.

The aim of this study was to determine the binding of highly-specific mu, delta, and opioid radioligands in rat spinal cord following unilateral dorsal rhizotomy. Using computerized grain counting, we quantified changes in mu, delta, and kappa opioid receptor binding in discrete areas of the rat spinal cord associated with the terminations of nociceptive primary afferent fibers.

Spinal segment L4 was obtained from control rats and from surgically prepared animals at 1, 2, 4 and 8 days after unilateral dorsal rhizotomy (N=8 rats/group). Autoradiographic studies of opioid binding sites were performed on spinal cord sections using tritiated sufentanil (0.3 nM), DPDPE (5 nM), and U-69593 (3 nM) to label mu, delta, and kappa sites, respectively. Specific binding was defined as total binding minus the non-specific binding obtained in the presence of unlabeled ligands. Preliminary data suggest a significant decrease for all three ligands in laminae I-II, lamina V, and lamina X after unilateral rhizotomy, with the greatest decreases observed at 4 days after the rhizotomy. Mu and kappa binding sites in laminae V and X returned to control values at 8 days after rhizotomy. In contrast, delta sites in all three regions of analysis remained significantly decreased throughout the 8 day time course of these experiments. The greatest percent decrease (>50%) was observed for kappa sites in laminae I-II, V, and X, at 4 days after dorsal rhizotomy. These results confirm previous studies demonstrating a significant decrease in spinal cord opioid receptors following unilateral dorsal rhizotomy. The decreases suggest the location of mu, delta, and kappa opioid receptors on the central processes of nociceptive primary afferent fibers. Additionally, these studies suggest that opioid receptors located on intrinsic spinal neurons or processes are altered in response to unilateral dorsal rhizotomy.

159.4

QUANTITATIVE AUTORADIOGRAPHIC MAPPING OF OPIATE (δ, μ and κ) BINDING SITES IN THE AUDITORY CORTEX OF THE MALE FERRET. M.I. Davila-Garcia , R. Lu and E. M. Leslie. Dept. of Pharmacology University of California, Irvine, Irvine, CA 92717.

The distribution of opiate binding sites in the auditory cortex of the ferret was analyzed using quantitative autoradiography. Adult male ferrets weighing between 900 and 1.2 Kg were anesthetized with nembutal (30mg/Kg) and perfused with 30% sucrose in 0.85% NaCl. Brains were removed and immediately frozen in dry ice for 30sec and placed at -70°F until used. The brains were cut in 15μm sections in a cryostat and processed for binding. Opiate binding sites were defined as follows: [^{3}H]DADLE in the presence of D-pro^{4}Morphiceptin for δ receptors, [^{3}H]DAGO for μ receptors and [^{3}H]Diprenorphine in the presence of DSLET and D-pro^{4}Morphiceptin for κ receptors. Non-specific binding was defined with 1μM levallorphan and specific binding was calculated as the difference between total and non-specific binding. Delta binding sites were identified in the upper layers of auditory cortex, being highest in layers III and IV, and lowest in layers V and VI. In contrast, μ binding sites were restricted to the lower cortical layers, layer V being most significantly labeled, followed by layers VI and IV. Kappa sites were distributed mainly in the border between layers II and III and in layer V, with layers III and IV having moderate and layers I and VI having negligible amounts. These results demonstrate a differential distribution of opiate binding sites in the auditory cortex of the ferret. This distinct pattern of opiate binding sites may correspond to a functional distribution as yet undescribed.

This research was supported by a 5P01 DC00450-03 grant.

159.5

EXERCISE-INDUCED CHANGES IN BETA-ENDORPHIN BINDING PARAMETERS TO EQUINE LYMPHOCYTES. J.G. Hamra*, S.G. Kamerling and J.D. Harkins.* Dept. Vet. Phys. Pharm. and Tox., Louisiana State Univ. Baton Rouge, LA 70803.

Recent studies from our laboratory have shown that racehorses release BE after strenuous exercise and that this opioid binds in a specific and saturable manner to equine lymphocytes (LYMs). In the present study 15 thoroughbred horses were exercised on an inclined treadmill at progressively increasing speeds for 20 minutes. Near maximal exertion heart rates (200 beats/min) were attained post-exercise. Blood samples were obtained prior to and immediately after exercise. LYMs were isolated using density gradient centrifugation and suspended in medium 199 supplemented with 0.1% BSA. LYMs,(7.5×10^6 cells/ml) were incubated with (3-[^{125}I] iodotrosyl^{27} BE (human) and increasing concentrations of equine BE (10^{-12}M - 10^{-6}M) for 4 hours at 25°C. Scatchard analysis of the binding data revealed a significant decrease in the dissociation constant (K_d) following exercise with a mean decrease of 0.68 pM which suggests a higher affinity for the receptor following exercise. There was also a significant decrease in the B_{max} suggesting a decrease in receptor number following exercise. These results suggest that increased BE levels following exercise modulate equine LYM receptor binding and therefore equine LYM function. (Supported by the Grayson Foundation).

159.6

Alterations in Opioid Receptor Binding Following Continuous ICV Administration of Butorphanol and Morphine. M. Makimura*, P.J. Horan*, B. Hoskins, and I.K. Ho, Dept. Pharmacol. and Toxicol., Univ. MS Medical Center, Jackson, MS 39216

Male Sprague-Dawley rats received continuous ICV infusions of saline (1 µl/hr), butorphanol or morphine (52.3 nmol/hr) for 3 days via s.c.-implanted osmotic minipumps (Alzet 2001). Withdrawal was precipitated in the butorphanol- and morphine-infused rats by administration of naloxone (5 mg/kg, s.c) or by terminating the infusions (abrupt withdrawal). Binding characteristics of opioid receptors were determined in membranes prepared from whole brains minus cerebella and frontal cortices using the µ receptor ligand, DAGO, the δ receptor ligand, DPDPE, the κ receptor ligand, U-69593, and the non specific ligand, naloxone. Abrupt withdrawal from butorphanol or morphine resulted in 107% and 51% increases, respectively, in naloxone binding. Binding of the other receptor ligands was not altered by abrupt withdrawal. Naloxone-precipitated withdrawal increased the dissociation constants (decreased affinities) of mu and kappa receptors in butorphanol-and morphine-infused animals. These results suggest that the properties of opioid receptors are altered by continuous presence of butorphanol or morphine and receptor sensitivity to antagonists appears to increase. (Supported by DA 05828).

159.7

INTERLEUKIN-2 SUPPRESSED 3H-OHMEFENTANYL AND 3H-DADLE BINDING TO RAT BRAIN OPIOID RECEPTORS. Z.Y.Li. Shanghai Institute of Materia medica, Academia Sinica, Shanghai 200031, P.R.China.

Interleukin-2 is a lymphokine, an important immune regulator and has been shown to act sommogenic and alagesic via opioid receptors. 3H-Ohmefentanyl and 3H-DADLE are the selectively specific mu and delta opioid receptor agonist respectively. By using the radioligand receptor binding assay and in vitro organ assays, I investigated the mechanism of the Alal25Il-2 (HrIL-2) acting on opioid receptors. In rat brain membranes, HrIL-2 (10 pM to 1 uM) competitively binds to receptor binding site with the 3H-Ohmefentanyl and 3H-DADLE. The inhibition of the two ligands binding is up to 90%, it is dose-response effect and partially reversed by Naloxone. The depressant effect of HrIL-2 on the electrically evoked contractions of mouse vas deference had EC50 37 nM, Ke 62 nM (Naloxone) and Ke 119 nM (Mr2266) respectively. In contrast with guinea pig ileum assays, the action on the mouse vas deference was slowly reversed by naloxone and Mr2266, indicating that it was mediated by delta receptor not mu opioid receptor.

159.8

DESIGN OF A POTENT DYNORPHIN-RELATED PEPTIDE WITH A HIGH SPECIFITY FOR THE κ-OPIOID RECEPTOR. G.P. Martinka*, K. Jhamandas, L. Sabourin*, C. Lapierre* and S. Lemaire. Department of Pharmacology, Faculty of Health Sciences, University of OTTAWA, Canada K1H 8M5.

An adrenomedullary dynorphin-related peptide (Dyn-A-(1-13), Tyr^{14}, Leu^{15}, Phe^{16}, Asn^{17}, Gly^{18}, Pro^{19}: Dyn I-(1-19)), was synthesized by the solid phase procedure. The synthetic peptide was tested for its ability to inhibit electrically-evoked contractions of the guinea pig ileum (GPI), to compete with the binding of prototype liqands selective for κ-,µ- or δ -opioid receptors in membrane preparations of guinea pig cerebellum and rat brain and to produce antinociception in the rat. In the GPI, Dyn I-(1-19) possessed a relative potency (IC_{∞}:0.5nM) that was comparable to that of [D-Pro^{10}]-Dyn A-(1-11) (IC_{50}:0.5nM) and Dyn A-(1-13) (IC_{50}:0.7nM). The affinity of Dyn I-(1-19) for the κ site in the membrane preparation of the guinea pig cerebellum (IC_{50}:0.2nM) was also comparable to those of Dyn A-(1-13) (IC_{50}:0.11nM) and [D-Pro^{10}]-Dyn A-(1-11) (IC_{50}:0.10nM). Its relative affinities for µ (IC_{50}:7nM) and δ (IC_{50}:71nM) receptors were much lower than those of [D-Pro^{10}]-Dyn A-(1-11) and Dyn A-(1-13), rendering the peptide the most selective for the κ opioid receptor. Low doses intrathecal Dyn I-(1-19) produced profound antinociceptive effects in the rat paw pressure assay but not in the tail flick test without affecting blood pressure and motor activity. Supported by MRC (PG-20).

159.9

WITHDRAWN

159.10

STRUCTURE-ACTIVITY RELATIONS OF NALOXONE BENZOYLHYDRAZONE (NalBzoH): EFFECTS ON SUBTYPE SELECTIVITY AND DISSOCIATION KINETICS. K.M.Standifer, L.Liu, R.Parameswaran, J.Z.Ginos, and G.W.Pasternak. Dept. of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY 10021.

We have recently reported on the synthesis and activity of a novel opiate derivative, NalBzoH which has several interesting characteristics. Among these are its prolonged duration of mu receptor antagonism ($\tau_{1/2}$ = 24 hr) and its potent kappa analgesic activity at doses above those necessary to antagonize mu actions. We have shown that the analgesic actions of NalBzoH are mediated through a novel kappa$_3$ receptor, which was identified using ^{3}H-NalBzoH.

As this compound is structurally similar to naloxone, its mu antagonism was expected; the analgesia at the kappa$_3$ site and the pseudoirreversible binding was not. Consequently, we are very interested in determining the structural entity responsible for each of these properties. While the 3-iodo,4-hydroxybenzoyl hydrazone derivative of ^{3}H-naloxone showed NalBzoH-like binding with similar K_D, B_{max}, and slow dissociation, the 4-hydroxybenzoyl hydrazone compound exhibited binding properties more similar to naloxone. The lack of correlation between affinity from competition studies and dissociation rates suggests an unusual binding model. Other unlabeled NalBzoH derivatives display interesting differences in the kappa$_1$/kappa$_3$ selectivity as well as mu$_1$/kappa$_3$. These and other structure-activity determinations will be shown.

159.11

OPIOID BINDING POTENCY AND INHIBITION OF NEUROTRANSMITTER UPTAKE BY THE ANALGESIC TRAMADOL. E.E. Codd, R.P. Shank, R.B. Raffa and J.L. Vaught. R.W.J. Pharmaceutical Research Institute at McNeil Pharmaceutical, Spring House, PA 19477.

The synthetic opioid analgesic tramadol is the most widely used opioid analgesic in Germany and is in Phase III clinical trials in the U.S. In addition to opioid activity, it appears to have a non-opioid component to its mechanism of action. In the mouse 48°C hot plate, for example, tramadol antinociception was only partially reversed by naloxone. In an attempt to enhance the understanding of the neurochemistry underlying tramadol's analgesic activity, tramadol's interaction with mu, delta and kappa opioid binding sites as well as it's inhibition of the synaptosomal uptake of several neurotransmitters was studied. Tramadol exhibited a 10-20 fold receptor selectivity for inhibition of mu (^{3}H-DAGO) vs. delta (^{3}H-DPDPE) or kappa (^{3}H-U-69,593) binding, with a K_i for inhibition of ^{3}H-DAGO binding of about 2μM. Tramadol inhibited norepinephrine and serotonin uptake in rat brain synaptosomes, with K_i values of 0.64μM and 1.01μM respectively. Tramadol, at a concentration of 10μM, did not inhibit the uptake of dopamine, adenosine, 5'-AMP or GABA. Tramadol's activities in inhibiting both opioid binding and monoamine uptake may be a partial explanation for its unique combination of analgesic efficacy and lack of clinically significant constipation, respiratory depression and abuse liability.

159.12

COMPETITIVE INTERACTIONS AT [^{3}H]DTG-DEFINED SIGMA RECOGNITION SITES IN GUINEA PIG BRAIN. D.L. DeHaven-Hudkins and L.C. Fleissner*. Dept. of Enzymology and Receptor Biochemistry, Sterling Research Group, Malvern, PA 19355.

The interactions of prototypic sigma compounds with the sigma recognition site in guinea pig brain were investigated in saturation binding experiments using [^{3}H]DTG as the ligand and haloperidol to define non-specific binding. (+)Pentazocine, (+)3-PPP, haloperidol and rimcazole were evaluated at the IC_{50} and two times the IC_{50} determined from competitive inhibition experiments. Although each compound demonstrated a competitive interaction at the sigma binding site when tested at its respective IC_{50} value, as expressed by a two-fold shift in the K_D, the decrease in affinity at higher concentrations of cold competing drug was much less than predicted. The compounds were not interacting with the binding site in a noncompetitive fashion since there were no significant alterations in B_{max} values. These data suggest one of the following possibilities: (1) interactions at the sigma recognition site may represent a case of hyperbolic competitive inhibition, or (2) the affinities of these compounds for other receptors are contributing to non-specific effects in the Scatchard analysis of binding to sigma recognition sites. Furthermore, caution is warranted when making conclusions based on Scatchard analysis about competitive vs noncompetitive interactions at binding sites when only one concentration of cold competing drug is evaluated.

159.13

HIGH-AFFINITY BINDING OF [^{3}H]ADAMANTYL-TOLYL GUANIDINE TO THE SIGMA RECEPTOR. J.B. Fischer, N.L. Reddy*, K.J. Burke-Howie*, E. Weber[1] J.F.W. Keana*[2] and A.C. Server. Cambridge NeuroScience Research, Inc., Cambridge MA 02139, [1]Dept. of Pharmacology, U.C. Irvine, Irvine CA 92717, and [2]Dept. Chemistry, U. of Oregon, Eugene OR 97403.

N-Adamantan-1-yl-N'-o-tolyl guanidine (ADTG) is a higher affinity analog of Di-o-tolyl guanidine (DTG), a well-characterized sigma receptor ligand. We have prepared [^{3}H]ADTG by catalytic tritiation of a brominated precursor and purification by HPLC, giving a product with a specific activity of about 26 Ci/mmol. Used as a radioligand with guinea pig brain membranes [^{3}H]ADTG exhibited a large saturable binding signal that was blocked by low concentrations of haloperidol, (+)pentazocine, (+)3-PPP, DTG and several other sigma ligands, showing a similar pharmacological profile to [^{3}H]DTG. The saturable binding signal defined by 10 μM haloperidol or ADTG was 90-95% of the total binding. Receptor saturation experiments indicated a single binding site with a Kd value of 4.0 nM and a Bmax of 2.3 pmol/mg protein (n=3). These results indicate that this compound primarily binds to the sigma receptor in guinea pig brain membranes.

159.14

A DTG-BINDING PROTEIN SOLUBILIZED FROM LIVER IS SIMILAR TO THAT FOUND IN BRAIN. M.S. Sonders,* J.A. Lee,* J.F.W. Keana,#* and E. Weber. Dept. of Pharmacology, UC Irvine, Irvine CA 92717 and #Dept. of Chemistry, Univ. of Oregon, Eugene, OR 97403

The sigma binding site is a putative receptor which has been characterized most extensively in brain though its presence has also been recognized in several peripheral tissues. Recent work in our laboratory has been directed towards purifying the protein comprising the sigma binding site from guinea pig liver since it appears to be 15 to 20-fold enriched as compared to brain. Here we detail the initial characterization of a solubilizable sigma binding site from liver.

Washed particulate liver fractions were solubilized and assayed with [^{3}H]-DTG according to the procedure of Kavanaugh et al. [J. Neurochem. (1989) 53: 1575]. Typically 20 mM cholate solubilized 70% of the protein and 20-30% of the sigma binding sites. Saturation analysis indicated the existence of two sites whose K_Ds were approximately 25 and 500 nM; their densities were 10 pmol/mg and 25 pmol/mg protein, respectively. An array of sigma ligands displayed a rank order of inhibitor potency identical to that seen in solubilized brain tissue [ibid.].

Photoaffinity labeling of the soluble liver sigma binding site with [^{3}H]-azido-DTG using the protocol of Kavanaugh et al. [PNAS (1988) 85: 2844] revealed specific incorporation of tritium into a single protein band of M_r 28,000 (compare 29 kDa from brain [ibid.]). Taken together, these data suggest the probable identity of soluble DTG-binding proteins from guinea pig brain and liver, and the feasibility of purifying the sigma binding site from liver.

This work was supported by a grant from NIDA.

159.15

CHARACTERIZATION OF "SIGMA-LIKE" SITES IN RAT LIVER MEMBRANES: FURTHER EVIDENCE FOR SIGMA-1 AND SIGMA-2 SITES. A.E. Bruce, S.B. Hellewell*, and W.D. Bowen. Sect. Biochem., Div. Biol. and Med., Brown Univ., Providence, RI 02912.

Hepatic membranes were prepared by homogenization of rat liver in 10 mM Tris-HCl, pH 7.4, containing 0.32 M sucrose at 4°C. Scatchard analysis of binding of the prototypic sigma ligands [3H]DTG and [3H](+)-3-PPP, revealed the presence of a high density of high affinity binding sites. Parameter values were: [3H]DTG, Kd = 17.9±2.1 nM, Bmax = 11,895±1033 fmol/mg protein; [3H](+)-3-PPP, Kd = 51.9±3.4 nM Bmax = 11,070±1117 fmol/mg protein. Competition studies gave the following rank order of potency: DTG = (-)-pentazocine > fluphenazine > (+)-3-PPP > haloperidol > (-)-3-PPP > (-)-SKF 10,047 > (+)-SKF 10,047 = (+)-pentazocine. [3H](+)-Pentazocine bound with Kd = 7.5±1 nM and Bmax = 2,929±548 fmol/mg protein, whereas competition with [3H]DTG revealed a Ki = 1,058±123 nM. In addition, displacement of [3H](+)-pentazocine revealed a pharmacological profile similar to that of sigma sites of guinea pig brain.

The majority of [3H]DTG and [3H](+)-3-PPP binding sites in liver resemble sites we have characterized in PC12 cells and designated "sigma-2" (Hellewell and Bowen, *Brain Res.*, in press). While sigma-1 sites are synonymous with the well-characterized sigma sites of guinea pig brain, putative sigma-2 sites are characterized by: 1) high affinity for haloperidol, DTG, and (+)-3-PPP, 2) low affinity for (+)-benzomorphans, 3) reversed stereoselectivity for opiates ((-) > (+)), and 4) lower molecular weight (18-21 kDa). The high affinity of [3H](+)-pentazocine and its profile suggests that liver contains both sigma-1 and sigma-2 sites. This was confirmed by photolabeling with [3H]azido-DTG, revealing labeled polypeptides of 25 and 21.5 kDa. (Supported by PHS Grant NS-26746. We thank Dr. Eckard Weber for his gift of [3H]azido-DTG.)

159.16

PHARMACOLOGICAL CHARACTERIZATION OF SIGMA-LIKE RECEPTOR/BINDING SITE IN RAT LIVER. **D.I. Schuster, G. Singh, G. Ehrlich, and R.B. Murphy. Dept. Chemistry, New York University, New York NY 10003.**

Previous workers have described a sigma binding site in peripheral tissues. We report the pharmacology of this site in rat liver membranes using [^{3}H]-haloperidol. The binding site is saturable and unitary. K_i values found were (nM): haloperidol 1.3 , DTG 63, (+)-cyclazocine 296, buspirone 301, (-)-butaclamol 322, (+)-SKF10,047 536, (-)-cyclazocine 1863, (+)-butaclamol 3500, (-)-SKF10,047 5279. Saturation studies in the presence of carbon monoxide gave statistically unchanged values of K_d (0.8 nM) and B_{max} (23,000 fMol/mg), indicating that the binding site is not a liver cytochrome P-450. Values of B_{max} are highest in neonatal rats and rapidly decline with age. These data suggest that the liver sigma-like binding site is similar to but not identical with the CNS site. Supported by NIDA RO1 DA 05728 (RBM).

159.17

A CORRELATION BETWEEN SIGMA RECEPTOR BINDING PARAMETERS AND BEHAVIORAL RESPONSIVENESS TO A SELECTIVE SIGMA LIGAND IN RATS OF VARIOUS AGES. MK Hemstreet, RR Matsumoto, WD Bowen, and JM Walker. Brown University, Providence, RI 02912

The functional significance of sigma binding sites in motor function was examined with both behavioral and biochemical assays. The behavioral paradigm involved unilateral microinjections of DTG into the red nucleus of male rats of various ages. This technique was previously shown to reliably produce an acute deviation of head angle (torticollis) in rats. For in vitro experiments, rat brain membranes were prepared from age-matched subjects and ligand-binding assays were conducted on these membranes using [^{3}H]-DTG as the radioligand; sigma receptor binding parameters were then determined from the binding data using Scatchard analysis.

In agreement with previous studies, intrarubral DTG induced significantly greater cervical dystonia than did control injections. The degree of torticollis varied with age, although no clear age-dependent pattern emerged. The amount of head deviation following intrarubral DTG was highly correlated with the amount of bound ligand in the brain membranes of the age-matched in vitro subjects ($r=0.87$). This correlation suggests a functional relationship between sigma receptor binding in the mammalian brain and normal maintenance of head posture.

159.18

CLORGYLINE AND SKF-525A INTERACT POTENTLY WITH SIGMA BINDING SITES G. Battaglia Dept. of Pharmacol., Loyola University of Chicago, School of Medicine, Maywood, IL 60153

The high density of sigma sites labeled by 3H-HAL (3H-Haloperidol) in cerebellum and liver suggests that some "sigma" sites may be on enzymes such as MAO (monoamine oxidase) or cytochrome P450. A pharmacologic profile of 12 MAO inhibitors (MAOI's) at 3H-HAL labeled sigma sites in cerebellar homogenates demonstrated that only clorgyline exhibited high affinity (Ki=3.4nM). Most other MAOI's had affinities greater than 10uM with the isomers of deprenyl being somewhat more potent but exhibiting a stereospecificity opposite that for MAO inhibition. Clorgyline administration (3mg/kg, s.c. 1x/day for 7 days) caused a 15% decrease in 3H-HAL labeled sigma sites in cerebral cortex but no change in liver. Tranylcypromine, an MAOI with markedly lower affinity for sigma sites, was without effect. The microsomal enzyme inhibitor, SKF-525A (Proadifen), also had high affinity (Ki=6nM) for 3H-HAL labeled sigma sites in liver and brain. Subcellular fractionation studies in liver revealed the presence of sigma sites in fractions P1 and P3, but not P2, as indicated by the affinities of pentazocine and (-)BTC. SKF-525A, exhibited high affinity for 3H-HAL labeled sites in the nuclear (P1) and other non-microsomal (P2 and P3) fractions. **These data indicate that clorgyline and SKF-525A interact potently with sigma sites.** These sigma sites are unlikely to be on mitochondrial MAO since most MAOI's had low affinity. In addition, it is unlikely that these sites are on cytochrome P450 since SKF-525A had high affinity for sigma sites in all non-microsomal fractions. These data do suggest that 3H-HAL labeled sigma sites may be present on mitochondrial components other than MAO, since mitochondria were present in all subcellular fractions investigated.

SECOND MESSENGERS I

160.1

ACTIVATION OF MUSCARINIC RECEPTORS IN HUMAN-SK-N-SH NEUROBLASTOMA CELLS ELICITS A SUSTAINED HYDROLYSIS OF POLYPHOSPHOINOSITIDES. S.K. Fisher, A.M. Heacock, E.B. Seguin* and B.W. Agranoff. Neurosci. Lab., Univ. of Mich., Ann Arbor, MI 48104

The contribution of polyphosphoinositides to muscarinic receptor (mAChR)-stimulated phosphoinositide turnover has been evaluated for both intact and digitonin-permeabilized cells. Addition of carbachol (CCh) to [^{3}H]inositol-prelabeled intact cells resulted in a rapid and sustained loss of [^{3}H]phosphatidylinositol 4,5-bisphosphate with the concomitant appearance of labeled I(1,4,5)P_3, I(1,3,4)P_3 and IP_4. Inositol mono- and bisphosphates steadily accumulated in response to mAChR activation and comprised > 95% of inositol phosphate formation at incubation times > 5 min. The major IP_2 isomer formed at all times was the (1,4) species.Of the two IP_1 isomers produced, [^{3}H]I(4)P increased continuously whereas label in [^{3}H]I(1)P/[^{3}H]I(3)P was delayed in appearance but thereafter progressively accumulated. Whole homogenates of SK-N-SH cells metabolized added I(1,4,5)P_3 to I(1,4)P_2 and I(4)P, whereas I(1,3,4,5)P_4 was degraded to I(1)P/I(3)P. We conclude that mAChR occupancy elicits the continuous breakdown of polyphosphoinositides, and that much of the label present in I(1)P/I(3)P fraction is derived from operation of the IP_3 kinase pathway. Although addition of CCh to digitonin-permeabilized cells also resulted in a sustained release of [^{3}H]inositol phosphates, the ratio of [^{3}H]I(4)P:[^{3}H]I(1)P/I(3)P produced was greater than that observed for intact cells. Measurement of IP_3 kinase and IP_3 5'-phosphatase activities revealed that digitonin permeabilization enhanced the phosphatase and diminished the kinase activity. Thus, the routes of degradation of I(1,4,5)P_3 differ between the intact and permeabilized preparations. Supported by NIH NS 23831, NS 15413 and NIMH MH 42652.

160.2

CELL-SURFACE RATHER THAN SEQUESTERED MUSCARINIC RECEPTORS ARE LINKED TO PHOSPHOINOSITIDE HYDROLYSIS. A.K. Thompson and S.K. Fisher. Neuroscience Laboratory and Department of Pharmacology, The University of Michigan, Ann Arbor, MI 48104

Addition of carbachol (CCh) to intact human SK-N-SH neuroblastoma cells results in a rapid sequestration of 50% of the cell-surface muscarinic acetylcholine receptors (mAChRs) into a lipophilic domain without a loss of stimulated phosphoinositide (PPI) turnover (Thompson and Fisher, 1990, *JPET* **252**:744). One explanation that may reconcile these inconsistencies is that the sequestered receptor is both recognized by the agonist and can couple to PPI hydrolysis. To address this issue, PPI hydrolysis was monitored at 10°C, a temperature at which the cycling of mAChRs between cell-surface and sequestered domains is prevented. Intact [^{3}H]inositol prelabeled cells were first exposed to CCh for 1 h at 37°C to induce mAChR sequestration, the agonist removed by washing at 0°C and agonist-stimulated PPI hydrolysis measured at 10°C for 2 h. When compared to controls, pretreatment of cells with CCh resulted in a subsequent 40-50% loss of [^{3}H]inositol phosphate formation when stimulated with either hydrophilic or lipophilic agonists in the presence of Li^+. In contrast, when CCh-pretreated cells were incubated at 37°C, a temperature at which receptor cycling occurs, only a 5-15% loss of activity was detected. Pretreatment of intact SK-N-SH cells with carbachol also resulted in the appearance of a population of mAChRs which exhibited a very low affinity for the hydrophilic full agonist, CCh, whereas lipophilic agonists detected the mAChR population in control and CCh-pretreated cells with equal affinities. Thus, whereas the lipophilic agonist can readily bind to both sequestered and cell-surface receptors, hydrophilic agonists only detect the latter population of mAChRs. We conclude that cell-surface mAChRs are preferentially coupled to PPI hydrolysis in SK-N-SH cells. For a sustained PPI hydrolysis to occur, it appears that the sequestered mAChRs must return to the cell surface. (Supported by NIMH Grant 42652 and NIH Training Grant 5 T32 GM07767-10.)

160.3

THE Na^+/Ca^{2+} EXCHANGER AND PHOSPHOINOSITIDE METABOLISM IN SYNAPTONEUROSOMES. M. Benuck, M.E.A. Reith and A. Lajtha. Center for Neurochemistry, N.S. Kline Institute, Ward's Island, NY, 10035.

We find evidence supporting the participation of the Na^+/Ca^{2+} exchanger in the inositol response to depolarization (30 mM KCl) or sodium channel activation (10 uM veratridine). In these studies, the release of [3H]inositol phosphates (IP_1, IP_2 and IP_3) is compared with the increase in $[Ca^{2+}]_i$ measured with fura-2-AM in mouse cerebral cortical synaptoneurosomes. The ratio in the inositol response to veratridine over that to KCl in time is different for IP_2 and IP_1; this may be related to the more rapid rise of $[Ca^{2+}]_i$ upon addition of KCl as compared to veratridine.

The participation of voltage-sensitive calcium channels appears to be minor, since the presence of ω-conotoxin did not significantly affect the inositol response or $[Ca2+]_i$ levels upon addition of KCl or veratridine. Benzamil and 5-(N,N dimethyl) amiloride, blockers of the Na^+/Ca^{2+} exchanger, inhibited the inositol response to KCl and veratridine. Omission of Na^+ from the external medium reduced the effect of KCl and veratridine on the inositol response and on $[Ca^{2+}]_i$ levels. These results give further indication of the participation of the Na^+/Ca^{2+} exchanger in the regulation of phosphoinositide metabolism.

160.4

THE RELATIONSHIP OF AGONIST- AND CAFFEINE-SENSITIVE CALCIUM POOLS IN SINGLE BOVINE ADRENAL CHROMAFFIN CELLS. K.A. Stauderman and M.M. Murawsky*. Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215.

Stimulation of single bovine adrenal chromaffin cells with histamine or angiotensin II (A_{II}) leads, initially, to at least one large "spike" of elevated intracellular free Ca^{2+} ($[Ca^{2+}]_i$). While IP_3-induced Ca^{2+} release is almost certainly involved in these spikes, the potential contribution of Ca^{2+}-induced Ca^{2+}-release (CICR) has not been addressed. To examine the role of CICR, we monitored $[Ca^{2+}]_i$ changes in single fura-2 loaded chromaffin cells treated with or without 10 mM caffeine, an agent that will discharge internal Ca^{2+} pools sensitive to CICR. With no external Ca^{2+} present, cells not treated with caffeine displayed an initial large spike of $[Ca^{2+}]_i$ after stimulation with either 10 µM histamine or 0.1 µM A_{II} (peaks 720 ± 103 and 746 ± 81 nM, respectively). Caffeine alone produced a large transient elevation of $[Ca^{2+}]_i$ (peak 796 ± 84 nM). In caffeine pretreated cells, the amplitudes of histamine- and A_{II}-induced increases of $[Ca^{2+}]_i$ were both decreased by 94%. Conversely, caffeine responses were inhibited 100% and 92% after pretreatment with histamine or A_{II}, respectively. Thus, over 90% of the Ca^{2+} released by either histamine or A_{II} arises from caffeine-sensitive stores, and are consistent with CICR playing a major role in the development of the initial Ca^{2+} spikes. The role of CICR in the later stages of agonist responses is under investigation.

160.5

INHIBITION OF CARBACHOL-STIMULATED PHOSPHOINOSITIDE TURNOVER BY KAPPA OPIOID AGONISTS IN RAT HIPPOCAMPUS. S. Periyasamy and W. Hoss. Dept. of Medicinal and Biological Chemistry, Univ. of Toledo, Toledo, OH 43606.

The effects of various subtype-selective opioid agonists on carbachol-stimulated phosphoinositide (PI) turnover response in rat hippocampal slices were examined. U-50,488H, a kappa-selective agonist that stimulates PI turnover in this preparation (Periyasamy and Hoss, Life Sci., in press, 1990), inhibited carbachol stimulated PI turnover in a concentration-dependent manner with an IC_{50} value of 33 ± 9.0 μM. Other kappa-agonists, including ketocyclazocine and D[Ala]^2-dynorphin-A (1-13) amide also produced concentration-dependent inhibition of carbachol-stimulated PI turnover, but with lower potency. On the other hand, the mu-selective agonist [D-Ala^2-N-Me-Phe^4-Gly^5-ol]enkephalin and the delta-selective agonist [D-Pen^{2,5}] enkephalin caused only a small decrease in carbachol-stimulated PI turnover. The inhibitiory effect of U-50,488H was not blocked by either kappa-selective antagonists nor-binaltorphimine and MR2266 (10 μM) or tetradotoxin (1 μM) suggesting that the U-50,488H effect is neither mediated through kappa-receptors nor through the release of endogenous neurotransmitters. A Lineweaver-Burke plot of the stimulation of PI turnover by carbachol in the presence and absence of U-50,488H showed that the K_m was not changed (11.4 ± 3.4 and 11.5 ± 2.6 μM) whereas V_{max} was reduced (1534 ± 31 and 3849 ± 460 cpm) indicating that the inhibition was non-competitive. U-50,488H also inhibited guanosine 5-[β, γ-imido]triphosphate (Gpp NHP)-stimulated PI turnover in rat hippocampal membranes in a concentration-dependent manner with an IC_{50} value of 33 ± 12 μM. However, U-50,488H had no effect on phospholipase-C-stimulated PI turnover. In receptor binding studies U-50,488H displaced the non-selective muscarinic antagonist [^{3}H]-1-quinuclidinyl benzilate ([^{3}H]-1-QNB) binding in a concentration dependent manner with an IC_{50} value of 4.5 ± 1.5 μM. A Scatchard plot of [^{3}H]-1-QNB binding in the presence and absence of U-50,488H showed that the K_d was changed (883 ± 250 and 364 ± 147 pM) where as B_{max} remained the same (1.66 ± 0.16 and 1.65 ± 0.18 pmol/ mg protein) suggesting that the inhibition was competitive. These data indicate that U-50,488H acts at the G-protein and muscarinic receptors but not kappa receptors or release of transmitters. Supported by DA04068 and DA06258.

160.6

P_2 PURINERGIC AND BRADYKININ RECEPTOR-INDUCED CALCIUM RESPONSE AND INOSITOL PHOSPHATE RELEASE IN NG108-15 CELLS. T.A. Lin, G.A. Weisman* and G.Y. Sun. Dept. Biochem., Univ. Missouri, Columbia, MO 65212.

Both ATP and bradykinin (BK) are known to transduce their signals through stimulation of poly-PI breakdown and release of inositol trisphosphate. In turn, this second messenger can cause an increase in intracellular [Ca^{2+}]i. The present study addresses effects of these two receptor types on [Ca^{2+}]i response and inositol phosphate release in the neuroblastoma-glioma hybrid cells (NG108-15). In the presence of extracellular Ca^{2+} (1 mM), both ATP (1-100 μM) and BK (1 nM-1 μM) could induce transient increases in [Ca^{2+}]i, reaching a peak within 10 seconds and subsequently declining to near basal level within 1 min. When cells were pretreated with ATP (100 μM), they failed to repond to a second challenge of ATP although a small response could be regained on removal of ATP by washing. Cells that were pretreated with ATP (1-2000 μM) responded well to BK (1 μM). On the other hand, cells that were pretreated with 1 μM BK failed to respond to a second challenge of BK (1 μM) or ATP (100 μM). Inositol monophosphate isomers in these cells were analyzed by ion chromatography. Cells stimulated by either ATP or BK (5 min in the presence of lithium) indicated a 4-5 fold increase in Ins(4)P and, to a smaller extent, an increase in Ins(1)P also. Information on inositol phosphate isomers will be used to decide whether these receptors use the same or different [Ca^{2+}]i pools in the cell.

160.7

THE BRADYKININ ACTIVATION OF PHOSPHOLIPASE D IN PC12 CELLS IS NOT MEDIATED BY PROTEIN KINASE C. J. Horwitz and S. Ricanati*. Dept. of Pediatrics and Kennedy Mental Retardation Res. Ctr., The University of Chicago, Chicago, IL 60637.

Bradykinin activates phospholipase D in PC12 pheochromocytoma cells. Since bradykinin also activates protein kinase C in these cells, the possible role of this kinase in mediating the action of bradykinin was investigated. Phospholipase D activity was assayed by measuring the formation of [^{3}H]phosphatidylethanol ([^{3}H]PEt) in cells prelabeled with [^{3}H]palmitic acid and incubated in the presence of ethanol. The phorbol ester phorbol dibutyrate (PdBu) mimicked the effect of bradykinin on [^{3}H]PEt. The enzyme activated by PdBu was similar to the enzyme activated by bradykinin in terms of its Ca^{2+} dependence and specificity for phosphatidylcholine. Other data suggested that protein kinase C was not involved in mediating the effect of bradykinin. The protein kinase C inhibitor staurosporine (10 μM) significantly attenuated the effect of PdBu (-70%) but it did not block bradykinin stimulated [^{3}H]PEt. In addition, the effect of PdBu was partially additive with that of bradykinin. Prolonged treatment of PC12 cells with PdBu is known to deplete cells of protein kinase C. PdBu pretreatment completely blocked bradykinin stimulated [^{3}H]PEt in intact cells. This treatment, however, also caused a down regulation of phospholipase D (-55%) as assessed by an *in vitro* assay. Thus, the effect PdBu pretreatment on bradykinin stimulated [^{3}H]PEt could not be attributed to the depletion of protein kinase C. In conclusion, although the activation of protein kinase C leads to an increase in phospholipase D activity, this pathway does not play a role in mediating the effect of bradykinin. (Supported by NS-22694, HD-04583)

160.8

POLARIZED ACTIVATION OF INTRACELLULAR CALCIUM RELEASE AND CALCIUM-DEPENDENT ION CHANNELS BY ACETYLCHOLINE AND CHOLECYSTOKININ. H. Kasai and G.J. Augustine. Max Planck Inst. for Biophysical Chemistry, Goettingen, FRG.

We have examined the spatial distribution of agonist-induced Ca release and activation of Ca-dependent ion channels in isolated cells from rat pancreatic acini, using digital Ca-imaging and whole-cell patch clamp methods. Acetylcholine (ACh) and cholecystokinin (CCK), which generate IP_3, initially triggered Ca release at the luminal pole of these cells even though the receptors for these agonists are thought to be localized in the other (basolateral) side of the cell. The Ca rise then spread toward the basolateral side and eventually made Ca levels highest at this pole. In contrast, CCK-JMV, an analog of CCK that releases Ca without generating IP_3, did not produce the initial, spatially-restricted rise in Ca at the luminal pole. The ACh-induced Ca signal initially caused a sharp and transient activation of Cl current, which was followed by a simultaneous activation of both Cl and cation currents. In CCK-JMV the relative magnitude of the early, transient Cl current was small. These data (1) demonstrate that different agonists can regulate Ca release and ion channel activation in spatially and temporally distinctive patterns, (2) are consistent with two agonist-sensitive Ca release mechanisms, one of which is independent of IP_3, and (3) suggest a novel push-pull mechanism for Cl secretion in exocrine gland cells.

160.9

CHRONIC HALOPERIDOL TREATMENT ATTENUATES RECEPTOR-MEDIATED PHOSPHOINOSITIDE TURNOVER IN RAT BRAIN SLICES. R. Li*, L.Wing, Y. Shen, R.J. Wyatt, D.G. Kirch and D.M. Chuang. Neuropsychiatry Branch, NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032 and Biological Psychiatry Branch, NIMH, Bethesda, MD 20892.

To examine intracellular molecular mechanisms of haloperidol, we studied neurotransmitter receptor-mediated phosphoinositide (PI) turnover in rats treated with biweekly saline or IM haloperidol decanoate (HAL-D; 1.5 mg/kg/day) for 6 weeks. Basal and receptor.agonist-stimulated PI hydrolysis were assayed by lithium-dependent accumulation of ^{3}H-inositol monophosphate (IP_1) in brain slices pre-labeled with ^{3}H-myo-inositol. Basal PI turnover was unchanged after HAL-D, preliminary data indicate HAL-D significantly decreased carbachol-induced ^{3}H-IP_1 accumulation in striatum and hippocampus with a similar trend in frontal cortex. Norepinephrine sensitive ^{3}H-IP_1 accumulation was also significantly decreased in frontal cortex and hippocampus. Chronic haloperidol attenuated muscarinic and α1-adrenergic receptor-mediated PI hydrolysis, a result similar to previously reported effects of chronic lithium treatment on the PI system.

160.10

ENHANCED VASOPRESSIN (AVP)-INDUCED PHOSPHOINOSITIDE (PI) HYDROLYSIS IN RATS PRETREATED WITH I.C.V. OXYTOCIN (OT). P. Poulin, S. Weiss and Q.J. Pittman, Neuroscience Research Group, Univ. of Calgary, Calgary, Alberta, Canada T2N 4N1

Activation of V1 type of AVP receptors in the ventral septal area (VSA) causes motor disturbances in rats. These receptors can be "sensitized" by previous exposure to AVP or OT. This "sensitization" phenomenon is not due to an increase in the number or affinity of the VSA V1 receptors. In the present study, we further investigated possible mechanism(s) of the VSA V1 receptor sensitization by studying the regulation of postreceptor events (PI hydrolysis). Male Sprague Dawley rats were either sensitized with 10 pmole OT i.c.v. or given saline. 24 h later we compared the dose-response curves for AVP-stimulation of [^{3}H]inositol-1-phosphate (IP_1) accumulation in VSA slices from sensitized or control animals. In sensitized rats, AVP-stimulated accumulation of [^{3}H]IP_1 was 35% greater than in controls, at AVP concentrations of 10^{-8} to 10^{-5}M. Carbachol (1.0 mM) stimulation of [^{3}H]IP_1 accumulation was not different between the two groups. These results indicate that the PI response of VSA AVP receptors is functionally increased in sensitized animals when compared to controls. Supported by the Medical Research Council of Canada.

160.11

REGULATION OF PHOSPHOINOSITIDE HYDROLYSIS VIA AN M_3 MUSCARINIC RECEPTOR IN NEURONS IN PRIMARY CULTURE. J. Ellis, J.H. Huyler*, J. Hovious*, and R.H. Lenox. Neuroscience Research Unit, Dept. of Psychiatry, UVM College of Medicine, Burlington VT 05405.

We have previously shown that the muscarinic phosphoinositide (PI) response of striatal neurons in primary culture is mediated by an M_1 receptor. The same study found that neurons obtained from the brainstem did not possess M_1 receptors, but were more efficiently coupled to a muscarinic PI response, leading us to suggest the involvement of an M_3 receptor. Further pharmacological analysis has found that carbachol is more potent in stimulating the PI response in brainstem neurons (EC_{50} 21 μM) than in striatal neurons (61 μM). Pirenzepine is considerably less potent in antagonizing the response of brainstem neurons (apparent K_i 210 nM, compared to 23 nM at striatal neurons), but 4-DAMP is equipotent in the two populations of neurons (apparent K_i 1 nM). This M_3 muscarinic receptor is regulated by protein kinase C (PKC) activation in much the same way as we have previously found for the M_1 receptor of striatal neurons. That is, brief exposure of brainstem neurons to phorbol dibutyrate (PDBu) leads to translocation of PKC from the cytosol to the membrane fraction and also attenuates the M_3-mediated PI response. Washout of PDBu reverses the translocation of PKC, but the attenuation of the PI response persists for at least several hours. (Supported by R01 AG05214).

160.12

IDENTIFICATION OF HISTAMINE RECEPTORS ON HUMAN ASTROCYTOMA-DERIVED CELLS (UC-11MG) AND THEIR ROLE IN THE REGULATION OF INTRACELLULAR FREE CALCIUM. M. J. Lucherini and E. Gruenstein. Dep't of Molecular Genetics, Biochemistry and Microbiology, University of Cincinnati College of Medicine, Cincinnati, OH 45267-0524.

Astrocytes comprise 20% - 25% of the cellular volume of the human brain. Their function in the central nervous system (CNS) involves the uptake and metabolism of neurotransmitters and the maintenance of low levels of extracellular K^+. We have found that these cells are capable of generating repetitive action potentials (unpublished data). This observation suggests the possibility that neurotransmitters might be involved in mediating astrocytic responses. We have tested this hypothesis by examining the ability of a variety of neurotransmitters to elevate levels of the important intracellular second messenger cytoplasmic free Ca^{2+} (Ca^{2+}_i).

Experiments were carried out on UC-11MG human astrocytoma cells, a continuous cell line that expresses a broad range of biochemical and electrophysiological properties of well-differentiated astrocytes (Lomneth et al, *Brain Research*, 1989;486:95-107). Ca^{2+}_i was monitored in substrate-attached cells with the fluorescent indicators fura-2 and indo-1 using digital imaging analysis and dual wavelength photometry, respectively. Of the eleven neurotransmitters tested, only histamine caused a significant elevation in Ca^{2+}_i. The Ca^{2+}_i transient in response to histamine occurred within seconds and was maximal at 300 μM histamine. The response is mediated through H_1 receptors and involves production of IP_3. Although cAMP is not elevated following histamine stimulation, administration of dBcAMP 24 hours prior to histamine stimulation caused a 60% increase in the peak Ca^{2+}_i response. We conclude that histamine causes a significant Ca^{2+}_i response in human astrocytes and that this neurotransmitter may play a role in astrocytic function in the human CNS. Supported in part by grants NIH NS27814 and National AHA ref. #890513.

160.13

Effect of the Benzodiazepine Midazolam on Calcium Signalling in Astrocytes. William E. Code, J. Steven White, Leif Hertz. Depts. of Anaesthesia and Pharmacology, Univ. of Sask., Saskatoon S7N OWO Canada and College of Pharmacy, Univ. of Utah, Salt Lake City, Utah

A potassium induced entry of labelled calcium has been demonstrated in primary cultures of astrocytes (Hertz et al., J Neurosci Res, 22, 209, 1989). This calcium uptake is inhibited by a low concentration of the calcium channel antagonist nimodipine, indicating that it occurs through a voltage dependent L-channel. The astrocytic benzodiazepine receptor may interact with calcium channels. Many clinically used benzodiazepines, e.g., diazepam and midazolam, a water soluble benzodiazepine frequently used in anaesthesia, have equal affinity for the neuronal and astrocytic benzodiazepine receptor and may thus exert at least part of their action on astrocytes. We have measured free intracellular calcium (Ca^{2+}_i) in primary cultures of astrocytes with the fluorescent drug Indo-1. When extracellular K^+ (K_o) was raised from 3.6 to 20 mM, Ca^{2+}_i increased by 4.0±1.1 arbitrary units (AU). Exposure to the same K^+_o in the presence of midazolam (10 nM) caused a considerably higher increase (18.4±4.1 AU). In the presence of the astrocyte specific benzodiazepine antagonist PK11195 (1 μM) the effect by 20 K^+ alone was unaltered (an increase of 4.55±0.90 AU). When PK11195 was added in the joint presence of 20 mM K^+ and 10 nM midazolam most of the stimulatory effect by midazolam was abolished (7.9±1.1 AU). Since astrocytes possess calcium dependent K^+ channels, these effects may be important in the regulation of channel mediated transport of K^+ in astrocytes, a process which is of importance for regulation of K_o^+ in brain and thus of neuronal excitability. These effects are more potent than the effects of benzodiazepines on neuronal chloride permeability.

160.14

LITHIUM-INDUCED LETHALITY AND CHANGES IN CEREBRAL INOSITOL, INOSITOL MONOPHOSPHATES AND CALCIUM. M.-R. Hirvonen and K.M. Savolainen. Natl. Publ. Hlth Inst., Dept. Env. Hyg. & Toxicol., P.O.B. 95, SF-70701 Kuopio, FINLAND.

Lithium inhibits the hydrolysis of inositol-1-phosphate (Ins1P), an intermediate in neuronal phosphoinositide (PI) cycle. Ins1P reflects the activity of receptor-coupled PI-signaling. Effects of 2.5, 5, 10, 12, 14, 16 or 18 meq/kg of LiCl on lethality, and brain regional inositol, Ins1P, inositol-4-phosphate (Ins4P), and Ca^{2+} levels were studied in male rats 24 h after a single dose of LiCl. Inositol and inositol monophosphates were measured by gas chromatography and mass spectrometry, and metals with AAS. LiCl at doses exceeding 10 meq/kg increased lethality with a LD50 dose of 14 meq/kg. LiCl decreased brain inositol, and brain Li^+ increased in response to LiCl dose. Low doses of LiCl increased Ins1P but doses exceeding 10 meq/kg decreased Ins1P; LiCl did not affect Ins4P. Lethal doses of LiCl decreased brain Ca^{2+} by 40-60%. Effects of LiCl on Ins1P were significant in all brain regions except cerebellum. Dual effects of LiCl on brain Ins1P provide evidence that several enzymes in PI cycle with different sensitivities to Li^+ may be inhibited by the ion. Thus, Ins1P and Ins4P accumulation does not explain LiCl-induced decreases in brain inositol, and Ins4P even seems to be insensitive to Li^+. Effects of LiCl on brain total Ca^{2+} are likely to be nonspecific because they only occurred at lethal LiCl doses. Supported by The Academy of Finland.

160.15

COUPLING OF MUSCARINIC RECEPTORS IN RESPONSIVE AND NONRESPONSIVE RAT SWEAT GLANDS. M. P. Grant and S. C. Landis Depts. of Pharmacology and Neurosciences. Case Western Reserve University, Cleveland, OH 44106.

Previous studies have demonstrated that acutely denervated rat sweat glands, or glands sympathectomized at birth with 6-hydroxydopamine do not secrete in response to muscarinic stimulation; however, they express control levels of muscarinic ligand binding sites. This observation raises the possibility that the receptor is uncoupled from its appropriate intracellular effector. Studies of secretory mechanisms in other exocrine gland tissues have shown that secretion is stimulated *via* the PI pathway through the generation of IP_3 promoting the release of intracellular Ca^{2+}; however, it is not known if this mechanism is responsible for secretion in rat sweat glands.

We have investigated the coupling of muscarinic receptors to PI turnover in rat sweat glands. Preliminary results from SDS-PAGE analysis of membranes ^{32}P-ADP-ribosylated with pertussis toxin (PTx) demonstrated the existence of a 40-41 kD pertussis toxin sensitive protein in both control and 6-OHDA membranes. Immunocytochemical and western blot analysis is in progress to determine if this protein is responsible for coupling the receptor to phospholipase C. Stimulation with carbachol (1 mM) of slices of gland-rich tissue isolated from control animals produced approximately a four-fold increase in the amount of total inositol phosphates, and the effect was inhibited by atropine (1 μM); this response was identical to that of a parotid gland control. Further, stimulation of slices isolated from 6-OHDA treated animals also produced a four-fold increase in the amount of total inositol phosphates. Thus, it appears that muscarinic cholinergic receptors present on sweat glands of both responsive and nonresponsive glands are coupled to PI turnover. Supported by NS 23678.

160.16

EFFECTS OF CLIMBING FIBRE DESTRUCTION ON INOSITOL 1,4,5-TRISPHOSPHATE RECEPTOR IN THE RAT CEREBELLUM. P.P. Li, M.A. Green*, J.J. Warsh. Clarke Institute of Psychiatry, University of Toronto, Toronto, M5T 1R8, Canada.

Inositol 1,4,5-trisphosphate ($InsP_3$) regulates an array of cellular function through binding to its receptor. While modulations of $InsP_3$ receptors in vitro have been reported, little is known of the regulation of these receptors in vivo. To investigate whether $InsP_3$ receptors could be trans-synaptically regulated in vivo, we examined the effect of eliminating the olivocerebellar projection, on cerebellar $InsP_3$ receptors in rat. Twenty-one days after an injection of 3-acetylpyridine [3-AP] (60 mg/kg, i.p.), which caused a degeneration of the inferior olivary climbing fibres, the density of $InsP_3$ receptor was significantly reduced by 20% with no apparent changes in the binding affinity. A significant reduction in the binding density was evident as early as at 7 days after 3-AP treatment. No further reduction in binding density was found in rats given a second dose at 7 days. The in vivo effect of 3-AP was not related to a direct action on the $InsP_3$ receptors as 3-AP (0.5 mM) failed to inhibit ^{3}H-$InsP_3$ binding in vitro. The above data suggest that some of the $InsP_3$ receptors in cerebellum may be localized on the climbing fibre terminals. Alternatively, the reduction in $InsP_3$ receptors may reflect an adaptive response to the heightened parallel fibre-Purkinje cell synaptic transmission which is known to occur following climbing fibre degeneration.

160.17

SUBSECOND KINETICS OF INOSITOL 1,4,5-TRISPHOSPHATE-INDUCED CALCIUM RELEASE REVEAL BIPHASIC REGULATION BY EXTRAVESICULAR CALCIUM. E.A. Finch[1,3], T.J. Turner[2], and S.M. Goldin[2,3]. [1]Program in Neuroscience and [2]Biol. Chem. Dept., Harvard Med. Sch., Boston, MA 02115; [3]Cambridge NeuroScience Research, Inc., Cambridge, MA 02139.

The kinetics and modulation by extravesicular Ca^{2+} (Ca_e) of IP3-induced release (IICR) from synaptosome-derived microsomes were studied using a superfusion system affording 50ms time resolution and the precise control of agonist and ionic concentrations. IP3 evoked a transient release of actively accumulated $^{45}Ca^{2+}$ (EC_{50}=250nM, n_H=0.9). Maximal rate of $^{45}Ca^{2+}$ release occurred within 100ms and decayed exponentially ($\tau \leq 160$ms). An invariant τ over a range of [IP3] (30nM-10μM) indicates that rapid decay of release is not simply due to depletion of releaseable $^{45}Ca^{2+}$, but results from attenuation of the release mechanism. Ca_e modulated the kinetics and magnitude of IICR. Elevation of [Ca_e] from 100nM to micromolar levels initially potentiated (EC_{50}=650nM) and subsequently inhibited $^{45}Ca^{2+}$ release. Potentiation of IICR by Ca_e developed within 100ms whereas inhibition by Ca_e required several hundred msec of exposure to elevated [Ca_e]. The kinetics and biphasic nature of the modulation by Ca_e suggest a mechanism for sequential positive and negative feedback regulation of IICR that may contribute to transients and/or oscillations of cytosolic free Ca^{2+} *in vivo*. (Supported by NIH grant GM35423.)

160.18

INTRACELLULAR PERFUSION TECHNIQUE SHOWS THAT Ca^{2+}_i SUPPRESSED I_A IN ISOLATED HIPPOCAMPAL PYRAMIDAL CELLS. Qiang X. Chen, Robert K.S. Wong. College of Physicians and Surgeons, Columbia University, N.Y. N.Y., 10032.

We have continued to examine intracellular calcium (Ca^{2+}_i) effects on hippocampal pyramidal cells by an intracellular perfusion technique (see Chen et al., J. Physiol., 420: 207). Experiments were carried out on acutely dissociated adult guinea-pig neurons. Intracellular and extracellular solutions were prepared to allow isolated activation of K^+ currents. Whole cell currents were recorded while intracellular solutions with low (> 10^{-8} M) or high (4.5×10^{-4} M) Ca^{2+} contents were introduced. Step depolarizations to above -45 mV from -95 mV activated outward currents consisting of a transient component (I_A) followed by delayed sustained currents. Perfusion with the low Ca^{2+} solution caused a gradual increase of I_A and decrease of sustained currents. In a second series of experiments stable recordings were obtained in low Ca^{2+} intracellular solution. Introduction of high Ca^{2+} potentiated the sustained K^+ currents, and suppressed A current. We attempted to confirm the latter observation by isolating A current using kinetic and pharmacological means. Firstly, A current, with a lower threshold, was activated in isolation by low voltage depolarization (-40 mV). Secondly, addition of Cs (10 mM) and TEA (10 mM) to the perfusate blocked delayed currents at all depolarizations but still allowed activation of A current. Suppression and blockade of A current by high intracellular Ca^{2+} were confirmed under both conditions. The results suggest that under different conditions, intracellular Ca^{2+} can either enhance or depress neuronal excitability via its modulatory actions on K^+ channels.

160.19

NO EVIDENCE FOR $INSP_3$ MEDIATED CALCIUM MOBILIZATION IN THE ASTROCYTE POPULATION OF THE ADULT RAT HIPPOCAMPAL SLICE. D. M. Leifer* and S. N. Murphy. Dept. of Radiology, University of Chicago, Chicago IL 60637.

Recent evidence has supported the presence of $InsP_3$ coupled receptors upon cultured astrocytes from various regions of the CNS including hippocampus. These receptors appear to have many of the properties of neuronal G-protein linked receptors including those for norepinephrine (NE), quisqualate (QUIS), and endothelin (ED), apparently resulting in Ca^{2+} mobilization from intracellular stores. We wished to investigate the role of these receptors in the adult rat brain. We therefore performed fura-2 microspectrofluorimetry upon thin hippocampal slices from adult rats. Slices were incubated in fura-2 ester under conditions which rarely resulted in neuronal uptake of the dye. Slices were perfused with glycine rich HCO_3^- buffered modified EARLES solution. Lack of neuronal dye loading was confirmed by negative responses to 100 μM veratridine (0/8). Basal $[Ca^{2+}]_i$ was 217±9 nM in 222 astrocyte fields. Each field contained approximately 45 astrocytes. Of these fields, 70% responded to a 50 mM K^+ depolarization with a $\Delta[Ca^{2+}]_i$ of 113±12 nM. 50 mM K^+ depolarization following the removal of Ca^{2+} and the addition 500 μM EGTA to the medium resulted in responses that were reduced by only 12±15%. Under these conditions Ca^{2+} influx induced by ionomycin was abolished. Therefore, the possibility that the release of Ca^{2+} stores mediates part of the depolarization induced $[Ca^{2+}]_i$ increase seems likely. We proceeded to test the responses of this preparation to ED (0/6), NE (0/6), QUIS (0/7), kainate (0/4), NMDA (1/34), glutamate (3/35), and carbachol (6/83), obtaining responses only in <10% of the astrocyte fields only to the last three agonists which we could not preclude to be due to neuronal contamination in the field. Therefore, although our evidence supports the existence of Ca^{2+} stores in adult rat astrocytes, the stores appear either not to be linked to $InsP_3$ receptors or the $InsP_3$ linked receptors for NE, QUIS, and ED are absent from the astrocyte fields. (DOE grant DE-FG02-86ER60438.)

160.20

IDENTIFICATION OF INOSITOL TETRAKISPHOSPHATE AND HEXAKISPHOSPHATE BINDING PROTEINS FROM RAT CEREBELLUM. A. Theibert, C. Ferris, S. Danoff, S. Supattapone, R. Barrow*, and S. Snyder. Dept. Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD 21205.

Recently, the InsP3 receptor from cerebellum was purified and reconstituted by researchers in our laboratory. It has been proposed that in addition to InsP3, other inositol polyphosphates such as InsP4, InsP5, and InsP6 may also have signalling functions. Using a similar approach to investigate the function of these potential messengers, we have employed ligand binding techniques to characterize and identify their cellular receptors. Both [3H]InsP4 and [3H]InsP6 bind with high affinity to cerebellar membranes. These binding sites can be solubilized using the detergent CHAPS and can be partially purified and separated using heparin-agarose affinity chromatography followed by ion-exchange chromatography. The InsP4 binding protein has an affinity of approximately 10 nm and is very selective for InsP4 over InsP3, InsP5 and InsP6. The binding correlates with a protein of approximately 180 kd. The InsP6 site has an affinity of approximately 20 nm but is less selective than the InsP4 site. InsP5 and InsP6 are 4-fold less potent at this site. The purified InsP6 binding protein migrates as a complex comprised of a doublet protein at 105-110 kd and a singlet at 50 kd. We are currently reconstituting these binding proteins into lipid vesicles to examine their physiologic function.

160.21

HIGH AND LOW AFFINITY RECEPTOR SITES FOR INOSITOL-1,4,5-TRISPHOSPHATE IN DETERGENT EXTRACTS OF MAMMALIAN CEREBELLUM

W.S. Agnew and S.R. Hingorani. Dept. of Cellular and Molecular Physiology, Yale University School of Medicine, New Haven, CT 06510

A rapid ion exchange binding assay was developed to measure IP_3 binding to sites in detergent extracts of mammalian cerebellum. Separation of bound from unbound ligand occurs within ~1 second resulting in detection of 3-4 fold higher receptor abundance than observed with spun column assays. Scatchard replots of complete saturation isotherms performed with extracts of rat or bovine cerebellar membranes were consistently non-linear, suggesting the presence of multiple classes of receptor sites. In both cases curves were well fit by assuming two classes of sites. In rat preparations one class displayed K_d = 5 nM, B_{max} = 8-10 pmol/mg protein, while the second had K_d ~ 120 nM and B_{max} = 22-30 pmol/mg protein; somewhat lower abundance was observed in bovine extracts. These sites exhibited differential inhibition by free Ca^{2+}, thermal stability and dissociation rate constants, and may represent distinct receptor molecules or different affinity states of the same molecule. The combined population of receptor sites are more than 25 times as abundant as the saxitoxin-binding sodium channel and, assuming subunit molecular weights of 243,000 Da, may constitute up to 1% of the total protein in unfractionated cerebellar membranes.

160.22

CARBACHOL STIMULATES PHOSPHOINOSITIDE (PI) TURNOVER IN CHICK BRAIN SLICES: EFFECT OF MELATONIN. J.M. Fang, K.N. McMasters* and M.L. Dubocovich, Dept. Pharmacol., Northwestern Univ., Med. School, Chicago, IL 60611

Melatonin exerts its potent biological effects through activation of specific receptors, however the cellular mechanism of its action is not well known. The aim of this study was to investigate the second messenger system linked to the melatonin receptor in the chick brain, where 2[^{125}I]-iodomelatonin binding sites are widely distributed. Activation of melatonin receptors by melatonin (10^{-14}-10^{-4}M) did not modify the forskolin-stimulated adenylate cyclase (AC) activity in homogenates of chick hypothalamus, neo/ectostriatum and nucleus basalis. Activation of the muscarinic receptor with carbachol increased the formation of ^{3}H-inositol phosphates (lithium, 10 mM) in slices of chick telencephalon in a concentration-dependent manner with EC_{50}=8.5μM. The carbachol-induced increase in PI turnover was higher in 1 day old (497 ± 43% of control) than in 1 week old (231 ± 11% of control) chick brain slices with no changes in EC_{50}. Melatonin (10^{-4} M) did not modify the carbacol-induced increase in PI turnover. We suggest that the melatonin receptor in chick brain may be linked to the second messenger system other than AC and phospholipase C. Supported by USPHS grant MH 42922.

161.1

EXTRACELLULAR GDPβS INHIBITS WHOLE-CELL CURRENT RESPONSES TO EXCITATORY AMINO ACIDS IN RAT RETINAL GANGLION CELLS. Paul S. Jackson, Andrew E. Budson*, and Stuart A. Lipton. Dept. of Neurology, Children's Hospital & Harvard Medical School, Boston, MA 02115.

In tissue binding studies, analogs of guanine nucleotides act extracellularly as competitive antagonists at the NMDA receptor (Butcher *et al., Biochem. Pharm.* 1986;35:991; Monahan *et al., Molec. Pharm.* 1988;34:111; Baron *et al., J. Pharm & Exp. Ther.* 1989;250:162). We used the patch-clamp technique to investigate the effects of the most potent of these, the GDP analogue GDPβS, on NMDA-activated currents. In addition, we studied the specificity of this effect, i.e., possible antagonism of other subtypes of glutamate receptors by GDPβS.

Postnatal rat retinal ganglion cells were isolated, identified, and recorded from with patch electrodes, as previously described (Lipton and Tauck, *J. Physiol.* 1987;385:361). GDPβS (500 μM) completely and reversibly blocked the response to NMDA (200 μM), whereas 100 μM GDPβS produced 30% inhibition, and 50 μM resulted in little or no blockade ($n = 6$). A preliminary dose-response curve gave an estimated IC_{50} of ~$2x10^{-4}$ M. However, 500 μM GDPβS also produced 90% blockade of the response to 125 μM kainate ($n = 3$) and 30% inhibition of current induced by 25 μM quisqualate ($n = 2$). Thus, GDPβS was a relatively non-specific EAA antagonist, but did manifest differential effects on NMDA/kainate versus quisqualate responses. In this regard, the pattern of blockade by GDPβS of EAA responses in retinal ganglion cells was reminiscent of that previously observed for kynurenate (Aizenman, Frosch & Lipton, *J. Physiol.* 1988;396;75).

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161.2

ACUTELY DISSOCIATED, SUPERFICIAL DORSAL HORN NEURONS OF THE ADULT RAT: A STUDY OF EXCITATORY AMINO ACID RECEPTOR PROPERTIES. O. Arancio, M. Yoshimura, K. Murase, and A.B. MacDermott, Dpt. Physiol., Ctr Neurobiol. & Behavior, Columbia Univ., New York, NY 10032

In vitro preparations for studying receptor properties of spinal cord neurons include slices of spinal cord, cultured embryonic neurons, and acutely dissociated neurons. Neurons from immature animals or neurons that mature in culture may not express the same receptors as those maturing *in vivo*. Studying receptor pharmacology in the adult spinal cord *in vivo* or in slice would overcome these difficulties, but additional problems arise including diffusion barriers, uptake of agonists, and drug-evoked transmitter release. Therefore, we have developed a new preparation of acutely-dissociated superficial dorsal horn neurons (laminae I-II) from the adult rat spinal cord. The properties of the excitatory amino acid (EAA) receptors on these neurons were tested; the effects of the EAA antagonist, CNQX, on quisqualate (Quis), kainate (Kai) and N-methyl-D-aspartate (NMDA) evoked responses were studied quantitatively. Cells were tested under whole cell conditions with rapid drug application. Each neuron tested was sensitive to all three agonists; the K_d's for Quis, Kai and NMDA were 4, 140, and 90 uM, respectively. Responses to NMDA and Quis desensitized, while responses to Kai did not. Glycine augmented the responses to NMDA in a dose-dependent manner while APV markedly reduced the NMDA-evoked current. CNQX acted as a competitive antagonist of the Kai and Quis responses and produced noncompetitive inhibition of the NMDA responses that could be partially reversed by increasing glycine concentration. Thus, acutely dissociated adult spinal cord cells have similar receptors, defined pharmacologically, to those found on other CNS neurons.

161.3

EVIDENCE FOR MG^{++} DEPENDENCE OF $GABA_B$ RECEPTOR-MEDIATED INHIBITION OF THE NMDA COMPONENT OF SYNAPTIC TRANSMISSION. W.A.Wilson, R.A.Morrisett, D.D.Mott, H.S.Swartzwelder, D.V.Lewis. Depts. of Med. (Neurol.), Pharmacol. and Pediatr. (Neurol.), Duke University and the VA Med. Ctrs., Durham, NC 27710.

We have utilized the non-NMDA receptor antagonist, DNQX, and the $GABA_A$ channel antagonist, picrotoxin, to study synaptic activation of NMDA receptors. Under these conditions, large, long-lasting NMDA EPSPs were recorded from s. radiatum of area CA_1 upon stimulation of the Schaffer collaterals. These NMDA EPSPs exhibited sensitivity to competitive and non-competitive NMDA antagonists including Mg^{++}. When NMDA EPSPs were paired at interstimulus intervals between 100 and 400 msec, we observed an almost complete block of the second response (see Morrisett et al., this volume). We have demonstrated that paired-pulse inhibition of NMDA EPSPs is dependent upon activation of $GABA_B$ receptors most likely located on CA_1 pyramidal cell dendrites.

We hypothesized that some $GABA_B$ receptors located on CA_1 dendrites were in close approximation to NMDA receptor-operated channels. Therefore, the late IPSP that occurred due to $GABA_B$ receptor activation may regulate the voltage across NMDA channels and consequently the Mg^{++} block of those channels. To test this hypothesis, Mg^{++} was removed from the bath, resulting in approximately 50 % reduction in paired pulse inhibition of NMDA EPSPs. Removal of Mg^{++} had minimal effects on non-NMDA EPSPs. These data suggest that $GABA_B$ receptors potently regulate NMDA channel operation on the dendrites of CA_1 pyramidal cells. (NS #17701, MH #15177-13 and Vet. Admin.).

161.4

EFFECTS OF pH AND PROTEIN MODIFYING AGENTS ON THE NMDA INDUCED CURRENTS IN ISOLATED CATFISH HORIZONTAL CELLS. X.G. WU* AND B.N. CHRISTENSEN. Dept. of Physiology and Biophysics, Univ. Texas Med. Branch, Galveston, TX 77550.

Reactive groups of histidine and cysteine that constitute functional components of the n-methyl-d-aspartate (NMDA) receptor/channel protein were investigated using pH titration and specific protein modifying reagents. A titration curve that measured NMDA induced membrane currents under voltage clamp as a function of pH over the range of 5.5 to 9.5 gave a single pK near 6.5 suggesting that histidine imidazoles provide for one site of functional importance. The histidine modifying reagent diethylpyrocarbonate (DEPC 200 μM, pH 7.5) had two effects on the NMDA response. Initially, the NMDA response was potentiated following a 1-2 min exposure to DEPC. However, the NMDA response decreased to below control values following longer exposure to DEPC. Increasing the glycine concentration had the effect of retarding the time necessary to produce inhibition of the NMDA response with DEPC.

The functional role of cysteines was investigated with iodoacetic acid and dithiothreitol (DTT). Reduction of disulfide bonds with DTT potentiated the NMDA response whereas oxidation of sulfhydryls by iodoacetic acid resulted in a depression. These results suggest that reactive groups on these amino acids play an important functional role in the regulation of the NMDA receptor/channel protein. Supported by grant NEI-01897.

161.5

EXCITATORY AMINO ACID INDUCED INTRACELLULAR CALCIUM CHANGES IN VOLTAGE CLAMPED ISOLATED CATFISH HORIZONTAL CELLS. C.L. LINN AND B.N. CHRISTENSEN. Dept. of Physiology and Biophysics, Univ. Texas Med. Branch, Galveston, TX. 77550

Isolated horizontal cells loaded with the calcium sensitive fluorescent indicator fura-2 were voltage clamped using the patch electrode in the whole cell mode. While voltage clamped at -65 mV, changes in intracellular calcium levels were measured during rapid application of glutamate analogs under concentration clamp conditions. Previous studies by O'dell and Christensen (1986, 1989) have demonstrated that isolated cone horizontal cells respond to n-methyl-d-aspartate (NMDA), kainate (KA) and quisqualate (QA). NMDA (50 μM), KA (20 μM) and QA (10 μM) each elicited an increase in intracellular calcium corresponding with the agonist induced inward current. The order of the relative efficacy for the three agonists is KA > NMDA > QA. An increase in intracellular calcium can be due to either an influx from the extracellular medium and/or release from intracellular stores. We are currently investigating the mechanisms responsible for the agonist induced increases in intracellular calcium in catfish cone horizontal cells. Supported by Grant 1F32EY-06246 and EY-01897.

161.6

NMDA-MEDIATED INCREASE IN INTRACELLULAR CALCIUM IN HIPPOCAMPUS: EFFECT OF ETHANOL. Laura C. Daniell. Dept. of Pharmacol. & Toxicol., Medical College of GA., Augusta, GA. 30912-2300.

The effect of N-methyl-D-aspartate (NMDA) and L-glutamate on the intracellular free calcium concentration (Ca_i) and on calcium uptake was determined in microsacs and synaptosomes isolated from mouse brain. L-glutamate and NMDA increased Ca_i in hippocampal microsacs but had little or no effect on Ca_i in microsacs isolated from cortex or cerebellum or in synaptosomes. NMDA also increased calcium uptake into hippocampal microsacs. The EC_{50} values for NMDA-stimulated increases in Ca_i and calcium uptake in microsacs were about 30 μM. Maximal responses were observed with 100 μM NMDA. NMDA-stimulated increases in Ca_i were dependent on extracellular calcium. The NMDA antagonists, 5-methyl-10,11-dihydro-5H-dibenzo[a,d]cyclohepten-5,10-imine (MK-801), 2-amino-5-phosphonopentanoic acid (AP-5), Mg, and Zn blocked NMDA responses. Ethanol inhibited NMDA responses in an uncompetitive manner with an IC_{50} of 51 mM. This is the first demonstration of NMDA-mediated effects on ion flux in a cell-free brain preparation. This preparation may be useful for study of the in vitro effects of drugs or toxins on brain NMDA receptors.

161.7

MEMBRANE RESPONSES OF CA1 PYRAMIDAL NEURONS TO L-HOMOCYSTEATE IN THE RAT HIPPOCAMPAL SLICE. L. Provini,* S. Ito,* Y. Ben-Ari and E. Cherubini. INSERM U29, 123 Bd Port-Royal, 75014 Paris, France.

In several brain areas, L-homocysteate (L-HC) is considered to be an endogenous transmitter which preferentially activates NMDA receptors. The aim of this study was to examine the membrane responses of CA1 pyramidal neurons to bath application of L-HC in the rat hippocampal slice, using intracellular recording and current and voltage clamp techniques. In normal ACSF, L-HC (100-200 μM) depolarized the membrane and induced a pronounced burst-like firing pattern which resembled that produced by NMDA. In ACSF containing TTX (1 μM), L-HC (100-200 μM) induced at -65mV a depolarization (4-12 mV) which was associated with a small apparent decrease in membrane conductance (5%). These effects were almost completely blocked (90%) by APV (50 μM, n=4). In voltage clamp experiments, at -60 mV L-HC (100-300 μM) induced an inward current, which was voltage dependent and became outward near 0 mV. These effects were similar to those obtained by NMDA. The inward current was reduced by APV (50 μM, to 48%) and fully blocked by APV and 6-cyano-7-nitroquinoxaline-2,3-dione (10 μM). In 4 cells the response to L-HC was studied at two different temperatures (33°C and 20°C). The L-HC-induced inward current was enhanced at 20°C (186 %, at -60mV, 3 out of 4) suggesting that uptake modulates L-HC mediated responses. In contrasts, the response to NMDA was unchanged or even decreased. It is concluded that L-HC acts in CA1 hippocampal neurons as a mixed excitatory amino acid agonist, with a clear preferential effect for NMDA receptors.

161.8

EFFECTS OF ADENOSINE ANALOGUES ON N-METHYL-D-ASPARTATE (NMDA)-INDUCED LETHALITY IN MICE. D. Lutz, R. Dean and R.T. Bartus. CORTEX Pharmaceuticals, Inc., Irvine, CA 92718

It is known that glutamate acts as an excitatory amino acid (EAA) in the CNS. A large body of evidence has shown that excessive accumulation of glutamate results in excitotoxic neuronal cell death mediated by the NMDA-sensitive EAA receptor. This excitotoxicity has been implicated in such neurodegenerative diseases as Huntington's disease and stroke, and may also participate in multi-infarct dementia, Alzheimer's disease, and Parkinson's disease. The endogenous neuromodulator, adenosine, acts presynaptically to inhibit the release of glutamate and postsynaptically by inhibition of evoked synaptic potentials. These inhibitory effects of adenosine are mediated through the A1 receptor, which is co-distributed with NMDA receptors, especially in the hippocampus.

Initial experiments demonstrated that a systemic injection of NMDA (200 mg/kg, ip) in mice causes severe convulsions, resulting in 85% mortality at 10 minutes post-NMDA administration. Pre-treatment (ip, 30 minute absorption) with various A1, A2, and mixed A1/A2 adenosine agonists delayed the NMDA-induced effects in many cases. Most of these analogues induced profound side effects such as sedation, hypothermia, vasodilation, etc., at pharmacologically active doses. To further elucidate the mechanisms of adenosine modulation of NMDA-induced lethality, both centrally and peripherally active adenosine antagonists were administered prior to analogue administration. These results with adenosine analogues will be compared with drugs which directly affect the NMDA-receptor system, in addition to general CNS acting compounds.

161.9

BETA-METHYLAMINO-ALANINE NEUROTOXICITY AS A MODEL OF ALS. **Z. Rakonczay*, Y. Matsuoka*, E. Giacobini and H. Konrad. Dept. of Pharmacology, Southern Illinois Univ. Sch. Med., Springfield, IL 62794 USA**

Recent studies have raised the possibility that the Guam-type of amyotrophic lateral sclerosis (ALS) may be related to food intake of unusual neurotoxic amino acids acting upon glutamate receptors. We have attempted to develop a rat model for ALS based on amino acid neurotoxicity. Two different methods of administration of beta-N-methylamino-L-alanine (L-BMAA), were used: single daiiy i.c.v. injections (500 μg) or continuous infusion via Alzet-minipumps (500 μg/day) for 16d, 30d, 40d and 60d. Both cholinergic and glutamatergic systems were investigated in the cerebral cortex. ^{3}H-glutamate and ^{3}H-glutamate binding in the presence of 2.5 μM quisqualate (N-methyl-D-aspartic acid, NMDA) and ^{3}H-AMPA binding was significantly decreased at 16 days. Following this period, binding progressively increased and at day 60 of administration was either similar (NMDA and AMPA) or higher (glutamate) than controls. There were no differences between L-BMAA injected and vehicle injected sides of the brain. In vitro, L-BMAA was able to displace either AMPA or glutamate binding. ^{3}H-nicotine binding was significantly decreased at day 40 and 60 of administration while ^{3}H-QNB binding was unvaried. Acetylcholinesterase activity was significantly decreased at 16 days but reached control values at day 60 while choline acetyltransferase activity was unvaried. These biochemical results are consistent with an early damage to cortical neurons carrying glutamate or NMDA as well as nicotinic acetylcholine binding sites. Behaviorally, only single i.c.v. injections produced significant side effects (splay, rigidity and jerking). The symptoms were present during at least 10 days. Our results are consistent with an effect of L-BMAA on both glutamatergic and NMDA receptors in the rat cerebral cortex. (Supported by ALS grant GL-314.00)

161.10

BETA-METHYLAMINO-ALANINE-INDUCED BEHAVIORAL CHANGES IN THE RAT. **Y. Matsuoka*, Z. Rakonczay*, E. Giacobini and J. Couch. Dept. of Pharmacology, Southern Illinois Univ. Sch. Med., Springfield, IL 62794 USA**

The naturally occurring amino acid, beta-N-methylamino-L-alanine (L-BMAA) is a neurotoxin possibly involved in the pathogenesis of amyotrophic lateral sclerosis (ALS) in Guam and Rota islands. We attempted to elucidate the characteristics of L-BMAA-induced behavioral changes in male rats (SD strain). A single injection of 500 μg/rat of L-BMAA into the lateral ventricle induced splay, repetitive jerking movements, and rigidity in succession lasting 10 min after the injection. A series of single daily injections induced these behavioral changes during a period of at least 3 days in responder rats. These symptoms were attenuated after 10 days. The effects of i.c.v. pretreatment with DNQX (20 or 10 μg), a non-NMDA receptor antagonist, AP-5 (50 or 10 μg), a NMDA receptor antagonist, and MK-801 (100 or 5 μg), a non-competitive NMDA antagonist were investigated in a crossing-over study. The duration of jerking movements induced by L-BMAA was reduced significantly by DNQX (20 μg) but not by AP-5 and MK-801, while the latency of the jerking movements was significantly prolonged by these antagonists. The duration of the splay was not changed by the pretreatment with DNQX or AP-5 but tended to be more prolonged with MK-801. The rigidity induced by L-BMAA was completely inhibited by MK-801 (100 μg) and partly by DNQX (20 μg) (84%) and AP-5 (50 μg) (85%). These behavioral results together with the effects seen with in vitro binding displacement experiments, suggest that L-BMAA may act upon two or more kinds of glutamate type excitatory amino acid receptors. (Supported by ALS grant GL-314.00)

161.11

THE VOLATILE ANESTHETIC, ENFLURANE, DISRUPTS GLUTAMATE-STIMULATED [^{3}H]MK-801 BINDING IN RAT BRAIN. D.C. Martin and R.S. Aronstam. Departments of Anesthesiology and Pharmacology & Toxicology, Medical College of Georgia, Augusta, GA 30912.

Substantial evidence has targeted N-methyl-D-aspartate (NMDA) receptors as a site of anesthetic action. We investigated the influence of enflurane on [^{3}H]MK-801 binding in rat brain membranes. Rat forebrains were homogenized, treated with Triton X-100 (0.4%) and washed in 5 mM Tris-HCl (pH 7.4). [^{3}H]MK-801 binding was measured following incubation at 37°C by vacuum filtration and liquid scintillation counting.

Enflurane was a potent inhibitor of glutamate-stimulated [^{3}H]MK-801 binding (IC_{50} = 0.7 mM) but a much weaker inhibitor of glycine-stimulated [^{3}H]MK-801 binding: Maximal inhibition of [^{3}H]MK-801 binding stimulated by 100 uM glycine was 22% with 2.7 mM enflurane. However, in the presence of both glutamate and glycine, enflurane did not affect [^{3}H]MK-801 binding. Moreover, the inhibition of glutamate-stimulated [^{3}H]MK-801 binding by 2.3 mM enflurane was markedly attenuated by glycine in a concentration-related manner; inhibition of [^{3}H]MK-801 binding by enflurane was 50% in the absence of glycine but only 14% in the presence of 100 uM glycine. Saturation analyses indicated that enflurane decreased the density of high affinity [^{3}H]MK-801 binding sites (B_{max} = 2356 ± 237 fmol/mg, control; 1329 ± 193 fmol/mg, enflurane) without affecting the K_D (2.8 nM).

The present results indicate that 1) enflurane disrupts NMDA receptor-regulated ion channels by interfering with glutamate stimulation of [^{3}H]MK-801 binding and 2) glycine reverses this action.

(Supported by GM37948 and AA07698)

161.12

A NOVEL POTENT NMDA AGONIST: THE (2S,3R,4S) ISOMER OF α-(CARBOXYCYCLOPROPYL)GLYCINE. H. Shinozaki, M. Ishida and Y. Kudo*+. The Tokyo Metro. Inst. of Med. Sci., Tokyo 113, and +Mitsubishi Kasei Inst. of Life Sci., Tokyo 194, Japan.

The (2S,3R,4S)isomer of α-(carboxycyclopropyl)glycine (L-CCG-IV) caused a depolarization in the isolated rat spinal cord more markedly than L-glutamate or NMDA. The depolarization induced by L-CCG-IV was completely blocked by Mg ions, CPP, MK-801 or D-APV, and the effect of the antagonists was fully reversible upon washout, suggesting that L-CCG-IV was a novel potent NMDA type agonist. It is known that the activation of NMDA receptors induces the Ca influx into the nerve cells. L-CCG-IV was about 300 times more potent than NMDA in increasing the intracellular Ca concentration in a molar basis in the cultured rat hippocampal neuron loaded with fura-2. When L-CCG-IV was substituted by methoxymethyl group at the C-6 position, the (6R) isomer only showed similar pharmacological properties to kainate and caused a depolarization of C-fibers in the rat dorsal root. As a possible mechanism for explaining the conversion of L-CCG-IV derivatives into a kainate-type agonist, steric repulsion of active groups of CCG are conceivable. Thus, CCG isomers provide useful information about the interaction of excitatory amino acids and their receptor subtypes.

161.13

DESENSITIZATION AND SENSITIZATION TO SUBSTANCE P- AND EXCITATORY AMINO ACID-INDUCED BEHAVIORS IN THE MOUSE. X. Sun and A. A. Larson. Dept. of Vet. Biol., Univ. of Minn., St. Paul, MN 55108.

Repeated intrathecal injections of SP and the excitatory amino acid (EAA) agonist N-methyl-D-aspartic acid (NMDA) result in desensitiza-tion to the caudally directed biting and scratching response (CBS) elicited by SP and NMDA. Repeated injection of kainic acid (KA) and quisqualic acid (Quis) result in sensitization to CBS. The goals of the present study were [1] to characterize the role of interneurons in these effects and [2] to examine possible interactions between excitatory transmitters. The NMDA antagonist DL-2-amino-5-phosphono-valeric acid (APV) failed to alter either KA or Quis CBS during sensitization but inhibited all behaviors produced by SP and NMDA, suggesting an NMDA-mediated component in SP-induced behavior. Concanavalin A, which blocks desensitization to electrophysiologic effects of Quis, blocked sensitization to the behavioral effects of both Quis and KA, suggesting downregulation of EAA receptors during behavioral sensitization. Pretreatment with either bicuculline or 5-aminovaleric acid had no effect on KA- or Quis-induced sensitization, but inhibited the development of desensitization to SP and NMDA, suggesting that desensitization is mediated by GABA interneurons. SP and NMDA exhibited *cross-desensitization* while KA and Quis exhibited *cross-sensitization*. Pretreatment with NMDA had no effect on subsequent KA- or Quis-induced CBS and pretreatment with Quis also had no effect on subsequent injection of NMDA or SP. Pretreatment with SP potentiated the CBS response to KA, and pretreatment with KA inhibited the response to a subsequent challenge with NMDA, and to a lesser extent SP. These data suggest a unique spectrum of modulatory actions for SP and EAAs. Supported by USPHS grants DA04090, DA 04190 and DA00124.

161.14

EXCITATORY AMINO ACID ANTAGONISTS AS ANTI-EMETICS. M.T. Price and J. W. Olney. Washington University, St. Louis, MO 63110.

The excitatory amino acid (EAA), glutamate (Glu), has emetic properties in various species, including humans. When present in elevated concentrations in the blood, Glu selectively penetrates a specific brain region (area postrema, AP) that lacks blood brain barriers and contains neurons that mediate an emesis chemoreceptor trigger (ECT) function; it is believed that Glu induces emesis by excitatory interaction with receptors on the dendrosomal surfaces of AP/ECT neurons. Glu is a mixed EAA agonist that can activate either N-methyl-D-aspartate (NMDA) or non-NMDA subtypes of EAA receptor. Experiments in dogs and ferrets were undertaken to better characterize the emetic properties of various EAA agonists and to evaluate the possibility that EAA antagonists might have useful anti-emetic properties. In dogs, NMDA, kainic acid (KA, a non-NMDA agonist) and Glu induced vomiting in a dose-dependent manner; the NMDA antagonist, D-AP5, blocked emesis induced by NMDA but not by KA or Glu; the broad-spectrum EAA antagonist, kynurenic acid, blocked emesis induced by any of the three agonists. In ferrets we studied the ability of EAA antagonists to prevent malaise and emesis induced by cisplatin, a cancer chemotherapy agent. A high dose of cisplatin (10 mg/kg iv) caused repeated emesis in all controls (n = 5) which was totally prevented in experimental ferrets (n = 5) by IV infusion of a mixture of 10 mg/kg/hr CNQX (non-NMDA antagonist) and 5 mg/kg/hr D-AP5 (NMDA antagonist). Since CNQX and D-AP5 readily penetrate the AP/ECT zone but not more general brain regions, we propose that they prevent cisplatin-induced emesis by blockade of EAA receptors on AP/ECT neurons that perform a central switchboard function connecting incoming and outgoing limbs of an emesis reflex arc. Thus, EAA antagonists may prove useful in the control of any type of emesis involving reflex mediation through an EAA synapse at the central switchboard level. Moreover, by selecting EAA antagonists that do not penetrate blood brain barriers, the therapeutic benefit can be achieved without CNS side effects.

161.15

DIFFERENCES IN ANTINOCICEPTIVE ACTIVITY BETWEEN NMDA *AND NON-NMDA ANTAGONISTS IN MICE. J. Näsström, U. Karlsson, C. Post. ASTRA PAIN CONTROL AB, R&D, Preclinical Research, S-151 85 SÖDERTÄLJE, SWEDEN.

Previous behavioural studies have indicated that excitatory amino acid (EAA) antagonists may posses antinociceptive activity. The effects after intrathecal administration of different classes of EAA antagonists have, however, not been thoroughly investigated. We found that competitive and selective NMDA antagonists produced dose dependent and reversible effects in the hot plate and formalin tests, being slightly more potent in the formalin test and with no or negligible effects in the tail-flick test. Non-selective EAA antagonists or non-NMDA (kainate-quis/AMPA) receptor antagonists were equally effective in the hot plate, tail-flick and formalin tests. This heterogenous group of antagonists was slightly more potent in the tail-flick test and slightly less potent in the formalin test than in the hot plate test. The non-competitive NMDA antagonists PCP, MK-801 and ketamine were by themselves without effect in all three tests. Ketamine and PCP had, however synergistic effects with the non-NMDA antagonist DNQX. When given in high doses, all of the EAA antagonists impaired the motor function of the mice.

The present results show that EAA antagonists acting at both NMDA and non-NMDA receptors produce dose dependent and reversible antinociceptive effects. Antagonists that selectively block the NMDA receptor complex have a different profile in the nociceptive tests than the non-selective antagonists.

161.16

QUISQUALATE SENSITIZATION OF HIPPOCAMPAL NEURONS. J.F.Koerner,E.R.Whittemore and R.L.Johnson*. Depts. of Biochem. and Medicinal Chem. and Neuroscience Grad. Program, Univ. of Minnesota, Minneapolis, MN 55455.

Sensitization of rat CA1 hippocampal neurons to excitatory agonists by quisqualic acid (QUIS-effect) is known to be reversed by L-homocysteinesulfinic acid, L-alpha-aminoadipic acid and L-serine-O-sulfate. We report that these compounds also act as "pre-blockers" which, when added and then removed from the medium, prevent subsequent sensitization by QUIS. This is consistent with a sequestration mechanism in which these compounds may either block QUIS uptake or antagonize its interaction with an intracellular binding site.

Quisqualic acid sensitizes neurons to a unique group of compounds, all of which have close homology to glutamic acid and which contain a phosphonate, phosphinate, or phosphate group. Although D- and L-2-amino-4-phosphonobutanoic acid (AP4) are equipotent, both the alpha amino and carboxylic acid residues are essential. This is demonstrated by the low activity of 4-phosphonobutanoic acid and 3-aminopropylphosphonic acid. The response to monoanionic 2-amino-4-methyl-phosphinobutanoic acid and a conformationally restricted cis-cyclopentyl-AP4 analogue are strongly potentiated, whereas other conformationally constrained cyclohexyl, cyclopentyl, and cyclopropyl AP4 derivatives are only minimally enhanced. Supported by NIH NS 17944.

161.17

INHIBITION OF HIPPOCAMPAL PATHWAYS BY CYCLOPROPYL ANALOGS OF 2-AMINO-4-PHOSPHONOBUTANOIC ACID (AP4). N.L. Peterson, H.B. Kroona*, R.L. Johnson*, J.F. Koerner. Dept. of Biochem., Dept. of Medicinal Chem., and Neuroscience Grad. Program, Univ. of Minnesota, Minneapolis, MN 55455.

AP4-sensitive glutamate receptors in rat hippocampal slices were studied using two synthetic cyclopropyl analogs of AP4: (E)- and (Z)-1-amino-2-(dihydroxyphosphinylmethyl)cyclopropane-carboxylic acid. Extracellular electrophysiological techniques were used to evaluate the effect of these constrained analogs on synaptic transmission. Cumulative concentration-response curves were obtained and the IC_{50} of each analog was determined. In the lateral perforant path (LPP), both analogs were found to be equipotent having an IC_{50} of 15μM. In the medial perforant path (MPP), E-cyclopropyl AP4 was found to be more potent than Z-cyclopropyl AP4 (IC_{50}=100μM vs. 1500μM). These data are similar to AP4 which is more potent in the LPP than the MPP (IC_{50}=2.5μM vs 2500μM). In the CA1 pathway, E-cyclopropyl AP4 was found to be more potent than Z-cyclopropyl AP4 (IC_{50}=450μM vs 2500μM). AP4 itself has an IC_{50}=1800μM in CA1. The data suggest that glutamate assumes an extended conformation in the LPP AP4 receptor rather than a folded conformation. Furthermore, comparison of rank potencies suggests that the AP4 receptors in the MPP and CA1 pathway, where the E analog was found to be approximately 10 fold more potent than the Z analog, are distinct from the AP4 receptors in the LPP, where the E and Z analogs were found to be equipotent. Supported by NIH NS 17944.

161.18

THE EFFECT OF EXOGENOUS GLUTAMINE (GLN) ON BASAL AND NEWLY SYNTHESIZED (NEW) EXCITATORY AMINO ACIDS AND GABA IN MOUSE HIPPOCAMPUS IN VITRO. I.M. Kapetanovic, W.D. Yonekawa and H.J. Kupferberg*. Epilepsy Branch, NINDS, NIH, Bethesda, MD 20892.

GLN plays an important role in ammonia, energy, and amino acid (AA) neurotransmitter metabolism and compartmentation often referred to as the 'GABA-GLN-glutamate cycle'. GLN is normally present in brain extracellular fluid and CSF in the range of 0.2 to 0.5 mM, but, is not included in artificial cerebrospinal fluid (ACSF) used for brain slices. This study examined levels of basal and NEW GLN, GABA, glutamate (GLU) and aspartate (ASP) and the effect of exogenous GLN in hippocampal slices under different experimental conditions. Isotopic enrichment, after incubation with $^{13}C_6$-glucose, was used to measure NEW AA. Basal and NEW AA were quantitated by gas chromatography-mass spectrometry of the dimethyl t-butyl silyl derivatives. Basal levels of AA in freshly cut slices were 75-89% of the intact hippocampus and decreased with time in ACSF. After 90 min, GLN levels were only ca. 10% of the intact hippocampus, while the other AA were ca. 65%. ACSF containing 0.5 mM GLN maintained basal levels of all AA much closer to those of in vivo hippocampus, and this concentration of GLN had no electrophysiological effects under control conditions. In the presence of increasing concentrations of a depolarizing agent, K^+, GLN helped maintain basal AA levels and increased levels of NEW AA, especially GLN. In the presence of 15 mM K^+, basal levels of all AA declined over the 30 min incubation period in ACSF without exogenous GLN, but not in ACSF with exogenous GLN. In addition, in ACSF containing GLN, the levels of NEW GLN were severalfold greater than in ACSF not containing GLN. The data suggest that GLN may function to maintain physiological levels of AA and its presence in ACSF may be important in neurochemical studies.

161.19

NMDA INDUCTION OF c-fos mRNA IN RAT BRAIN: A QUANTITATIVE in situ HYBRIDIZATION STUDY.

P.F. Morgan and M. Linnoila. Laboratory of Clinical Studies, NIAAA, Blg10 3C102, 9000 Rockville Pike, Bethesda, MD 20892, USA.

The excitatory amino acid N-methyl-D-aspartate (NMDA) binds to the NMDA sub-population of excitatory amino acid receptors and thereby increases the transmembrane flux of calcium ions. The proto-oncogene transcription factor, c-fos is expressed in response to increases in intracellular calcium. We therefore mapped and measured c-fos mRNA in response to NMDA administration in order to identify NMDA-activated brain structures.

Rats were injected with NMDA (0-375 mg/kg, i.p.). Thirty minutes later the animals were sacrificed and brains rapidly removed and cooled in isopentane at -30oC. Brains were then sectioned (12 um) at -15°C, air dried, fixed in paraformaldehyde, then hybridized with a ^{35}S-c-fos murine oligonucleotide probe for c-fos mRNA. Specificity of the probe was assessed by conventional northern techniques. Hybridized message was visualized by exposure to X-omat photographic film. Acute NMDA induced a highly discrete brain distribution of c-fos mRNA which was stereospecifically blocked by MK801 (10 mg/kg, i.p., 20 minutes prior to NMDA).

These results reveal that the dentate gyrus of the hippocampus and the piriform cortex are particularly activated by acute NMDA administration.

GABA$_A$ RECEPTORS II

162.1

Lack of involvement of protein kinases A and C in the GABA$_A$ receptor-gated Cl channel desensitization in mice spinal cord cultured neurons. A.K. Mehta and M.K. Ticku. Department of Pharmacology, University of Texas Health Science Center, San Antonio, Texas 78284-7764.

GABA and muscimol produced desensitization of GABA$_A$ receptor-gated Cl channels, as measured by ^{36}Cl-influx assay, in mice spinal cord cultured neurons. The desensitization phenomenon was time- and concentration-dependent, and reversible. Both active and inactive forms of forskolin decreased GABA-induced ^{36}Cl-influx alone as well as when preincubated in conjunction with GABA. Furthermore, 8-bromo-adenosine, 8-bromo-adenosine cAMP, dibutyryl cAMP and cAMP also directly inhibited GABA-induced ^{36}Cl-influx, without preincubation. However, the inhibitor of protein kinase A, H-8, did not reverse the effect of cAMP analogs on the inhibition of GABA-induced ^{36}Cl-influx. Moreover, active phorbol ester failed to modify GABA-induced desensitization. These results suggest that cAMP analogs inhibit GABA-induced ^{36}Cl-influx by acting via an extracellular site without the involvement of protein kinase A.

162.2

MAINTENANCE OF GABA$_A$ RECEPTOR ACTIVATED CURRENT MAY NOT REQUIRE PHOSPHORYLATION IN ADULT RAT PRIMARY SENSORY NEURONS. **F.F. Weight and G. White, Sect. of Electrophysiology, Nat. Inst. on Alcohol Abuse and Alcoholism, Rockville, MD 20852.**

Chen et al. (J. Physiol. 420:207, 1990) reported that within 10 minutes of gaining access to the interior of isolated hippocampal neurons GABA$_A$ receptor activated current was reduced to less than 10% of the initial value when ATP was not added to the internal recording solution under conditions of whole-cell recording. In contrast, we report that when ATP was not added to the internal solution used for recording from freshly isolated dorsal root ganglion (DRG) neurons the amplitude of the GABA$_A$-activated current remained stable. Responses to GABA were evoked within 1 min of rupture of the cell membrane and at 5 min intervals thereafter. At the 20 min time point, current was 94±6% (n=6, 10 μM GABA) and 95±4% (n=6, 100 μM GABA) of current evoked at the 1 min time point when ATP was not included in the internal solution (p>0.1, paired t-test). Similar values were obtained at time points of 40 minutes or more. With 4 mM ATP in the internal solution, current was 95±8% of the initial amplitude (p>0.1, n=5, paired t-test) after 20 min. However, in the presence of alkaline phosphatase (100 μg/ml) a decrement occurred over the initial 10 minutes of recording (70±4%) but not between 10 and 20 minutes (70±4% vs 67±3%) in 4 neurons sampled. These results suggest that in DRG neurons a component of GABA$_A$-activated current is sensitive to dephosphorylation but that the current does not require ATP to be maintained. The difference between hippocampal and DRG neurons may lie in GABA$_A$ subunit composition or in intracellular regulators of channel function.

162.3

cAMP ANALOGS INHIBIT GABA-GATED CHLORIDE FLUX AND ACTIVATE PROTEIN KINASE A IN RAT BRAIN SYNAPTONEUROSOMES Patricia P. Edgar, Gunter Heuschneider*, Jonathan A. Cohn*, and Rochelle D. Schwartz Depts. of Pharmacology & Medicine, Duke University Med. Ctr, Durham, NC

Recent studies of structure and function indicate that the GABA$_A$ receptor can be phosphorylated by a cAMP-dependent protein kinase (PKA; Kirkness et al., 1989) and that GABA$_A$ receptor-mediated ion flux is inhibited by cAMP analogues (Heuschneider and Schwartz, 1989). We have investigated the regulation of GABA$_A$ receptor function by cAMP analogues in more detail. Cerebral cortical synaptoneurosomes were incubated with the permeant cAMP analogues, dibutyryl, 8-bromo, and 8-chlorophenylthio (CPT) cAMP, 10 min prior to the measurement of muscimol-induced ^{36}Cl uptake. Each analogue inhibited the maximal muscimol-induced ^{36}Cl uptake (CPT> dibutyryl> 8-bromo); the potency of muscimol was not altered. cAMP had no effect, suggesting that the permeant analogues act intracellularly to inhibit the muscimol response. The potency (IC_{50} = 1mM) and maximal inhibition by CPT-cAMP were similar in cortex, hippocampus, striatum and cerebellum. The effect of CPT-cAMP on benzodiazepine potentiation of muscimol-induced ^{36}Cl uptake was also studied. In other studies PKA activity was measured in broken synaptoneurosomes incubated with cAMP analogues. In cortex, cAMP analogues activated PKA with the following order of potencies: (CPT = 8-bromo>>dibutyryl). The potency and maximal effect of CPT-cAMP on PKA activity differed in the 4 brain regions. Studies to determine whether CPT-cAMP stimulates protein phosphorylation in intact synaptoneurosomes are in progress. These results suggest that GABA$_A$ receptor function can be modulated by cAMP under conditions which activate PKA, although a causal relationship remains to be established. Supported by NSF Predoctoral Fellowship (P.P.E.) and by NS24577 and PMA Foundation (R.D.S.)

162.4

Direct Effects of Second Messenger System Modulators on the GABA A Receptor Complex. N.J. Leidenheimer, L.D. Hahner*, M.D. Browning, R.A. Harris. Dept. Pharmacol., Univ. Colorado HSC and Denver VAMC, Denver, CO, 80262

Mouse brain microsacs were incubated with low millimolar concentrations of cAMP, 8BrcAMP or CPTcAMP for 10 min on ice prior to measurement of muscimol-stimulated $^{36}Cl^-$ uptake (3 sec). These compounds inhibited muscimol-stimulated $^{36}Cl^-$ uptake by 14%, 12% and 76%, respectively (p<0.05). Pretreatment with the protein kinase inhibitor H8 (30-300uM) failed to antagonize the CPTcAMP effect suggesting that CPTcAMP inhibition of $^{36}Cl^-$ flux may be independent of phosphorylation. To further investigate this possibility, microsacs were osmotically lysed and resealed in the presence of EDTA (3mM) to inhibit phosphorylation. Inhibition of phosphorylation was confirmed using microsacs loaded with (^{32}P)-ATP. Under these conditions CPTcAMP inhibited muscimol-stimulated $^{36}Cl^-$ flux by 86%. In both EDTA-treated lysed/resealed microsacs and intact microsacs not treated with EDTA, low millimolar concentrations of cAMP, 8BrcAMP or CPTcAMP significantly (p<0.05) inhibited binding of the GABA A receptor ligand [^{3}H] SR 95531 as did the protein kinase inhibitor H7. IBMX and forskolin were found to inhibit binding of the Cl^- channel ligand [^{35}S] TBPS.

These results indicate that compounds commonly used to alter second messenger systems can affect the receptor sites and function of the GABA A receptor complex by mechanism which do not involve protein phosphorylation.

162.5

POLYCLONAL ANTIBODYS THAT INHIBIT THE PHOSPHORYLATION OF THE BOVINE GABA$_A$ RECEPTOR. E.M. Dudek[1], S. Endo[2], M.D. Browning[1], Olsen, R.W.[2] [1]*Dept. of Pharmacology, Univ. of Colorado Hlth. Sci. Cntr., Denver, CO 80262*; and [2]*Dept. of Pharmacology, UCLA Sch Med., Los Angeles, CA 90024*

The GABA$_A$ receptor (GABA$_A$-R) is a member of the family of ligand-gated ion channels. Considerable attention has been focussed on the possibility that this class of receptors could be regulated by phosphorylation. We have recently shown that the β-subunit of the GABA$_A$-R could be phosphorylated by both the cAMP-dependent protein kinase (PKA) and by the Ca2+ and phosphatidylserine-dependent protein kinase (PKC) (Browning et al., PNAS 87:1315,1990). The two kinases appeared to phosphorylate distinct β-subunits based on the migration pattern in SDS-PAGE. We have prepared polyclonal antibodies against the consensus sequence for PKA substrates that is found in the cytoplasmic loop of the bovine GABA$_A$-R β-subunit. Antibodies were raised in rabbits and purified on an affinity column made with the peptide. We have examined the ability of this antibody to influence the phosphorylation of the GABA$_A$-R *in vitro*. The antibody produces essentially complete inhibition of the phosphorylation of the bovine GABAα_A-R by PKA. A concentration of antibody that produces >90% inhibition of the bovine GABA$_A$-R produces partial (<30%) inhibition of the phosphorylation by PKA of the rat GABA$_A$-R. We have also examined the ability of this antibody to inhibit the phosphorylation of the GABA$_A$-R by PKC. We found that the antibody was equally effective at inhibiting both PKA and PKC phosphorylation of the GABA$_A$-R. These data suggest that this antibody may be a useful tool for *in situ* studies of the physiological significance of GABA$_A$-R phosphorylation. Moreover, the fact that the antibody could inhibit both PKA and PKC phosphorylation of the receptor suggests the possibility that the substrates for PKC and PKA are within close proximity to the cytoplasmic loop domain used to make the antibody. Supported by PHS grants NS26377 and DK40483 to MDB and by NS22071 to RWO.

162.6

ANTISERA AGAINST SYNTHETIC PEPTIDES CORRESPONDING TO SPECIFIC DOMAINS WITHIN GABA$_A$ RECEPTOR SUBUNITS. M. E. Charlton, P. M. Sweetnam* and J. H. Neale, Dept. of Biology, Georgetown Univ., Wash. D.C. 20057 and *Nova Pharmaceutical Corp. Baltimore MD 21224.

A series of synthetic peptides have been produced which correspond to specific domains within subunits of the GABA$_A$ receptor, as deduced from the analysis of bovine and rodent nucleotide sequences. Antisera were prepared by immunization of rabbits with peptides as well as peptide-carrier conjugates. These antisera were characterized for their reactivity with the synthetic peptides, brain membranes, brain membrane protein resolved by SDS-PAGE, affinity purified receptor and with brain tissue fixed for immunohistochemistry. Additionally, the ability of antisera and affinity purified antibodies to immunoprecipitate the receptor complex or individual subunit proteins was determined. Initial results suggest that this approach will provide immunological reagents which are useful in the analysis of GABA$_A$ receptor structure and diversity.

162.7

[3H]FLUNITRAZEPAM PHOTOLABELS THE N-TERMINAL 30 KILODALTONS OF THE GABA-A RECEPTOR ALPHA SUBUNIT. Geoffrey Smith and Richard W. Olsen. Dept. of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024.

The α subunits of the GABA$_A$ receptor are prominently labeled by benzodiazepine photoaffinity ligands. We have been attempting to identify the domain of this subunit which is involved in ligand binding by generating peptide maps of purified receptor photolabeled with the benzodiazepine ligand [3H]flunitrazepam ([3H]FLU). Receptor was purified from bovine cortex by benzodiazepine affinity chromatography, equilibrated with 10 nM [3H]FLU, then exposed to UV light at 365 nm, resulting in the covalent incorporation of [3H]FLU in a protein band of relative molecular weight (M_r) 52 kD, as determined by SDS-PAGE. After digestion of the labeled protein with Staph. aureus V8 protease, the resulting peptides were separated by SDS-PAGE and transferred to PVDF membrane for N-terminal sequence analysis. This procedure produced four labeled fragments of M_r 30kD, 19kD, 13 kD, and 9kD. Sequence analysis of the 30kD fragment revealed a peptide beginning at Leu_8 of the published bovine α_1 sequence, however, sequencer fractions up to residue 22 demonstrated no radioactivity. Thus, it can be shown that the site for photoaffinity modification by benzodiazepine agonists is within the N-terminal 30 kD of the α subunit sequence thereby extending up to the cytoplasmic end of the proposed first transmembrane spanning domain. The smaller fragments ought to allow more precise localization.

162.8

SUBUNIT SUBTYPE-SPECIFIC ANTIBODIES REVEAL BRAIN REGIONAL HETEROGENEITY OF GABA$_A$/BENZODIAZEPINE RECEPTORS. Shuichi Endo and Richard W. Olsen. Dept. of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024.

Alpha subunit-subtype specific antibodies were obtained to small synthetic peptides with amino acid sequences specific for bovine α1, α2, α3, and rat α4 subunits of the GABA A/benzodiazepine receptor (GABA-R) deduced from cDNAs. Two GABA-R ß subunit-specific antibodies were also obtained:one to a putative extracellular sequence(ßex), the second to a cytoplasmic sequence(ßcyt) which encompasses the consensus phosphorylation site for the cyclic AMP-dependent kinase on the bovine ß1 subunit.Both the ßex and ßcyt antigen peptide sequences have homology among ß1,ß2, and ß3 subtypes. GABA-Rs purified from bovine cortex, hippocampus, and cerebellum were electrophoresed, transferred to nitrocellulose, and probed with these six antibodies (Western blot). In cortex, α1 (53 kDa), α2 (55 kDa), α3 (60kDa), and α4 (61kDa) were detected with α subunit subtype-specific anti-bodies, and four bands (61, 59, 57, and 55kDa) were detected with both anti-ßex and ßcyt antibodies. In hippocampus, α1, α2, and α4, and three ß bands (61, 59, and 55kD) were detected (but no α3 or ß57). In cerebellum, only α1 and one major ß band (59 kD) were detected. In these three brain regions, the number of α subunit subtypes detected corresponds to the number of ß bands detected. It is possible that each respective α subunit subytpe is associated with a particular ß subunit subtype of GABA-R. Supported by NIH grant NS22071

162.9

DEVELOPMENTAL DISTRIBUTION OF THE mRNAs ENCODING THE α1, α2, AND α4 POLYPEPTIDES OF THE RAT GABA$_A$ RECEPTOR. M. Khrestchatisky, K.F. Greif, R. Weatherwax*, and A.J. Tobin, Dept. of Biology, UCLA, Los Angeles, CA 90024

GABA$_A$ receptors result form the combination of several structurally distinct components including α, β, γ, and δpolypeptides. Some of these polypeptides are essential for the allosteric modulation of the GABA$_A$ receptor by therapeutically important drugs, such as Benzodiazepines (BZ). BZ receptor subtypes, characterized pharmacologically, have distinct localizations and distinct ontogenetic patterns. Pritchett et al. (Science 245, 1989) have shown that different γ-polypeptides, when combined with β- and γ-polypeptides, largely determine the BZ binding properties of reconstituted receptors. The developmental distribution of GABA$_A$ receptor mRNAs was studied using in situ hybridization histochemistry with ^{35}S-labeled rat α1, α2, and α4 antisense cRNAs. The developmental stages chosen were P0, P4, P7, P10 and P14. In the GABAergic regions examined, the α2 RNAs are present at birth or appear first, closely followed by the α4-RNAs where they are coexpressed. The α1 RNAs appear later in development (P10), predominate in the cerebellum, and become the major α-RNAs along the neuroaxis after that stage, in agreement with the notion that the α1 polypeptides contribute to the BZ type I receptors. Supported by NS21908 and NS 22256 to AJT.

162.10

SINGLE CHANNEL PROPERTIES OF HETEROOLIGOMERIC RAT GABA$_A$ RECEPTORS EXPRESSED USING DIFFERENT ALPHA SUBUNIT VARIANTS. T.A. Verdoorn, R. S. Kass*, P.H. Seeburg*, and B. Sakmann*, Max-Planck-Insititut für med. Forschung and ZMBH Heidelberg, West Germany.

We examined the extent of functional heterogeneity produced in GABA receptors constructed from different combinations of subunits. Receptors were expressed in transiently transfected mammalian fibroblasts and functional properties studied using patch clamp techniques in the whole cell and outside-out configurations. The amplitudes of single channels opened by GABA depended on the presence or absence of the γ2 subunit. Exchanging α3 for the α1 subunit did not alter the effect that γ2 had on conductance. For example, receptors comprised of the α1γ2 and α1β2γ2 subunit combinations had main states with single channel conductances of 30.8 ± 1.0 pS (n = 7) and 31.5 ± 0.8 pS (n = 5) respectively. GABA receptors constructed from α1β2, subunits showed a markedly smaller conductance (11.3 ± 0.23 pS, n = 6). The pattern of variation in channel conductance was preserved when the α1 subunit was replaced with α3. Thus, α3β2 GABA receptors had a small main conductance state of 13.0 ± 0.4 pS (n = 3), whereas the γ2 containing combinations gave larger conductances. GABA receptors comprised of α3γ2 subunits showed a conductance of 26.7 ± 0.4 (n = 3) and the conductance of α3β2γ2 GABA receptor channels in two patches was 25.4 and 29.7 pS. Larger conductance channels were also observed in two patches from cells expressing the α2β2γ2 subunit combination (24.0 and 29.5 pS). Noise analysis of whole cell currents indicated that the kinetics of the α2β2γ2 GABA receptor channels were similar albeit slightly slower than that of α1β2γ2 GABA receptors. Two cells transfected with α2β2γ2 subunits gave two component power density spectra (slow: 79.5 and 48.4 mS; fast: 4.08 and 2.59 mS). Two kinetic components are also observed in cells expressing the α1β2γ2 combination (35.7 ± 4.8 and 2.10 ± 0.21 mS, n = 4). These data suggest that the functional heterogeneity contributed by the α1, α2 or α3 subunits at the single channel level is limited. Further work is necessary to determine if other functional features such as desensitization or allosteric modulation by drugs are affected by exchanging α subunit variants.
T.A.V. was supported by the Alexander vonHumboldt Foundation.

162.11

KINETIC PROPERTIES OF CLONED $\alpha_1\beta_1$ GABA$_A$ RECEPTOR CHANNELS: REGULATION BY PENTOBARBITAL AND PICROTOXIN.

N.M. Porter+, T.P. Angelotti+*, R.E. Twyman+, and R.L. Macdonald+#. Depts. of Neurology+, Physiology#, and Pharmacology*, Univ. of Michigan, Ann Arbor, MI 48104

Chinese hamster ovary cells stably transfected with plasmids containing bovine α_1 and β_1 subunit cDNAs (Moss *et al.*, Euro. J. Pharm., *in press*) were used to record GABA$_A$ receptor currents from excised outside-out patches. GABA evoked single channel currents that opened predominantly to a 19 pS conductance state in contrast to the native spinal cord neuronal GABA$_A$ receptor which opens primarily to a 27 pS main conductance state and much less frequently to 19 and 11 pS sub-conductance states. Opening frequency increased by 50% as GABA concentration was increased from 5 to 25 μM. However, mean open time, burst duration, and openings per burst did not change. Open time and burst duration frequency histograms were best fit with 2 exponential functions that did not vary with GABA concentration. In spinal cord neurons, mean open and burst properties increased with GABA concentration and 3 or 4 exponential functions were required to fit the frequency histograms (Macdonald *et al.*, J. Physiol. (Lond.) 410:479,1989). The $\alpha_1\beta_1$ GABA-evoked current was enhanced by pentobarbital by increasing mean channel open time and current was reduced by picrotoxin by decreasing opening frequency. Diazepam did not enhance GABA receptor current.

These studies suggest that the $\alpha_1\beta_1$ GABA$_A$ receptor contains domains that form functional channels with conductance and gating properties that differ from those recorded from mouse spinal cord neurons. In addition, sites are present on the $\alpha_1\beta_1$ GABA$_A$ receptor for allosteric regulation by barbiturates and picrotoxin-like drugs.

162.12

SINGLE CHANNEL KINETIC PROPERTIES OF CLONED $\alpha_1\beta_1\gamma_2$ AND $\alpha_3\beta_1\gamma_2$ GABA$_A$ RECEPTOR CHANNELS: COMPARISON TO THE NATIVE SPINAL CORD GABA$_A$ RECEPTOR.

T. Ryan-Jastrow+, T.P. Angelotti+@, R.E. Twyman+ and R.L. Macdonald+#. Depts. of Neurology+, Physiology# and Pharmacology@, Univ. of Michigan, Ann Arbor, MI 48104.

COS and HEK 293 cells transiently transfected with plasmids (SV40 promotor; obtained from E. Barnard) containing GABA$_A$ receptor bovine α_1 and β_1 and human γ_2 or bovine α_3 and β_1 and human γ_2 subunit cDNAs were used to record GABA-evoked single channel currents from excised outside-out patches. Low concentrations of GABA evoked single channel currents to multiple conductance states that were similar to currents recorded from the native spinal cord GABA$_A$ receptor. The dominant or main-conductance state was about 27 pS and was the same as the main-conductance state found in the native receptor. Open, closed and burst durations were analyzed using single channel analysis techniques.

The results suggest that the $\alpha_1\beta_1\gamma_2$ and $\alpha_3\beta_1\gamma_2$ GABA receptors contain domains that form functional channels with conductance and gating properties that were similar to the native mouse spinal cord receptor. However, the open state properties of the $\alpha_1\beta_1\gamma_2$ and $\alpha_3\beta_1\gamma_2$ receptors differed, suggesting that the open state regulatory mechanisms were different. The open state properties of the $\alpha_1\beta_1\gamma_2$ were the most similar to the native GABA$_A$ receptor. Microscopic kinetic reactions schemes consistent with the single channel properties of $\alpha_1\beta_1\gamma_2$ and $\alpha_3\beta_1\gamma_2$ receptors are presented and compared to the native GABA$_A$ receptor.

162.13

SINGLE CHANNEL KINETIC PROPERTIES OF THE GLYCINE RECEPTOR MAIN- AND SUB-CONDUCTANCE STATES OF MOUSE SPINAL NEURONS IN CULTURE.

R.E. Twyman+ and R.L. Macdonald+#. Depts. of Neurology+ and Physiology#, Univ. of Michigan, Ann Arbor, MI.

Single channel kinetic properties of the 42 pS main- and the 27 pS sub-conductance states of the strychnine-sensitive glycine receptor were investigated using outside-out patches in symmetrical chloride recording media. At low concentrations (0.5, 1 and 2 μM), glycine evoked a concentration-dependent increase in average current, opening frequency, mean open duration and mean burst duration of each conductance state.

Analysis of open and closed durations show that at least three open and four closed states existed for each conductance state. Although opening and closing rates differed for each conductance state, open time constants for each conductance state did not vary with glycine concentration. Open state properties suggested that a singly liganded open state existed for both conductance states and that the resultant increased current and mean open time with glycine concentration was due to an increased probability of opening to longer, more stable, fully liganded open states. Similar results were found for bursts.

The data suggested that since the two conductance states have similar general gating characteristics, those protein domains conferring these properties of the receptor were similar. However, both conductance states differed in opening and closing rates and suggested that the receptor domains regulating entry into and exit from each conductance state were different. Although not known, these functional differences may be due different subunit composition. Kinetic schemes for the conductance states will be presented.

162.14

Inhibitory efffects of pregnenolone sulfate on cloned GABA$_A$ receptor subunits expressed in *Xenopus* oocytes.

R. Shingai, M. Sutherland, S.Zaman, R. Harvey M. Darlison and E. A. Barnard. Mol. Neurobiol. Unit, Med.Res. Council Cambridge, England

An endogenous steroid pregnenolone sulfate (PS) has an inhibitory effect on GABA$_A$ receptors. Messenger RNAs were made by *in vitro* transcription from cloned bovine GABA$_A$ receptor subunits (α1,α2,α3 and β1) and the human GABA$_A$ receptor subunit γ2. Combinations of one of the α subunits with β (two subunits) or combinations of one of the αs with the β and γ (three subunits) were injected in *Xenopus* oocytes for expression of receptor complexes. GABA at 0.2-10 μM, which corresponds to 40-50% of the maximum dose-response, was applied simultaneously with PS to oocytes. In all combinations PS showed an inhibitory effect in the concentration range above 10^{-7} M. The degree of depression by the steroid was in the order of, from a strong to a weak effect, $\alpha2+\beta+\gamma$ (ED50=4×10^{-7}), $\alpha1+\beta$, $\alpha1+\beta+\gamma$, $\alpha2+\beta$, $\alpha3+\beta$, $\alpha3+\beta+\gamma$ (ED50=5×10^{-6}). Thus addition of the $\gamma2$ subunit changed the sensitivity to the steroid in different directions depending on the subunit combinations. In about 25 % of total oocytes expressing combinations with $\alpha2$ or $\alpha3$, a small augmentation (110-150 %) of the response was observed when PS at 10^{-9}-10^{-8} M was co-applied with GABA. Pre-application of PS for a longer period and at higher concentration enhanced the inhibitory effect in all combinations of subunits, suggesting that the steroid may have both a direct and indirect effect on GABA receptors; the latter is in the manner such that the PS trapped in the lipid membrane interacts laterally with the receptors. PS alone at above 10^{-6} M produced an outward current in $\alpha_i+\beta$ (i=1,2,3) combinations. Experiments which change extracellular ion concentrations suggest that this current may be due to inhibition of spontaneous openings of kinetically deficient receptors.

162.15

WHOLE-CELL RECORDINGS OF PRE-NATAL RAT HIPPOCAMPAL NEURONS IN CULTURE REVEAL EMBRYONIC GABA$_A$ RECEPTOR FUNCTIONS.

A.Y. Valeyev* and J.L. Barker. Lab. of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

Cells were cultured from embryonic (E) day 17-19 rat hippocampal tissue for one or more days in serum-supplemented growth medium. Intracellular recordings in the whole-cell configuration were made at room temperature using Na^+_o-free and K^+_i-free electrolytes. GABA$_A$ receptor agonists and antagonists were either applied by pressure pulses from closely positioned pipettes or by gradual diffusion in the extracellular medium. Most of the cells recorded under these conditions responded to one or another ligand. When the equilibrium potential for Cl^- was set near 0 mV current responses to GABA or muscimol reversed polarity near 0 mV, suggesting a dominant role for Cl^- ions in the conductance response. Dose-response studies of membrane responses to brief pulses of agonist showed that micromolar concentrations of GABA were required for evoking consistently detectable responses. Many cells also responded to the general anesthetics (-)pentobarbital and alphaxalone. Micromolar concentrations of these drugs, like the transmitter GABA, elicited membrane current responses that reversed near 0 mV. Sequental exposure of individual cells to both anesthetics revealed cells sensitive to one but not the other agent as well as sells responding to both. These results indicate that GABA$_A$ receptor function emerges pre-natally in rat hippocampal neurons and that barbiturate and steroid binding occurs at different sites on the receptor/channel complex.

162.16

ENFLURANE AND HALOTHANE ENHANCE GABA-ACTIVATED CHLORIDE CURRENTS IN CULTURED RAT HIPPOCAMPAL NEURONS.

P.A. Brooks[1], M.V. Jones, J.L. Barker[2] and N.L. Harrison. Dept. Anes. and Critical Care, Univ. of Chicago, Chicago, Il. 60637; [1]Dept. of Physiology, The Royal Free Hospital Sch. of Med., London, U.K.; [2]Lab. Neurophysiol., NINDS, NIH, Bethesda, MD 20892; SPON: Brain Research Association

We have studied the effects of the inhalant anesthetics enflurane (ENF) and halothane (HAL) on GABA$_A$-receptor mediated chloride currents in cultured rat hippocampal neurons. Recordings were made at 25°C using the whole-cell voltage-clamp technique with an intracellular solution based on K gluconate, and externally perfused with HEPES-buffered saline. Neurons were voltage-clamped at - 40mV. GABA was applied in close proximity to the soma by 10-100ms pressure applications from micropipettes containing 25 or 50μM GABA. A pulse of GABA elicited transient outward currents (I_{GABA}), that were blocked by bicuculline and potentiated by diazepam, and had reversal potentials (E_{GABA}) between -65 and -80mV. Anesthetics were applied either from a vaporizer or via the perfusion medium, and their concentrations were measured using gas chromatography of samples withdrawn from the bath during the experiment.

In 19 of 20 experiments in which ENF was present at 0.4 - 1.5mM, application of ENF increased the peak amplitude of I_{GABA} with no change in E_{GABA}. The enhancement of I_{GABA} increased with [ENF] and was fully reversible; up to three-fold enhancements were observed at higher concentrations. Similar increases of I_{GABA} were produced by HAL (0.09 - 0.7mM). In addition, both ENF and HAL alone produced small and reversible increases in membrane conductance. We conclude that clinically relevant concentrations of ENF and HAL modulate the GABA$_A$-receptors on cultured rat hippocampal neurons to produce a marked increase in GABA-activated chloride conductance.

P.A.B. was supported by a Medical Research Council Travelling Fellowship.

162.17

ZINC AND CADMIUM INHIBIT GABA SENSITIVITY WITH DIFFERENT EFFICACIES. J. J. Celentano*, M. Gyenes*, T. T. Gibbs, D.H. Farb. Dept. of Anatomy and Cell Biology, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203.

It has recently been shown that the GABA receptor chloride ionophore complex is sensitive to modulation by a variety of divalent cations. We have studied the effects of two such ions, zinc and cadmium, on GABA sensitivity in cultured chick spinal cord neurons. We find that both cations inhibit GABA sensitivity in a saturable manner but neither cation achieves 100% inhibition at saturating concentrations. The maximum inhibition induced by Zn (90%) is greater than that induced by Cd (72%). High concentrations of both cations (3 mM Zn, 5 mM Cd) applied in combination result in an intermediate level of inhibition. This is consistent with a common divalent cation modulatory site at which Zn has a higher intrinsic efficacy than Cd. Inhibition induced by lower concentrations of both Cd and Zn was partially reversed by 5 mM barium which had no significant effect by itself.

The residual GABA response in the presence of high Zn or Cd was pharmacologically similar to the full GABA response. Further inhibition was acheived with bicuculline, SR 95531, picrotoxin, and pregnanolone sulfate. In addition the residual GABA response was subject to stimulation by a benzodiazepine, a barbiturate, and a steroid. These positive modulators did not significantly alter the cation dose response curves. The divalent cation site is therefore distinct from the sites used by other known GABA modulators.

162.18

EFFECTS OF PROGESTERONE AND 3α-OH-DHP ON β-ALANINE-INDUCED CURRENTS IN CULTURED NEURONS. F.-S. Wu, T.T. Gibbs, and D.H. Farb. Dept. of Anatomy and Cell Biology, SUNY Health Science Center at Brooklyn, Brooklyn, NY 11203.

We have previously shown that progesterone enhances GABA-induced chloride currents and antagonizes those induced by glycine, and that its reduced metabolite 5α-pregnan-3α-ol-20-one (3α-OH-DHP) dramatically potentiates GABA-induced currents, but produces little effect on glycine responses. Here we examine the effects of these two steroids on currents induced by β-alanine in cultures of chick spinal cord neurons. Using whole-cell recording methods cells were voltage-clamped at -70 mV. Drug solutions were applied to single neurons by pressure ejection from 7-barrel pipettes. While pressure application of progesterone (100 μM) rapidly and reversibly reduced (by 31 ± 2.0%, in 6 cells) responses to 200 μM β-alanine, application of 3α-OH-DHP (1 μM) potentiated (by 116 ± 18%, in 6 cells) β-alanine responses. In the presence of 1 μM strychnine, a selective glycine receptor antagonist, both progesterone and 3α-OH-DHP potentiated β-alanine-induced currents. In 6 cells, the average potentiation produced by progesterone and 3α-OH-DHP was 264 ± 46% and 1621 ± 277%, respectively. In contrast, in the presence of 100 μM SR-95531, a selective GABA$_A$ receptor antagonist, inhibition of β-alanine-induced currents by progesterone was increased to 54 ± 4.6% (in 6 cells) and 3α-OH-DHP produced a small inhibitory effect (10 ± 2.8%, in 5 cells) on β-alanine responses. These results suggest that β-alanine activates both the glycine receptor and the GABA$_A$ receptor.

162.19

DESENSITISATION OF GABA$_A$ RECEPTOR CHANNELS IN PATCHES OF MEMBRANE EXCISED FROM CULTURED SPINAL CORD NEURONS. D.A. Mathers and Y. Wang. Physiology Department, University of British Columbia, Vancouver, B.C. V6T 1W5 Canada.

Recent studies have shown that elevated intracellular Ca^{2+}, $[Ca^{2+}]_i$ decreases the sensitivity of rat hippocampal neurons to externally applied γ-aminobutyric acid (GABA) (Stetzler et al., Science, 242:339, 1988). In this study, we have examined the effect of intracellular calcium ions on the kinetic properties of GABA$_A$ receptor channels. Embryonic mouse spinal cord neurons were removed at day E13 and maintained in Minimum Essential Medium for 14-21 days prior to use. Outside-out patches were bathed on both faces in solutions containing 145 mM chloride. $[Ca^{2+}]_o$ was 1 mM, while $[Ca^{2+}]_i$ was varied from 0.1 nM to 10 μM using Ca-EGTA buffers. 5 μM GABA induced a response which showed desensitisation at all $[Ca^{2+}]_i$ levels tested. The open times of GABA induced single channel currents were fit by the sum of multiple exponentials. At high $[Ca^{2+}]_i$ (1μM), the mean open time of GABA induced channels was significantly reduced when compared to values obtained in the presence of low $[Ca^{2+}]_i$.

REGULATION OF CATECHOLAMINE RECEPTORS

163.1

ACTIVATION OF DIACYLGLYCEROL-PHOSPHOINOSITOL PATHWAY BY DOPAMINE D2B RECEPTORS R.D. Todd, J. Hickock, and K. O'Malley. Depts. Psychiatry, Genet. & Anat. & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

The intercellular signal dopamine interacts with two pharmacologically distinct types of neural receptors termed D1 and D2. The D1 receptor has been linked to activation of the cyclic AMP second messenger system. Contradictory reports have appeared about the inhibitory effects of D2 receptor on the cyclic AMP and the diacylglycerol-phospoinositol (DAG-PI) second messenger pathways. Bunzow et al. (Nature 336:783-787, 1988) reported the cloning of a cDNA for a rat D2 receptor which is a member of the G-protein coupled receptor gene family. This receptor, called here $D2_A$, is capable of interacting with G-proteins and inhibiting adenylate cyclase activity. We recently reported on the isolation of a transfected cell line, DA10-4, which expresses a rat D2 receptor (Todd et al., PNAS 86:10134-10138, 1989) distinct from that found by Bunzow et al. Here, we report association of this novel D2 receptor (called $D2_B$) with activation of the DAG-PI second messenger systems. Stimulation of $D2_B$ receptors with either PPHT or bromocriptine results in increases in intracellular calcium in DA10-4 cells. This increase can be blocked or reversed by application of D2 antagonists or inorganic calcium blockers (Todd et al, 1989). Growth of DA10-4 cells for up to 12 hours in 0-0.5 μg/ml pertussis toxin or 0-1.0 μg/ml cholera toxin had no effect on calcium level changes, suggesting that the expressed receptors are not linked to Go, Gi, or Gs proteins. In contrast, the calcium increases were blocked in a dose dependent fashion by the protein kinase C inhibitor sphingosine ($E.C._{50}$ = 1 μM). PPHT stimulation also resulted in increases in free inositol-phosphate levels (IP1, IP2, and IP3) with the same time course as the calcium level changes. These results suggest that $D2_B$ receptors can activate the DAG-PI second messenger systems.

163.2

DOPAMINE D-2 RECEPTOR REGULATION BY Na^+ AND H^+ B. Tester and K.A. Neve. VA Medical Center and Oregon Health Sciences University, Portland, OR 97207.

Regulation of ligand binding to dopamine D-2 receptors by Na^+ may be due to sodium-dependent isomerization of D-2 receptors. We now describe selective regulation of ligand binding by H^+. As shown in the table below, $[H^+]$ had little effect on the binding of the antagonists spiperone and butaclamol to D-2 receptors. In contrast, K_I values for inhibition of the binding of [^{3}H]spiperone by sulpiride and epidepride varied widely according to the pH of the binding assay. The affinity of agonists for D-2 receptors was also decreased by increasing $[H^+]$. Binding of dopamine to D-1 receptors was not altered by changing pH from 6.5 to 8. Two lines of evidence suggest that effects of Na^+ and H^+ on D-2 receptors are related. First, greater pH-induced shifts in affinity were observed in the absence of Na^+ than in the presence of 50 mM Na^+. Second, the most pronounced changes in affinity induced by high $[H^+]$ were for drugs sensitive to Na^+, such as substituted benzamides and agonists.

Affinity values (nM)

Drug	pH 6.5	pH 8.0	6.5+Na^+	8.0+Na^+
spiperone	0.038	0.016	0.024	0.017
butaclamol	0.2	0.2		
epidepride	16	0.36	0.68	0.052
sulpiride			290	16
dopamine	26,000	1,000	16,000	1,800
(+)3-PPP	45,000	7,000		

(Supported by the VA Merit Review Program and MH 45372.)

163.3

EFFECTS OF ADRENALECTOMY AND GLUCOCORTICOIDS ON RAT BRAIN DOPAMINE RECEPTORS. D.Biron*,T.Di Paolo, School of Pharmacy,Laval University and Dept. of Molecular Endocrinology, CHUL, Québec, Canada G1V 4G2

The behavioural responses to dopamine (DA) agonists has been shown to be influenced by adrenalectomy (ADX) (Eur.J. Pharmacol.152:255-261,1988). In order to investigate the possible mechanism of this response, we studied the effects of adrenalectomy (ADX) (28 days) and Dexamethasone (DEX) on the amount of D1 and D2 DA receptors by autoradiography using the D1 and D2 antagonists(^{3}H-SCH 23390 and ^{3}H-Spiperone) in regions of the nigrostriatal and mesolimbocortical pathways. D1 and D2 receptors decreased in anterior striatum (AS) by 6% and 17% (P< 0.05 and 0.01),in median striatum (MS) by 10% (D2,P< 0.01) and of 31% (D1,P< 0.01) in substancia nigra (SN) in ADX rats vs control ovariectomized rats(OVX). DEX treatment (1mg/kg/d,I.M.,10 days) reversed these effects in AS (D1,D2,P< 0.01) in MS (D2,P< 0.01) and in SN (D1,P< 0.05) vs ADX rats. DEX also increased the amount of D1 receptors in accumbens and MS by 10% (P< 0.01) vs OVX rats.It is known that ADX elevates and DEX decreases ACTH levels. However,our findings are unlikely due to ACTH since our results in striatal homogenates of rats treated with ACTH and corticosterone showed increased amount of D1 receptors such as following DEX treatment.Our results suggest a role of the adrenals in the modulation of DA activity in the rat brain that could be implicated in the behavioural changes occuring in stress .(Supported by the MRC of Canada)

163.4

STIMULATION OF PHOSPHATIDYLINOSITOL TURNOVER BY DOPAMINE D2 RECEPTORS IN RAT STRIATUM. S. Kito, R. Miyoshi and T. Nomoto*. Division of Health Sci., Univ. of the Air, Chiba 260 and Dept. of Pharmacology, Tokyo Women's Medical College, Tokyo 162, Japan.

Dopamine receptors are classified into D1 and D2 subtypes on the basis of their pharmacological and biochemical characteristics. The intracellular signalling system of D2 receptors has not been determined conclusively. It has been described that as well as the inhibition of adenylate cyclase, D2 receptors are linked to other signalling system. The authors investigated effects of dopamine on phosphatidylinositol (PI) turnover in the rat striatum. Overall agonists of D1 and D2 receptors, both dopamine and apomorphine, caused stimulation of PI turnover. The effect of dopamine was dose-dependent. The dopamine effect was prevented by the D2 receptor antagonist, spiperone, while it was not by the D1 receptor antagonist, SCH23390. Pretreatment of striatal slices with islet-activating protein (IAP) caused a partial inhibition of the effect of dopamine. Neither forskolin nor tetrodotoxin affected stimulation of PI turnover elicited by dopamine. Above-mentioned results suggest that D2 receptors activate PI turnover in the rat striatum through an IAP-sensitive GTP binding protein. The second messenger system of D2 receptors is more complicated than previously thought.

163.5

DOPAMINE D1 AND D2 RECEPTORS IN RAT CORTEX: AUTORADIOGRAPHIC LOCALIZATION FOLLOWING NEUROTOXIC LESIONS. S. Giddings, M. Al-Tikriti, R. Kessler, R.H. Roth, and R.B. Innis. Dept. Psychiatry, VA Medical Center and Yale University, West Haven, CT 06516.

Relative to the dopaminergic (DA) innervation of cortex, the dopamine D1 and D2 receptors may be located "presynaptically" on DA terminals and/or "postsynaptically" on neurons intrinsic to cortex. To examine the relative neuronal distribution of these sites, we performed quantitative in vitro receptor autoradiography following a "presynaptic" lesion of DA terminals (with 6-hydroxydopamine (6-OHDA) injected into the medial forebrain bundle) or a "postsynaptic" lesion in cingulate and medial prefrontal (MP) cortex (with local injection of ibotenic acid).

Groups of male Sprague-Dawley rats received injections of 6-OHDA (pretreated with desipramine for relative sparing of noradrenergic innervation); ibotenic acid; or vehicle. Approximately 10 days after injection, animals were killed, the brains removed, and 1 mm coronal tissue slabs cut in a brain mold. Cryostat sections (20 µm) were used for quantitative receptor autoradiography with ^{125}I-epidepride (D2 ligand) and ^{3}H-SCH23390 (D1 ligand). In a parallel group of animals, the cingulate and MP cortical areas were dissected from the 1 mm slabs for measurement of DA and DOPAC as a neurochemical characterization of the lesions. Results with D1 receptor autoradiography are pending.

D2 receptors are concentrated in cortical layers V and VI of cingulate and MP cortex. Local injection of ibotenic acid caused a reduction of at least 70%, although the concentrations of DA and DOPAC were not significantly changed. In contrast, injection of 6-OHDA into the medial forebrain bundle caused no significant change in D2 receptor binding, but was associated with a 60% decrease of DA and a 40% decrease of DOPAC in cingulum.

These results suggest that the vast majority of D2 receptors in cingulate and MP cortex are not located on dopaminergic terminals, but rather "postsynaptically" to these terminals on neurons whose cell bodies are intrinsic to the cortex.

163.6

QUANTITATION OF ISOTYPES OF D2 RECEPTORS USING SOLUTION HYBRIDIZATION. R.P. Artymyshyn,* R.R. Luedtke, B.R. Monks,* J.L. Neisewander, and P.B. Molinoff. Dept. of Pharmacology, University of Pennsylvania, Philadelphia, PA 19104.

Solution hybridization was used to quantitate the expression of two isotypes of the D2 receptor. Sequences corresponding to 450 bases of the coding region of the long form of the third intracellular loop (i3) and full-length coding sequences of both the long and short forms of the D2 receptor were amplified using PCR, cloned into a Bluescribe (pBS+) plasmid vector, and sequenced. T3 RNA polymerase was used to synthesize a radiolabelled antisense riboprobe using the sequence of the long form of the third intracellular loop as a template. Sense RNA synthesized using the full-length coding sequence of the long and short isotypes of the D2 receptor was used to define specific hybridization. Hybridization was carried out overnight using one of the sense control RNA templates or total RNA isolated from tissue or cells. After hybridization and digestion with nucleases, hybridization products were size-fractionated on a urea/acrylamide sequencing gel. Autoradiographs of gels containing control RNA as template demonstrated distinct banding patterns. The i3 probe hybridized to the long-form sense RNA to reveal a single band of 410 bases. When the same probe was hybridized to the short-form sense RNA the probe was split into two bands, one of 290 bases and one of 68 bases. The pattern seen when total RNA from tissue was hybridized with the i3 probe revealed two major bands: one band corresponding to the long form of the receptor and the other corresponding to the short form. The migration of these bands was identical to that seen with the sense RNA controls. Computer-assisted densitometry was used to quantitate the amount of RNA present in each band. The long form of the receptor is expressed at higher levels than the short form in the striatum, while in a cell line containing dopamine receptors derived from the pituitary (Sup1), the long form predominates to an even greater degree (>10:1). The development of this technique allows us to accurately measure the individual isotypes of mRNA coding for the D2 receptor. Supported by the American Heart Association and USPHS NS 18591.

163.7

BOTH ISOFORMS OF THE D2 DOPAMINE RECEPTOR COUPLE TO A G PROTEIN-ACTIVATED K^+ CHANNEL WHEN EXPRESSED IN GH_4 CELLS. L.C. Einhorn, P. Falardeau[1], M. Caron[1], O. Civelli[2] and G.S. Oxford. The Neurobiology Curriculum, University of North Carolina, Chapel Hill, NC 27599, [1]Dept. of Cell Biology, Duke University, Durham, NC 27710, and [2]Vollum Institute, Portland, OR 97201.

Recent evidence suggests that the D2 dopamine (DA) receptor gene encodes two receptor isoforms (415 vs. 444 amino acids). The 444 a.a. splice variant differs from the short variant by an additional 29 a.a. insert in a region thought to couple to G-proteins. In pituitary lactotrophs, D2 receptors activate K channels via a PTX-sensitive G-protein. To examine whether the two receptor isoforms differentially regulate such an effector mechanism, cDNA encoding either receptor isoform was transfected into prolactin-secreting GH_4 cells which lack D2 receptors. Receptor density was assayed by ^{3}H-spiroperidol binding and two clones with equivalent levels of each isoform (2.0 pmol/mg protein) were used for patch clamp studies. Application of DA to cells expressing either receptor isoform results in a rapid membrane hyperpolarization (5-10mV) halting spontaneous spiking activity. Under voltage clamp, D2 agonists activate a current which reverses near E_K and shifts with extracellular $[K^+]$. The K current response to DA was independent of $[Ca^{++}]$ or intracellular cAMP. DA responses were never observed in non-transfected cells, although similar K currents could be induced by somatostatin (SRIF). The DA response was slightly greater in cells expressing the long rather than the short isoform, however K current responses to SRIF differed similarly. Both the SRIF- and DA-activated K currents are sensitive to blockade by $[TEA]_o$ and are abolished by PTX pretreatment. We suggest that both forms of the D2 receptor can functionally couple to K channels, and share a common signal transduction pathway with SRIF receptors.

163.8

NON-STEADY-STATE MEASUREMENT OF IN VIVO RECEPTOR BINDING WITH POSITRON EMISSION TOMOGRAPHY: SPECIFICITY ANALYSIS. J. S. Perlmutter, S. Moerlein, D-R. Hwang* and R. Todd. Departments of Neurology, Radiology, Psychiatry and Genetics, Washington Univ. Sch. of Med., St. Louis, MO 63110.

The purpose of this study is to determine the specificity of our non-steady-state method for in vivo measurement of radioligand binding in brain with positron emission tomography (PET) and ^{18}F-spiperone (^{18}F-SP). Three to 6 studies were performed in each of 4 male baboons. Animals were pretreated with either ketanserin (S_2), eticlopride (D_2) or unlabeled SP to compete with ^{18}F-SP for specific binding sites. We measured regional blood flow, blood volume and total binding in blood of ^{18}F-SP. After i.v. injection of ^{18}F-SP, sequential PET scans and arterial-blood samples were collected for 3 hrs. Data were analyzed with a 3-compartment model that considered the accumulation of radiolabeled metabolites in arterial blood. Four baboons were sacrificed and SP binding was measured with in vitro homogenate techniques. There was no detectable in vitro or in vivo specific binding of SP in cerebellum. In vitro specific binding of SP in striatal tissue was 76% to D_2 sites and 24% to S_2 sites, whereas, about 34% of apparent in vivo specific binding in striatum could be blocked by ketanserin and 70% blocked by eticlopride. The close relationship between in vitro and in vivo findings further validates this PET method although the modest differences may result from the relatively poor resolution of PET.

163.9

DOPAMINE-STIMULATED LOW K_M GTPASE ACTIVITY IN RAT AMYGDALOID COMPLEX. J.E. Lachowicz, S.E. Eldon and C.D. Kilts. Dept. of Pharmacology, Duke Univ. Med. Ctr., Durham, N.C. 27710.

Striatal D_1 and D_2 dopamine receptors are functionally classified by their ability to stimulate and inhibit adenylate cyclase activity via interaction with the guanyl nucleotide binding proteins, G_s and G_i, respectively. We have demonstrated that in the amygdaloid complex, neither D_1 nor D_2 receptors influence adenylate cyclase activity. To determine whether amygdaloid dopamine receptors are coupled to G-proteins despite their apparent non-coupling with adenylate cyclase, the effects of dopamine on low K_m GTPase activity were assessed. Dopamine (100 uM) stimulates GTPase activity in the amygdala to the same degree as in the striatum. The δ -opiate agonist D-pen,D-pen enkephalin (10 uM) and the muscarinic cholinergic agonist carbachol (1 mM) also increase low K_m GTPase activity in the amygdaloid complex as well as the striatum.

These results suggest that amygdaloid dopamine receptors regulate the activity of G-proteins coupled to effectors other than adenylate cyclase. Characterization of the pharmacology of the dopamine receptor and G-protein subtypes involved will be presented. (Supported by NIGM-5T32GM07105 and MH-39967).

163.10

HETEROLOGOUS DESENSITIZATION OF D_1 DOPAMINE RECEPTOR-COUPLED ADENYLYL CYCLASE ACTIVITY IN NS20Y NEUROBLASTOMA CELLS. Anne C. Barton, Lauren E. Black, and David R. Sibley. Experimental Therapeutics Branch, NINDS, NIH, Bethesda, MD 20892.

8-(4-chlorophenylthio)-adenosine-3':5'-cyclic monophosphate (CPT-cAMP), a membrane permeable analogue of cAMP, induced heterologous desensitization of D_1 receptor-coupled adenylyl cyclase (AC) activity in NS20Y neuroblastoma cells. Membranes prepared from these cells showed a 55-75% reduction in maximal DA-induced stimulation of cAMP production following 24 hr pretreatment with 1 mM CPT-cAMP. The maximal responsiveness to adenosine was also decreased by 60%, while AC responsiveness to PGE_2, sodium fluoride and forskolin was decreased 15-20%. Desensitization of D_1 receptor-coupled AC activity by CPT-cAMP was time-dependent, exhibiting a maximal effect by 20 hr and an approximate $t_{1/2}$ of 6 hr. CPT-cAMP treatment also induced down-regulation of D_1 receptor binding activity, the time course and extent of which matched AC inactivation. Saturation analysis with ^{3}H-SCH-23390 after 24 hr CPT-cAMP treatment indicated that the loss of binding activity was predominantly due to a decrease in the B_{max}. Similarly, following 24 hr CPT-cAMP treatment, DA dose response curves for stimulation of cAMP production indicated that the predominant desensitizing effect is a reduction in the maximal responsivesness of the enzyme to DA. In addition, agonist/^{3}H-SCH-23390 competition curves following CPT-cAMP treatment demonstrated a loss in high affinity agonist binding. The effect of CPT-cAMP on D_1 dopamine receptor coupled AC activity and binding was dose-dependent, exhibiting an IC_{50} of $\approx$ 50 μM, and was specific for cAMP as 8-bromo-AMP produced negligible effects on both AC activity and binding. These data suggest that cAMP mediates a heterologous form of desensitization of the D_1 receptor involving functional uncoupling and receptor downregulation.

163.11

REGULATION OF D_1 DOPAMINE RECEPTOR FUNCTION IN NS20Y CELLS: ROLE OF G-PROTEINS.
T.W. Lovenberg[1], D.E. Nichols[3], E.J. Nestler[4], R.H. Roth[4], R.B. Mailman[1,2]. Pharmacology[1] and Psychiatry[2], Univ. North Carolina, Chapel Hill, NC 27599; Med. Chem. and Pharmacognosy[3], Purdue Univ., West Lafayette, Indiana 47907; Pharmacology and Psychiatry[4], Yale Univ., New Haven, CT 06510.

NS20Y neuroblastoma cells possess D_1 receptors and muscarinic receptors that are linked to the stimulation and inhibition of adenylate cyclase, respectively. To study these processes, cyclic AMP was measured in intact cells following either cholera or pertussis toxin treatment. Pretreatment with pertussis toxin (100 ng/ml), which ribosylated >95% of G_{i}, caused the complete loss of muscarinic induced inhibition. Dihydrexidine (a D_1 agonist) or forskolin (a direct activator) stimulated cAMP accumulation were unaffected. Conversely, cholera toxin dose-dependently induced large accumulations of cAMP. At low cholera toxin concentrations, dihydrexidine (300 nM) was an effective stimulus. However, when cholera toxin was >100 ng/ml, dihydrexidine was ineffective, whereas forskolin (1 μM) could still enhance cAMP accumulation. These data suggest that adenylate cyclase in NS20Y cells is regulated by both stimulatory and inhibitory G-proteins linked to D_1 and muscarinic receptors, respectively. Interestingly, dihydrexidine-stimulated cAMP accumulation was additive with forskolin-stimulated cAMP accumulation at low forskolin concentrations (10 nM-3 μM), but synergistic at high forskolin (3-100 μM) concentrations, suggesting possible interactions between activated G_s and the catalytic unit of adenylate cyclase in this cell type.

CELL BIOLOGY OF CATECHOLAMINE RECEPTORS

164.1

DEVELOPMENTAL EXPRESSION AND REGIONAL DISTRIBUTION OF A FAMILY OF ALPHA ADRENERGIC RECEPTORS IN THE RAT BRAIN. Susan K. McCune and Mark M. Voigt. Lab. of Developmental Neurobiology, NICHD, and Lab. of Mol. Biol., NINDS, NIH, Bethesda, MD 20892.

Multiple subtypes of adrenergic receptors have been identified by a combination of molecular biological and pharmacologic techniques. However, it has been difficult to differentiate various subtypes of these receptors in tissues based on rank-order potencies of agonists and antagonists. In order to examine the regional and developmental expression of multiple alpha adrenergic receptor subtypes in the brain, we have isolated clones from a rat brain cDNA library for the α1B, α2A, and α2D adrenergic receptors.

Northern blots of mRNA from various brain regions in the adult rat were probed with random-primed DNA derived from each of the subtypes. These blots indicated that all three subtypes are present in the cortex, hippocampus, hypothalamus, midbrain, brainstem and cerebellum. The α2D receptor message was strongest in the cortex, hippocampus, hypothalamus and cerebellum and weakest in the midbrain and brainstem. The mRNA transcript for the α2A receptor was most abundant in the hypothalamus with an equivalent but lower representation in the other areas. The α1B receptor message showed a fairly uniform distribution throughout the brain regions examined. The ontogenic expression of these receptors was also studied using these three probes. The mRNA transcripts for the α2D and α1B clones showed an increase in abundance from embryonic day 18 through adult while the transcript for the α2A remained constant prenatally and then declined into adulthood.

These results document a unique regional and developmental expression for three subtypes of adrenergic receptors. This receptor diversity may play an important role in modulating the physiologic responses to catecholamines.

164.2

GENERATION OF A DNA PROBE FOR THE ALPHA$_2$-ADRENERGIC RECEPTOR IN AN OPOSSUM KIDNEY CELL LINE (OK). H.S. Blaxall and D.B. Bylund. Department of Pharmacology, University of Nebraska Medical Center, Omaha, NE 68198-6260.

Previous studies from our laboratory have characterized an alpha$_2$-adrenergic receptor in the OK cell (Murphy, T.J. and Bylund, D.B., J. Pharmacol. Exp. Ther., 244:571-578, 1988). As a preliminary step in cloning the receptor, the polymerase chain reaction (PCR) was utilized to generate a probe for this receptor. Using the published sequences of the human α_2 receptors C_4 and C_{10}, oligonucleotide primers were developed to transmembrane regions I and VII. RNA was isolated from OK cells using an Applied Biosystems nucleic acid extractor. Between 1.5-2 μg of this RNA was reverse transcribed using Moloney Murine Leukemia Virus reverse transcriptase. The resulting cDNA's subjected to 30 cycles of PCR with the above primers, yielding a product of the expected size of approximately 1 Kb. PCR was also used on HPα2GEN (a plasmid containing alpha$_2$ C_{10} to produce a 0.6 Kb fragment which includes transmembrane regions I to V. This 0.6 Kb fragment labeled by the random primer method hybridized strongly to the 1 Kb PCR product from the OK cDNA. This 1 Kb product is currently being used as a probe in a lambda gt11 OK cell cDNA library (Supported by NIH grant GM 40784).

164.3

NORADRENERGIC AND NON-NORADRENERGIC RECEPTOR BINDING OF [125I]IODOCLONIDINE (ICLO) IN HUMAN TISSUE. J.E. Piletz and G.A. Ordway, Depts. Psychiatry, Neuroscience & Pharmacology, Case Western Reserve Univ. Cleveland, OH 44109.

LIGAND computer analysis revealed two high affinity sites for ICLO in normal human platelet purified plasma membranes and in frontal cortex lysates. The two sites were highly comparable between tissues: in platelets KD1= .09 nM(±.05 SD) & Bmax1= 55 fmol/mg prot.(±23 SD), while KD2= 1.9 nM(±1.7 SD) & Bmax2 = 200 fmol/mg (±54 SD)(n=3). In frontal cortex, KD1=.12 nM(±.07 SD) and Bmax1=56 fmol /mg(±38 SD) while KD2=1.7 nM(±1.1SD) & Bmax2 =191 fmol/mg (±57 SD)(n=7). Both sites were GTP sensitive. Site1 was displaced at high affinity by (-)norepinephrine (NE),clonidine(CLO),and yohimbine(YOH). However,site2 was not significantly displaced by NE even at 10uM; apparently an imidazoline-preferring (IZP) site. Expressed as percent of specific 0.3nM ICLO binding (± 10uM YOH), 10uM NE displaced only 60% (=A2 adrenoceptors), but 10uM CLO or YOH displaced the additional 40% (=IZP sites) in cortex and platelets. Autoradiography at 0.3nM ICLO revealed the same in entorhinal cortex (EC) layer 1. However, in EC layers 2-4,and 5 & 6, IZP sites predominated (>80%). In dentate gyrus and hippocampal layer CA4, ICLO was displaced equally by 10 uM of NE, CLO or YOH (100% adrenoceptors). In pyramidal and molecular CA1 layers ICLO was about 80% displaceable by NE and CLO (80% adrenoceptors,no IZP sites)but 20% more was displaced by YOH. Relevance is to the design of ICLO binding studies in pathological states.

164.4

Subnuclear Distribution of Alpha$_2$-Binding Sites in the Rat Parabrachial Nucleus. H.Herbert[1], G.Flügge[2], and E.Fuchs[2]; [1]Univ. of Tübingen, Dept. Animal Physiol., Morgenstelle 28, 7400 Tübingen, FRG; and [2]German Primate Center, Kellnerweg 4, 3400 Göttingen, FRG.

The parabrachial nucleus (PB) is composed of several subnuclei which have distinct cyto- and chemoarchitectonic properties and specific sets of afferent and efferent connections. We investigated the distribution of ^{3}H-rauwolscine (^{3}H-RAUW) binding sites to elucidate whether the anatomical diversity of PB subnuclei is also reflected by a differential distribution of alpha$_2$-adrenoceptors.

Five different areas of the PB complex were analyzed: the Kölliker-Fuse nucleus (KF), the medial (m)PB, the lateral (l)PB, the external lateral (el)PB, and the "waist area". The largest number of binding sites were found in the elPB (B_{max}:280 fmol/mg), and in the "waist area" (220 fmol/mg), followed by the lPB (180 fmol/mg). The mPB (150 fmol/mg) and the KF (130 fmol/mg) exhibited only few binding sites for ^{3}H-RAUW. Scatchard analyses revealed that the binding sites in different PB subnuclei also have different affinities for the ligand. K_D-values were lowest in the elPB, the lPB and the "waist area" with K_D:6.8nM, 7.3nM, and 10.6nM, respectively. Fairly high K_D-values were found in the mPB and the KF (K_D:15.7nM and 16,1nM, respectively), indicating a low affinity for the ligand.

To summarize, ^{3}H-RAUW binding demonstrates that each subnucleus in the PB exhibits a distinct pattern of alpha$_2$-adrenoceptors. The highest B_{max}-values were found in those PB subnuclei that receive catecholaminergic afferents from the medial nucleus of the solitary tract. Experiments are currently performed to examine the response of alpha$_2$-binding sites to different types of deafferentiation such as the selective depletion of the noradrenergic innervation.

164.5

IDAZOXAN BINDS "IMIDAZOLINE-PREFERRING" SITES AND ALPHA$_2$ NORADRENERGIC RECEPTORS IN HYPOTHALAMUS. P. A. VINCENT, C. P. REBOULLEAU* AND H. H. FEDER Biol. Sci. Rutgers University, Newark, NJ 07102

The imidazoline clonidine, an alpha$_2$ noradrenergic (NA) receptor agonist, has been used to study reproductive behavior in rodents. We have shown that another imidazoline, idazoxan, a putative alpha$_2$ NA receptor antagonist, blocks steroid-dependent female reproductive behavior in guinea pigs. In the present work, we investigated the binding properties of idazoxan and the alpha$_2$ NA antagonist yohimbine in guinea pig hypothalamus. We demonstrated that: 1) A number of alpha$_1$ and beta NA selective drugs compete with only low affinity for idazoxan binding sites. 2) Yohimbine, epinephrine and norepinephrine displace idazoxan poorly (IC_{50} = 5.5 x 10^{-6} M, 2.5 x 10^{-3} M and 7.5 x 10^{-3} M, respectively). 3) Imidazolines such as tolazoline and guanidine derivatives such as guanabenz displace idazoxan with high affinity. This evidence suggests that idazoxan and other imidazolines bind to hypothalamic alpha$_2$ NA sites as well as non-alpha$_2$ NA sites. Brain "imidazoline-preferring" sites are probably involved in the central regulation of blood pressure and may also be involved in modulation of steroid-dependent reproductive behavior.

164.6

DETECTION OF α_2-ADRENOCEPTOR SUBTYPE GENE EXPRESSION IN CULTURED MAMMALIAN CELLS M. Scheinin*, A. Marjamäki*, M. Perälä*, R. Voutilainen*, B. Sjöholm*, J.W. Regan[1]*. Dept. of Pharmacol., Univ. of Turku, SF-20520 Turku, Finland, and [1]Dept. of Pharmacol. & Toxicol., College of Pharmacy, Univ. of Arizona, Tucson, AZ 85721 (SPON: European Neuroscience Association)

Chinese hamster lung fibroblasts, transfected with the dexamethasone-inducible mammalian expression vector pMAMneo, have been used to express human platelet (α_2-C10) and human kidney-type (α_2-C4) α_2-adrenergic receptors. Expression levels in the resulting cell lines were quantitated by two different assays. Ligand binding studies with ^{3}H-atipamezole (0.03-4 nM), a novel subtype-nonselective α_2-antagonist, showed 4 to 5-fold increases in receptor density in crude membrane preparations from dexamethasone-treated cells. Parallel increases in receptor subtype gene expression were also seen in Northern blot analysis of both total and poly-A$^+$-RNA. With the full length α_2-C4 and α_2-C10 cDNAs as probes, subtype-specific mRNA species of about 4.5 kb and 3.5 kb were recognized.

Recombinant DNA techniques are increasingly used to express neurotransmitter and drug receptors in cell cultures for studies on the molecular mechanisms of drug action. This approach necessitates the development of practical and sensitive detection methods for receptor subtype gene expression in small samples.

164.7

^{3}H-ATIPAMEZOLE, A NEW α_2-ADRENOCEPTOR RADIOLIGAND FOR RECEPTOR BINDING STUDIES T. Miettinen*, R. Voutilainen*, J-M. Savola[1]*, B. Sjöholm*, L. Ahtee[2], M. Scheinin*. Dept. of Pharmacol., Univ. of Turku, SF-20520 Turku, [1]Farmos-Group Ltd., Turku, [2]Dept. of Pharmacy, Univ. of Helsinki, Finland

Atipamezole (ATI, 4-(2-ethyl-2,3-dihydro-1H-inden-2-yl)-1H-imidazole) is a novel potent, specific α_2-adrenoceptor antagonist with a high α_2/α_1-selectivity ratio. We have now carried out a preliminary investigation of the suitability of ^{3}H-ATI (65 Ci/mmol, NEN) as a radioligand for in vitro receptor binding studies, performed essentially as described by Bylund (JPET 245:600, 1988). ^{3}H-ATI, and for comparison, ^{3}H-rauwolscine (RAU) (both at 0.02-4.0 nM), were incubated with the membranes in the absence and presence of phentolamine (PHEN, 10 uM) or norepinephrine (NE, 50 uM) to determine the specificity of the observed binding. ^{3}H-ATI binding equilibrated rapidly, and was also readily reversible upon addition of competing drugs. Scatchard analysis indicated that ^{3}H-ATI labelled one population of sites, with Hill coefficients near unity. Compared to ^{3}H-RAU, ^{3}H-ATI labelled significantly more PHEN-defined sites in rat brain cortical (B_{max} 460 vs. 80 fmol/mg prot) and neonatal rat lung membranes (230 vs. 100), whereas in human blood platelets the number of binding sites detected was in closer agreement (250 vs. 190). With NE, both radioligands yielded approximately equal B_{max} values. The affinity of ^{3}H-ATI was somewhat higher than that of ^{3}H-RAU (K_D 0.1-0.8 vs. 0.4-0.8 nM), particularly in rat brain cortex. ^{3}H-ATI thus appears to possess promising potential as an α_2-adrenoceptor radioligand, but further validation with competing drugs is required before its introduction into routine use. The higher PHEN-defined B_{max} values compared to ^{3}H-RAU in some tissues probably reflect binding to imidazole-preferring sites.

164.8

ALPHA$_2$-ADRENERGIC INHIBITION OF NEURALLY-MEDIATED SECRETION IN PORCINE DISTAL JEJUNUM (PDJ). Keith R. Hildebrand* and David R. Brown. Dept of Vet Biol, U of Minnesota, St Paul, MN 55108

Electrical transmural stimulation (ETS) delivered to muscle-stripped PDJ mucosa mounted in Ussing chambers evokes a neurally-mediated increase in short-circuit current (Isc) attributable to active anion secretion. In this study we examined the effects of norepinephrine (NE) on the ETS-evoked increases in Isc. Serosal NE inhibited ETS-evoked increases in Isc with an EC_{50} of 23 nM and complete inhibition at 10 µM. The secretory actions of carbachol which acts directly on epithelial cells remained unaffected by NE. The α-adrenergic antagonists, phentolamine and yohimbine, at 1µM produced respective 8 and 38-fold decreases in NE potency. Propranolol and prazosin, β and α_1-adrenergic antagonists respectively, at 1 µM did not alter the NE potency. Desipramine (10 µM), a blocker of neuronal NE reuptake, enhanced NE potency by 1000-fold. In the presence of desipramine, the α_2-adrenergic agonist p-aminoclonidine was extremely potent (EC_{50} = 5 pM) in inhibiting ETS responses relative to the α_1-adrenergic agonist phenylephrine (EC_{50} = 40 nM). Dopamine and isoproterenol, a β-agonist, were weak agonists with EC_{50}'s > 10 µM. Tyramine, a releaser of endogenous NE, like NE produced a concentration-dependent inhibition of ETS-evoked responses which was antagonized by phentolamine (1 µM). These results suggest that (i) NE inhibits electrically-evoked anion secretion by acting at α_2-adrenergic receptors located on submucosal neurons, and (ii) PDJ submucosal neurons receive functional NE inputs.

164.9

MULTIPLE MECHANISMS OF CATECHOLAMINE INDUCED GLYCOGENOLYSIS IN BRAIN TISSUE: INVOLVEMENT OF α1-ADRENORECEPTORS. T.T. Quach, M. Dam*, F. Brion*, C. Rose* and A.M. Duchemin. INSERM U109, 2ter Rue d'Alesia. Paris 75014. France.

We have previously shown that noradrenaline stimulated the hydrolysis of ^{3}H-glycogen in mouse brain slices. This effect is selectively mediated by β1-receptors. However, in the liver, the glycogenolytic effect of catecholamine can be mediated either by the β-adrenergic receptor or by a typical cAMP-independant α1-adrenergic receptor. Based on these findings, we propose to examine whether similar plasticity can be observed in brain tissue. To eliminate the β1-adrenergic receptor component, adrenergic drugs selective for α receptors were used to explore the particitation of the α-adrenoreceptors. Addition of phenylephrine and methoxamine, two relatively potent α1-adrenoreceptor agonists produced a clear concentration-dependent hydrolysis of newly synthetized 3H-glycogen. Their apparent Ka values were 2.0±0.1 μM and 15±0.1 μM respectively. This effect was selectively inhibited by α1-receptor antagonists, such as WB401, prazocin and phentolamine. A selective α2-adrenoreceptor agonist, clonidine, 0.1 μM, did not produce any significant glycogenolytic response. The glycogenolytic response to α1-adrenoreceptor agonists was markedly reduced when the extracellular calcium ion was lowered to 0.3 μM. Thus, the glycogenolytic response to α1 receptor agonists could involve calcium as a second messenger as other responses mediated by α1 adrenoreceptors.

164.10

SITE-DIRECTED MUTAGENESIS OF HUMAN ALPHA$_{2A}$-ADRENERGIC RECEPTORS IDENTIFIES AMINO ACID RESIDUES INVOLVED IN LIGAND BINDING AND AGONIST ACTIVATION. Cheng-Dian Wang*, Melissa A. Buck* and Claire M. Fraser. Section on Mol. Neurobiology, LPPS, NIAAA, Rockville, MD 20852.

The functional significance of conserved aspartic acid (Asp) and serine (Ser) residues in transmembrane domains (TMD) of human α_{2A}-adrenergic receptors (α-AR) was examined by site-directed mutagenesis and permanent expression of mutant receptor genes in CHO cells. Substitution of Asp^{79} with Asn had no effect on antagonist or imidazoline agonist binding but increased α-AR affinity for ß-phenethylamine agonists. Asn^{79} mutant α-AR were unable to mediate agonist-induced changes in forskolin-stimulated cAMP levels. Substitution of Asp^{113} with Asn eliminated the ability of mutant α-AR to bind [^{3}H]-yohimbine, consistent with previous findings on the role of this Asp in ligand binding to ß-AR and muscarinic receptors. Replacement of Asp^{130} with Asn had no effect on ligand binding but altered cellular responses to epinephrine. Wild type α-AR expressed in CHO cells mediate both inhibition and potentiation of forskolin-stimulated cAMP accumulation; Asn^{130} mutant α-AR only inhibit cAMP production in response to agonist. Ser^{200} in the 5th TMD appears to be involved in interaction with the *meta*-hydroxyl group of catecholamines. These data together with our previous work indicate differences in ligand binding to α-AR and ß-AR, but suggest common mechanisms of receptor activation.

164.11

HUMAN CEREBRAL MICROVASCULAR ENDOTHELIUM ANALYSIS OF ADRENERGIC RECEPTORS LINKED TO ADENYLATE CYCLASE. F. Bacic*, S. Uematsu*, R. McCarron* and M. Spatz. LNNS, NINDS, NIH, Bethesda, MD 20892 and Johns Hopkins Hospital, Baltimore, MD.

Receptors for neurohormones coupled to quanine nucleotide regulatory protein were detected in cultured endothelial cells (EC) derived from three fractions of human cerebral microvessels (Spatz, et al., 1989). This report characterizes adrenergic receptors linked to adenylate cyclase (AC) activity in cultured EC dissociated from capillaries (CA) small [SM (< 50 μM)] and large [LM (> 50 μM)] arterioles and venules. Catecholamines and their analogues dose-dependently increased endothelial production of cAMP in all cultures. The specific antagonists for β_1/β_2 (propranolol), β_2 (butoxamine), β_1 (atenolol), α_1 (prazosin) and α_2-agonist (clonidine) dose-dependently blocked the stimulatory effect of isoproterenol. EC_{50} for isoproterenol was 5.27 x 10^{-8} M, 3.16 x 10^{-8} M and 1.9 x 10^{-9} M for EC derived from CA, SM and LM, respectively. The β_1/β_2, β_2- and β_1-type antagonists inhibited the isoproterenol AC stimulation with IC_{50} for propranolol 9.1 x 10^{-11} M (CA), 2.4 x 10^{-11} M (SM) and 1.9 x 10^{-11} M (LM); for butoxamine 1.8 x 10^{-13} M (CA), 1.1 x 10^{-11} M (SM) and for atenolol 5.8 x 10^{-11} M (CA) and 1.1 x 10^{-11} M (SM). Endothelial AC responsiveness to adrenergic agonists and antagonists is segmental in nature. Only endothelial β_2- and α_2-adrenergic receptors linked to AC activity were reported in cultured brain EC. This is the first demonstration of β_1- and α_1-adrenergic receptors coupled to AC activity in microvascular EC of human brain.

164.12

BIOCHEMICAL STUDIES OF BETA ADRENOCEPTOR FUNCTION IN VIVO IN THE RAT CEREBRAL CORTEX. E.A. Stone and S.M. John*, Dept. Psychiatry, New York University Sch. Med., New York, NY 10016.

Previous research in brain slices has shown that the cAMP response to beta receptor activation in the rat cortex is mediated primarily by beta-1 receptors and appears to occur largely in glial cells. Whether these findings hold for the brain in vivo is not known. To examine this question we have employed a newly devised method for studying beta receptor function in vivo which involves microdialysis measurement of extracellular brain cAMP. Rats implanted with microdialysis probes in the prefrontal cortex were infused with norepinephrine in the presence or absence of a beta-1 antagonist (ICI 89,406), a beta-2 antagonist (ICI 118,551) or a selective glial metabolic inhibitor, fluorocitrate (FC). It was found, in preliminary experiments, that the beta-1 blocker was more effective than the beta-2 in attenuating the cAMP response to NE. FC, however, had equivocal effects, producing a significant reduction when given alone but not when given with a NMDA receptor blocker (aminophosphonovaleric acid) to inhibit FC-induced convulsions. The results suggest that the in vitro finding of beta-1 predominance for the cAMP response in the cortex holds for in vivo conditions but the role of the glia in the in vivo response is as yet unclear. Supported in part by grants AFOSR 89-0208 and MH45265.

164.13

DIURNAL AND GLUCOCORTICOID REGULATION OF BETA ADRENERGIC RECEPTORS IN THE RAT HIPPOCAMPUS. H.M.Chao, H.Coirini, T.T.Silverstein* and B.S. McEwen. Rockefeller University, N.Y.,N.Y.10021.

In the hippocampus, which is highly sensitive to diurnal changes in serum glucocorticoids, there is a circadian rhythm to the noradrenaline stimulated cAMP accumulation that is abolished by adrenalectomy (ADX). We investigated the hypothesis that the diurnal rhythm in cAMP is mediated by glucocorticoid regulation of the β adrenergic receptors in the hippocampus. The expression of the β1 and the β2 adrenergic receptor (β1AR and β2AR) in the hippocampus was determined by receptor autoradiography (to assess receptor binding levels) and by in situ hybridization (to assess receptor mRNA levels). Animals were intact or ADX and were sacrificed at intervals throughout the light/dark cycle. There was less β2AR binding in the hippocampus than β1AR binding and this was mirrored in the expression of the respective receptor mRNAs. The levels of β2AR binding and mRNA were not affected by the time of day or by ADX. However there was a diurnal rhythm in the expression of the β1AR, both in receptor binding and in mRNA levels, that was altered by ADX. (Supported by MH41256.)

164.14

CHRONIC EXPOSURE OF RAT GLIOMA C_6 CELLS TO OXAPROTILINE REDUCES THE DENSITY OF BETA ADRENOCEPTORS. D.H. Manier*, F. Sulser. Vanderbilt Univ. Sch. of Med., Nashville, TN 37232.

The (+)-enantiomer of oxaprotiline (O) blocks the neuronal uptake of norepinephrine (NE), down-regulates the density of beta adrenoceptors (BAC) in brain and de-amplifies the NE signal while the (-)-enantiomer is devoid of these pharmacological properties. In cultured rat glioma C_6 cells (C_6), both enantiomers of O reduce the B_{max} value of BACs with equal potency without a change in K_D values. Since nonlinear regression analysis of competition binding curves revealed that the bulk of BACs in in C_6 displays high nanomolar affinity for isoproterenol (I), a deamplification of the BAC signal following both enantiomers is implied. While the down-regulation by I occurs rapidly with a significant effect at 15 minutes and a maximal effect at 2 hours, at least 48 hours are required for the enantiomers of O to elicit a significant reduction in BAC density. I still elicits the same degree of down-regulation of BACs in (-)-O treated cells as in control preparations and is blocked by co-incubation with propranolol. Though the beta antagonist seems not to affect the (-)-O induced down-regulation of BACs, drug interactions preclude an unequivocal interpretation of the data. The results with (-)-O suggest a novel BAC deamplification that is independent of an agonist receptor interaction (Supported by USPHS Grant MH-29228).

164.15

PERIPHERAL ADRENERGIC RECEPTOR FUNCTION IN DEPRESSED PATIENTS E.S. Werstiuk, M. Coote*, and M. Steiner. **Departments of Biomedical Sciences and Psychiatry, McMaster University, Hamilton, Ontario, Canada, L8N 3Z5.**

Alterations in adrenergic receptor function have been implicated in depression, and modulation of receptor sensitivity is thought to be the mechanism of action of effective antidepressant treatments. The present study was undertaken to evaluate platelet α_2- and leukocyte, β_2-adrenergic receptors in depressed patients and to assess the effect of ECT treatment on these parameters. Subjects were 13 acutely depressed patients, treated with ECT, and 18 subjects served as controls. We measured adrenergic receptors binding parameters (Bmax and Kd) in intact platelets (^{3}H-Yohimbine) and in intact leukocytes (^{125}I-Cyanopindolol) and receptor function, β-agonist stimulated, and α_2-agonist inhibited cAMP levels in leukocytes and platelets respectively. All parameters were determined pre-treatment and 14 days after the last ECT. Bmax of ^{3}H-Yohimbine binding was significantly elevated in untreated patients compared to controls ($p<0.05$). We found no patient/control differences in any other parameters measured. Treatment with ECT led to enhanced receptor-mediated cAMP levels in both platelets, and leukocytes, but this did not reach statistical significance. Binding affinity for ^{3}H-Yohimbine increased in platelets of ECT treated patients and was significantly different from those of controls ($p<0.01$). Our findings support the hypothesis that in depressed patients platelet α_2-receptor densities are elevated. The effects of ECT, however will require further clarification. **Supported by the Ontario Mental Health Foundation.**

164.16

SPECT IMAGING OF THE DOPAMINE D2 RECEPTOR IN HUMAN AND MONKEY BRAIN. M. Al-Tikriti, S.W. Woods, S. Zoghbi, J. Seibyl, R.H. Roth, D.S. Charney, G.R. Heninger, A. Alavi†, P.B. Hoffer, H.F. Kung†, and R.B. Innis. Dept. Psychiatry, VA Medical Center and Yale Univ., West Haven, CT and †University of Penn., Philadelphia, PA.

The brain uptake of the specific dopamine D2 receptor probe ^{123}I-IBZM (iodobenzamide) was studied with SPECT (Single Photon Emission Computed Tomography). A series of twenty scans were performed on 10kg female baboons (Papio anubis) injected with 2-16 mCi of ^{123}I-IBZM and five healthy human subjects with 5 mCi injection. The uptake of radioactivity measured in the Strichman 810X Brain Imager was very similar in humans and monkeys. "Specific" uptake (defined as the concentration of radioactivity overlying striatum minus that in a posterior cortical area) was concentrated in the striata, reached maximal levels in 45-90 min, and was relatively stable for the following 60-100 min. Pharmacological specificity was demonstrated in monkeys by displacement of radioactivity with haloperidol (20 µg/kg i.v.), which caused 65-90% decrease of specifically bound radioligand in 30-60 min.

HPLC analysis of arterial blood samples in humans and monkeys showed that ^{123}I-IBZM was metabolized rapidly (more than 50% within 10 min) into two less lipophilic compounds. Levels of non-protein bound ^{123}I-IBZM reached a stable concentration at the same time as the "Specific" brain uptake measured with SPECT.

Endogenous dopamine appears capable of displacing IBZM binding. D-amphetamine (1.0 mg/kg i.v.) caused 75% decrease of "Specific" uptake. The effect of d-amphetamine (but not that of haloperidol) could be blocked by pretreatment of the animal with reserpine. Quantitative ex vivo autoradiography following injection of ^{123}I-IBZM into monkeys confirmed the concentration of radioligand in caudate and putamen with low levels in globus pallidus. However, autoradiograms also showed receptor binding in substantia nigra, an area presumably too small to be seen in the SPECT image.

164.17

CO-DISTRIBUTION OF D_1 RECEPTOR mRNA AND D_1 RECEPTOR BINDING IN THE RAT BRAIN: AN IN SITU HYBRIDIZATION RECEPTOR AUTORADIOGRAPHIC ANALYSIS. A. Mansour, J.H. Meador-Woodruff, Q.Y. Zhou *, O. Civelli*, H. Akil, and S.J. Watson Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0702; Vollum Institute for Advanced Biomedical Research, The Oregon Health Sciences University, Portland, OR 97201

The recent cloning of the D_1 dopamine receptor (Zhou et al, Nature, submitted) suggests that it is structually similar to the D_2 receptor and is a member of the G-protein-coupled seven transmembrane receptor family. This study examines the co-distribution of D_1 receptor mRNA in relation to D_1 binding sites in rat brain using a combination of in situ hybridization and receptor autoradiographic techniques. Adjacent slide-mounted brain sections were examined for D_1 receptor binding using [^{3}H] SCH23390 (in the presence of 1 µM Ketanserin) and D_1 mRNA was visualized with a 530 bp riboprobe corresponding to a region between the third and sixth transmembrane domain of the D_1 receptor. D_1 receptor- mRNA and binding sites appear to be co-distributed in the caudate-putamen, nucleus accumbens, olfactory tubercle, hippocampus, and cerebellum. Regions demonstrating a lack of correspondence include the substantia nigra and suprachiasmatic nucleus where high levels of D_1 binding are observed in conjunction with no D_1 mRNA. These results suggest that the binding observed in these latter regions is localized on terminals arising from distant somata. This is supported by the finding that unilateral ibotenic lesions of the caudate-putamen produce a marked loss of D_1 receptor binding in the ipsilateral substania nigra, pars reticulata. These studies were supported by MH00818, DA02265, MH422251, and MH45614.

164.18

DISTRIBUTION OF D_1 AND D_2 DOPAMINE RECEPTOR mRNAs IN THE RAT BRAIN: AN *IN SITU* HYBRIDIZATION STUDY. J. H. Meador-Woodruff, A. Mansour, Q.Y. Zhou*, J.R. Bunzow*, O. Civelli*, and S. J. Watson Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720; Vollum Institute for Advanced Biomedical Research, The Oregon Health Sciences University, Portland, OR 97201

The dopamine receptor has been resolved into at least two distinct subtypes, D_1 and D_2. Neuroanatomical localization of these subtypes, until quite recently, has been limited to visualization of the binding sites by the use of radiolabelled pharmacological ligands. The recent cloning of the D_1 and D_2 receptors now allows the direct visualization of the mRNAs that code for these dopamine receptors. We have mapped these mRNAs in the rat brain with *in situ* hybridization, using a 530 base riboprobe complementary to rat D_1 mRNA, and a 495 base riboprobe that recognizes both $D_2\alpha$ and $D_2\beta$ mRNA. The distribution of D_1 and D_2 mRNAs was fairly similar in most traditional dopaminoceptive regions of brain, with high levels of both visualized in the caudate-putamen, nucleus accumbens, and olfactory tubercle, with distinct labelling in the septum and limbic cortex. Some regions had markedly different patterns of distribution of these mRNAs, such as in the hippocampal formation. D_2 mRNA could be visualized in dopamine-containing regions as well, most notably in the substantia nigra, ventral tegmental area, and zona incerta; D_1 mRNA was not appreciated in any of these structures, suggesting that the autoreceptors in these areas are exclusively D_2. Detailed comparisons of these mRNAs will be presented for various brain levels. This work was supported by MH00818, DA02265, MH422251, and MH45614.

164.19

CLONING AND EXPRESSION OF A HUMAN AND A RAT D1 DOPAMINE RECEPTOR. Q.Y.Zhou, D.K.Grandy, L.Thambi, J.Kushner, H.H.M.Van Tol, J.Salon, J.R.Bunzow and O.Civelli, Vollum Inst., OHSU, Portland, OR 97210.

The importance of the dopaminergic system in brain function has been emphasized by its association with neurological and psychiatric disorders such as Parkinson's disease and schizophrenia. Based on their biochemical and pharmacological characteristics, dopamine receptors are classified into D_1 and D_2 subtypes. As the most abundant dopamine receptor in the central nervous system, D_1 receptors appear to mediate some behavioral responses; modulate activity of D_2 dopamine receptors; and regulate neuron growth and differentiation. The D_2 dopamine receptor has been cloned by low stringency screening. We report here the cloning of human and rat D_1 dopamine receptors by applying an approach based on the polymerase chain reaction. The cloned human D_1 dopamine receptor has been characterized on the basis of four criteria: the deduced amino-acid sequence which reveals that it is a G-protein-coupled receptor; the tissue distribution of its messenger RNA which is compatible with that of the D_1 dopamine receptor; its pharmacological profile when transfected into COS-7 cells; and its ability to stimulate cAMP accumulation in human 293 cells.

165.1

CHOLINERGIC MODULATION OF ORAL MOVEMENTS INDUCED BY DIFFERENT CHRONIC NEUROLEPTIC ADMINISTRATION REGIMENS G. D. Ellison, A. S. Keys*, and Y. Bohn*. Department of Psychology, UCLA, 405 Hilgard Ave., Los Angeles, CA 90024

Oral movements (OMs) of rats given chronic haloperidol (HAL) pretreatment using either weekly HAL injections (HI), pillow implants of HAL (HP), or a combination of HI and HP (IP) were measured in a computerized video analysis system following acute injections of several doses of physostigmine (PHY), scopolamine (SCO), or saline. Previous findings of different behavioral syndromes induced by the two HAL regimens were replicated. PHY treatment resulted in an augmentation of OMs across all regimens, but especially in HI animals. SCO had different effects depending upon HAL regimen, with large, steep-sloped OMs increased in control & HI animals but attenuated in HP & IP animals. Surprisingly, IP rats were quite similar to the HP group and in fact showed the least severe syndrome. The results confirm the importance of drug regimen effects and the need for a multivariate approach in the study of neuroleptic induced OMs.

165.2

ACUTE SYSTEMIC ADMINISTRATION OF CHOLINERGIC AGONISTS AND ANTAGONISTS PRODUCE UNIQUE PATTERNS OF ORAL ACTIVITY IN RATS. Mary Ann Chapman and Ronald E. See, Department of Psychology, Washington State University, Pullman, WA, 99164-4820.

Cholinergic manipulation of oral movements in rodents has been extensively utilized in attempts to model various types of oral dyskinesias, including dystonia and tardive dyskinesia. Groups of female, Sprague-Dawley rats (N=6-8) were habituated to being placed in Plexiglas tubes and jaw movements monitored by a computerized video detection system. Each group was tested with a single cholinergic agonist, antagonist at 3 different doses. Drugs used (dose range in mg/kg) included the cholinergic agonists pilocarpine (.50, 1.0, 2.0), oxotremorine (.10, .50, 1.0), and physostigmine (.05, .20, .50) and the cholinergic antagonists scopolamine (.01, .05, .10) and trihexyphenidyl (.50, 1.0, 2.0). Oral movements were automatically recorded and analyzed according to amplitude and slope. Fast fourier analysis was also used to determine percentage of power at various frequencies.

Each agonist tested produced a unique profile of increased oral activity. Oxotremorine increased small-amplitude oral movements and increased the percentage of oral movements at higher frequencies (9-11 Hz). Pilocarpine increased middle-amplitude oral movements and increased the percentage of oral movements in the middle frequency range (5-9 Hz). Physostigmine increased oral movements at all amplitudes measured, and showed a shift in the frequency spectrum similar to pilocarpine. In contrast, the antagonists did not potently enhance oral activity but did produce unique changes in oral movements. Concurrent administration of scopolamine completely blocked the pilocarpine-induced oral movements. The relationship of these data to cholinergic mechanisms mediating oral activity will be discussed.

165.3

BEHAVIORAL EFFECTS OF IC ADMINISTRATION OF THE NICOTINE ANALOGUE CYTISINE IN RATS. E.J. Cline and C. Ksir, Department of Psychology,University of Wyoming, Laramie, WY, 82071.

Different investigators have suggested that the locomotor activation caused by nicotine may be mediated by mesolimbic dopamine projections. Other studies have shown that the nicotinic agonist cytisine can also produce increases in behavioral activity when infused into the VTA. Previous studies have reported sensitization to the locomotor effects of repeated nicotine injections. One purpose of this study was to determine whether nicotine pre exposure would alter the behavioral response to cytisine. VTA cannulas were implanted in 24 rats. 12 received 0.2mg/kg nicotine and 12 received saline S.C. for 7 days. On day 8 rats were tested for behavioral activity after bilateral IC infusion of 1 ul cytisine (3,10, or 30 nm/2ul) or saline. Drugs were given in a counterbalanced order over 4 days. Each rat was put in a plexiglass chamber divided into quadrants, and number of quadrant crossings was counted for 20 min. On day 12, 1/2 the rats were injected with 0.2mg nicotine and their behavior monitored. Only the 30 nm dose of cytisine significantly increased quadrant crossings. There was no effect of pretreatment with nicotine on the subsequent response to cytisine. There was, however, a significant effect of daily nicotine injections on the subsequent response to nicotine in these animals. These results show that 30nm cytisine can cause increases in locomotor activity. Results suggest that locomotor sensitization to nicotine after repeated exposure is a separate phenomenon from mesolimbic DA involvement in the acute effects of nicotine.

165.4

DRUG-INDUCED EMOTION: BEHAVIORAL VERSUS INTEROCEPTIVE EFFECTS OF NICOTINE (NIC) AND PENTYLENETETRAZOL (PTZ). C.M. Harris, Dept. Pharmacol., NY Col. Osteo. Med., Old Westbury, NY, 11568.

NIC substitutes in a systematic manner for the anxiety-like discriminative stimulus produced by PTZ (Harris et al., Psychopharmacol. 98:460-464, 1989). NIC also produces a pattern of emotional behaviors identical to that of PTZ: in 27 drug naive rats, both NIC, 0.64 mg/kg and PTZ, 20 mg/kg increased incidence of defecation (29% and 79% respectively), increased urination (45% and 62%), decreased food consumption (29% and 45%), and both failed to increase vocalization or escape attempts in response to handling. In 24 rats trained to discriminate PTZ, 20 mg/kg from saline, 1 ml/kg, in a two-lever choice task with food reward, the visceral responses to PTZ, as well as a decreased rate of lever pressing for food reward, continued with no sign of tolerance or sensitization for 70 pairs of PTZ and saline sessions, and NIC also produced these visceral and rate-suppressant effects. However, although both overt and interoceptive effects of NIC and PTZ were dose-dependent, occurrence of the overt behaviors did not reliably predict detection of the drugs' interoceptive effects.

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165.5

DOSE DEPENDENT BEHAVIORAL CHANGES INDUCED IN SELECTED MEMBERS OF A PRIMATE SOCIAL COLONY BY ACUTE ADMINISTRATION OF THE 5-HT_{1A} AGONIST 8-HYDROXY-2-(DI-N-PROPYLAMINO) TETRALIN (8-OHDPAT). R.F. Schlemmer, Jr., J.E. Young*, and J.M. Davis. Dept. of Pharmacodynamics, University of Illinois at Chicago and Research Dept., Illinois State Psychiatric Institute, Chicago, IL 60612.

This study sought to determine the behavioral effects induced in socially housed monkeys by 8-OHDPAT, a highly selective agonist at the 5-HT_{1A} receptor. The study was conducted using a stable social colony of 5 adult stumptail macaques (Macaca arctoides). Following observation of baseline colony behavior, 6 acute doses of (+)8-OHDPAT HBr, 0.005-0.30 mg(base)/kg, were administered to the 4 females of the group in a crossover design. The dominant male remained untreated. Only 2 monkeys received drug treatment per day and at least 72 hr. separated drug treatment to the same animal. Each monkey received an i.m. injection of drug or normal saline 15 min. prior to the start of each observation. Each day of the study, a 60 min. observation session was conducted by a "blind" observer who recorded the behavior of the colony from a checklist of over 40 social, solitary and drug-induced behaviors. 8-OHDPAT reduced initiated social activity, including social grooming, at doses $\geq$ 0.05 mg/kg. Although checking (visual scanning) was significantly increased, resting (eyes open) was increased and self grooming and feeding were decreased. Interestingly, 8-OHDPAT, 0.1-0.3 mg/kg, induced movement abnormalities similar to those induced by neuroleptic drugs (tremor, bradykinesia, rigidity, posturing). Conversely, 8-OHDPAT failed to induce limb jerks (myoclonus) and body shakes as do hallucinogens in this species. The results of this study implicate 5-HT_{1A} receptors in the mediation of important social and solitary behaviors in primates.

165.6

CENTRAL ADMINISTRATION OF 8-OH-DPAT OR MIDAZOLAM INTO THE ARCHISTRIATUM DIFFERENTIALLY EFFECTS RESPONDING UNDER A ANTICONFLICT PROCEDURE IN PIGEONS S.T. Ahlers & J.E. Barrett, Environmental Medicine Dept., Naval Medical Res. Inst. & Dept of Psychiatry, Uniformed Services Univ. of the Health Sciences, Bethesda, MD 20814

Key pecking of pigeons was maintained under a two-component multiple schedule in which every 30th response produced food. During one component, correlated with a red keylight, every 30th response also produced an electric shock that suppressed responding during this component. Administration of 8-OH-DPAT (0.03-0.3 mg/kg, IM), or administration of 0.1-30.0 µg 8-OH-DPAT injected into the archistriatum, increased responding in the punishment component. In general, increases in punished responding with 8-OH-DPAT injected into the archistriatum were as high as those observed with systemic administration. Higher doses (1.0-3.0 mg/kg given IM tended to decrease responding in both components whereas nonspecific decreases in response rate were not observed when 8-OH-DPAT was administered into the archistriatum. Relative to 8-OH-DPAT, the short acting benzodiazepine midazolam increased punished responding over a fairly narrow dose range from 0.1-1.0 mg/kg when given IM, and also decreased unpunished responding at higher doses. In most, but not all animals, the magnitude of increased punished responding seen with systemic administration of midazolam were comparable to those observed with 8-OH-DPAT. In contrast, administration of midazolam (0.1-10 µg/3µl) into the archistriatum produced marginal increases in punished responding but and did not systematically affect unpunished responding. These data suggest that the archistriatum may be one of several discrete structures mediating 5HT_{1A} anticonflict behavior in the pigeon. Supported by PHS Grant DA-02873.

165.7

ACUTE EFFECTS OF FENFLURAMINE AND PARA-CHLOROAMPHETAMINE ON COPULATORY PERFORMANCE OF MALE RATS. M. M. FOREMAN, R. L. LOVE* AND J. L. HALL*, The Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN, 46285.

The augmentation of serotonergic neuron activity has a variety of effects on components of sexual response. Experimental treatments that increase serotonergic receptor activity suppress the expression of sexual behavior but can also induce erection and ejaculation in the male rat. The present studies have evaluated the effects of 0.1-3 mg/kg fenfluramine and 0.01-1 mg/kg para-chloroamphetamine (pCA) on copulatory rate (CR), copulatory efficiency (CE) and ejaculatory latency (EL) of male rats of the Sprague-Dawley strain. These test compounds were dissolved in a vehicle containing 1 mM ascorbic acid and 1 mM acetic acid and given by s.c. injection 30 minutes prior to testing. Both compounds produced significant, dose related decreases in CR and CE and significant, dose-related increases in EL. The administration of 3 mg/kg fenfluramine or 1 mg/kg pCA suppressed the capacity of approximately 80 % of the rats to achieve ejaculation. In all of these responses, pCA appeared to be 3 times more potent than fenfluramine. The effects of these 5-HT releasing agents were blocked by pretreatment with LY53857, a 5-HT_2 antagonist. These data are suggestive that the augmentation of 5-HT release suppressed the sexual drive (e.g. decreased CR) and decreased the capacity to achieve erection (e.g. decreased CE) and ejaculation (e.g. increased EL) of male rats. The antagonism of the responses to fenfluramine and pCA by pretreatment with LY53857 is indicative of an involvement of 5-HT_2 receptors in the endogenous control of sexual response.

165.8

TOOTH PULP-EVOKED JAW-OPENING REFLEX IN CAT IS SUPPRESSED BY MEDETOMIDINE, AN ALPHA-2-ADRENORECEPTOR AGONIST, BUT NOT BY COCAINE. P. Kemppainen*, A. Pertovaara and T. Kauppila* (SPON: European Neuroscience Association). Dept. of Physiology, Univ. of Helsinki, Helsinki, Finland.

The effects of medetomidine, an alpha-2-adrenoreceptor agonist, and cocaine on tooth pulp-elicited jaw-opening reflex was studied in pentobarbitone-anaesthetized cat. The threshold of the jaw-opening reflex was determined by constant current pulses (duration: 2 ms) applied at a frequency of 0.25 Hz. Medetomidine elevated the threshold in a dose-dependent way (30-100 μg/kg, i.p.), and this elevation was significantly reduced by atipametzole (1 mg/kg, i.p.), an alpha-2-adrenoreceptor antagonist. The inhibitory interaction between two successive dental stimuli (in-field inhibition) was suppressed by a lower dose of medetomidine (30 μg/kg) than the threshold to single electric pulses (55 μg/kg). Only the highest dose of medetomidine (100 μg/kg) reduced significantly the temporally facilitated (in-field facilitation) response. In comparison, cocaine (1-25 mg/kg, i.p.), a non-specific monoaminergic agent, had no marked effect on the tooth pulp-evoked jaw-opening reflex. It is concluded that systemic medetomidine, through an action on alpha-2-adrenoreceptors, can suppress a predominantly nociceptive trigeminal reflex in anaesthetized cats. In-field inhibition and in-field facilitation display different sensitivities to medetomidine effects.

165.9

A DOSE-RESPONSE FOR ISOPROTERENOL AND BEHAVIORAL THERMOREGULATION IN RATS. C. Chancellor-Freeland* and H.J. Carlisle. Department of Psychology, University of California, Santa Barbara, CA 93106.

The present experiment examines the effects of pharmacological augmentation of nonshivering thermogenesis (NST) on behavioral thermoregulation in rats. Peripheral administration of the beta agonist, isoproterenol(isotonic saline, 20ug/kg, 65ug/kg, 100ug/kg) was used to stimulate NST. The effects of the different drug doses on an operantly-conditioned response were examined. Contrary to predictions, isoproterenol did not enhance NST, but rather impaired heat production in a cold environment. Behavioral heat intake increased with isoproterenol treatments, while core temperature continued to drop in a dose-dependent manner. These results suggest that the use of isoproterenol as a thermogenic agent should be reconsidered.

165.10

THE EFFECTS OF NOVELTY STRESS ON DISCRIMINATIVE CONTROL BY LOW DOSES OF CHLORDIAZEPOXIDE IN PIGEONS. P.L. Shultz and A. Tomie*. Rutgers University, New Brunswick, N.J. 08903.

To examine the effects of novelty stress on chlordiazepoxide (CDP) discrimination, 12 homing pigeons previously tested on CDP (4.0, 2.8, 2.0, 1.4, 1.0, .7 and .5mg/kg) vs. saline discrimination were given ten minutes of novelty stress followed immediately by administration of either a CDP test solution (1.4, 1.0, .7 or .5mg/kg) or saline. One hour post-injection, the pigeons were placed in a two key operant chamber and the drug discrimination procedure was initiated. Results revealed that novelty stress increased the mean percent of drug appropriate choices for all four test doses by 15-20% above levels of discriminative control observed in the non-stress condition. This effect occurred rapidly and was very durable. These results are not inconsistent with present neurophamacological findings (Paul, 1988) which show a stress-induced increase in benzodiazepine receptor number.

165.11

UNCONTROLLABLE BUT NOT CONTROLLABLE STRESS PRODUCES ENDURING ANXIETY IN RATS DESPITE ONLY TRANSIENT BENZODIAZEPINE RECEPTOR INVOLVEMENT. K. R. Short and S. F. Maier. Dept. of Psychology, Univ. of Colorado, Boulder, CO 80309.

Anxiety was measured by changes in social interaction (File & Hyde, 1978) independent of locomotion changes 24 hrs after 100 escapable or inescapable shocks or restraint. Subjects with no control over the stressor exhibited significantly higher anxiety than either non-shocked subjects or subjects that maintained control over stressor termination, which did not differ from one another. The anxiety increases could be prevented by treatment with the benzodiazepine (BZ) receptor antagonist Ro15-1788 prior to shock treatment, but could not be reversed by antagonist treatment prior to interaction testing. A similar pattern of decreased interaction could be produced by treating restrained rats with the anxiogenic BZ receptor inverse agonist FG-7142 24 hrs prior to interaction. These results are consistent with the hypothesis that anxiogenic rather than anxiolytic endogenous BZ receptor ligands (Ferrero et al., 1984) are involved in some anxious behavior. The results also provide evidence for separate receptor systems involved in the generation, maintenance, and/or expression of anxiety. BNS 8808840

165.12

CONFLICT TESTING FOR EFFECTS ON MEMORY AND ANXIETY IN AN APPROACH-AVOIDANCE TEST DEMONSTRATES SELECTIVE ADVANTAGES OF BUSPIRONE AND GEPIRONE OVER DIAZEPAM AND LORAZEPAM. Martin E. Judge, Novo Nordisk, Ferrosan CNS Division, Sydmarken 5, DK-2860 Soeborg, Denmark.

Conflict testing is widely used to demonstrate the cognitive effects of anxiolytic drugs. Anxiolytic effects are frequently inferred from increased tolerance to shock in a drinking conflict test, while a decreased latency to enter an area where shock was previously received is interpreted as indicating amnesia.

In an approach-avoidance system (Judge and Quartermain, Neuroscience Letters; 24: 313-317, 1981) using thirsty mice and a water-shock (0.33 mA) motivated two session conflict test, it was possible to demonstrate both the anxiolytic and the amnesic effects of clinically efficacious minor tranquilizers given i.p. 30 min. before the first session. There were clear differences between the benzodiazepines diazepam and lorazepam, which gave only a weak "anxiolytic" effect but potent amnesic effects, while buspirone and gepirone gave clear anxiolytic effects (100% increase in day 1 shocks) as well as a significant amnesia, but with a much greater separation of anxiolytic and amnesic effects.

165.13

LOW-DOSE INTERACTIONS OF THE IMIDAZOBENZODIAZEPINE RO 15-4513 WITH ETHANOL ON WHEEL-RUNNING BEHAVIOR IN THE RAT. M.A. Bixler, R. Hughes and M.J. Lewis Department of Psychology, Howard University, Washington, D.C. 20059.

Previous work has demonstrated that Ro 15-4513 (3.0 mg/kg, ip) pretreatment can augment ethanol-induced (0.75 g/kg, ip) suppression of wheel-running behavior in the rat (Bixler,M.A. and Lewis,M.J.,Drug Dev. Res., in press). Moreover, Ro 15-4513 administration alone suppresses spontaneous locomotor activity, as assessed via wheel-running behavior. At doses as low as 0.078 mg/kg Ro 15-4513 suppresses wheel-running in rats. In the present study, subjects were maintained on a 12:12hr light/dark cycle, and allowed 10 minute daily access to a running wheel during the dark phase. To examine its interaction with ethanol, Ro 15-4513 was administered in an ascending dose series according to an A-B-A repeated measures design. Relatively low doses of Ro 15-4513(0.078 to 0.312 mg/kg) were found to antagonize ethanol-induced suppression of wheel-running in a dose-dependent manner, and independently suppress wheel-running when administered alone. This suggests a possible biphasic action of Ro 15-4513 with respect to its antagonism of the motor-incapacitating effects of ethanol.

(Supported in part by NIAAA grants AA06263 and RR08016)

165.14

DRUG DISCRIMINATION IN BABOONS TRAINED TO DISCRIMINATE ß-CARBOLINE-3-CARBOXYLIC ACID ETHYL ESTER (ß-CCE) OR PENTYLENETETRAZOLE (PTZ) N.A. Ator and R.R. Griffiths* Johns Hopkins Univ. Sch. of Med. Baltimore, MD 21205.

ß-CCE and PTZ both act through the benzodiazepine/γ-aminobutyric-acid (BZ/GABA) receptor complex but through different sites: ß-CCE through the BZ receptor and PTZ through the picrotoxinin site. Both have subjective effects characterized as anxiogenic in humans. Baboons were trained to discriminate either ß-CCE (0.18 mg/kg i.m.) or PTZ (10 mg/kg i.m.) from the no-drug condition. Both discriminations were acquired relatively slowly compared to such training with sedative/anxiolytics in this species. In previous work the PTZ response was occasioned by ß-CCE and by the novel anxiolytic buspirone (a drug having little effect in the BZ/GABA complex). In the present study the ß-CCE response was occasioned by PTZ and buspirone suggesting overlap in the stimulus generalization profiles of PTZ and ß-CCE. Yohimbine, a drug with high α-2 adrenergic activity, has been reported to have anxiogenic effects but did not occasion drug lever responding reliably in either training group. The discriminative stimulus effects of ß-CCE but not PTZ were blocked by the BZ-receptor antagonist flumazenil (flumazenil alone, up to 18 mg/kg p.o., had no effect). Neither phenytoin nor valproic acid blocked the PTZ discriminative stimulus. To date neither a putative anxiogenic effect nor a particular molecular mechanism appears to account for commonalities in discriminative stimulus effects of PTZ and ß-CCE. (NIDA Grant DA04133)

165.15

TOLERANCE AND BEHAVIOURAL DEPENDENCE STUDIES IN RATS WITH BUSPIRONE AND IPSAPIRONE. A.J.GOUDIE and M.J. LEATHLEY*. Psychology Dept., Liverpool Univ., Liverpool, L69 3BX, U.K.

Chronic effects of buspirone (4 mg/kg. b.i.d) and ipsapirone (15 mg/kg b.i.d), and of withdrawal, were studied in rats run for 2 operant sessions/day on a DRL 15 sec schedule. When administered pre-session, both drugs suppressed responding by c. 50%. During 22 days of treatment (b.i.d) full tolerance developed to both drugs. On withdrawal, subjects treated pre-session with buspirone and ipsapirone showed significant (c. 50%) increases in response rates, which were highest on the first withdrawal day and then decreased to baseline over 4 further days. However, subjects which had always received ipsapirone (15 mg/kg b.i.d) after operant sessions showed no withdrawal-induced increases in responding. The withdrawal effects observed cannot have been due to physical dependence, as they were only seen in subjects receiving chronic pre-session treatment. The withdrawal effects recorded were due presumably to state-dependent learning induced by chronic pre-session administration of behaviourally disruptive drug doses. These data indicate that tolerance develops to effects of buspirone and ipsapirone on DRL responding when the drugs are administered at high doses twice daily. They also suggest that state dependent learning may be of critical importance in "behavioural dependence" procedures when such procedures are used to assess withdrawal effects.

165.16

RO 15-1788 REVERSES PHARMACOLOGIC TOLERANCE, BUT NOT CONTINGENT TOLERANCE, TO DIAZEPAM'S ANTICONVULSANT EFFECT. Michael J. Mana and John P.J. Pinel. *Dept. of Psychology, Univ. of British Columbia, Vancouver, B.C., Canada. V6T 1Y7.*

The development of tolerance to the anticonvulsant effect of diazepam (DZP; 2 mg/kg, IP) administered once every 48 hr for 20 days is contingent upon amygdaloid kindled rats receiving a convulsive stimulation 1 hr after each drug injection; accordingly, we refer to this as contingent tolerance. Tolerance to DZP's anticonvulsant effect also develops when the same dose is administered every 8 hr over a 10-day period, even though the rats are not stimulated during this time; we refer to this as pharmacologic tolerance because its development is a function of drug exposure. The present experiment examined the effect of the benzodiazepine antagonist RO 15-1788 (Flumazenil) on the retention of contingent and pharmacologic tolerance to DZP's anticonvulsant effect. On the tolerance-test trial that followed each tolerance-development regimen, when each rat received DZP 1 hr prior to a stimulation, the mean forelimb clonus duration (FLC) was not significantly different for the two groups (42 sec for the Contingent-Tolerance Group and 51 sec for the Pharmacologic-Tolerance Group). The rats from both groups were administered RO 15-1788 (5 mg/kg, IP) 24 hr later. When these rats were retested 24 hr later after the RO 15-1788 injection, there was a signficant loss of tolerance in the Pharmacologic-Tolerance Group (FLC = 24.6 sec) but not in the Contingent-Tolerance Group (FLC = 43.8 sec). RO 15-1788 did not affect the convulsions of nontolerant control rats. These data extend an earlier report that RO 15-1788 reverses pharmacologic tolerance to DZP's anticonvulsant effect, presumably by restoring the sensitivity of GABA-A receptors associated with benzodiazepine receptors (Gallager & Gonsalves, IPET, 244: 79-83, 1988), and suggest that contingent and pharmacologic tolerance to DZP's anticonvulsant effect are both phenomenologically and physiologically distinct. ****Supported by an MRC of Canada postgraduate fellowship to MJM, a BCHS Grant to JPJP, and by a donation of RO 15-1788 from Hoffman-LaRoche Canada****

165.17

SHIFTS IN NONDRUG BASELINE FOLLOWING ACQUISITION OF DIAZEPAM-PENTYLENETETRAZOL DISCRIMINATION REFLECT ADAPTIVE PROCESSES. R.L. Smith and R.J. Barrett*. Dept of Psychology, Vanderbilt University and VA Medical Center, Nashville, TN 37203

Rats were trained to discriminate diazepam (DZ) (0.3 mg/kg) from pentylenetetrazol (PTZ) (10-20 mg/kg). Training doses of the drugs were adjusted such that when animals were tested on saline 48 h after the last acquisition session they made 50% of their responses on each lever. Similar tests at subsequent intervals (7, 27 and 90 days) indicated a drift in the nondrug baseline from 50% DZ lever choice to 48%, 34% and 22% for the 7, 27 and 90 day intervals, respectively. If the drift in the nondrug baseline reflects the gradual dissipation of adaptive changes induced by chronic injection of the training drugs, then it should be possible to reinstate the original nondrug baseline (50%) by simply administering chronic injections of the training drugs. Animals were assigned to one of three groups and received either DZ-PTZ discrimination training, DZ-PTZ injections but no training, or saline injections for 14 days. Tests 48 h after the final treatment indicated that in addition to retraining, injections alone were effective in reinstating the original nondrug baseline. These results support the explanation that the instability in the nondrug baseline reflects the gradual dissipation of adaptive processes.

165.18

DIAZEPAM-INDUCED AMNESIA IN A DELAYED NONMATCHING-TO-SAMPLE TASK MAY BE THE RESULT OF SEDATION RATHER THAN MEMORY LOSS. L.E. Kalynchuk* and C.H.M. Beck. Psychology, University of Alberta, Edmonton, AB, Canada T6G 2E9.

Acute administration of benzodiazepines to rats impairs acquisition of avoidance tasks. It is not clear whether this represents an amnesic effect generalizable in a delay-dependent fashion to nonaversive tasks, nor whether the effect would be observable in chronically treated animals. To explore these issues we examined the effects of diazepam (2.0 mg/kg,ip) administered acutely or chronically (x6) on the performance of rats on a food-reinforced delayed nonmatching-to-sample task (DNMTS) with nonrepeating objects. Compared to saline controls, acutely treated rats made more errors but this deficit was delay-independent. Chronically treated rats did not differ from controls in the number of errors but did use a different strategy. Whereas control animals ran to the test object, examined it, switched to the other object, and displaced the object, the chronic rats paused in front of the objects, looked at both, moved to one and displaced it. These rats were generally less active than the control rats. Mobility was reduced even further in acutely treated rats and errors were related to a tendency to proceed directly to the nearest object and to increasing immobility throughout the session. In conclusion, diazepam-induced impairment in performance of DNMTS may be a consequence of sedation rather than amnesia.

165.19

REPEATED ADMINISTRATION OF DIAZEPAM BUT NOT OF CLONAZEPAM REDUCES 3H-RO 5-4864 BINDING IN THE MITOCHONDRIAL FRACTION OF RAT CEREBRAL CORTEX.

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We previously reported that rapid tolerance to the EEG synchronization and behavioral sedation can occur after large doses of diazepam (DIAZ) but not of clonazepam (CLN). The effect of DIAZ is abolished when the drug is coadministered on a chronic basis with PK 11195. The effects of CLN undergo to tolerance when the drug is coadministered on a chronic basis with Ro 5-4864 (Ph Bioch Beh 35,933,1990). These data suggest that the peripheral benzodiazepine (BZ) receptor can modulate the occurrence of rapid tolerance to the sedative effects of BZs.

To verify this possibility, 3H-Ro 5-4864 binding was determined in the mitochondrial fraction of cerebral cortex of rats treated for 5 days (once a day) with DIAZ (10 mg/kg iv), CLN (2.5 mg/kg iv) or Ro 5-4864 (4 mg/kg iv). In naive rats, Kd and Bmax of 3H-Ro 5-4864 were 3.2 nM and 0.82 pmol/mg prot, respectively. No change was observed after single injection of all these drugs. After 5 days of treatment, a decrease of the Bmax was obtained in rats receiving DIAZ (-48%) and Ro 5-4864 (-74%), but not CLN or vehicle.

These data are consistent with the hypothesis that a down regulation of peripheral BZ receptor may play an important role in the development of rapid tolerance to the sedative effect of BZs.

165.20

ISOLATION SUPRESSES BOTH IMMUNE AND NEUROENDOCRINE FUNCTIONING AND DECREASES THE LOCOMOTOR ACTIVITY OF NORMAL AND IMMUNOSUPPRESSED C3H/HeN MICE.

D.L. Collins, S.J. Cohen*, D.E. Gruber*, M.M. D'Alesandro*, and D.A. Mickley. Armed Forces Radiobiology Research Institute, Bethesda, MD 20814-5145.

Environmental variables can dramatically influence behavioral, neuroendocrine, and immune system parameters. We examined the effects of housing on locomotion, circulating lymphocytes, and catecholamines (i.e., Norepinephrine [NE] of 12-week-old female C3H/HeN mice. Mice (10/group) were either immunocompromised (following 0.5 or 4.5 Gy ^{60}Co irradiation) or normal (sham-irradiated) and were housed in isolation or groups (10/cage) for 25 days following radiation exposure. Individual spontaneous locomotion measures and blood samples were obtained 25 days following the radiation and housing manipulations. Blood samples showed that the catecholamines of isolated mice were more divergent (i.e., NE=73% and EPI=81%) from normal values than were the catecholamines of group-housed mice (i.e., NE=38% and EPI=62%). Across doses, mice housed in isolation had 50% less circulating EPI and 43% less circulating NE than did group-housed mice. This study suggests that the regulatory processes of the neuroendocrine system are influenced not only by exposure to immunosuppressive radiation but also by environmental factors, such as different housing conditions.

PEPTIDES: BIOSYNTHESIS AND METABOLISM I

166.1

EFFECT OF COLCHICINE ON RAT HYPOTHALAMIC VASOPRESSIN, OXYTOCIN AND SOMATOSTATIN BIOSYNTHESIS IN VIVO.

B.Liu*, R.P.S.Kwok, and J.D.Fernstrom. Neuroendocrine Program, Dept. Psychiatry, Univ. of Pittsburgh, Pittsburgh PA 15213.

Colchicine (COL) blocks axonal transport and causes peptides to accumulate in their neuronal cell bodies of origin. This accumulation has recently been used to estimate vasopressin (AVP) synthesis rate in hypothalamus (*Am.J.Physiol. 257* :R109,1989). We have examined this method for possible use in estimating somatostatin (SRIF) synthesis as well. Groups of rats received COL (8 μg, third ventricle), and were killed 10 or 24 hr later. Whole hypothalamus, or hypothalamic regions and posterior pituitary were assayed for immunoreactive (IR) AVP and/or SRIF. COL treatment clearly increased IR-AVP levels at 10 and 24 hr, in whole hypothalamus and supraoptic and para/periventricular nuclei, as previously shown. However, *no* changes were noted in IR-SRIF content, in whole hypothalamus, para/periventricular nucleus or median eminence. Synthesis of AVP and SRIF peptides (SRIF-14 and SRIF-28) was also estimated *in vivo* in hypothalamus 4 hr after third ventricular ^{35}S-cysteine injection, administered 6hr and 20 hr after COL. ^{35}S-AVP, ^{35}S-SRIF-14 and ^{35}S-SRIF28 were isolated on HPLC; the accumulation of label in each peptide 6-10 hr after COL was increased over control, while at 20-24 hr, peptide labeling was clearly reduced. At this latter timepoint, no alteration in the mRNA level for proSRIF or proAVP was apparent. The results suggest that COL treatment coupled with IR-SRIF measurements is *not* a useful method for estimating SRIF synthesis in hypothalamus. In addition, they show that COL can suppress SRIF and AVP labeling within 24 hr, indicating either that the build-up of peptide in the cell body ultimately inhibits synthesis, or that COL lowers synthesis by some other mechanism. The effect is presumed to be post-transcriptional, since the mRNA levels were unaltered. Because of the COL-induced changes in labeled AVP synthesis, care must be taken in the use of the COL method for estimatling AVP synthesis.

166.2

LOCALIZATION OF OXYTOCIN mRNA IN AXONS OF THE HYPOTHALAMO-NEUROHYPOPHYSIAL TRACT.

G.F. Jirikowski, P.P. Sanna and F.E. Bloom. Dept. of Neuropharm. Scripps Clinic and Res. Fnd., La Jolla, CA 92037

With in situ hybridization, using 5'bromo-2'deoxyuridine (BRDU)-labelled oligoprobes complementary to oxytocin (OT) mRNA, we found hybridization in some of the Herring bodies in the median eminence and the posterior lobe of 6 days lactating rats. In lactating rats, whose pups had been removed 5 hours before sacrifice, we observed hybridization in axons in the lateral hypothalamus and increased hybridization in both the median eminence and the posterior lobe, while hybridization in the magnocellular perikarya seemed to be reduced. With electron microscopy we could demonstrate that the axonal hybridization signal was present in secretory vesicles. Several hypothetical explanations are under functional evaluation: The shift of mRNA into the axonal compartment could represent a mechanism for rapid downregulation of synthetic activity. Since neurons are generally thought to have low RNAase activity, it also seems possible that neurons are capable of reducing mRNA levels by terminal release. RNA might also be stored in axons and transported retrogradely to reinitiate translation upon certain stimuli without de novo transcripiton. Preliminary experiments seem to provide evidence that certain target neurons can take up mRNA to translate the respective message, thus representing an novel principle of interneuronal communcation.

166.3

MODULATION OF STRIATAL PREPROENKEPHALIN MRNA LEVELS BY SCOPOLAMINE.

A.E. Pollack and G.F. Wooten. Dept. of Neurology, University of Virginia, Charlottesville, Va. 22908.

Eight days post lesion (dpl) preproenkephalin (PPE) mRNA levels increased 2.5-fold in the striatum ipsilateral to a 6-hydroxydopamine (6-OHDA) lesion of the substantia nigra pars compacta. Administration of the D2 agonist, quinpirole, or the D1 agonist, SKF 38393, for 7 d following a 6-OHDA lesion attenuated the increase in striatal PPE mRNA by 30% and 15% respectively as compared to the ipsilateral striatum of 8 dpl control rats (Pollack and Wooten, Soc. Neurosci. Abst., 15:368, 1989). In striatal slices, application of a D2 dopamine agonist decreased the K^+-induced release of acetylcholine (Stoof et al., Eur. J. Pharm., 84:211, 1982) suggesting that dopamine agonists could regulate striatal PPE mRNA levels indirectly via effects on cholinergic interneurons. In this study we examined the effects of scopolamine (a muscarinic cholinergic antagonist) treatment on striatal PPE mRNA following a 6-OHDA lesion. Rats received a unilateral stereotaxic injection of 6-OHDA (2 μl, 4 mg/ml) and their striata were processed separately for RNA extraction. PPE mRNA of the ipsi- and contralateral striata were compared by dot blot hybridization using a ^{32}P-labeled 30mer oligonucleotide probe complementary to PPE mRNA. Administration of scopolamine (10 mg/kg/3xd, s.c.) to intact rats for 14 d resulted in a 25% reduction of striatal PPE mRNA compared to control animals ($p<0.01$), whereas a 7 d course of treatment was without effect. Administration of scopolamine (10 and 50 mg/kg/3xd, s.c.) for 7 d following a 6-OHDA lesion attenuated the increase of PPE mRNA in the striatum ipsilateral to a nigral lesion by 40% ($p<0.05$). In contrast, scopolamine administration did not alter PPE mRNA in the contralateral striatum. Thus, dopamine may, at least in part, regulate PPE mRNA expression via effects on striatal cholinergic interneurons.

166.4

EXPRESSION AND PROCESSING OF PRO-OPIOMELANOCORTIN IN A NEURONAL CELL LINE.

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We examined the expression and processing of monkey pro-opiomelanocortin (POMC) following transfection into a mouse neuronal cell line, Neuro 2A (N2A). Two POMC vectors were compared for their level of expression and pattern of peptide processing: one which employed the Zn^{2+}-inducible metallothionein promoter (Mtneo-POMC) and another which employed the cytomegalovirus immediate early gene promoter (CMV-POMC). It was found that POMC expression, as assessed by total β-Endorphin levels, was markedly higher in cells transfected with CMV-POMC than with Mtneo-POMC. Molecular sieving of cells transfected with either vector revealed that the precursor was processed at the correct sites to give β-Lipotropin and β-Endorphin. While both cell lines produced ACTH and α-melanocyte-stimulating hormone (α-MSH, ie. $ACTH_{1-13}$), the levels of fully processed peptides were much lower than those found in tissues which normally produce POMC, such as the pituitary.

We also investigated the structural parameters dictating processing of the mid-ACTH cleavage site (sequence; -lys-lys-arg-arg-). A series of mutants of this region were subcloned into the Mtneo vector, transfected into N2A cells, and α-MSH production measured. Preliminary data suggest that one of the mutant vectors (cleavage site sequence; -lys-lys-arg-gly-) produces higher levels of α-MSH in N2A cells than the wild-type vector. This implies that the arg in position 4 of the wild-type cleavage site sequence may prevent cleavage by the N2A protease. (Supported by NIDA DA02265 and the Lucille P. Markey Charitable Trust).

166.5

DECREASED EXTRACELLULAR CONCENTRATION OF GLUTAMATE AND ELEVATED TISSUE CONTENT OF DYNORPHIN IN THE HIPPOCAMPUS OF AGED RATS. W. Zhang, L. Thai, W. Mundy, M. Gallagher, H. Tilson and J. S. Hong. LMIN, NIEHS/NIH, Research Triangle Park, NC and Dept. of Psychology, Univ. of North Carolina, Chapel Hill, NC.

The purpose of this study was to examine the effects of aging on extracellular glutamate and tissue dynorphin A(1-8)(DYN) contents in the hippocampal formation of Fischer-344 rats. Data collected for glutamate using an *in vivo* microdialysis perfusion method were compared to DYN tissue content in the same brains. The present investigation showed a selective reduction (40%) of extracellular, but not tissue, concentration of glutamate in aged (24 mo) rats compared to young (3 mo) rats. Furthermore, the decrease in extracellular glutamate was inversely correlated with an elevated hippocampal content of DYN. This study extends the earlier finding of elevated dynorphin in the aged hippocampus to another rat strain. In addition, the results suggest that the dysregulation of dynorphin occurs in conjunction with reduced excitatory transmission within the hippocampal formation. This interpretation is also consistent with our observation that glutamate antagonist administration prevents a decrease in hippocampal dynorphin content induced by perforant path stimulation in the young adult rat. (Supported by grants MH39180 and BNS-8719881.)

166.6

STORAGE AND PROCESSING OF PROENKEPHALIN IN ADRENAL CHROMAFFIN CELLS. S.P. Wilson. Dept. of Pharmacology, Univ. of South Carolina Sch. of Med., Columbia, SC 29208

The relationship between proenkephalin (PE) storage and processing was examined in primary cultures of bovine adrenal medullary chromaffin cells (purity >95%). Cultures were labeled with [^{35}S]methionine with or without prior exposure to secretagogues to enhance PE synthesis. A crude chromaffin vesicle (CV) fraction obtained by differential centrifugation of cell homogenates was subjected to density gradient centrifugation. [^{35}S]Met^5-enkephalin sequences in gradient fractions were quantitated following digestion with trypsin + carboxypeptidase B and reversed-phase high performance liquid chromatography (RPHPLC). Within 1 h after labeling all PE-derived peptides were incorporated into structures with properties of newly synthesized CV. These vesicles required 9-12 h to achieve the buoyant density of mature CV. The processing of PE was examined in whole cell extracts by first separating the major PE-derived peptides by RPHPLC with subsequent analysis of [^{35}S]Met^5-enkephalin content. Maximum formation of free pentapeptide occurred within 2-4 h after labeling, with a corresponding loss of [^{35}S]Met^5-enkephalin from PE-derived peptides of ≥15 kD. No appreciable processing of other PE-derived peptides, including species of 3-10 kD, was observed over 24 h. The results show that PE is rapidly packaged into CV and that PE processing is complete before CV maturation. These studies also suggest that many of the PE-derived peptides found in the adrenal medulla are final secretory products. This research was supported by NSF grant BNS-8719149.

166.7

PROTEOLYTIC ACTIVITY IN BOVINE ADRENAL CHROMAFFIN GRANULES USING [^{35}S]-METHIONINE LABELLED PROENKEPHALIN COPOLYMERIZED INTO SDS-PAGE. S.F. Roberts*, J.W. Irvine*, and I. Lindberg. Dept. of Biochem. and Mol. Biol. LSU Medical Center, 1901 Perdido St., New Orleans, LA 70112.

A novel method was used to characterize proenkephalin cleaving activities in subcellular fractions. Purified [^{35}S]-methionine labelled recombinant proenkephalin was added to SDS polyacrylamide gels prior to polymerization. Samples were applied to polymerized gels which were run on ice, washed with 2.5% Triton X-100 to remove SDS, and then incubated at 37 C to allow renatured enzymes to cleave *in situ* proenkephalin into diffusible peptides. Enzymatic activity was revealed as clear bands against a dark background by autoradiography of dried gels.

A 1 M NaCl extract of membranes pelleted from freeze/thawed crude (P2) chromaffin granules contained two dominant bands of activity at pH 8.2, a 75 kDa and a calcium-dependent 60 kDa. A 30 kDa band could be discerned as a minor component.

Sucrose gradient fractionation of P2 granules assayed at pH 8.2 showed the 75 kDa enzyme in granule and lysosomal fractions while the 60 kDa enzyme was concentrated in the lysosomal and mitochondrial fractions. Neither of these bands were inhibited by DFP or E-64. At pH 5.0 a 66 kDa band was evident that is possibly lysosomal. A 30 kDa activity may be granule-associated but is at the limit of detection. Preliminary results also indicate that a 46 kDa enzyme may be associated with both chromaffin granules and anterior pituitary secretory granules.

166.8

[LEU]ENKEPHALIN HYDROLYSIS IN CRUDE MEMBRANE FRACTIONS OF CHICK BRAIN. G. Schulteis[1], S. Shibanoki*[2], D. Beniston*[1], K. Ishikawa*[2], E.L. Bennett[1], M.R. Rosenzweig[1], & J.L. Martinez, Jr.[1]. [1]Dept. Psych., Univ. Calif., Berkeley, CA, 94720, & [2]Dept. Pharm., Sch. Med., Nihon Univ., Tokyo, Japan.

Hydrolysis of [leu]enkephalin (LE) in crude (P2) membrane fractions of selected chick brain regions was examined using HPLC/electrochemical detection. LE (32.4 µM) was added to P2 from ventral (V) or dorsal (D) forebrain, tectum (T), brainstem (B), or cerebellum (C). The only detectable products of LE hydrolysis were free Tyr and Tyr-Gly-Gly (YGG). Tyr formation did not differ markedly among brain regions; differences in YGG formation were noted, however, with V > T > D=B > C. A somewhat similar profile was noted for LE concentrations in these regions (Bennett et al., this volume). In P2 of whole forebrain, bestatin (IC50=2µM) and puromycin (IC50=6µM), aminopeptidase inhibitors, inhibited Tyr formation; in combination with earlier reports of bestatin and puromycin affinity for aminopeptidases M and MII (Molec Pharmacol, 29:281, 1986), our data suggest that MII activity is predominant in chick forebrain. YGG formation was potently inhibited by captopril (IC50=0.003µM), an angiotensin converting enzyme (ACE) inhibitor, but poorly inhibited by thiorphan (IC50=0.6µM), an 'enkephalinase' inhibitor, suggesting that ACE activity accounts for most YGG formation in chick forebrain. This contrasts with data from rat forebrain or striatum, in which thiorphan is considerably more potent than captopril. (Supported by PHS grants DA04795, DA04195, DA05334, from NIDA).

166.9

DETERMINATION OF THE DISTRIBUTION OF LEUCINE-ENKEPHALIN IN BRAIN REGIONS OF THE TWO-DAY-OLD CHICK BY RADIOIMMUNOASSAY. E.L. Bennett, P.J. Colombo, J.Y. Sun*, G. Schulteis, M.R. Rosenzweig, and J.L. Martinez Jr. Dept. of Psychology, Univ. of California, Berkeley, CA 94720

The brain of the two-day-old chick was dissected into the following regions: the cerebellum, optic tectum, brain stem, and dorsal and ventral aspects of the forebrain. The forebrain was dissected at the level of the lamina medullaris dorsalis such that the dorsal portion contained the visual wulst, neostriatum, and ectostriatum. The ventral forebrain contained the lobus parolfactorius and paleostriatum augmentatum. Tissue samples were immediately homogenized and prepared for radioimmunoassay of leucine-enkephalin (LE). Preliminary values of pmol LE-like immunoreactivity/mg protein in the regions tested, based on 13 individual samples, were as follows: cerebellum = 0.042 ± 0.033, dorsal forebrain = 0.437 ± 0.188, optic tectum = 1.234 ± 0.290, brain stem = 2.699 ± 0.916, and ventral forebrain = 4.836 ± 1.169.

Supported by PHS grants DA04795 and DA05334 from NIDA.

166.10

RECIPROCAL EFFECTS OF CENTRAL AND PERIPHERAL AXOTOMY ON CALCITONIN GENE-RELATED PEPTIDE CONTENTS IN RAT DORSAL ROOT GANGLION CELLS. Y. Kashihara*, Y. Inaishi* and M. Sakaguchi* and M. Kuno. National Institute for Physiol. Sciences, Okazaki 444, Japan.

The synthesis of calcitonin gene-related peptide (CGRP) in cultures of adult rat dorsal root ganglion (DRG) cells is known to be up-regulated by nerve growth factor (NGF; Lindsay et al., Neurosci. 33:53, 1989). We measured the CGRP content in the rat lumbar DRG *in vivo* by a two-site enzyme immunoassay 9 days after rhizotomy or peripheral nerve section. Section of the sciatic nerve (peripheral axotomy) on one side reduced slightly (by 15%) but significantly the CGRP content in the lumbar DRG compared with the control side. In contrast, section of the lumbar dorsal roots (central axotomy) caused a 2·7-fold increase in the CGRP content of the lumbar DRG. In order to test the possible effects of cut motor fibers associated with section of the sciatic nerve, the lumbar ventral roots were transected. Unexpectedly, this procedure caused a 1·7-fold increase in the CGRP content of the lumbar DRG. This was not attributed to section of ventral root afferent fibers since section of the 4th lumbar ventral root increased the CGRP content in the 5th lumbar DRG as well as in the 4th lumbar DRG. It is concluded that, in addition to NGF, at least two other factors are involved in regulation of the synthesis of CGRP in sensory neurons.

166.11

Somatostatin mRNA during development of the Rat Retina

DM Ferriero, VA Head*, RH Edwards, SM Sagar

We have previously shown marked developmental regulation of somatostatin(SS) concentrations during retinal development. SS appears as early as embryonic day 16(E16) in high quantities and decreases to barely detectable levels at an early postnatal age, gradually returning to adult levels after eye opening. To determine whether these variations in retinal SS content reflect variations in synthesis, we determined SS mRNA levels using Northern blot hybridization as a measure of the regulation of SS transcription accumulation. SS RNA probe was prepared from a SS cDNA clone in SP65. When relative SS mRNA hybridizing signal is plotted as a function of age, the pattern of gene expression in the developing retina paralleled the peptide concentration throughout development, both showing higher concentrations prenatally than postnatally. There appears to be only one species of SS mRNA present throughout development. These data suggest that there is control of SS peptide expression throughout development at the level of SS mRNA; the early appearance of message and peptide suggest a role for SS in the developing retina.

166.12

CORRELATION OF ANGIOTENSIN II BINDING AND METABOLISM IN RAT BRAINS.

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We are using the micropunch technique to investigate the correlation of angiotensin II (AII) binding and metabolism of AII to angiotensin III in Wistar-Kyoto (WKY) and Sprague-Dawley (SD) rats. Using a 1-2 mm thick midsagittal slice, we are punching out 5 areas known to bind AII (nucleus of the solitary tract, paraventricular nucleus, subfornical organ, suprachiasmatic nucleus and the cortex of the superior colliculi) and 3 areas as negative controls (the frontal region of the cortex, the posterior region of the cerebellum, and the ventromedial nucleus of the hypothalamus). We are also including the adrenal cortex as a positive control for binding. Tissue punches from several animals are pooled and divided into aliquots for binding and metabolism. Binding studies are a single point determination using ^{125}I (Sarcosine^{1})(Isoleucine^{5}) AII to quantitate binding. Metabolism studies are done with ^{125}I-AII, and use HPLC to separate ^{125}I-AII and its metabolites. Detection and quantification are done with radioligand detection. Preliminary binding data agrees with the distribution of AII receptors in brain regions.

166.13

VASOPRESSIN AND ANGIOTENSIN II DISAPPEAR AND REAPPEAR IN POST-MITOTIC SOLITARY HYPOTHALAMIC CELLS OF THE HOMOZYGOUS BRATTLEBORO RAT.

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The mutation of the homozygous Brattleboro rat is due to a single base deletion in exon B resulting in a completely different C-terminus of the vasopressin (VP) precursor. This altered precursor cannot be transported from the endoplasmic reticulum towards the Golgi apparatus and is therefore not packaged in granules. For this reason the axonal transport of VP granules is blocked. The expression in VP cells of two co-existing peptides, Angiotensin II (Ang II) and dynorphin (DYN), is, respectively, totally disturbed and not affected.

Paradoxically, a small number of solitary hypothalamic neurons of di/di rats synthesize the wild-type VP precursor (i.e. VP, neurophysin and glycopeptide) (Van Leeuwen et al., PNAS, 86:6417, 1989). During life an increasing number of these somatic post-mitotic neurons (up to 3% of the VP cells) undergoes a switch to a genuine heterozygous phenotype (i.e. exhibiting both the wild-type and mutated VP precursors). Here we report the coexistence of Ang II and glycopeptide in these heterozygous cells indicating that for the expression of Ang II a normal VP precursor is necessary. It is hypothesized that in the Golgi apparatus the various neuropeptides present in VP cells, and in the same granule, are packaged in the following order: first DYN, followed by VP and Ang II. Alternatively, protein sorting in the trans-Golgi network is possible.

166.14

METABOLISM OF CCK-8 AND ITS ANALOGUE BY BRAIN SLICES OF FRONTAL CORTEX AND HIPPOCAMPUS.

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Cholecystokinin-8 (CCK-8) is widely distributed throughout the brain and is involved in the neural control of several biological processes. The role which CCK-8 plays is linked to the mechanisms which regulate biosynthesis and metabolism. Brain slices from frontal cortex and hippocampus, rich in CCK-8, were incubated with CCK-8 or an analogue of CCK-8, SNF-8702 (Asp-Tyr-[N-Me]Nle-Gly-Trp-[N-Me]Nle-Asp-Phe-NH2), to study extracellular metabolism by membrane bound peptidases. Rats were ether anesthetized and perfused with cold buffer. Brains were removed and placed into a rat brain matrix. A 2 mm coronal slab was taken at Bregma 4.20 - 2.20 mm and 2 mm punches were taken from frontal cortex. A 2 mm coronal slab was taken at Bregma -4.16 mm - -6.30 mm and punches were taken from the CA3 region. Brain slices (240 μm) were washed in O_2/CO_2 (95:5%) buffer at 4°C to remove soluble enzymes. 4 Slices per tube were time-course incubated with 50 μM CCK-8 or SNF-8702 at 37.5°C. CCK-8 was metabolized at a high rate with a half-life of 60 min for both cortex and CA3. Fragments were identified as formed by several proteases. SNF-8702 was stable to metabolism with a $t_{1/2}$ of > 400 min. This peptidase resistant, biologically active analogue, containing modifications of Met^{3} and Met^{6} was not recognized by serine proteases or metalloendopeptidase 3.4.24.15. Neutral endopeptidase-24.11 cleavage at Gly^{4}-Trp^{5} and Asp^{7}-Phe^{8} appears to be inhibited by [N-Me]Nle modifications at the 3 and 6 positions of CCK-8. These data provide information concerning structural requirements for CCK-8 metabolism by specific proteases. (Supp. by NIH DA 06284, DK 36289 and MH 42600)

166.15

PARTIAL PURIFICATION OF A CCK CLEAVING ENDOPROTEASE FROM RAT BRAIN SYNAPTOSOMES.

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An endoproteolytic activity which specifically cleaves CCK-33 to produce CCK-8 has been partially purified from a rat brain synaptosome preparation. This activity was assayed by incubating with substrate and separating the product on DEAE Sephadex ion exchanger. The assay product co-eluted with authentic CCK-8 on reverse phase HPLC. Both soluble and membrane bound activities were present in synaptosomes. A two step purification of the soluble activity involved anion exchange chromatography over DEAE Sephacel, followed by gel filtration on a G-150 Sephadex column. These steps resulted in a 320 fold enrichment of activity relative to the original brain homogenate. Because the CCK-33 cleaving activity of the crude preparation was marginally inhibited by aprotinin (25 μg/ml), kallikrein activity as assayed by the fluorogenic substrate Pro-Phe-Arg-AMC was followed through the purification. The two activities were separated by the procedure. The CCK-33 cleaving activity was not inhibited by PMSF (5 mM), soybean trypsin inhibiter (200 μg/ml), pCMB (5 mM) or EDTA (2mM). The activity would not cleave CCK-12 desulfate. Because of its subcellular localization, this non-trypsin, non-kallikrein activity is a good candidate for a CCK converting enzyme. Supported by NIH Grant NS18667

167.1

AUTOCRINE REGULATION OF SELECTED PITUITARY GONADOTROPE FUNCTIONS BY ACTIVIN.

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Inhibins are gonadal dimeric proteins comprising an α and one of two β (βA or βB) subunits, that suppress FSH secretion by the anterior pituitary. Potent FSH-releasing proteins, activins, have been purified from gonadal fluids and characterized to be dimers of the inhibin β subunits. We have shown previously that inhibin/activin α and βB subunit mRNA's are expressed in the anterior pituitary and that the two proteins can be localized by immunohistochemistry to the gonadotropes which produce both LH and FSH. We report here that α and βB subunit proteins were immunoprecipitated and resolved on SDS-PAGE from ^{35}S-cysteine labeled lysates of cultured rat anterior pituitary cells. We have considered that the pituitary gonadotrope might produce either inhibin B (αβB) or activin B (βBβB) and have explored their possible autocrine roles within primary rat anterior pituitary cultures by using antibodies that neutralize either dimer. The addition of mouse monoclonal antibodies against βBβB (MAb-βB) that neutralize the effects of activin B strongly suppressed the spontaneous secretion of FSH while not influencing the release of LH. The inhibition of FSH secretion by MAb-βB was dose and time dependent. Treatment of cells with MAb-βB sensitized cells to activin A (βAβA), which is not bound by this antibody, while the response to hypothalamic gonadotropin releasing factor was attenuated. The addition of affinity purified rabbit antibodies directed towards the inhibin α subunit (rAb-α) that neutralize the biological activity of inhibins had no effect on basal or GnRH stimulated FSH or LH. We propose that the pituitary gonadotropes produce activin B which selectively enhances the secretion of one of the cells' products, FSH, and not LH.

167.2

TRANSFORMING GROWTH FACTOR-β: A POTENT INHIBITOR OF PROLACTIN SECRETION AND LACTOTROPIC GROWTH IN PRIMARY CULTURES OF RAT ANTERIOR PITUITARY CELLS.

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Transforming growth factor-β (TGF-β) has been shown to be an important regulator of growth and differentiation of various normal epithelial cell types. We report here, for the first time, that this growth factor inhibits both the secretion and growth of anterior pituitary (AP) lactotropes in primary cultures.

Enriched populations of rat AP cells were obtained from estradiol (E_2)-treated ovariectomized female rats and grown in culture, in serum-free Macoy-5A media for the secretion studies or grown in Macoy-5A media containing 2.5% fetal calf serum for the growth studies. Acute treatment (4 h) of TGF-β (0.002-20 ng/ml) dose dependently inhibited the secretion of basal and TRH-induced prolactin release. The basal secretion of other AP hormones was not affected by similar treatment. Furthermore, 2.0 ng/ml of TGF-β potentiated the inhibitory action of 1 μM but not 10 and 100 μM of dopamine on prolactin secretion. Long-term treatment (1-6 days) with 2.0 ng/ml of TGF-β completely suppressed the basal and E_2-induced growth of lactotropes in culture, as assessed by counting viable cell numbers and ^{3}H-thymidine incorporation into cellular DNA. The growth-inhibiting action of the growth factor was prevented by a monoclonal antibody to TGF-β. These results suggest that TGF-β may be important in controlling pituitary lactotrope growth and function.

167.3

INHIBIN, ESTROGEN AND PROGESTERONE EFFECT IN OVINE PITUITARY CULTURE ON GNRH RECEPTOR mRNA ACTIVITY.

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A *Xenopus* oocyte expression assay was used to study the effect of hormonal treatment on GnRH receptor mRNA activity in primary ovine pituitary cultures. Cultures were treated for 48 hours with drugs or vehicle and RNA was isolated. *Xenopus* oocytes, when injected with RNA isolated from cells expressing the GnRH receptor, develop an electrophysiological response to GnRH. The size of the response obtained was used to assay the GnRH receptor mRNA activity in the samples. Oocytes were each injected with 250 ng of total RNA. Two days later, using standard two-electrode voltage clamp, the maximum clamp-current in response to 2 x 10^{-7} M GnRH was measured. RNA injection from control cultures led to a mean response to GnRH of 8 nA(n=5). The response obtained after the following treatments were: 10 ug/ml crude inhibin, 90±18 nA(n=5); 10 nM estradiol, 63±32 nA(n=3); inhibin + estradiol, 334±88 nA(n=5); inhibin + 100 nM progesterone, 30±15nA(n=5). These data suggest that estrogen and inhbin have a complementary effect in increasing GnRH receptor mRNA activity and progesterone prevents the inhibin induced increase. (Supported by NIH Grant K11 DK01854 and USDA Grant 86-CRCR-1-2181.)

167.4

GnRH DECREASES GnRH RECEPTOR mRNA ACTIVITY IN A RODENT PITUITARY CELL LINE.

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Gonadotropin releasing hormone (GnRH) is a hypothalamic decapeptide that stimulates luteinizing hormone and follicle-stimulating hormone secretion from the anterior pituitary gland. Activation of pituitary gonadotrophs by GnRH is dependent on the level of GnRH as well as the responsiveness of GnRH receptors to GnRH and other agonists. GnRH receptor expression in Xenopus oocytes injected with RNA isolated from alpha-T3 cells, a gonadotrope cell linederived from a transgenic mouse, has been demonstrated (Mol. Endocrinol. 4: 119, 1990). Using Xenopus oocyte as a bioassay, GnRH receptor mRNA activity levels from alpha-T3 cells treated with GnRH, inhibin, testosterone, progesterone, and estrogen were examined. No apparent change was found in GnRH receptor mRNA activity from alpha-T3 cells treated with inhibin, estrogen, progesterone, and testosterone for 48 hours. Exposure to GnRH, however, led to a decline in GnRH receptor mRNA activity, similar to that seen in the thyrotropin releasing hormone receptor regulation in GH3 rat pituitary cells by thyrotropin releasing hormone (Science 238: 1406, 1987) . A 40 % decrease was observed in mRNA activity isolated from alpha-T3 cells treated with 1 uM GnRH for 48 hours. These data suggest GnRH receptor regulation by altered biosynthesis in response to continuous exposure to GnRH in alpha-T3 cells.

167.5

Possible role of tubulin in GnRH receptor-G protein coupling in the rat anterior pituitary lobe.

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We have recently demonstrated that a GnRH agonist stimulated G protein GTPase activity in the rat anterior pituitary in a concentration-dependent manner; moreover, this stimulation was blocked by a GnRH antagonist (*J. Neuroendocrinology*, in press). Colchicine and taxol, which are known to stimulate tubulin-induced GTPase activity, were used in the present study to delineate the possible role of tubulin in signal transduction in the anterior pituitary lobe. Plasma membranes from anterior pituitary lobes obtained from adult male rats were prepared by a discontinuous sucrose gradient method and stored at -80°C. Colchicine and taxol stimulated the G protein GTPase activity in the pituitary membranes in a concentration-dependent manner. For example, 100 nM of colchicine and taxol stimulated the GTPase activity by up to 80% and 70%, respectively. Although these results suggest that these drugs interact with tubulin present in association with the pituitary membranes, the possibility of a direct action on G proteins can not be ruled out. In another experiment, GnRH agonist (100 nM) stimulated the GTPase activity by up to 70%; in the presence of 100 nM of either colchicine or taxol, the ability of GnRH agonist to stimulate the GTPase activity was completely abolished. These drugs may 1) inhibit the GnRH binding to its receptor, or 2) disrupt GnRH receptor-G protein coupling. Studies are in progress to evaluate these two possibilities. (Supported by GM-37948 and AA-07698).

167.6

MODULATION OF NEURAL OXYTOCIN mRNA BY GONADAL STEROIDS DURING SEXUAL MATURATION.

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We have previously demonstrated (Miller, F.D. et al P.N.A.S. 86;2468) that in female rats, oxytocin (OT) mRNA increases during puberty and is decreased in adult females following ovariectomy. We have investigated the factors that regulate neural oxytocin mRNA in the male and female rat brains during puberty. Prepubertal male and female rats were gonadectomized and replaced with estradiol and testosterone. Forty days later animals were sacrificed and total cytoplasmic RNA preparations from the brain were analyzed on Northern blots using 32P labelled oxytocin riboprobes. These experiments indicate that the pubertal increase in OT mRNA is partially inhibited by gonadectomy. This inhibition is completely reversed by administration of exogenous steroids. Estadiol had greater effects on OT gene expression than testosterone in both male and female rats. To determine whether oxytocinergic neurons were equally responsive to steroid hormones at all developmental stages, 10 day old male and female rats were treated with estradiol, testosterone or dihydrotestosterone for 10 days. Steroids had no effect on the expression of OT mRNA in the prepubescent rats suggesting that neuronal maturation is a requisite for sensitivity to gonadal steroids.We also determined levels of OT mRNA during the estrus cycle in mature female rats: OT mRNA was approximately two-fold higher during proestrus versus metestrus or diestrus. These studies suggest levels of circulating gonadal steroids regulate neural OT mRNA but that additional factor(s) also play a role in the observed developmental increase.

167.7

OXYTOCIN MEDIATES THE ANGIOTENSIN-INDUCED RELEASE OF LH. C. A. Johnston, J. B. Gelineau-van Waes* and M. V. Templin*. College of Pharmacy, Wash. State Univ., Pullman, WA 99164-6510.

Recent evidence from our laboratory using the cycling female rat has demonstrated a physiologically important stimulatory role for central oxytocin (OXY) neurons in regulating the preovulatory release of luteinizing hormone (LH). This effect requires the presence of physiologically high levels of plasma estradiol (E2). Central injection of angiotensin II (ANG II) also stimulates LH release by an E2-sensitive mechanism (Steele et al., Endo. 111:722, 1982). To determine whether the influence of ANG II on LH secretion was mediated by OXY neurons, the ability of intracerebroventricular (icv) injection of ANG II (10 ng) to affect plasma LH was examined on both proestrus (when plasma E2 is high) and on metestrus (when plasma E2 is low) in rats pretreated with either saline or an OXY antagonist, [1-(ß-mercapto-ß, ß-cyclopentamethylene propanoic acid) 2-O-methyltyrosine, 8-orni-thine] vasotocin (45 ug/kg BW, iv) 50 min prior to the icv injection. ANG II or vehicle was administered at 15:00 h on proestrus or metestrus and plasma samples were obtained via chronic jugular cannulae from the unanesthetized, freely moving rats at 10 min before and 5, 15, 30 and 60 min after the icv injections. ANG II increased plasma LH on proestrus and tended to decrease LH on metestrus. The OXY antagonist blocked the increase in LH caused by ANG II on proestrus, and exerted a slight stimulatory influence on plasma LH on metestrus. The data demonstrate that the ANG II-induced effects on plasma LH may be mediated by OXY neurons, and that the influence of OXY neurons on LH may switch from inhibitory to stimulatory under the influence of E2.

167.8

HIGH LEVELS OF BRAIN ANGIOTENSIN PRECEDE THE LUTEINIZING HORMONE SURGE IN FEMALE RATS. I. Phillips, B. Kimura*, P. Borra*, M. Reitman*, S.P. Kalra and P. Kalra. Dept. of Physiology, University of Florida, Gainesville, FL 32610.

Brain angiotensin (b Ang II) has several functions in fluid balance and has been implicated in reproductive function. To test if brain ang II is elevated during the reproductive cycle, 3 experiments were carried out in rats. 1) Normal, cycling in female rats was tested by vaginal smears and brain tissue removed at metestrus, diestrus, proestrus and estrus. The results showed occasional but inconsistent high levels of b Ang II during the proestrus phase. 2) Female rats were ovariectomized and given replacement estrogen. On the day of testing, rats were injected with progesterone at 10 a.m.. This results in an LH peak at 3 p.m. At 10 minute intervals from 12 a.m. to 3 p.m., rats were sacrificed and brain Ang II measured by RIA and HPLC. Brain Ang II in the hypothalamus is normally 100 pg/gm tissue. Between 1:20 p.m. and 1:45 p.m., 4 rats had levels of b Ang II in the ug or ng range in the hypothalamus. These high increases in b Ang II occurred prior to the LH peak. Plasma Ang II levels did not rise. 3) Female rats treated the same as in Exp. 2, were anesthetized and CSF collected through an intraventricular push-pull perfusion technique. Samples of CSF were collected every 15 minutes, 2 hours after progesterone injection. A peak of Ang II in CSF was found 3.5 hr. after progesterone. The results indicate that b Ang II peaks in high quantity before the LH release and may contribute to the beginning of the LHRH-LH surge. These are the highest levels of Ang II we have ever detected in the brain.

167.9

SEXUALLY DIMORPHIC DISTRIBUTION OF NEUROTENSIN mRNA IN THE MEDIAL PREOPTIC REGION OF THE RAT. M.J. Alexander, Z.J. Kiraly*, and S.E. Leeman, Univ. of Mass. Med. Ctr., Worcester, MA 01655.

We have previously reported that estrogen induces neurotensin (NT) mRNA in the anterior medial preoptic nucleus (AMPN) (*Endocrinology* 125:2111, 1989), a cell group essential for the preovulatory surge of luteinizing hormone in the rat. To investigate the possibility of sexually dimorphic expression of the NT gene in this region, we used *in situ* hybridization histochemistry with a ^{35}S-labeled cRNA probe to detect NT mRNA in adult male (n = 6) and female (n = 12) rats. Brains were obtained from female rats at diestrus 1 (n = 6) and proestrus (n = 6), the estrous cycle stages during which circulating estradiol levels are at a minimum and maximum, respectively. In both males and females, labeled cells were present in a continuum beginning in, or immediately lateral to, the AMPN and extending caudally through the medial preoptic nucleus (MPN). However, a marked sex difference in the distribution of NT mRNA was observed. A single coronal section (20 µm) through the caudal AMPN of each rat was examined by an investigator blinded to the rat's sex, and bilateral cell totals were obtained. The AMPN cross section from proestrous rats contained four times (36 ± 3) as many labeled cells as did that of male rats (9 ± 1). Cell totals for diestrous rats were intermediate and more variable (18 ± 5) but were nevertheless significantly different from the proestrous totals. There was also a sex difference in the distribution of NT mRNA in the rostral portion of the medial subdivision of the MPN, where labeled cells in the female were located closer to the midline than those of the male. These results i) provide evidence of sexually dimorphic expression of the NT gene in the rat preoptic region, ii) indicate that NT mRNA abundance in the AMPN varies across the estrous cycle in parallel with the circulating estradiol level, and iii) are consistent with the view that NT neurons in the AMPN are involved in the neural regulation of the preovulatory surge of luteinizing hormone.

167.10

INTRAVENTRICULAR ADMINISTRATION OF NEUROPEPTIDE Y (NPY) INHIBITS LH RELEASE IN OVARIECTOMIZED SHEEP. P. V. Malven, S. A. Haglof* and H. DeGroot*. Dept. Animal Sciences, Purdue Univ., West Lafayette, IN 47907.

We investigated whether the elevated secretion of pituitary LH in ovariectomized ewes could be suppressed by intracerebral infusion of NPY via permanently implanted guide cannula. Human NPY in dosages of 20 µg peptide/infusion (20 µl volume and 20 sec duration) was infused unilaterally into the brain of 9 ewes (13 different sites) on 46 days. Equal volumes of saline were infused 1 day before or after NPY. Each daily infusion occurred after 90 min of blood sampling at 10 min intervals, and this sampling continued for an additional 100-190 min. Concentrations of serum LH were quantified by radioimuunoassay and were presumed to reflect LHRH secretion. To infer that exogenous NPY suppressed LHRH, post-NPY concentrations of LH had to be lower than (1) pre-NPY levels as well as (2) post-saline levels for all trials at a particular site. These criteria were satisfied in 13 NPY trials representing 5 cannula sites. Four of these five sites were in or near the ventricular system. Two sites were in the anteroventral portion of one lateral ventricle while two other effective sites were in the rostral part of the 3rd ventricle. The fifth effective site was within the tissue of the hypothalamic ventromedial nucleus. Ineffective sites of NPY infusion included posterior hypothalamus and the lateral and dorsolateral parts of anterior hypothalamic area. Although the site of NPY action cannot be precisely localized after intraventricular infusion, these results suggest that NPY may suppress LHRH by acting on circumventricular tissues in and around the medial parts of the rostral hypothalamus. In summary, intracerebral administration of NPY suppressed release of LHRH/LH in ovariectomized ewes, and this effect is consistent with published observations in ovariectomized rats and rabbits. Supported in part by USDA grant 87-CRCR-1-2538.

167.11

SERUM LH AS INFLUENCED BY NEUROPEPTIDE Y (NPY) IN SHEEP. T. M. McShane*, J. L. Miner, D. K. Keisler* and J. A. Paterson*. Dept. Anim. Sci., Univ. of Missouri, Columbia, MO 65211.

In experiment 1, the effect of centrally administered NPY on tonic secretion of LH was investigated. Six yearling ewes were fitted with lateral cerebroventricular (LCV) and indwelling jugular cannulas. Ewes were synchronized using progestagen sc implants. Twenty-two hours after implant removal, feed was removed and blood was collected every 15 min for 6 h for LH and estradiol 17-ß (E) analysis. At 26 h after implant removal, ewes were injected LCV with either 13 µg of NPY (NPY, n=3) or .9% saline (SAL, n=3). Feed intake between 2 and 3 h post treatment was greater (P<.02) in NPY (483.3 g) vs SAL (176.7 g). Serum conc. of E (pg/ml) did not differ between NPY (4.12) and SAL (3.96). All ewes exhibited episodic secretion of LH prior to LCV injection, whereas during 2 h post-treatment, 3/3 SAL and 0/3 NPY exhibited a pulse of LH. In exp. 2, 30 days following exp. 1, ewes were re-randomized and treated as in exp. 1. Fifteen min following LCV treatment, 50 µg GnRH was given iv to each ewe. Blood samples were obtained every 15 min, from immediately prior to until 2 h after LCV treatment. Concentrations of LH did not differ between NPY and SAL over time. In summary, NPY may act to suppress tonic secretion of LH in the presence of E (ranging in serum conc. from 2.50 to 5.33 pg/ml), but does not influence GnRH-induced LH secretion.

167.12

NALOXONE ENHANCES LHRH RELEASE FROM THE ISOLATED HYPOTHALAMUS OF OVARIECTOMIZED, ESTROGEN-PRIMED, MIDDLE-AGED RATS. B.S.Rubin,J.C.King and R.A.Strauss*. Department of Anatomy and Cellular Biology, Tufts Health Science Schools, Boston, MA 02111.

Endogenous opiates are important for the maintenance of regular estrous cyclicity, and changes in opiate tone on proestrus may be integral to the preovulatory LH surge. Experimental evidence suggests that opiate neuronal activity may change with age. Such changes could contribute to the eventual loss of spontaneous LH surges in female rats. The present study tests the ability of an opiate receptor blocker to influence LHRH release from hypothalamic fragments from ovariectomized, estrogen-primed, middle-aged (MA) female rats. Young and MA animals received 3 days of estrogen priming, and were sacrificed prior to the expected afternoon LH surge. Brains were removed and the MBH was dissected and placed into 0.5 ml chambers for in vitro perifusion. Fragments were oxygenated, maintained at 37° C, and effluents collected continuously at 10 minute intervals. After an initial 2 hour period, tissues were stimulated sequentially at hourly intervals with pulses of naloxone (0.4 mg/ml), norepinephrine (600 uM), and potassium chloride (ll0 mM). LHRH output from hypothalamic fragments from all of the MA females was enhanced by at least 50% after exposure to naloxone. Naloxone enhanced LHRH output from 4 of the 6 hypothalami taken from young females. These data suggest that endogenous opiates continue to exert an inhibitory influence on LHRH release in aging females prior to an expected LH surge. The possibility that opiate influence remains elevated in MA females and contributes to age-related alterations in the LH surge is being examined in vivo. Supported by NIH HD 19174.

167.13

NALOXONE AUGMENTS LH PULSE FREQUENCY IN GONADECTOMIZED MALE AND FEMALE FERRETS BEFORE, BUT NOT AFTER, ESTRADIOL REPLACEMENT.
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Male and female ferrets were purchased in breeding condition and gonadectomized. Ten days later, subjects were implanted with double intrajugular catheters. Blood samples were collected every 5 minutes from each subject via one catheter tube, while saline or the opioid receptor antagonist, naloxone (600 µg/kg/hr) was continuously infused for 2 hours via the other catheter. Intravenous infusions of naloxone caused a significant rise in LH pulse frequency in males and females, while mean LH concentrations and LH pulse amplitudes were not affected. Gonadectomized ferrets were then given 2 daily injections of a low dose of estradiol (1.25 µg/kg) for one week which produced plasma levels of estradiol similar to those of intact estrous females. Naloxone failed to stimulate LH pulse frequency, or other LH parameters in gonadectomized ferrets of either sex after they received estradiol. Our results for the ferret resemble those previously obtained by other investigators in another induced ovulator, the rabbit. They contrast, however, with the results of numerous studies using spontaneously ovulating species in which gonadal steroids, if anything, facilitate the ability of naloxone to stimulate LH secretion.
(Supported by RO1 HD21094, RO2 MH00392 and MH09812)

167.14

ß-ENDORPHIN REGULATION OF LHRH RELEASE IN EWES: IMMUNOCYTOCHEMISTRY, TISSUE CONTENT AND IN VIVO ANALYSIS.
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ß-endorphin (ßE) and other endogenous opioids have been implicated in the neuroendocrine control of LHRH release. We analyzed: **a)** the distribution of both peptides immunocytochemically; **b)** their tissue content in median eminence (ME) and immediately adjacent hypothalamic areas, and **c)** their *in vivo* release from ME by push-pull cannula (PPC) sampling during the estrous cycle of the ewe. ßE containing perikarya are located in and around the arcuate nucleus and fibers are present in the diagonal band, medial septal nucleus, and medial and lateral hypothalamic areas, including the preoptic region and the posterior ME. LHRH-containing perikarya located in the preoptic area project also to the ME, providing opportunities for synaptic interactions between ßE- and LHRH-containing axons at this level. In vivo release of ßE from the postero-lateral ME in the same ewes (n=5) was higher in late luteal (day 16) than in mid-luteal (day 11) stages of their estrous cycle (30±8 vs 139±30, mean ± sem, pg/200 µl PPC perfusate/20 min). *In vivo* LHRH content in these same PPC samples also increased from day 11 through16 of the estrous cycle (16±5 vs 76±20 pg/200 µl PPC perfusate/20 min). Administration of ßE through the PPC (5 µg/ 200 µl PPC perfusate) decreased *in vivo* LHRH release in late luteal ($P<0.01$) but not in mid-luteal ewes. Changes in ßE tissue content were observed in ewes killed during these reproductive stages (58% reduction in ME-stalk: 45±7 vs 26±6 pg/µgP and 500% increase in infundibulum/ arcuate: 5±3 vs 25±6 pg/µgP, n=12, mid-luteal vs late luteal, respectively). These results indicate that ßE might effect an increasing tonic inhibition on LHRH release until the onset of the preovulatory surge of LHRH
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167.15

CHANGES IN OPIOID INHIBITION OF LUTEINIZING HORMONE (LH) SECRETION IN PREPUBERTAL EWES.
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Previous research indicates that opioid antagonists increase LH secretion in prepubertal ewes. We have found that the LH response to a single dose of the opioid antagonist WIN44,441-3 (WIN) decreased with age prior to puberty. The purpose of the current study was to determine : 1) a more complete dose-response curve for WIN at various ages and 2) pituitary response to gonadotropin-releasing hormone (GnRH). Prepubertal ewes were given 0, .0625, .125 or .25 mg WIN/kg body weight, (n=6/group) at 16, 22, and 28 weeks of age. Blood samples were taken at 15 min intervals for 3 h before and after WIN injection (iv). Ewes were then given 5 ng/kg body weight of GnRH (iv) and blood samples taken for 1 h. At 16 weeks of age only the .25 mg/kg dose increased LH secretion. In contrast, in 22 week old lambs both the .125 and .25 mg/kg doses were effective in increasing LH pulse frequency. At 28 weeks, there was no LH response to any dose. Pituitary response to GnRH was greater ($P < .05$) at 22 weeks than at other ages. Experiment 2 was conducted to determine if a diurnal variation existed in response to WIN at these ages. Lambs on a natural photoperiod were injected with .25 mg/kg WIN at either 0900, 1200 or 1500 h. Blood samples were taken at 15 min intervals for 1 h before and 5 h after WIN injection. Half of the ewes (n=8) at each time received dextrose as a vehicle. At 16 and 22 weeks of age, lambs in the 0900 and 1500 h groups had increased LH pulse frequency ($P < .01$) following WIN injection. However there was no response in the 1200 h group. At 28 weeks there was no effect of WIN at any time. These results suggest a decrease in opioid inhibition of LH secretion as lambs mature. Further it appears that at 16 and 22 weeks of age inhibitory opioid tone varies during the day.

167.16

EFFECT OF ESTRADIOL (E_2) ON LHRH AND POMC mRNA LEVELS DEPENDS UPON THE INTERVAL BETWEEN OVARIECTOMY AND HORMONE REPLACEMENT.
S.L. Petersen, S. Shores* and S. McCrone*. Dept. of Anat. & Neurobiol., Univ. Missouri Med. Sch., Columbia, MO 65212.

Estradiol (E_2) exerts both negative and positive feedback effects on LH release, in part, by altering LHRH neuronal activity. β-endorphin (synthesized from proopiomelanocortin mRNA; POMC) has been implicated as a negative modulator in the transduction of E_2 signals into the LHRH system. We have shown previously that in ovariectomized (OVX) animals, **positive** feedback effects of E_2 on LH release are temporally associated with changes in LHRH mRNA and POMC mRNA levels which, in turn, appear to reflect changes in neuronal activity. In the present studies we tested the hypothesis that **negative** feedback effects of E_2 on LH release are also reflected in changes in LHRH and POMC mRNA levels. We used in situ hybridization histochemistery to measure mRNA changes resulting from E_2 treatment in long-term (LT) versus short-term (ST) OVX females, models exhibiting different levels of negative feedback. Rats were OVX and implanted s.c. either immediately (ST), or after 4 weeks (LT) with E_2. Three days after implantation, animals were sacrificed between 0800 and 0900 H. POMC mRNA levels were markedly decreased in LT OVX rats compared with E_2-treated LT OVX rats. Surprisingly, LHRH mRNA levels were also markedly decreased in the LT OVX rats compared with estrogenized counterparts. In contrast, in LT OVX rats, E_2 decreased LHRH mRNA levels, but did not alter POMC mRNA levels. These results suggest that the activity of neurons encoding LHRH and β-endorphin may be decreased in LT OVX rats and that previously-reported differences in the effect of E_2 on LHRH mRNA levels may be a result of differences in animal models.

167.17

AN INCREASE IN ß-ENDORPHIN IN ARCUATE NEURONS IS CORRELATED WITH REPRODUCTIVE MATURATION IN MALE FERRETS.
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Steroid negative feedback on luteinizing hormone (LH) secretion is mediated in part via endogenous opiate inhibition of LH-releasing hormone. In male ferrets, the low circulating levels of LH observed prepubertally are solely due to gonadal steroid inhibition, and a decrease in responsiveness to steroid permits the pubertal rise in pulsatile LH release. We tested whether production of the endogenous opiate ß-endorphin is greater in prepubertal than in postpubertal ferrets. ß-endorphin-producing neurons of the arcuate nucleus were identified immunocytochemically in sexually immature (n=4) and mature (n=4) male ferrets. Two animals in each group received a cerebroventricular injection of 200 µg colchicine in 2 µl artificial CSF; the other 2 ferrets in each group received vehicle injections. Ferrets were anesthetized and perfused with Zamboni's fix 24 hr later. Coronal sections (40 µm) were processed for immunocytochemical visualization of ß-endorphin using R. Benoit's ßendo-2 as first antibody, Vecastain ABC-Elite Kit reagents, and diaminobenzidine as chromagen. In both groups, the number of labelled cell bodies was increased by colchicine pretreatment. The number of immunopositive arcuate neurons was 15-fold higher in colchicine treated adults compared to colchicine treated prepubertal ferrets. These results do not suggest that a decrease in production of ß-endorphin mediates the pubertal decrease in responsiveness to steroid negative feedback, but indicate an alternative role for this neuropeptide during reproductive maturation. Supported by HD26483.

167.18

AGE-RELATED CHANGES IN PROOPIOMELANOCORTIN (POMC) GENE EXPRESSION IN THE ARCUATE NUCLEUS OF OVARIECTOMIZED RATS ARE INDEPENDENT OF REPRODUCTIVE STATUS.
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The transition to reproductive acyclicity in the rat is associated with altered hypothalamic function. This results in subtle changes in the pattern of pulsatile LH secretion. Opiate peptides are thought to play a role in maintaining normal patterns of LH secretion; however, it is unknown whether they are involved in age-related reproductive decline. The present study was designed to assess whether POMC gene expression changes with age, and whether any changes are related to the reproductive status of the animal. Seven groups of animals were used: young (3-4 mo) regularly cycling; middle-aged (10-12 mo) regularly cycling, irregularly cycling and constant estrus; and, old (17-19 mo) regularly cycling, constant estrus and persistent diestrus. Three weeks after bilateral ovariectomy (OVX) animals were sacrificed at 1000 h and the brains rapidly removed and stored at -70°C. Brain sections (8µm) were taken through the arcuate nucleus and prepared for *in situ* hybridization with a ^{35}S-labelled POMC riboprobe. Slides were exposed to film and POMC mRNA levels were quantitated by measuring the relative optical density within a fixed window placed over the arcuate nuclei. POMC mRNA levels were highest in the young animals and were decreased in the middle-aged group. No further decline was noted in old animals. This age-related decline in POMC mRNA was independent of the reproductive status of the animals before OVX. These data suggest that hypothalamic POMC mRNA levels decline by the time animals are middle-aged and may contribute to reproductive decline. (NIH AG 02224).

167.19

MEDIAL PREOPTIC μ-OPIATE RECEPTOR DENSITY BEFORE AND AFTER PARTURITION IN RATS. A.R. Mateo*, R.P. Hammer, P.M. Ronsheim*, and R.S. Bridges. Dept. Anat. & Reprod. Biol., Univ. Hawaii, Honolulu, HI 96822 and Dept. Anat. & Cell. Biol., Harvard Med School, Boston, MA 02115.

Activation of μ-opiate receptors disrupts maternal behavior in rats (Bridges & Grimm, 1982). μ-Receptor density in the medial preoptic area (MPOA) is elevated during pregnancy and reduced during lactation (Hammer & Bridges, 1987). Moreover, the density of MPOA μ-receptors is cyclical and gonadal steroid hormone-dependent (Hammer, 1990). The present study examined MPOA μ-receptor density during the periparturitional period. Brains were removed from ovariectomized or timed-pregnant Sprague-Dawley rats on days 18, 20 or 22 of pregnancy, and 1 hr or 1 day postpartum. Cryosections were prepared, preincubated for 30 min at 4°C in buffer, incubated for 60 min at 25°C in 50 mM Tris HCl, 3 mM MnAc, 0.1% BSA and 2 nm [^{3}H]DAGO, rinsed, dried and exposed to X-ray film. Density of MPOA μ-receptors was then determined using quantitative autoradiography. Receptor density was greatest on day 18 of pregnancy, when it was significantly higher than after ovariectomy ($p < 0.01$). Thereafter, receptor density declined gradually. There was, however, no abrupt change in density after parturition. It would appear that μ-receptor density decreases gradually *following* parturition, and that this decrease is not directly affected by the prepartum decline in plasma progesterone. Rather, receptor level may decline over time due to receptor turnover in the absence of sufficient hormonal stimulus promoting continued receptor expression. The subsequent reduction of MPOA μ-receptor level later during lactation may facilitate continued expression of maternal behavior. Supported by USPHS Awards DA04291 and MH00536 to RSB and NS01161 and RR03061to RPH.

HYPOTHALAMIC-PITUITARY-GONADAL REGULATION: GnRH

168.1

DIM LIGHT DURING THE NIGHT DECREASES HYPOTHALAMIC LHRH CONTENT OF RATS. T.Porkka-Heiskanen, M-L.Laakso*, D.Stenberg. Institute of Physiology, University of Helsinki, Finland

We have previously reported that the hypothalamic LHRH content of rats decreases under constant light (Porkka-Heiskanen, Acta Physiol Scand 1990, 138:187-192). The melatonin synthesis of the rat is known to be inhibited in such conditions. In the present experiment we studied the effect of dim night light on the hypothalamic LHRH and pineal melatonin contents of rats. The rats were kept under 12/12 illuminance schedule, where during the day the illuminance was 300 lx and during the night for the experimental group 1 lx and for the control group complete darkness. After one week the rats were decapitated in groups of 10 animals during 24 hours every three hours beginning at 1000 h. In the night light group the nocturnal pineal melatonin was significantly decreased (2-way ANOVA, $p < 0.0001$), but not to the daytime levels, the peak appeared at 0400 h in both the control and the night light groups. The hypothalamic LHRH content was significantly decreased in the night light group (2-way ANOVA, $p < 0.0001$) throughout the day. We conclude that the decrease of hypothalamic LHRH content is as sensitive to illuminance as the pineal melatonin synthesis. The decrease appears also in periodically altering light (300 vs. 1 lx) and thus seems to be dependent on the intensity of the night illuminance rather than on the lack of periodicity under constant light.

168.2

HYPOTHALAMIC GONADOTROPIN-RELEASING HORMONE (GnRH) GENE EXPRESSION IN LACTATING RATS. O.-K. Park, S. Gugneja*, and K. E. Mayo*, Department of Biochemistry, Molecular Biology and Cell Biology, Northwestern University, Evanston, IL 60208.

Suppression of LH secretion in lactating rats is thought to occur by a decrease in hypothalamic GnRH secretion. To address the question of whether a suckling stimulus plays a role in regulating the hypothalamic GnRH system, we examined GnRH gene expression utilizing *in situ* hybridization. Pregnant rats were housed individually with a standard photoperiod (14L:10D). Four groups of animals were included in this study: intact with or without pups, and ovariectomized (OVX) with or without pups. The day of delivery was designated day 0. On day 2, bilateral OVX was performed and litter size was adjusted to 9. On day 8, pups were removed from appropriate groups. At 1000-1100 h on day 10, rats were sacrificed, brains were rapidly frozen for *in situ* hybridization and trunk blood was collected for gonadotropin RIAs. 20 μm coronal sections of the brain in a region encompassing the diagonal band of Broca (DBB), the septal area, the organum vasculosum of the lamina terminalis (OVLT), and the preoptic area (POA) were hybridized to an ^{35}S-UTP-labeled antisense RNA probe synthesized from a full-length rat GnRH cDNA. The number of GnRH mRNA-expressing cells in the OVLT-POA in both intact and OVX rats with pups was decreased to 68.6 ± 6.7 % and 69.6 ± 5.3 % of control values, respectively. In these animals, serum LH levels were significantly decreased while serum FSH levels remained unchanged. No significant changes in the number of GnRH mRNA-expressing cells were noticed in the DBB and the septal area, suggesting that GnRH gene expression in subsets of GnRH neurons located in the OVLT-POA area is inhibited by the suckling stimulus. No noticeable differences were found between intact and OVX groups, indicating that the effect of suckling does not require ovarian steroids. Our results suggest that a suckling stimulus suppresses pituitary LH secretion, at least in part, by decreasing GnRH gene expression in the OVLT-POA area.

168.3

EVIDENCE THAT CAESARIAN-SECTION-INDUCED OVULATION IN THE RAT IS MEDIATED BY THE PELVIC AND HYPOGASTRIC NERVES. S.T. CUNNINGHAM, J.S. ROSENBLATT* AND B.R. KOMISARUK
Inst. Animal Behavior, Rutgers Univ., Newark, NJ 07102

In the rat, a spontaneous ovulator, mating can induce ovulation reflexively (Endoc. 79:1130,'66). Furthermore, reflexive ovulation in rats in response to afferent stimulation of the reproductive tract is indicated by findings that Caesarian section (CS) induces early ovulation (latency approx. 18h) that is blocked by uterine ligation (N.P. Johnson, Diss. Abst.,'72). To test whether the innervation of the reproductive tract mediates CS-induced ovulation, tubal ova and hemorrhagic ovarian follicles were counted 24h after bilateral transection of the pelvic (PX) and/or hypogastric (HX) nerves or sham neurectomy, performed on gestation day 22 (8-11 AM). Immediately upon completion of this procedure, all rats received CS. Results: (Mean and [range] #ova, and #hemorrhagic follicles, resp.): Sham group, n=8: 4.5 [1-11], 3.5 [1-5]; PX, n=7: 0 [0-2], 0 [0-9]; HX, n=5: 0 [0], 0 [0]; PX+HX, n=5: 0 [0], 0 [0]. All nerve transection groups were significantly lower in both measures than the sham group (Mann-Whitney U-tests, p's <0.01-0.05). These findings suggest that CS activates the pelvic and hypogastric nerves to trigger ovulation, implying that normally the afferent stimulation produced during parturition may reflexively stimulate ovulation. Support: (BRK):NIH NLS-2 5RO1 NS 22948, GRS 5 SO6 RR08223, and Busch Fdtn.

168.4

EFFECTS OF SHORT-TERM CASTRATION ON LHRH PATTERNS IN INTRA-HYPOPHYSIAL MICRODIALYSATES. J.E. Levine and J.M. Meredith. Dept. Neurobiol & Physiol., Northwestern Univ., Evanston, IL 60208

In a recent study using push-pull perfusion of the hypothalamus, we demonstrated that frequency of pulsatile luteinizing hormone-releasing hormone (LHRH) release is increased in short-term castrate rats. It was proposed that hypothalamic feedback actions of testicular hormones are manifest primarily through retardation of the activity of the LHRH pulse generator. We sought to confirm this hypothesis by measuring LHRH in the extra-cellular fluid of the anterior pituitary. Rats received guide cannulae implants with stylettes extending into the adenohypophysis. Animals underwent intra-hypophysial microdialysis procedures on day 4 following castration or sham-surgery. Analysis of microdialysate LHRH levels revealed that patterns in both groups were pulsatile, with mean pulse frequency being moderately higher ($p<.05$) in castrates (1.30±0.26 pulses/h, n=6) vs. sham-castrates (0.87±0.06 pulses/h, n=11). Castration produced no significant effect on LHRH pulse amplitude and mean LHRH levels. Our results using intrahypophysial microdialysis are strikingly similar to those previously obtained in hypothalamic push-pull perfusion experiments. Moreover, they are entirely consistent with the hypothesis that rapid responses (<24h) to castration arise primarily from disinhibition of pituitary responsiveness, while more slowly-developing changes in LHRH pulse generator activity, such as those observed in this study, underlie the continued post-castration rise in LH secretion. (NIH RO1-HD20677, PO1-HD21921, and KO4-HD00879)

168.5

DYNAMIC CHANGES IN PROGNRH AND GNRH DURING PROESTRUS IN THE FEMALE RAT. Y.J. Ma, M.J. Kelly and O.K. Ronnekleiv. Dept. of Physiology, OHSU, Portland and ORPRC, Beaverton, OR 97201.

The contents of proGnRH and GnRH in preoptic area (POA) and basal hypothalamus (BH) were measured by RIA using ARK2 and EL14 antisera, respectively. Plasma levels of LH, estrogen and progesterone were also determined. Female rats on a 12:12 light:dark schedule, lights off at 1630 h, were sacrificed at hourly intervals during proestrus (0830-1730 h). ProGnRH was significantly ($P < 0.01$) elevated in the POA (mean peak value: 150 ± 20 fmoles/POA, N=6), but not in the BH (mean value: 22 ± 2 fmoles/BH, N=6), 6-7 h before lights off. GnRH was significantly ($P < 0.01$) elevated in the BH (mean peak value: 2160 ± 110 fmoles/BH, N=6), but not in the POA (mean value: 147 ± 2 fmoles/POA, N=6), 3 h before lights off and immediately preceding the proestrous surge of LH h. Plasma levels of estrogen were elevated during the morning and declined at the time of the LH surge. Plasma levels of progesterone were low during the morning, and increased at the time of the LH surge. These data indicate that there is an increase in synthesis of proGnRH in the POA and increase in processing to GnRH during transport to the median eminence prior to the LH surge during proestrus. The mechanisms by which these changes occur are presently being investigated. (PHS HD 16793, P30 HD 18185).

168.6

PHORBOL MYRISTATE ACETATE-INDUCED LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) RELEASE FROM PREOPTIC AREA-MEDIOBASAL HYPOTHALAMIC EXPLANT CULTURES: A ROLE FOR PROTEIN KINASE C IN LHRH NEURON ACTIVATION. Richard W. Clough, Dept. of Anatomy, Southern Illinois University School of Medicine-Carbondale, Carbondale, IL. 62901

Phasic gonadotropin secretion in female rats is controlled by preoptic area neurons which project to and release LHRH into the vasculature of the median eminence ultimatley to gain access to the gonadotrophs of the anterior pituitary gland. Although stimulation of LHRH release by a cAMP dependent mechanism (Hartter et al., Neuorendo, 40, 1985) and a calmodulin (CaM) dependent mechanism Drouva et al, Neuroendo, 38, 1984) have been described, and, a preliminary report of phorbol ester induced LHRH release has been offered (King et al., SON ABST 15:258.14, 1989), characterization of intracellular signaling mediating LHRH release remains ill-defined. The present study has evaluated LHRH release in vitro using explants comprised of preoptic area-mediobasal hypothalamus as previously described (Clough, et al, Brain Res 446 1988). Explants were removed from decapitated rats and placed into a perifusio n tissue culture apparatus and subsequently perifuesd at a flow rate of 25 ul/min with an F12 culture media. Following 2 hours of equilibration, explants were sequentially exposed to PMA (10^{-4} M) in dimethylsulfoxide (DMSO), DMSO alone and a final challenge with potassium chloride (KCl). Fractions of effluent were collected every 15 minutes, frozen and subsequently assayed for LHRH by radioimmunoassay PMA, which is a potent stimulant of Protein Kinase C resulted in a dramatic and prolonged increase in the release of LHRH ($p<0.05$). Subsequent experiments evaluating the calcium dependancy of LHRH release in vitro suggest that there is an extracellular-calcium-independant mechanism operative in LHRH neurons. Taken together, these studies demonstrate that, in addition to the previously described LHRH release in response to dibutryl cyclic AMP, there are alternate second messenger sytems with the capability to mediate LHRH release. Supported by NIHCHDD grant 24426.

168.7

EFFECTS OF INCUBATION TEMPERATURE ON THE *IN VITRO* SECRETION OF TRH, CRH, GnRH. G. Cizza* [1,2], A.E. Calogero* [1,2], G. P. Chrousos [2], P. W. Gold* [1], and M. A. Kling [1]. CNE NIMH [1], and DEB NICHD [2], National Institutes of Health (NIH) Bethesda, M. D. 20895

The hypothalamus has a major role in the control of temperature. On the other hand, several hypothalamic hormones can affect body temperature. In the present study we assessed whether ambient temperature directly affects the secretion of thermoregulatory peptides. Hypothalami explanted from 8-10 week old S-D male rats and incubated in medium M 199 in a 48-well plate were exposed to plain medium first, then to 80 mM KCl at temperatures ranging from 0° to 42°. We measured directly, by RIA, the amount of TRH, CRH, GnRH released in the medium.

T R H : spontaneous release remained quite stable; KCl-stimulated release showed a plateau between 22° and 37°, a decrease at higher temperatures. At temperature above 0° there was a significant stimulation by KCl (Basal 0° 18.6±1.6; 22° 14.1±1.4; 28° 22.3±4.0; 32° 13.6±0.7; 37° 14.0±0.6; 39° 9.8±0.9; 42° 13.9±1.0.KCl: 0° 16.9±1.5; 22° 28.1±2.2; 28° 32.9±3.7; 32° 32.5±3.6;37° 30.1±1.5; 39° 21.2±2.0; 42° 23.1±2.1).

C R H : CRH showed a pattern similar to TRH (Basal 0° 8.8±1.2; 28° 7.9±0.9; 32° 5.3±0.5; 37° 5.1±0.3; 39° 3.7±0.1; 42° 4.2±0.1. KCl: 0° 4.6±0.4; 28° 67.5±7.1; 32° 51.8±20.8; 37° 68.5±9.9; 39° 37.1±10.0; 42° 34.5±11.1).

G n R H : both basal and KCl-stimulated secretion showed, at all temperatures tested, an inverse correlation between the temperature and the amount of GnRH released (Basal: 0° 5.9±1.6; 28° 3.2±0.7; 32° 1.1±0.2; 37° 1.3±0.2; 39° 1.7±0.7; 42° 0.7±0.2. KCl 0° 5.6±1.9; 28° 5.0±1.3; 32° 3.1±1.1; 37° 3.0±0.6; 39° 1.6±0.8; 42° 0.9±0.3). In conclusion : A) for all cases we found no stimulation of secretion by KCL at 0°, suggesting a common, energy-dependent, mechanism; B) at higher temperatures, we found two different patterns of secretion, perhaps reflecting the respective roles of these peptides in thermoregulation. C) incubation temperature is a critical factor to control in studies of hormone release *in vitro*.

168.8

LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) AND GONADOTROPIN-RELEASING HORMONE ASSOCIATED PEPTIDE (GAP) AND THE APPEARANCE OF LUTEINIZING HORMONE (LH) AND FOLLICLE-STIMULATING HORMONE (FSH) IMMUNOREACTIVITY IN THE RAT ANTERIOR PITUITARY GLAND (APG). C.A. Blake, G.T. Campbell*, F. Mascagni*, M.D. Culler* and A. Negro-Vilar*. Dept. of Anatomy, USC Sch. of Med., Columbia, SC 29208; Reproductive Neuroendocrinology Section, Lab. of Molecular and Integrative Neuroscience, NIEHS, NIH, Research Triangle Park, NC 27709.

We investigated the effects of anti (A)-LHRH serum or A-GAP serum on the development of gonadotrophs in the APG. Non-immune serum or A-LHRH, A-LHRH/GAP (which bound LHRH and GAP), or A-GAP serum were injected sc into neonatal female and male rats on days 1,3,5 and 7 after birth. Pups were killed on day 9. A-GAP had no effects on the parameters measured. A-LHRH and A-LHRH/GAP had similar effects. In both sexes, they reduced the percentage of APG cells immunoreactive for LH or FSH. In females, immunoneutralization of LHRH decreased the size of LH and FSH cells. No treatment had any significant effect on the size of LH or FSH cells in males. LH and FSH cells were of similar size within each group. The results suggest that LHRH but not GAP plays a role in the development of the number of LH and FSH cells in the APG of female and male rats and the size of gonadotrophs in female rats. Supported by a grant from the NIH (HD 22687).

168.9

LOCUS COERULEUS-PERIVENTRICULAR GRAY SUBSTANCE COMMUNICATION PARTICIPATES IN THE CONTROL OF THE ESTROUS CYCLE IN THE RAT. L.P. Solano-Flores, M. P. Rosas-Arellano* and R. Guevara-Guzmán. Dep. Fisiología, Fac. Medicina. U.N.A.M. Ap. Postal 70250, 04510-México, D.F. México.

We have shown that electrolytic damage of locus coeruleus (LC) causes a transient cessation of the estrous cycle and experimentally-induced persistent estrous in the naive female rat. Simultaneous damage of the periventricular gray substance (PVG) adjacent to the LC prevented those suppression effects. Histological evidences indicate a LC-PVG communication. These facts suggest a physiological role of LC-PVG communication in the modulation of neuroendocrine events for ovarian function control. To assess whether the LC-PVG communication participates in the modulatory systems for gonadotropic activity, the LC was anatomically separated from the PVG by means of knife cuts. Done that, the estrous cycling was totally suppressed during 10-13 days, then, 3-6 irregular cycles were observed. The regular cycling was reestablished 30-40 days after the cuts. These data suggest that the LC-PVG communication is part of a modulatory circuit for the gonadotropic neuroendocrine mechanisms. CONACyT Fortalecimiento al Posgrado N° 121.

168.10

CHANGES IN GONADOTROPIN-RELEASING HORMONE DURING METAMORPHOSIS AND FINAL MATURATION IN SEA LAMPREY. S.A. Sower, T.G. Bolduc*, and Y.H. Youson*. Dept. of Zoology, Univ. of New Hampshire, Durham, NH 03824 and Dept. of Zoology, Univ. of Toronto, Toronto, Ont.

Gonadotropin-releasing hormone (GnRH) concentrations were measured in brains of sea lampreys during metamorphosis (early developmental) and the final reproductive period with a homologous radioimmunoassay using lamprey GnRH antiserum (JAK 1467). Larval lampreys were collected in May and were sacrificed between July and November during the seven stages of metamorphosis. One of the four larval samples showed detectable levels of lamprey GnRH, but consistent levels were not detected until stage 6, and reached highest concentrations at stage 7. A second GnRH-like molecule was detected and occurred at stage 4, and persisted in small concentrations to the end of metamorphosis. The time of appearance of GnRH within the brain is consistent with the time in which the gonads of lampreys enter a rapid period of growth. In adult female lampreys, there were significant increases in brain GnRH (1989) and plasma estradiol (1988, 1989) through time during the final maturational period. Changes in brain GnRH and estradiol covaried through time. These data provide further evidence for an association between GnRH and estradiol during reproduction. Supported by NSF DCB-8808946 and DCB-8904919 (SAS) and NSERC (JHY).

168.11

CHANGES IN LHRH NEURONAL POPULATIONS ASSOCIATED WITH THE LH SURGE IN YOUNG AND MIDDLE-AGED FEMALE RATS. J.C.King, B.S.Rubin and P.Yao* Dept. of Anatomy and Cellular Biology, Tufts Health Science Schools, Boston, MA 02111.

LHRH neurons were examined at two time points on proestrus in middle-aged and young cycling female rats in an attempt to reveal dynamic changes associated with the preovulatory surge of LH. In addition, it was anticipated that these studies might yield insight into the nature of the deficit that leads to disruption of LH surges in aging females. Immunocytochemistry was performed using 2 different antisera to LHRH, 419 (A. Arimura) and 1076 (R. Millar). These antisera have been used extensively in the laboratory and have consistently revealed a difference of approximately 20% in the number of immunoreactive [+] perikarya detected on the morning of each day of the estrous cycle. The data obtained thus far reveals larger numbers of + cell bodies in animals sampled on the evening of proestrus compared to those sampled prior to the LH surge. This increase was observed with each antiserum, but not with either antiserum in aging females. If an increase in LHRH+ neurons on proestrus evening is indicative of secretion-synthesis coupling, then perhaps the absence of an increase in detectable LHRH perikarya in aging females suggests a disruption of this regulation. Comparison of cell counts of LHRH+ perikarya from each animal with the two LHRH antisera revealed the expected relationship in young females, but not in middle-aged animals. We hypothesize that the differences in cell number detected with these two antisera reflect different rates of processing of prohormone. These data suggest that the dynamic biosynthesis/ processsing activities associated with the LH surge in young animals are not as rapid or as robust in aging females. NSF DCB8702388; NIH HD19174.

168.12

PRO-GnRH mRNA AND PEPTIDE IN AGED AND YOUNG FEMALE RATS. O.K. Ronnekleiv, J.E. Thornton and K.C. Chambers. Dept. of Physiology, OHSU, Portland and ORPRC, Beaverton, OR 97201 and Dept of Psychology, USC, Los Angeles, CA.

The distribution and number of neurons containing proGnRH mRNA, proGnRH and GnRH were investigated in aged (20-21 months) and young (4-5 months) Fischer 344 female rats. Aged (N=4) and young (N=5) animals were perfused during estrus with 4% paraformaldehyde, the brains sectioned at 50μm and reacted for proGnRH (ARK-2) and GnRH (EL14). Aged (N=5) and young rats (N=5) were prepared during estrus for in situ hybridization using a 42-mer oligonucleotide cDNA probe to detect proGnRH mRNA as described (Ronnekleiv and Resko, Endocrinology, 126:498, 1990). The number of neurons that contained GnRH was lower in the aged as compared to young animals ($P=0.053$). The intensity of cell stain as well as the GnRH fiber stain in the median eminence, were similar between the two groups. The number of neurons containing proGnRH was significantly decreased ($P<0.02$) and the intensity of the stain was highly reduced in the old compared to young rats. The number of neurons containing proGnRH mRNA in 10-μm sections (N=20) through the POA was not different between the old and young rats (149 $\pm$ 56 and 184 $\pm$ 32, respectively). These results suggest that proGnRH synthesis is reduced in the aged female rat. (PHS HD 16793, HD 20970).

168.13

SEX DIFFERENCES IN SPINE DENSITY IN VMN NEURONS OF PERIPUBERTAL RATS. Annabell C. Segarra and Bruce S. McEwen. The Rockefeller University, 1230 York Ave., N.Y., N.Y. 10021.

The ventromedial nucleus of the hypothalamus (VMN) is one of the main regulatory sites of female sexual behavior. Using the single-section Golgi impregnation technique, sex differences in VMN neurons in gonadectomized juvenile and peripubertal rats were assessed. The effect of estrogen treatment on VMN neurons was also investigated. Juvenile rats were gonadectomized at 16 and peripubertal rats at 36 days of age. At day 5 post-surgery rats were injected with estradiol benzoate (EB) (20 ug/kg) or oil for 2 days after which they were perfused and the brains processed for Golgi staining. EB treatment significantly increased dendritic and soma spine density in juvenile and peripubertal male and female rats. A sex difference was observed in oil and in EB treated rats, with females exhibiting higher dendritic spine density. Moreover, dendritic and soma spine density was significantly higher in juvenile vs peripubertal rats. It is possible that the sex differences observed in dendritic and soma spine density and in the response to EB treatment is due to an organizational effect of sex steroids. During this critical period, sex steroids might bias synaptogenesis of select steroid sensitive cells and ultimately affect neuronal circuitry.

168.14

IMMUNOCYTOCHEMICAL DEMONSTRATION OF NEURAL CELL ADHESION MOLECULE (NCAM) ALONG THE MIGRATION ROUTE OF LUTEINIZING HORMONE-RELEASING HORMONE (LHRH) NEURONS IN MICE. M. Schwanzel-Fukuda, S. Abraham, K. L. Crossin, G. M. Edelman and D. W. Pfaff. Rockefeller University, New York, New York 10021.

Neural cell adhesion molecule (NCAM) is a cell surface glycoprotein produced by all nerve cells early in development. An integral membrane protein, it mediates cell-to-cell adhesion and is present on the cell body, neurites and on the tips of growing axons (Edelman, Ann.Rev. Cell Biol., 2:81-116, '86). Examination of Bouin's-fixed, paraffin-embedded serial sections of fetal mice, with antisera to NCAM and LHRH, using a double-label procedure, showed 1) by day 11 NCAM-immunoreactive fibers form a "scaffolding", either side of midline, appearing to anchor the tip of the rostral forebrain to the epithelium of the olfactory placode and 2) shortly thereafter, LHRH-immunoreactive neurons, which originate in the medial olfactory placode (Schwanzel-Fukuda and Pfaff, Soc. Neurosci. Abstr. p. 984, '88; Nature, 338:161-164, '89; Wray et al., Devel.Brain Res., 46:309-318, '89), migrate out of the placode, cross the nasal septum and enter the forebrain, along and within the NCAM-immunoreactive fascicles that also arise from the medial part of the placode. These fascicles appear to be central projections of the terminalis and vomeronasal nerves, and often surround a cord of migrating LHRH neurons. Antisera to cytotactin, CTB proteoglycan, fibronectin or laminin, used on adjacent sections, showed no immunoreactive cells or fibers within the migration route. Since no migrating LHRH cells were seen independent of the NCAM-immunoreactive fascicles, we raise the possibility that an NCAM scaffold provides a guide for migration of LHRH cells into the brain. Supported by NIH grant NS 19662 and funds from the Whitehall Foundation (M.S.-F.).

168.15

SYNAPTIC ARRANGEMENTS OF GONADOTROPIN-RELEASING HORMONE (GnRH) NEURONS IN BREEDING SEASON SHEEP: REGIONAL COMPARISONS. J.J. Xiong* and M.N. Lehman. Dept. Anat. & Cell Biol., Univ. Cincinnati Coll. Med., Cincinnati, OH, 45267.

GnRH neurons and their projections play a pivotal role in the control of seasonal reproduction in the Suffolk ewe. We previously described seasonal changes in the density of synaptic input onto preoptic GnRH neurons in intact ewes (Karsch et al.,*Neurosci. Abstr.*, 13: 1527). We now compare the synaptic arrangements of GnRH neurons located in different brain regions in intact (n=5) and ovariectomized estradiol-implanted ewes (OVX+E) (n=5) perfused during the breeding season. Brains were processed for EM immunocytochemistry using LR-1 antiserum (gift of Dr. R. Benoit) and an avidin-biotin-HRP procedure. GnRH neurons were analyzed from the medial preoptic area (POA), medial septum-dorsal preoptic area (MS), and anterior hypothalamic area (AHA). No significant regional differences, nor differences between intact and OVX+E ewes, were observed in the density of innervation among GnRH neurons, although GnRH cells in all regions received significantly fewer inputs than adjacent non-identified cells. Previously unreported observations from these animals include the presence of immunoreactive GnRH axon terminals in the POA and AHA, one example of direct contact between 2 GnRH somata in the MS, and what appears to be an autosynapse emanating from a GnRH cell. Comparison of this data with that from ewes perfused during anestrus will reveal whether seasonal alterations in GnRH ultrastructure are due to changing photoperiodic or hormonal signals. [Supported by NIH HD21968 (MNL)]

168.16

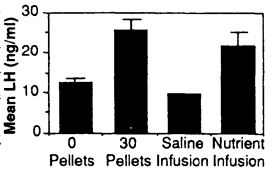
HYDROCEPHALUS RESULTS IN PERSISTENT DISRUPTION OF THE ESTROUS CYCLE IN THE HAMSTER (Mesocricetus auratus). S. D. Ham*, A. I. Canady*, R. Johnson* and J. A. Mitchell. Departments of Neurosurgery and Anatomy & Cell Biology, Wayne State University School of Medicine, Detroit, MI 48201

Hydrocephalus (HC) is a major neurological disorder in children and often results in diverse endocrinopathies including altered hypothalamic-pituitary-ovarian function. The effects of HC on the estrous cycle in hamsters maintained in a photoperiod of 12 h light/12 h dark were determined. Regularly cycling animals were assigned to control (C) (saline injected) and kaolin treated (K) groups. Injections of 0.03ml sterile kaolin in saline (250mg/ml) or vehicle were made into the cisterna magna. Body weight, food and water intake and estrous cycles were monitored daily. At selected intervals, animals were anesthetized and perfused with fixative: brains were examined, ovaries and uteri were weighed. Ventricular distention was apparent by 3 days, maximal by 21 days and persisted beyond 50 days post-K. Relative to controls, K animals experienced acute reductions in body weight, and food and water intake but were equivalent to controls by 50 days. Alterations in the estrous cycle were immediate and persistent; % C vs. K: normal = 80% vs. 30%; irregular = 20% vs. 40%; persistent diestrus = 0% vs. 30%; persistent estrus = 0% vs 0%. Mean ovarian weights did not differ in K vs. C animals. However, some uteri were atrophic and others within normal range. Studies are in progress to determine the anatomical correlates of HC induced disruption of normal ovarian function. (Supported by a grant from the Children's Hospital of Michigan).

168.17

EVIDENCE THAT THE SUPPRESSION OF HYPOTHALAMIC-PITUITARY-GONADAL AXIS ACTIVITY DURING FASTING RESULTS FROM A NUTRITIONAL SIGNAL AND NOT THE PSYCHOLOGICAL STRESS OF FOOD DEPRIVATION. D.A. Schreihofer, D.B. Parfitt*, and J.L. Cameron. *Depts. of Behavioral Neuroscience, Psychiatry, Physiology, and the Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

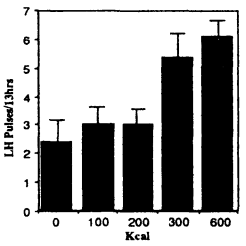
We have previously shown that short periods of fasting (1-2 days) significantly suppress the frequency of pulsatile LH secretion in male rhesus monkeys, and that this appears to result from a decrease in GnRH stimulation of the pituitary. Refeeding a normal meal rapidly restores pulsatile LH secretion. We have hypothesized that restricting food intake may suppress the central drive to the reproductive axis via "metabolic signals" resulting from the state of undernutrition or via "stress signals" resulting from the psychological stress of food deprivation. To differentiate between these two possible types of signals we have examined whether feeding through an indwelling gastric catheter can restore pulsatile LH secretion in fasting monkeys. We reason that this type of refeeding would relieve the undernutrition without relieving psychological stress. Adult male rhesus monkeys with indwelling gastric and venous catheters were fasted for one day, and on the following day they received a a liquid nutrient infusion (equal in caloric content and macronutrient composition to a normal meal) at the time they are normally fed (11:00 AM). Blood samples were collected for 5 h prior to infusion and 13 h after infusion at 20 min intervals. Nutrient infusion caused a rapid increase in pulsatile LH secretion, and therefore mean LH, similar to that caused by refeeding a normal meal of 30 Purina monkey chow pellets, as shown in the figure. In contrast, a saline infusion of equal volume did not restore pulsatile LH secretion in a pilot study with a single monkey. These results indicate that relieving undernutrition caused by fasting, without relieving the psychological stress of food deprivation, can restore the activity of the HPG axis. These findings support the hypothesis that "metabolic signals" occurring in undernourished states cause the suppression of the central drive to the reproductive axis.



168.18

FASTING-INDUCED SUPPRESSION OF PULSATILE LH SECRETION IS RAPIDLY REVERSED BY REFEEDING IN RHESUS MONKEYS *(Macaca mulatta)*. D.B. Parfitt*, K.R. Church*, and J.L. Cameron. *Depts. of Behavioral Neuroscience, Psychiatry, Physiology, and Ctr. for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

We have previously shown that brief periods of fasting (1-2 days) cause a slowing of pulsatile LH secretion in adult male rhesus monkeys that appears to result from a decrease in GnRH stimulation of the pituitary. The present study was designed to investigate how rapidly this effect of fasting can be reversed and whether a correlation exists between the amount of food provided during refeeding and the rate of restoration of pulsatile LH secretion. Adult male rhesus monkeys (5-7/group) were fasted for one day and the next day they were refed different amounts of food (0-600 kcal of Purina monkey chow) at the normal time of feeding (11:00 am). Blood samples were collected from 8:00am to 12:00 midnight at 20 min intervals. Refeeding the monkey's standard meal (600 kcal) resulted in the immediate stimulation of LH pulse frequency (6.14 ± .55 pulses/ 13 h). Moreover, the degree of restoration of LH pulse frequency was directly correlated with the amount of food provided in the refeeding meal, as shown in the figure below. These results indicate that the suppression of the central drive to the reproductive axis can be immediately relieved by refeeding a normal meal and that the restoration of pulsatile LH secretion is dependent on the amount of food consumed. These findings suggest that a "metabolic signal" related to the degree of undernutrition of the body modulates the central drive to the reproductive axis.



168.19

DECREASED AVAILABILITY OF METABOLIC FUELS OR FOOD DEPRIVATION ATTENUATES THE PREOVULATORY LH SURGE IN SYRIAN HAMSTERS R. W. Dickerman, J. E. Schneider*, and G. N. Wade Neuroscience and Behavior Program. Dept. of Psychology, University of Massachusetts, Amherst, MA 01003.

Food deprivation or treatment with the metabolic inhibitors 2-deoxy-D-glucose (2DG) plus methyl palmoxirate (MP) reduces the rate of ovulation and estrous behavior in female hamsters (Schneider, J.E. and Wade, G.N., Science 244:1326,1989). 2DG and MP inhibit utilization of glucose and fatty acids respectively. We examined the effect of either food deprivation or 2DG+MP on plasma levels of LH, E_2, and FSH in hamsters. On days 1 and 2 of the estrous cycle, hamsters were either food deprived, given food and treated with 2DG+MP in vehicle (2DG+MP), or given food and treated with vehicle alone (controls). Serum was collected at -10 and -4 hours before lights out. The expected time of the preovulatory LH surge is at -4 hours before lights out.

In controls serum LH was elevated ($p<0.01$) at -4 compared to -10 hrs (3.2 ± 0.8 vs 0.8 ± 0.2ng/ml). In food deprived hamsters LH levels were not different at -4 compared to -10 hrs (1.1 ± 0.5 vs 0.8 ± 0.1ng/ml). In 2DG+MP treated animals, serum LH was higher ($p<0.05$) at -4 compared to -10 hrs (1.4 ± 0.4 vs 0.6 ± 0.1ng/ml), but the levels at -4 hrs were less than in controls. Levels of E_2 did not change in any group over time. However, serum E_2 concentrations in controls were higher ($p<0.01$) than in 2DG+MP or food deprived animals. Significant fluctuations in serum FSH concentrations were not detected.

These data indicate that similar to food deprivation, reduced availability of metabolic fuels is associated with an attenuated LH surge. Reduced availability of metabolic fuels may act to reduce LH by a peripheral action or through a direct central effect.

Supported by NS10873, DK32976, and MH00321.

168.20

SUPPRESSION OF PUBERTY BY PROLONGED EXERCISE IN FEMALE RATS: EFFECTS ON HORMONE LEVELS, AND RECOVERY ON REPRODUCTIVE FUNCTION WITH GONADOTROPIN-RELEASING HORMONE (GnRH). J.M. Manning and F.H. Bronson*. Dept. of Zoology, University of Texas, Austin, TX 78712.

Loss of menstrual cyclicity is common in athletes engaged in endurance training. In an effort to better understand the relationship between prolonged exercise and ovulation, we made a variety of comparisons between female rats in two treatment groups: 1) Prolonged Exercise, in which growth and reproductive development were arrested at a peripubertal stage be requiring rats to run for prolonged periods of time in order to obtain food, vs. 2) Voluntary Exercise, in which same-aged control rats were fed ad lib and given free access to a running wheel. The pulsatile secretion of luteinizing hormone (LH) was completely suppressed by the Prolonged Exercise treatment, but FSH levels were unaffected. Rats in the Prolonged Exercise regime exhibited elevated corticosterone titers, and the circadian secretory pattern of this steroid was shifted out of phase with running activity. GnRH levels in the hypothalamus and LH levels in the pituitary were enhanced, not suppressed, by prolonged exercise. Most importantly, rats subjected to the Prolonged Exercise treatment achieved normal pubertal development when given pulsatile infusions of GnRH, despite a total lack of body growth. The results of this study clearly show that the suppressive effects of prolonged exercise occur at or above the level of the hypothalamic GnRH pulse generator.

INVERTEBRATE SENSORY SYSTEMS

169.1

5-HT MODULATES ACCOMMODATION/ADAPTATION IN STATOCYST HAIR CELLS OF *HERMISSENDA*. T. Crow. Dept. of Neurobiology and Anatomy, Univ. of Texas Medical School, Houston, Tx. 77030.

Serotonin (5-HT) alters the excitability of identified photoreceptors within the eyes of *Hermissenda* and has been proposed to contribute to cellular plasticity associated with *in vivo* conditioning (Crow, 1988; Forrester and Crow, 1989). Immunohistochemical studies have identified serotonergic neurons in the cerebropleural ganglia and immunoreactive fibers and varicosities in the region of the neuropil near photoreceptor and statocyst hair cell synaptic terminals (Land and Crow, 1985). In order to further characterize the role of 5-HT as a modulator in *Hermissenda*, its effects upon statocyst hair cell accommodation were examined.

5-HT (10^{-4}M) produces a slow depolarization of hair cells accompanied by an increase in input resistance and an increase in the amplitude and frequency of hair cell noise. Hair cell excitability is enhanced by 5-HT as expressed by larger amplitude responses produced by extrinsic constant current depolarizing pulses and less apparent spike frequency adaptation. In order to rule out the influence of synaptic input from photoreceptors contributing to the effects of 5-HT on accommodation, hair cells were either surgically isolated from all synaptic input or photoreceptors were surgically eliminated. After elimination of synaptic input from the visual system, 5-HT still produced less accommodation in hair cells to the same depolarizing current pulse. In surgically isolated hair cells, 5-HT resulted in an increase in the amplitude of electrotonic potentials evoked by hyperpolarizing and depolarizing current pulses.5-HT produced an enhancement of multiple regenerative events evoked by depolarizing current pulses. The multiple regenerative events are TTX insensitive, and are blocked or reduced in amplitude by 15mM Ni^{2+}. 5-HT may affect hair cell accommodation by decreasing an outward K^+ current or enhancing an inward Ca^{2+} current.

169.2

RECEPTIVE FIELDS AND PROPERTIES OF A NEW CLUSTER OF MECHANORECEPTOR NEURONS INNERVATING THE MANTLE REGION AND THE BRANCHIAL CAVITY OF THE MARINE MOLLUSK *APLYSIA CALIFORNICA*. B. Dubuc and V.F. Castellucci. Lab. de Neurobiologie, Institut de Recherches Cliniques de Montréal, Montréal, (Québec), Canada. H2W 1R7.

The rostral LE cluster (rLE) is a new set of mechanoreceptor neurons innervating the mantle area, the branchial cavity, the gill and the siphon of the marine mollusk *Aplysia californica*. We have compared the organization of their receptive fields with those of LE, RE and RF sensory neurons of the abdominal ganglion which have been reanalyzed.

There is extensive receptive field overlap among the four populations of sensory cells; the most exposed areas of the mantle are the most densely innervated. The sensory threshold is similar for all groups. The neurons of the LE and rLE clusters have common physiological properties. Their actions potentials are broadened by serotonin and SCP peptides and narrowed by dopamine and FMRFamide. Their synaptic outputs undergo synaptic depression, homosynaptic and heterosynaptic facilitation.

More than one hundred mechanoreceptor neurons innervate the entire mantle and siphon skin, branchial cavity and gill of *Aplysia*. The degree of their divergence to various interneurons and motor neurons mediating the gill and siphon withdrawal reflex and other reflexes is under investigation. (Supported by MRC grant MA10047 and Strauss Canada Foundation).

169.3

PERIPHERAL AND CENTRAL RESPONSES OF THE LATERAL LINE ANALOGUE OF THE CUTTLEFISH *SEPIA* TO LOCAL WATER MOVEMENTS. B.U. Budelmann[1], H. Bleckmann[2,*] and T.H. Bullock[3]. [1]Marine Biomed. Inst., Univ. Texas. Med. Branch, Galveston, TX 77550, [2]Div. Biol. Univ. Bielefeld, D-8400 Bielefeld, FRG and [3]Neurobiol. Unit, A-001, SIO, UCSD, La Jolla, CA 92093.

The epidermal head lines of *Sepia* and other cephalopods are composed of rows of hair cells. They respond to local water movements in a way similar to the amphibian and fish lateral lines (Budelmann, B.U. & Bleckmann, H., *J. Comp. Physiol. A*, 165:1, 1988).

The response of the lines to stimulation with sinusoidal water waves has three components: (i) a large phasic ON-response, (ii) a tonic microphonic response, frequency-doubling within a wide frequency range, and (iii) a slow OFF-wave. Slowly rising and falling stimuli lack the ON and OFF components. The amplitudes of the receptor potentials increase nonlinearly with stimulus amplitude (tested at 6-100 Hz) and finally reach saturation. Best displacement frequency is about 100 Hz and lowest threshold recorded was 0.06 μm peak-to-peak water displacement (18.8 μm/s as velocity), calculated at the skin surface. Abrupt modulation of stimulus frequency or amplitude elicits large responses at the onset and offset of modulation and marked changes in the tonic microphonic response. Change of stimulus direction relative to the course of the line has only little effect on the summed response, perhaps due to the four directions of polarization of the hair cells in each line. Preliminary recordings from subesophageal brain areas show only little change of the, presumably first-order, afferent signals of the epidermal lines. [Support: NIH, DFG and NSF grants]

169.4

ARE THERE CHOLINERGIC PRIMARY AFFERENT SYNAPSES ON CRAYFISH LATERAL GIANTS? E. T. Vu, M. W. Miller, and F. B. Krasne. Dept. of Psychology and Brain Res. Inst., University of California, Los Angeles, CA, 90024.

Primary afferents (PAs) of the crayfish lateral giant (LG) circuit make cholinergic synapses having nicotinic ganglionic pharmacology on sensory interneurons. The PAs also monosynaptically innervate the LGs via junctions that are thought from latency and following ability to be electrical. However, the monosynaptic EPSPs are diminished by LG depolarization and increased by hyperpolarization. This could result from (1) a chemical component to transmission, (2) spread of polarizing current into PA terminals, or (3) biassing of rectifying electrical junctions (see Edwards et al., Neurosci. Abstr., 14:999, 1988). A similar observation on monosynaptic inputs to the medial giants (Glantz & Viancour, J. Neurophys., 50:1122, 1983) has been explained by mechanism (1). We find that the LGs have cholinergic receptors that are pharmacologically similar to those of sensory interneurons, also encouraging interpretation (1). However, mecamylamine, which blocks those receptors, has no effect on monosynaptic EPSPs. (USPHS grant NS08108).

169.5

BRANCHING PATTERNS OF SINGLE OLFACTORY AFFERENTS IN THE CRAYFISH BRAIN. DeF. Mellon, V. Alones, & M. Riddick. Dept. of Biology, Univ. of Virginia Charlottesville, Virginia 22903.

In crustaceans primary olfactory afferents are associated with batteries of unique antennular sensilla called aesthetascs. Each sensillum houses the ciliary dendrites of several hundred bipolar sensory neurons, many of which are narrowly tuned to specific odorants. A current hypothesis regarding the central organization of olfactory input regards each olfactory glomerulus as a functionally unique subunit, within which information about specific odorants is refined and amplified. We have suggested previously that primary olfactory afferent projections to the brain in crayfishes are not spatiotopically distributed; instead afferent fibers from each antennular olfactory sensillum distribute themselves among all glomeruli in the ipsilateral olfactory lobe. We speculate that afferent fibers having similar odorant sensitivities terminate in the same glomeruli. These conclusions are valid, however, only if each afferent fiber terminates in just one glomerulus. We have now obtained evidence from Golgi preparations for a univalent pattern of terminal distribution in the deutocerebrum of the crayfish, Procambarus. Observations in numerous preparations suggest that primary fibers approach the olfactory lobe without branching and that each penetrates only a single glomerulus, within which each fiber typically subdivides into four or five filaments that terminate at the central apex of the glomerular column. Varicosities which we interpret as presynaptic sites are a prevalent feature of the columnar terminals and occur as well as in the peripheral cap region of each glomerulus. These findings are not inconsistent with our recent observations that the glomerular columns are sites of contact between unidentified presynaptic neurons and Biocytin filled olfactory-globular tract interneurons. Supported by the Whitehall Foundation.

169.6

AFFERENT PROJECTIONS TO THE MIDBRAIN OF THE SPINY LOBSTER REVEALED BY BIOCYTIN. M. SCHMIDT*, B.W. ACHE. Whitney Lab. and Depts. of Zoology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086.

The projection of afferent fibers in the antennular nerve to the midbrain (deutocerebrum) of the Caribbean spiny lobster, *Panulirus argus*, was characterized by applying the recently introduced intracellular marker, biocytin (J. Neurosci. Meth. 25: 1-11, 1988), to cut nerve bundles as an anterograde tracer. Three deutocerebral neuropils receive input from the antennular nerve. Numerous fibers (0.3 - 2 μm diam.) project to the olfactory lobe (OL), surround it, and penetrate its column-like glomeruli. The fibers extend into the base of the glomeruli where they terminate in small boutons (1 - 2 μm diam.), presumably representing presynaptic sites. Typically, the thicker fibers branch outside the OL and project into several glomeruli. In contrast, the thinner fibers only innervate single glomeruli. A second, very heterogeneous population of afferent fibers (0.3 - 8 μm diam.) projects into both lobes of the lateral antennular neuropile. The thickest of these fibers also branch to the medial antennular neuropile where they terminate in large boutons (5 μm diam.). (Supported by DFG award Schm 738/1-1 and NSF award # 88-10261).

169.7

THE AFFERENT PROJECTION PATTERN OF CERCAL BRISTLE HAIRS IN THE COCKROACH. Darryl I. Daley. Department of Physiology, National College of Chiropractic, Lombard, Il 60148.

The projection pattern of the largest bristle hairs (BH's) located on the ventral surfaces of the cerci of adult cockroaches (Periplaneta americana) was examined by bringing the broken off end of a BH in contact with a 200 mM cobaltic hexamine chloride solution for 18-24 hours at 5° C. The cobalt was then precipitated as a sulfide and silver intensified.

The largest BH's appear to be singly innervated since only one axon is stained by the above method. The sensory neurons innervating BH's project to a broad region of neuropil and show some overlap with the projection area of filiform hairs (Daley, D. L., Soc. Neurosci. Abst.,15,1287, 1989). Those BH afferents from proximal cercal segments tend to have smaller arbors and terminate more posteriorly. The arbors of BH afferents located more distally on the cerci are more extensive and project more anteriorly when compared to BH afferents more proximally located. When small groups of neighboring BH afferents were stained they all arborize in the same region of neuropil. Thus the afferent projection of the BH's is somatotopic.

169.8

A MISSING LINK IN INSECT AUDITION. S.R. Shaw, Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia, Canada.

When a spine on a cockroach leg is deflected by an amount subthreshold for its own mechanoreceptor neuron, a vibrationally evoked potential (VEP) is still recorded from the leg nerve. If the leg is pulsed with airborne sound, a damped oscillatory acoustic EP results (AEP), consisting of initially coherent volleys of impulses at a fixed frequency around 300 Hz, regardless of tone frequency or intensity, implying resonant or electrical tuning. The AEP originates from an internal organ, not external hairs, since covering the leg with Vaseline fails to abolish it. Local cooling or cautery pinpoint its origin in the tibia below the knee, the site the subgenual organ (SGO), previously identified as a sensitive mechanoreceptor tuned to surprisingly high vibrational frequencies (H. Schnorbus, 1971, Z. vergl. Physiol. 71: 14). Local SGO ablation abolishes both VEP and AEP, and cross-modal adaptation is highly effective, indicating that VEP and AEP both originate in the same receptors. Threshold acoustic tuning curves (CF 1.8-2.2KHz) sometimes peak below 50dB SPL, a sensitivity commensurate with bona fide insect ears. Threshold activation energy is $4x10^5$ greater for VEP than AEP, suggesting that these SGOs are specialized for audition. Thus the tibial ears of crickets may have evolved from SGOs that were not just pure vibration detectors, as previously supposed, but from SGOs that already possessed high acoustic sensitivity, like the SGOs in cockroach legs. (Supported by NSERC A9593, Canada)

169.9

QUANTITATIVE ANALYSIS OF TOPOGRAPHIC MAPPING IN THE CRICKET CERCAL SENSORY SYSTEM. G.A.Jacobs and N.S.J.Poy*. Dept. of Molecular and Cell Biology, Univ. of CA, Berkeley, CA 94720

Wind sensitive mechanosensory afferents form a topographic map of wind direction in the cricket cercal glomerulus. We have analyzed this sensory map by staining sensory neurons with cobalt and reconstructing their terminal arbors in 3-D using a computer-based reconstruction system. Filiform hairs have been shown to be segregated into seven principle directional sensitivity classes. At least five afferents from each class were reconstructed for the map. The location and volume of neuropil filled by the afferent arbors were measured and scaled to a "mean" standard ganglion. A computer atlas of the positions of the afferents' terminal varicosities was constructed by dividing the cercal glomerulus into cubical volume elements, or "voxels", and calculating the density of varicosities of each type of afferent within each voxel. The resolution of the atlas could be varied by changing the dimensions of the voxels. Afferents with directional sensitivities separated by at least 90° showed very little overlap, whereas those separated by 45° or less showed significant overlap. The variability in the density distribution of varicosities from different afferents of the same identified type was also assessed. These results will be discussed in terms of possible substructure in the map.

169.10

CODING AND DECODING IN THE CERCAL FILIFORM HAIR RECEPTORS OF THE CRICKET. David Warland*†, Michael A. Landolfa*, John P. Miller, William Bialek*†, and Robert M. Olberg. Department of Physics†, and Department of Molecular and Cell Biology, University of California at Berkeley, Berkeley, California 94720

The cercal filiform hair receptors of the cricket *Acheta domesticus* encode information about near-field air current stimuli. Behavioral studies have shown that crickets can discriminate among different kinds of air-current stimuli within 100 msec, based on information obtained from the deflection of filiform hairs. Traditional approaches to the study of neural coding attempt to predict average neural response to particular stimuli. The cricket, however, faces nearly the opposite task: based on a limited number of spikes, the cricket must make real-time estimates of air movement. Here we present the results of a study which allows the generation of real-time estimates of filiform hair motion based on observations of the spike train from single sensory cells. The analysis yields a characterization of an equivalent noise spectrum. It is ultimately the uncertainties in the estimate of hair movement that limit the resolution with which the cricket can distinguish features of air current stimuli. We calculate the threshold for reliable detection of movement in a real-time estimation task and the information coded by a single afferent.

169.11

INFORMATION THEORETIC CALCULATION OF DIRECTIONAL RESOLUTION AND OPTIMAL WIDTH OF TUNING CURVES OF FOUR PRIMARY INTERNEURONS IN THE CRICKET CERCAL SYSTEM. F.E. Theunissen* and J. P. Miller. Dept. of Molecular and Cell Biology, Uni. of California, Berkeley, CA 94720.

The activity patterns of primary sensory interneurons in the cricket cercal sensory system encode information about the direction of air current stimuli in the animal's immediate environment. In previous studies, we have characterized the directional sensitivity of several types of these interneurons. On the basis of this data, principles of information theory were used to calculate the maximum directional resolution attainable by the sub-system consisting of four low velocity threshold interneurons. This analysis was limited to a consideration of the mean independent firing rates of the interneurons. These interneurons had broad symmetrical directional sensitivity curves that could be accurately represented as truncated sine waves. We have demonstrated that the system could achieve a directional resolution of 4.22 bits. The directional resolution was also calculated for hypothetical situations in which the widths of the tuning curves were varied from the observed values. These simulations showed that the width of the tuning curve of the real cells were close to optimal, and that this resolution was robust to changes in the relative spacing of the tuning curves. Analyses incorporating information available in the higher order statistics and covariance of activity in different cells allow for even greater resolution.

169.12

SENSORY AFFERENTS AND INTERNEURONS DEFINE DISCRETE SYNAPTIC NEUROPILS IN THE LEG NEUROMERES OF *Drosophila*
R. Naresh Singh[1], Shubha R. Shanbhag*[1], and Nicholas J. Strausfeld[2]. [1]Molecular Biology Unit, Tata Institute for Fundamental Research, Homi Bhabha Road, Bombay 400 005, India and [2]ARL Division of Neurobiology, and Center for Insect Science, Univ. of Arizona, Tucson AZ 85721

Golgi- and Cobalt-silver staining demonstrates the neuroarchitecture and cellular organization of thoracic neuropils receiving sensory afferents from, and giving rise to motor neurons to the legs of *Drosophila melanogaster*. Selective uptake of HRP containing sucrose or KCl (or NaCL) has resolved specific chemosensory afferents into leg neuromeres. Sensory terminals occupy specific zones of the leg neuromere, where they terminate in laminar or glomeruli-like domains arranged concentrically around coarse core neuropil. Specific domains of terminals are visited by the dendritic trees of inter- and intrasegmental interneurons. The neuroanatomy suggests that motor neurons supplying leg muscles receive the great majority of their sensory afferents from the leg of the same side and segment. The same motor neurons are richly supplied by local and interganglionic interneurons. Interganglionic connections are also provided by certain sensory afferents which extend between leg neuromeres. However, unlike the direct primary afferent supply to the brain from the wings, the passage of sensory information from the legs to the brain may be mediated by interneurons with segmentally repeated dendritic trees, the axons of which ascend to the descending neuron dendrites originating in the dorsolateral deutocerebrum. The organization of leg neuromeres is compared to their segmental homologues in the brain; chemosensory antennal lobes and antennal mechanosensory neuropils of, respectively, the ventral and dorsolateral deutocerebrum. **Supported by NSF Biological Centers Grant DIR 82-20082**

169.13

NEURAL CORRELATES OF DIRECTIONAL SELECTIVITY IN THE VISUAL SYSTEM OF THE FLY.
H. Öğmen. Dept. of Elect. Eng., University of Houston, Houston, TX 77204.

Recent efforts in the understanding of neural correlates of motion detection and directional selectivity in the visual system of the fly includes electrophysiological studies using single photoreceptor stimulations (Riehle & Franceschini, *Exp. Brain Res.*, 54:390-394, 1984) and a combination of electrophysiology and neuropharmacology (Schmid & Bülthoff, *Biol. Cybern.*, 59:71-80, 1988). Results of the former have been interpreted in favor of facilitatory models while results of the latter in favor of inhibitory models thus creating an apparent conflict. Predictions of a neural network model proposed for directionally selective cells in the visual system of the fly (Öğmen & Gagné, *Neural Networks*, 1990) for single photoreceptor stimuli as well as for neuropharmacological manipulations offer a possible resolution to the paradox and suggest that motion detection and directional selectivity should not be confined into a single synaptic interaction.

169.14

ANALYSIS OF ADJACENCY AMONG NEURONS IN THE FLY OPTIC LOBE. A. Fröhlich. Biol. Dept., MSVU, Halifax, NS, Canada B3M 2J6.

The relationship between the architecture of a neuropile and the synapse classes found therein was analyzed from serial EM-sections of the first optic neuropile, or lamina, of the fly Musca domestica. As many profiles of cell processes as possible were identified uniquely within a defined proximal area of the lamina. Then the frequency was scored with which profiles of certain cells are direct neighbours to profiles of other cells. Results show that at least half of all processes directly adjacent to neuronal processes are those of epithelial glial cells. Adjacency between neurons is most frequent between profiles of those neurons that occur together at synapses, be it as pre- and postsynaptic partners, or as postsynaptic processes opposite a shared presynaptic site of a multiple-contact synapse. Adjacency is least frequent between profiles of the same cell or cell type. Self-avoidance is absolute in processes of neurons L1 and L2. Some contact (<5%) occurs between axons of different photoreceptors, alpha processes of amacrine cells and beta processes of T1 cells. The results imply that the contact preferences of cells that determine the architecture of the lamina influence the composition of its synapses as well.

169.15

SINGLE UNIT ANALYSIS OF VISUAL INTERNEURONS IN THE MESOPELAGIC MYSID, Gnathophausia ingens. J.F. Moeller and J.F. Case*. Univ. of Calif., Santa Barbara, CA, 93106.

Gnathophausia ingens inhabits a daytime depth of 600 m, dispersing between 400 and 1000 m at night. To determine visual capabilities of this deep-sea mysid, sensory neurons traversing the optic stalk were examined electrophysiologically with tungsten electrodes. Based upon their response to a single monochromatic light flash (10-1000 msec duration), interneurons were classified into two types. One class resembled sustaining fibers common to most crustaceans; the other class exhibited a unique "on" response without sustained firing. Both classes showed a 150 msec delay between stimulus and onset of spikes. Latency was inversely proportional to stimulus intensity. Response characteristics of these neurons may be indicative of a sensory system optimized for photon capture. Light levels of 10^4 photons sec^{-1} cm^{-2} could elicit a response.

169.16

AN INTERSTITIAL MATRIX BELT: ACTIN NETWORK FOR TUNING OPTICS IN NEURAL SUPERPOSION EYES. M. Järvilehto, R. Harjula*, and M. Weckström*. Dept. of Zoology, Univ. of Oulu, Linnanmaa, 90570 Oulu, Finland.

The interommatidial space (IOS) connecting the rhabdomeres of one ommatidium in the compound eye of the blowfly contains actin filaments (Järvilehto, M. et al. Soc. Neurosci Abstr., Vol. 15, Part 2, p.1292, 1989). The optical axes of the photoreceptors in seven different ommatidia feeding the same second order neuron are precisely aligned. Changes in deep pseudopupil (DPP), an indicator for the parallelity of the optical axes, were recorded in white mutant blowflies with low aperture optics using a video recorder. Actin was localized in EM by immuno-cryo-techniques (PAG). In detail we have located actin to a narrow region in the distal part of the IOS. Filaments are frequently found connecting R(3,4) and R(7) receptor cells. We dis rupted actin filaments by allowing weak (1-0.01 M) KI in Ringer solution diffuse into retina via small corneal hole. The DPP disappeared in a few minutes depending on concentration. Thus the actin network in distal IOS seems to play an important role in maintaining the optical alignment of the neuro-ommatidium.

169.17

DIFFERENTIATION OF PHOTORECEPTOR CELLS IN MANDUCA SEXTA. R.H.White and R.R.Bennett. Dept. of Biol., Univ. of Mass./Boston, Boston, MA 02125.

We are studying the differentiation of photoreceptor microvilli (rhabdomere) and smooth endoplasmic reticulum (SER) in relation to rhodopsin synthesis and the availability of the chromophore, 11-cis 3-hydroxy retinal (R3), in the pupal tobacco hornworm moth. The initiation of microvillus formation at the center of the clustered retinula cells, early in the pupal instar, is accompanied by the elaboration of adjacent tubular SER oriented parallel to the axes of the microvilli. Although SER is intimately associated with with the microvilli throughout their development - fingers of SER later extend deep into the growing microvilli - SER does not appear to contribute directly to microvillus formation by membrane flow. SER proliferates and becomes organized in myeloid bodies as development proceeds into the late pupa. The synthesis of opsin is regulated by the availability of R3. Normal rhodopsin levels are not required for microvillus differentiation; microvillus P-face particle density is reduced in R3 deficient moths at all stages of development. In the transition to the adult, SER fingers withdraw from the microvilli leaving a more limited system of subrhabdomeric cisternae, and much of the extensive pupal SER is degraded. In R3 deficiency, fingers and myeloid bodies remain. Thus R3 and/or rhodopsin deficiency affects the maturation of the SER, probably in relation to chromophore metabolism or transfer of rhodopsin from endomembrane to photoreceptor membrane. Supported by NSF grant BNS-8510087.

169.18

PHYSIOLOGY AND MORPHOLOGY OF GUSTATORY INTERNEURONS IN THE SUBESOPHAGEAL GANGLION OF THE FLESHFLY SARCOPHAGA BULLATA. B.K. Mitchell* and H. Itagaki. ARL, Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

The peripheral coding of gustatory information and the behaviors resulting from gustatory stimulation have been particularly well-documented in some Cyclorrhaphous Diptera by numerous investigators. Each gustatory sensillum on the labellum of the mouthparts is innervated by four chemosensory neurons. Three of these have been well-characterized, with one neuron preferentially sensitive to sugars and some amino acids, a second to cations, and the third to water. Despite the wide interest in the peripheral taste system and feeding behavior of these insects, the central processing of gustatory information leading to the control of feeding has not been directly studied. To address this, we have begun to record intracellularly from gustatory interneurons in the subesophageal ganglion of the fleshfly while stimulating the labellar sensilla with water and solutions of sucrose and of KCl. Since nothing is known about specific connections between the gustatory afferents and central cells, the first requirement is a catalogue of central cell types involved. So far, we have evidence for cells 1) stimulated by sucrose, but not KCl 2) stimulated by sucrose and inhibited by KCl 3) inhibited by sucrose. We hope to describe the morphology of these cell types, and others that respond to different taste stimuli using fluorescent dye techniques.

(Supported by USDA Grant 87-CRCR-1-2362, NIH Postdoctoral Fellowship NS07990, NSERC Canada, and Monsanto Co.)

169.19

WITHDRAWN

170.1

MODELLING THE SALAMANDER OLFACTORY BULB: SINGLE CELL AND NETWORK INTERACTIONS. J. White, S.N. Neff*, A. Cinelli, and J.S. Kauer. Neurosci. Program, Tufts/NEMC, Boston, MA 02111.

Anatomical, physiological, and behavioral data obtained in the tiger salamander now allow modelling of odor encoding properties of the peripheral olfactory system: the olfactory epithelium (OE) and bulb (OB). Available data include single receptor and bulbar cell responses, topography of OE and OB connections, OE and OB response distributions using voltage-sensitive dyes (VSD), and behavioral odor responses. We have used two different modelling approaches that incorporate these data. The first models the temporal and spatial response properties of single OB cells. Cell responses are calculated with a series of linked differential equations representing activity and connectivity of various OE and OB cell types. The model computes the output in each cell type after simulated odor or electrical stimulation. Outputs computed for mitral/tufted cells compare very well with those observed in intracellular and VSD recordings. The second model uses a back propagation neural network algorithm from McClelland and Rummelhart (1988) to relate receptor cell loci with activity in their OB projection sites. The model is trained with OE to OB electrical mapping data and then uses known patterns of OE odor sensitivities to calculate OB odor response distributions. Computed patterns compare well with OB responses seen in VSD recordings.

Supported by grants from USPHS, ONR, Pew Freedom Trust, and the Dept. of Neurosurgery.

170.2

EXCITATORY ACTIONS OF GABA IN THE RAT OLFACTORY BULB. B. K. Rhoades and W. J. Freeman. Department of Molecular and Cell Biology, Univ. of Cal. at Berkeley, Berkeley, CA 94720.

GABA is an intrinsic neurotransmitter of the vertebrate olfactory bulb, released primarily by the superficial periglomerular (PG) cells and the deeper granule cells. GABAergic actions of granule cells are inhibitory, at A-type receptors of reciprocal dendrodendritic synapses with mitral and tufted projection neurons. GABAergic periglomerular cells are also generally considered to have inhibitory actions at both A- and B-type receptors. However, electrophysiological evidence, and subsequent ensemble- level modelling, from our laboratory strongly indicates that periglomerular cells are mutually excitatory and excite mitral and tufted cells.

GABA actions in the rat olfactory bulb were investigated in barbiturate anesthetized animals with a unilaterally exposed main olfactory bulb, primary olfactory nerve (PON), and lateral olfactory tract (LOT). Averaged evoked potential (AEP), spike post-stimulus time histogram (PSTH), transbulbar DC potential, and ongoing surface EEG responses to local and regional application of GABAactive neurochemicals were recorded.

GABA and baclofen (B-agonist) increased the slow negative PON-induced AEP component and LOT-induced AEP frequency; both indicative of increased PG cell activity levels and excitation of mitral/tufted cells. Picrotoxin (Cl^- channel uncoupler) and 5-aminovaleric acid (B-antagonist) had opposite AEP effects; indicative of decreased periglomerular excitation. Muscimol (A-agonist) and bicuculline (A-antagonist) had effects attributed to action at granule cell synapses. These results were confirmed by DC recording and periglomerular and mitral cell PSTHs. Ongoing EEG was altered by regional, but not local, neurochemical applications. Established neuropharmacology suggests disinhibition, but these electrophysiological results indicate direct GABAergic excitation by PG cells.

Research supported by NIMH grant MH06686.

170.3

GABAERGIC CIRCUITS OF DISSOCIATED MOUSE OLFACTORY BULB CELL CULTURES J. Weil and S.P. Fracek, Jr. Department of Biological Sciences and Center for Network Neuroscience, University of North Texas, Denton, TX 76203

The effects of g-amino butyric acid (GABA) and the GABA antagonist, bicuculline (BIC), on the dynamics of neural networks of mouse olfactory bulb monolayer cultures are examined. The cells are grown on multimicroelectrode plates that have 64 extracellular electrodes in a 16x4 array; within two weeks these cultures form networks that spontaneously generate bursting activity. Fast Fourier Transforms (FFTs) of single-unit baseline data show a broad peak between 1 and 3 Hertz (Hz). Phase-space reconstructions (PSR) are a method of determining the degree of order in a time series. PSRs of various parameters (e.g. burst duration) do not show any apparent patterns. GABA ($\geq$ 25 μM) consistently inhibits the spontaneous bursting; GABA (< 25 μM) causes either short term pauses (lasting only a few minutes), or decreases the bursting frequency. BIC ($\geq$ 5 μM) consistently generates a simple periodic pattern, the bicuculline pattern. FFTs of these data indicate a peak frequency of approximately 0.5 Hz. PSRs show a simple lobe pattern indicative of periodicity. At 5 μM BIC, this pattern can be changed back to the baseline state with several washings. At 100 μM BIC, this pattern persists through subsequent washes and within an hour decays to another, more complex state, that is similar to the original baseline state. These cultures are sensitive to the sequential order and concentration of both these compounds. Bursting activity is inhibited or becomes periodic if the sequence addition starts with GABA or 5 μM BIC, respectively. Addition of 100 μM BIC first and subsequent additions of GABA (up to 100 μM) show no inhibition of the bicuculline pattern. However, PSRs of these data indicate that subsequent GABA addition causes the system to enter a higher periodic state.

This research is supported by NSF Grant BNS-8719319, an ONR Contract N00014-90-J-1445, and a Faculty Research Grant (to SPF). The Center for Network Neuroscience is supported by grants from the Hillcrest Foundation of Dallas TX founded by Mrs. W.W. Caruth Sr. and the Texas Advanced Research Program (to G.W. Gross in whose laboratories these experiments were conducted).

170.4

IMMUNOCYTOCHEMISTRY OF DISSOCIATED MOUSE OLFACTORY BULB CELL CULTURES L. Guo*, T. Thomas*, R. Schafer and S.P. Fracek, Jr. Department of Biological Sciences and Center for Network Neuroscience, University of North Texas, Denton, TX 76203 USA.

The morphology and immunocytochemistry of intact murine olfactory bulbs (adult and embryos) was compared to that of dissociated mouse olfactory bulb cell cultures using antibodies to microtubule associated protein 2 (MAP2), tau protein (Tau), neurofilament 200 (NF200), and glial fribrillary acidic protein (GFAP). These analyses were carried out using both light microscopy (LM) and transmission electron microscopy (TEM). For LM, the streptavidin-biotin method (ABC) and 3.3'-diaminobenzidine was used to visualize the antibodies; for TEM, the streptavidin-biotin immuno-gold labelling technique was used. Cultured murine bulb cells were essentially similar to those found in intact bulbs, as judged by morphology and expressed proteins.

MAP2 antibodies heavily stained cultured nerve cell bodies and their thick, dendrite-like processes, when viewed with LM. At the TEM level, immuno-gold particles were found adjacent to microtubules in the soma and the dendritic-like processes. Non-specific gold staining of the background and glial cells was far lower than that associated with neurons. LM revealed that Tau antibodies lightly stained the soma. Some thick fascicles of cell processes were also lightly stained. TEM revealed Tau staining nerve cell soma, but always much less than MAP2. Some axon-like processes within thick fascicles had lightly stained regions adjacent to microtubules. NF200 heavily stained the cultured neurons, especially the soma and fascicles. GFAP, as expected, was found in glial cells only.

This research is supported by NSF Grants CHE-8509557 (to RS), BNS-8719319, ONR Contract N00014-90-J-1445, and a Faculty Research Grant (to SPF). The Center for Network Neuroscience is supported by grants from the Hillcrest Foundation and the Texas Advanced Research Program.

170.5

ANALYSIS OF CALCIUM INVOLVEMENT IN OPTICAL SIGNALS FROM THE IN VITRO SALAMANDER OLFACTORY BULB. A.R. Cinelli and J.S. Kauer. Neuroscience Program, Tufts/NEMC, Boston, MA 02111.

The long-lasting depolarizations previously seen in subglomerular levels of the olfactory bulb (OB) after ortho-(OD) and antidromic(AD) electrical stimulation have been attributed to Ca++ currents (Cinelli and Salzberg, Nrsci. Abst., 1988). In the present study these slow components were recorded in OB slices from waterphase Ambystoma tigrinum using the voltage-sensitive dyes RH414 and RH795. Video-rate imaging was used to evaluate the spatial properties of the signals and to examine effects of manipulating Na+ and Ca++ currents. Electrical stimuli applied locally to the glomerular layer elicit these signals in 10uM TTX or low [Na+] medium, while OD or AD stimuli do not, but lateral and central spread of the signals were reduced and the time courses of depolarization and suppression were increased. Since signals were still observed after 3.0 mM Ba++, it appears they did not arise from glia. These data suggest that these depolarizing signals are Ca++ current related and that Na+ affects their spatial distribution. These signals are presently being investigated using fluorescent calcium indicators.

Supported by the NIH, Pew Freedom Trust, and Dept. of Neurosurgery.

170.6

EVOKED NEURAL ACTIVITY IN THE SALAMANDER OLFACTORY BULB MONITORED WITH CALCIUM, pH, AND VOLTAGE-SENSITIVE PROBES. D.M. Senseman, J.-Y. Wu, and L.B. Cohen. Division of Life Sciences, University of Texas at San Antonio, San Antonio, TX, 78285 and Department of Physiology, Yale University School of Medicine, New Haven, CT, 06510, .

In the Tiger salamander (*Ambystoma tigrinum*) brief electrical stimulation of the olfactory nerve evokes a prolonged activation (>1000 ms) of the *in vitro* olfactory bulb and more central cortical structures that can be monitored optically with millisecond temporal resolution using voltage-sensitive probes in conjunction with a 124-element photodiode array. We recently found that evoked neural activity in this preparation can also be monitored optically using probes that are selective for changes in intracellular calcium (rhod-2 AM), extracellular calcium (murexide) and pH (phenol red). Nerve stimulation produced a transient increase in resting fluorescence after loading the *in vitro* preparation with the membrane permeant calcium indicator rhod-2 AM for 70 min. The fluorescent increase continued for 300 ms followed by a slow decay to base-line lasting several seconds. Significant differences in waveform were observed between the olfactory nerve, glomerular and granular layers. Qualitatively similar waveforms, but opposite in sign, were recorded with transmitted light when the preparation was bathed in saline containing the extracellular calcium indicator murexide (0.75 mg/ml). These data suggest that neural activity in the olfactory bulb is associated with a rise in intracellular calcium that is derived in part from extracellular sources. Nerve stimulation was also found to generate a transient decrease in light intensity when the preparation was bathed in unbuffered saline containing the pH indicator, phenol red (1 mg/ml). Unlike the 'calcium signals' which were monophasic, the initial decrease in light intensity was followed by a transient increase. In addition to voltage-sensitive dyes, calcium and pH probes may also prove useful for monitoring activity in the mammalian cortex.

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170.7

PARABRACHIAL GUSTATORY RESPONSES IN AWAKE RATS: AN EXTENDED STIMULUS ARRAY. H. Nishijo* and R. Norgren Dept. Physiol, Fac. Medicine, Toyama Med. Pharm. Univ., Toyama 930-01 JAPAN and Dept. Beh. Science, Col. Medicine, The Pennsylvania State University, Hershey, PA 17033.

Previously we reported that parabrachial (PBN) gustatory responses to 4 standard stimuli were equivalent whether the fluids were delivered via intraoral infusion or by licking (Neurosci. Abst. 15: 930, 1989). In the present study, a total of 74 single PBN neurons were tested with up to 15 sapid chemicals delivered by intraoral cannula. Of these, 70 responded differentially to one or more of the stimuli; the remaining 4 responded similarly to both water and tastants. Of the 70 taste neurons, 44 were tested with all 15 chemicals. Cluster analysis revealed 5 stimulus groups with mean intergroup correlations of less than 0.4. These groups consisted of the 3 Na-salts, the 3 Cl-salts, the 3 acids, quinine HCl, and 5 normally preferred chemicals. In this last group, polycose had an average correlation of 0.61 with 4 'sweet' chemicals. Factor analysis of the same data produced 4 factors that accounted for 85.6% of the variance. With the exception of QHCl, the individual factors grouped the stimuli as did the cluster analysis. The lowest loading for a chemical within a group was 0.75. Excluding quinine, the highest loading for a chemical outside a group was 0.39. Quinine had loadings of 0.52 and 0.43 in the factors that contained the high Cl-salt loadings and the high acid loadings, respectively. Supported by PHS grants NS 00240, MH 43787, and MH 00653.

107.8

GUSTATORY RESPONSES FROM THE NUCLEUS OF THE SOLITARY TRACT IN AWAKE RATS. K. Nakamura and R. Norgren Dept. Behavioral Science, College of Medicine, The Pennsylvania State University, Hershey, PA 17033.

Single neurons were isolated from the nucleus of the solitary tract of alert rats using previously described techniques (Nishijo & Norgren, **J Neurophys.** 63: 707, 1990). Fluid stimuli consisted of 50 ul samples of water, sucrose, NaCl, citric acid (CA), and quinine HCl (QHCl) delivered via intraoral cannulae. From a total of 78 neurons, 70 responded significantly to at least one of the sapid stimuli. The mean spontaneous rate of the taste neurons was 4.0±5.9 (SD) spikes/sec; the mean response to water (above spont. rate), 3.7±5.4 spikes/sec. Gustatory responses were considered significant if the activity elicited by a sapid chemical differed from the mean, uncorrected water response for each neuron by ± 2.5 SD. Based upon their largest response, taste neurons were classified as follows: 29 sucrose-best, 21 acid-best, 17 NaCl-best and 3 QHCl-best. Fifty percent of the neurons (35/70) responded significantly to only one of the four sapid stimuli, but the degree of specificity varied with best-stimulus category. Of the 21 acid-best neurons, 19 (90%) responded only to CA. For sucrose-best cells, specific sensitivity was less common (45%, 13/29), and for NaCl-best neurons, it was relatively uncommon (18%, 3/17). None of the QHCl-best neurons was specific. In general, gustatory neurons in the NST of awake rats resemble acutely recorded, peripheral taste cells more than chronically recorded parabrachial ones. Supported by PHS grants NS 00240, MH 43787, and MH 00653.

107.9

PROJECTIONS OF ELECTROPHYSIOLOGICALLY-IDENTIFIED LOCI IN THE ORALLY-RESPONSIVE SOLITARY NUCLEUS D. Becker* and S. Travers. Depts. of Psychology and Oral Biology, The Ohio State University, Columbus, OH 43210.

The nucleus of the solitary tract (NST) receives primary afferent terminations from gustatory and somatosensory afferents innervating the oral cavity. Recent neurophysiological experiments in this laboratory have demonstrated that NST neurons are organized as a function of modality and receptive field location (Travers and Norgren, '88). The present experiments examined efferent projections of small regions of NST that responded to specific stimulations of the oral cavity. Iontophoretic injections of horseradish peroxidase (HRP) or Phaseolus vulgaris leucoagglutinin (PHAL) were made through one side of a double-barrelled pipette (Dia=15u), whose second barrel was used for recording. When injections were made into regions of the NST that responded to gustatory or mechanical stimulation of the foliate and/or circumvallate papillae, a distinct pattern of label was seen in the reticular formation and NST. Terminal labelling was observed in the reticular formation directly subjacent to the NST, both rostral and caudal to the injection site. Terminal labelling was also present in the NST and was prominent caudal to the injection site in the medial and ventral subnuclei but not in the central subnucleus. The intrasolitary projections include regions implicated in processing visceral afferent signals (e.g., Hamilton and Norgren, '83) and in triggering the pharyngeal phase of swallowing (Hashim et al., '89). These projections provide an anatomical basis for the interaction of oral afferent information with visceral afferent signals and ingestion. Supported by NIH R29 DC00416.

107.10

SIMULATION OF THE MEMBRANE CURRENTS REVEALED BY CURRENT SOURCE DENSITY ANALYSIS IN PIRIFORM CORTEX PROVIDES INSIGHT INTO THE DYNAMICS OF PROPAGATION IN EXCITATORY FIBER SYSTEMS. K.L.Ketchum and L.B.Haberly. Dept. of Anatomy, Univ. of Wisc., Madison, WI 53706.

The membrane currents in a model of piriform cortex (PC) replicated those revealed by current source density analysis of the response to stimulation of afferent fibers in the lateral olfactory tract (LOT) in vivo. The model reproduced the timecourses of membrane currents by incorporating known excitatory and inhibitory synaptic inputs and with voltage dependent conductances. As a consequence of distributed conduction velocities and the large number of axons, activity in excitatory fiber systems was described as a continuous wavefront that disperses as it propagates. The timecourse of synaptic input was calculated by convolution of this dispersive wavefront and a synaptic conductance waveform. The resulting input waveforms were appropriately distributed in depth over a discrete cable of variable caliber that simulated the predominate pyramidal cell population. This reproduced capacitative effects on the timecourses and spatial distributions of membrane currents. It was found that the wavefront evoked by the afferent fiber volley could be described by a single dispersive wave originating at the caudal end of the LOT. This suggests that the wavefront remains coherent as it propagates in the LOT but disperses as it travels in slower LOT collaterals. It was found that the response component mediated by association fibers that originate in anterior PC could be described by a sum of dispersive wavefronts originating across the rostral to caudal extent of anterior PC. As a further validation of the model, the same parameters used to generate the afferent and association waveforms at the initial site successfully predicted changes in the waveforms at different recording sites. It is proposed that dispersive propagation within excitatory fiber systems provides a mechanism for analysis of temporally distributed information. While from the viewpoint of the site of origination information becomes spatially dispersed as it propagates, from the viewpoint of the postsynaptic neuron information is concentrated at a particular instant from sources activated at different times. Supported by NINCDS grant NS19865 to LBH and NRSA grant NS08328 to KLK.

107.11

THETA STIMULATION AND LONG-TERM POTENTIATION IN THE PIRIFORM CORTEX. J. S. Stripling, M. Paz Galupo*, and D. K. Patneau. Department of Psychology, University of Arkansas, Fayetteville, AR 72701.

Stimulation of association fibers in the piriform cortex (PC) using 10-pulse trains repeated at 10 sec intervals produces a selective long-term potentiation (LTP) of period 2 in PC evoked potentials (Stripling and Patneau, Soc. Neurosci. Abstr. 14: 1189, 1988). In contrast, stimulation with 4-pulse trains repeated at theta frequency potentiates transmission at either lateral olfactory tract (LOT) or association fiber synapses in the PC slice (Kanter and Haberly, Soc. Neurosci. Abstr. 15: 929, 1989), or, when applied to the LOT and used as a discriminative cue in intact animals, produces LTP of the A_1 component of the evoked potential, signifying increased transmission at LOT synapses (Roman, Staubli, and Lynch, Brain Res. 418: 221-226, 1987). The present experiment compared the effects of theta vs. spaced stimulation applied to the LOT or PC association fibers in intact animals in the absence of a learning task.

Male Long-Evans rats were stimulated in the LOT or layer Ib of the PC using 4-pulse 100 Hz trains. Layer Ib stimulation at theta frequency (200 ms inter-train interval) produced LTP of period 2 with no change in A_1. Stimulation of the LOT at theta frequency or layer Ib with trains at 2 sec intervals had no effect. Theta stimulation was more effective than spaced stimulation in triggering an afterdischarge, but this also had no effect on A_1. These results indicate that theta stimulation by itself is relatively ineffective in altering LOT transmission in intact animals, but can potentiate period 2 if cortical association fibers are activated.

(Supported by NSF Grant BNS 85-19700 and a grant from the Marie Wilson Howells Fund.)

171.1

PHOTORECEPTOR INNER AND OUTER SEGMENTS IN TRANSPLANTED RETINA. Y. Liu*, M.S. Silverman and S.E. Hughes. Central Institute for the Deaf and Department of Ophthalmology and Visual Science, Washington University School of Medicine, St. Louis, MO 63110.

Previously we reported transplantation of the outer nuclear layer (ONL) from the developing and mature rat retina as well as the ONL from mature human retina to adult albino rats with light-induced loss of photoreceptors (Silverman and Hughes, IVOS 30:1684; PCBR 314:687, 1989). While the transplanted photoreceptors stained positive for opsin and formed synapses with host retina (Hughes, Valentino & Silverman, IVOS 31:594 Suppl., 1990) inner segments (IS) and outer segments (OS) were rare and were primarily seen within rosettes that formed in areas where the organization of the transplanted ONL was disrupted.

To investigate the role of inner retina and developmental constraints on the expression of IS and OS we transplanted sheets of 1) mature rat retina, 2) mature retina in which the ganglion cell layer was removed by vibratoming, 3) immature retina (8 day old) or 4) immature retina minus ganglion cells to the subretinal space of mature light-damaged rats using a transcorneal approach (IVOS, 30:1684).

Two weeks following transplantation we found that in each group the transplant showed photoreceptors with a complement of IS and OS that were apposed to the underlying pigment epithelium. The degree of ONL, IS and OS organization as well as the amount of segment expression seen in these groups was different, with mature transplanted retina approximating normal retina > immature retina > mature retina minus ganglion cells > immature retina minus ganglion cells.

These results show that transplanted photoreceptors can express a comparatively normal complement of IS and OS. The degree of organization of the ONL and the expression of photoreceptor IS and OS is strongly influenced by inner retinal components as well as the developmental maturity of the transplant.

Supported by NIH EY 07547 and Retinitis Pigmentosa International.

171.2

TRANSPLANTED PHOTORECEPTORS FORM SYNAPSES IN RECONSTRUCTED RCS RAT RETINA. T.L. Valentino*, S.E. Hughes, and M.S. Silverman. Sensory Neuroscience Laboratory, Central Institute for the Deaf, St. Louis, MO 63110.

Inherited retinal degeneration afflicts a variety of animals, including humans. Several animal models exist for inherited retinal dystrophy, including the RCS rat. By three months of age the RCS retina is devoid of photoreceptors, presumably due to a deficit in the retinal pigment epithelium. We have previously shown that it is possible to isolate the immature outer nuclear layer from 8 day old nondystrophic congenic rats and transplant the resulting photoreceptor layer to the subretinal space of adult dystrophic animals (Hughes and Silverman, *Soc. Neurosci. Abstr.* 14: 1277, 1988; Silverman and Hughes, *PCBR* 314: 687, 1989). The transplanted photoreceptors integrate with the host retina, forming a new outer nuclear layer.

In a recent study involving transplantation of photoreceptors to light-damaged retina, we have shown that a new outer plexiform layer forms between the transplanted photoreceptors and the host inner nuclear layer (Hughes et al., *Invest. Ophthalmol. Vis. Sci.* 31 (Suppl): 594). The new outer plexiform layer contains ribbon synapses characteristic of photoreceptor synapses. We thus sought to determine whether similar events occur in the reconstructed RCS retina.

In transplants to the dystrophic RCS retina, a new outer plexiform layer is visible at the interface of the transplanted outer nuclear layer and the host inner nuclear layer. Ribbon synapses are frequently seen within this outer plexiform layer. These synapses are characteristic of rod spherules, with an electron dense ribbon surrounded by a cluster of vesicles. These synapses persist for at least three months. Ribbon synapses are extremely rare in control dystrophic retina.

These results indicate that transplanted photoreceptors form connections with elements of the host inner nuclear layer and may be used to reconstruct the dystrophic RCS retina.

Supported by NIH EY-07547 and the National Retinitis Pigmentosa Foundation.

171.3

IN SEARCH OF A RECEPTOR FOR OUTER SEGMENTS IN RAT RETINAL PIGMENTED EPITHELIUM. L. Tien and N.G.F. Cooper. Dept. of Anatomy and Neurobiology, Univ. of Tenn, Memphis, TN 38163

The phagocytosis of photoreceptor outer segments is thought to be a receptor-mediated process. A procedure has been developed to study membrane-associated glycoproteins of pigment epithelial cells to look for receptors of photoreceptor outer segments. The method involves the oxidation of the carbohydrate moieties of the RPE apical membrane glycoproteins. The resultant aldehyde groups are then labeled with biotin hydrazide. Biotinylated glycoproteins are then demonstrable through interaction with an avidin-based enzyme or avidin-based FITC probe. Three biotinylated glycoproteins with the MWs of 175 kD, 150 kD and 130 kD are detected with peroxidase linked streptavidin in Western blots. At the electron microscope level, the biotinylation sites are distributed on the microvilli of the RPE. The biotinylated RPE protein mixture was incubated with the neural retina and binding was detected through the use of streptavidin-FITC. The tips of the outer segments (OS) of the retina are fluorescently labeled and the inner retina is not labeled. Control sections which were not pretreated with the biotinylated RPE-proteins do not show any fluorescent labeling. Our results suggest that at least one of the three biotinylated RPE apical membrane glycoproteins seen in our blots can bind to the outer segments in tissue sections and they are therefore candidates for outer segment receptors.

171.4

ULTRASTRUCTURAL LOCALIZATION OF RPE EPITOPES IN IN SITU AND CULTURED RPE CELLS AND THEIR EXPRESSION IN FIBROBLASTS IN VITREOUS CULTURE. S.A.Vinores,P.A.Campochiaro*,W.Orman*, J.J.Hooks*,and B.Detrick*,Dept.Ophthalmol.,Univ.Virginia Sch.Med.,Charlottesville,VA and NEI,NIAID,NIH,Bethesda,MD.

Two distinct monoclonal antibodies (MAbs) against human retinal pigment epithelial (RPE) cells specifically stained RPE cells in situ by light microscopic immunohistochemistry. By electron microscopic immunocytochemistry, the labelling patterns of both MAbs were indistinguishable. In normal human RPE, the MAbs labelled the cytoplasm and surface and intracellular membranes. Other retinal and choroidal cells were negative. In vitro studies were performed to evaluate the effect of vitreous fluids on cell phenotype. Human RPE cells cultured on irradiated bovine vitreous demonstrated prominent staining on filaments of processes and on ribosomes in addition to cytoplasmic and membrane positivity. Human fibroblasts, which were normally negative, reacted with the RPE MAbs in the same pattern as RPE cells when maintained in vitreous culture. Unrelated MAbs failed to label RPE cells or fibroblasts. The induction of the two RPE-associated epitopes in fibroblasts suggests that their expression is related to metabolic or structural differences regulated by a component of bovine vitreous. These studies determine the cellular locations of specific RPE cell epitopes and demonstrate the effect of the vitreous environment on fibroblasts and RPE cells. These vitreous-induced alterations may also occur in ocular disorders such as proliferative vitreoretinopathy.

171.5

ULTRASTRUCTURE AND TRANSDUCTION IN THE CAUDAL PHOTORECEPTOR OF CRAYFISH. B. Kruszewska and J.L. Larimer. Zoology Dept., University of Texas, Austin, TX 78712

The well-known caudal photoreceptor (CPR) of crayfish (Prosser, J.Cell. Comp.Phys. 4:363,1934) has recently been assigned a command role for a particular behavior (Edwards, JEB 109:291,1984). Little is known, however, regarding its ultrastructure or the molecular basis of its transduction of light. Recording and injection techniques were combined with electron microscopy to determine whether the cell is a primary photoreceptor and to elucidate the transductive mechanism. The cell was impaled and pressure injected with 100 μM inositol(1,4,5,)trisphosphate (IP_3). IP_3 is known to play a role in signal transduction by stimulating release of internal calcium stores. Injection of IP_3 into the soma or dendrites mimicked the response to light, resulting in depolarization and increasing the firing frequency in proportion to the strength of injection. The effect was reversible and the cell continued to be light-sensitive between injections. Injection of 2mM $CaCl_2$ had the same effect. Control injections of IP_1, cGMP and KCl were sometimes accompanied by a change in membrane potential, but had no effect on firing rate. Control injection of IP_3 into other (non-CPR) cells showed no effect on firing rate in approximately 87% of cells impaled. The number of cells responding to $CaCl_2$ was higher.

The ultrastructure of the cell was also examined with the goal of determining whether it contained any phototransductive organelles. The cell was labeled by pressure injection with 0.01% 2nm colloidal gold particles and processed using standard electron microscopy techniques. Several processes of the labeled cell contained structures composed of layers of concentric membrane, structures that would be useful for maximization of photon capture by visual pigments. These structures, together with the clear response to injection of IP_3, suggest that the CPR is a primary photoreceptor and that transduction takes place by means of a second messenger system. (Supported by NIH NS05423)

171.6

LIGHT-REGULATED PROTEIN PHOSPHATASE ACTIVITY IN LIMULUS VENTRAL PHOTORECEPTORS. S.C. Edwards. Dept. of Biology, Univ. of South Florida, Tampa, FL 33620.

Exposure of dark-adapted (DA) Limulus ventral photoreceptors to an adapting light flash stimulates the phosphorylation of a 46 kD protein (46A), a major substrate for a calcium\calmodulin dependent protein kinase (CaCAM PK). Okadaic acid (OKA), a potent inhibitor of protein phosphatases 1 and 2A (PrP 1; PrP 2A), blocks the subsequent 46A dephosphorylation that normally occurs following the flash. This suggests that light increases the activities of both the kinase (presumably CaCAM PK) and the protein phosphatase that catalyzes 46A dephosphorylation (46A PrP), but with a latency of activation compared to the kinase. The level of phosphorylation of 46A in long term light adapted (LA) and DA cells is the same even though Ca_i in LA cells is elevated sufficiently to increase CaCAM PK activity. Addition of OKA to these preparations results in the selective increase in the level of 46A phosphorylation in LA, but not DA cells suggesting that the activities of both 46A PrP and CaCAM PK are elevated in LA cells. The identity of 46A PrP and how it is activated by light are presently unknown. While it is possible that it is either PrP 1 or PrP 2A since the concentration of OKA utilized so far in this study was 1 uM, a concentration sufficient to activate either enzyme, the narrow specificity for 46A is not characteristic of either enzyme. (Supported by NIH EY08765.)

171.7

DOES SEAWATER CAUSE STRUCTURAL LIGHT ADAPTATION IN *LIMULUS* VENTRAL PHOTORECEPTORS *IN VITRO* ? - A STUDY OF ORGAN CULTURE VS. SEAWATER INCUBATION. Robert N. Jinks* and Steven C. Chamberlain. Department of Bioengineering and Institute for Sensory Research, Syracuse University, Syracuse, NY 13244-1240.

Although most studies have utilized excised ventral photoreceptors maintained in seawater, Bayer and Barlow (*J. Gen. Physiol.* 72:539) showed that excised photoreceptors maintained in organ culture retain functional properties more characteristic of the intact state. For example, they possess a greater dynamic range with better threshold sensitivity than those maintained in seawater. To examine possible structural bases for this difference, ventral optic nerves of four animals were dissected in light and then maintained at 4° C *in vitro* in darkness for 5 hrs before fixation. One nerve of each animal was incubated in seawater; the other was incubated in organ culture. Preparation for examination with TEM followed our standard procedures (*J. Gen. Physiol.* 80:839).

Contrary to our expectations we observed no consistent differences in the overall size or organization of the light-sensitive rhabdomere; however, we found a consistent and significant ultrastructural difference in its microvilli. In organ culture, as *in vivo*, adjacent microvilli of dark adapted cells were joined by fused, quintuple-layered membrane. By contrast, in seawater, these quintuple-layered junctions had disappeared and all microvillar profiles were completely surrounded by extracellular space, both in the external and internal rhabdom.

Similar breakdown of the fused contacts between adjacent microvilli occurs late in the afternoon in the lateral eye of intact animals (*J. Neurosci.* 4:2792), suggesting that loss of fused junctions correlates with extensive light adaptation. Perhaps changes to the microvillar organization in ventral photoreceptors maintained in seawater underlie functional changes observed under these experimental conditions.

Supported by NIH grants EY03446 and EY00667.

171.8

DO DOUBLE CONES MEDIATE VERTEBRATE POLARIZATION SENSITIVITY? D.A. Cameron*, M. Rowe*, N. Engheta* & E.N. Pugh,Jr. Inst. of Neurological Sciences, and Depts. of Electrical Engineering & Psychology, Univ. of Pennsylvania, Philadelphia, PA 19104.

Light-adapted green sunfish (*Lepomis cyanellus*) sensitivity to light linearly polarized at different angles (Θ) was measured in a Maxwellian-view optical apparatus with a classical conditioning paradigm (heart rate suppression). Threshold sensitivity was a W-shaped function of Θ with maxima at 0° (vertical) and 90° (horizontal), and up to 0.8 log units less-sensitive at oblique angles. A dielectric waveguide model of the sunfish double cone has been developed, and shows promise in accounting for the threshold data. The propagation characteristics of optical waves inside this guide for electric field vectors oriented parallel to either the long or short axis of the waveguide cross-section have been analyzed numerically. Preliminary results reveal a dependence of mode propagation on the optical wave polarization in the model. We hypothesize that (1) the individual double cone acts as a polarization analyzer, and (2) the orthogonal, tetradic arrangement of the double cone mosaic determines the polarization-sensitivity curve in this vertebrate. Anatomical work in progress is examining the prediction that the double cone tetrads under the Maxwellian image are aligned along the axes (0° and 90°) of maximum polarization sensitivity. Theoretical work is aimed at demonstrating that the severe drop in sensitivity at oblique angles requires a neural polarization-opponent mechanism.

Supported by NIH grant EY-08260

171.9

EVIDENCE THAT HYPERPOLARIZATION DESENSITIZES TURTLE CONES BY REDUCING INPUT IMPEDANCE

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Previous experiments using the eyecup preparation have shown that adaptation may spread laterally between cones in the turtle retina (Copenhagen & Green, 1987; Itzhaki & Perlman, 1987). Using an isolated turtle retina preparation and pairs of microelectrodes we now show that the sensitivity of a cone may be reduced when it is hyperpolarized indirectly by current that is injected into a neighboring cone. The same result holds if current is injected directly into the cone itself.

In order to better understand the effects of directly applied current we used 50pA test pulses to measure cone input impedance in the presence and absence of direct, steady hyperpolarizing current. Under both conditions the voltage response was high-pass in nature, indicating the presence of a voltage-sensitive conductance not noted by others using a dark adapted eyecup preparation. Furthermore, hyperpolarizing current caused a decrease in both the peak and steady state resistance, suggesting that hyperpolarization-induced desensitization to light is a reflection of lower input impedance rather than a decreased photocurrent response. (Supported by NEI grant 00379.)

171.10

MODULATION OF CALCIUM-ACTIVATED CHLORIDE CURRENT IN CONE PHOTORECEPTORS WITH CHANGES OF EXTERNAL pH. Steven Barnes and Quynh Bui*, Department of Medical Physiology, University of Calgary Faculty of Medicine, Calgary, Alberta, Canada T2N 4N1.

Isolated larval tiger salamander cone photoreceptors exhibit robust Ca^{2+}-activated Cl^- tail currents following step depolarizations under whole-cell voltage-clamp when bathed in 10 mM HEPES-buffered media at pH 7.4 with 90 mM NaCl, 2.5 mM KCl, 3 mM $CaCl_2$ and 8 mM glucose (patch pipette: 100 mM CsCl, 3.5 mM $MgCl_2$, 1.5 mM Na_2ATP, 10 mM NaHEPES and 1 mM EGTA at pH 7.4). Decreasing bath pH (7.0) reduces Cl^- tail currents to nil, while increasing pH (7.8) leads to larger, sustained tail currents. Peak Ba^{2+} currents (20 mM Ba^{2+}) are reduced by decreasing pH (33% reduction, pH 6.8) and are enhanced by increasing external pH (25% increase, pH 7.8). The Ba^{2+} current activation range is also shifted positive 9 mV at pH 6.8 and negative 4 mV at pH 7.8, however neither the change in peak current nor activation shift accounts for the modulation of Cl^- current with pH.

Long depolarizations or repeated depolarizations in control solutions (pH 7.4) give slow Cl^- tail current decays similar to those in basic media (pH 7.5-7.8) suggesting that the enhancement of tail currents occurs as a result of an intracellular [Ca^{2+}] increase. However, EGTA's ability to buffer Ca^{2+} is reduced by decreasing pH, opposite to the reduction in Ca^{2+}-activated tail current seen with decreasing pH. Also, the $Na^+/Ca^{2+}/K^+$ exchanger of photoreceptors is probably not involved as block of exchange by internal Cs^+ has no effect on the pH dependence of tail current timecourse. Gating mechanisms intrinsic to the channel may be modified at different pH's.

Supported by the Alberta Heritage Foundation and MRC of Canada.

171.11

RESPONSES OF CULTURED CHICK EMBRYO PHOTORECEPTOR CELLS TO LIGHT: A ROLE FOR NEUROMODULATORS. **D. Stenkamp*, E. Adler-Graschinsky and R. Adler. Depts. of Neuroscience and Ophthalmology, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.**

We are investigating possible regulatory and developmental roles of light using cultures of chick embryo retinal cells which differentiate *in vitro* into neurons and photoreceptors (PhR). Cells grown in the absence of pigment epithelial and glial cells, with minimal intercellular contacts, were maintained from the time of seeding on a 12 hr light cycle (80 lux, from a tungsten or fluorescent source). Over 50% of the PhR contracted during the dark period and elongated during the light period. PhR elongation was accompanied by myoid constriction, resembling photomechanical movements *in vivo*. Some of the neuromodulators and second messenger systems known to affect photomechanical responses *in vivo* are also present in our cultures, and are now under investigation for possible regulatory roles *in vitro*. Ongoing studies show inhibitory effects of melatonin (10 μM) upon light-dependent elongation, and suggest that dopamine and cyclic nucleotides may also play a role.

Supported by USPHS grant EY04859, and an NSF graduate fellowship.

171.12

ADDITIONAL EVIDENCE SUPPORTING THE PRESENCE OF D2-LIKE RECEPTORS ON PHOTORECEPTORS OF THE MOUSE RETINA. A.I. Cohen. Dept. of Ophthal. and Visual Sciences, Washington University Sch. of Med., St. Louis, MO 63110.

Dark adapted (DA) mouse retinas isolated in dim red light and quick-frozen possessed about 60% more cAMP than did light adapted (LA) quick-frozen retinas. A similar disparity in cAMP and the ability of light to reduce the cAMP of dark incubated (DI) retinas were only preserved if retinas were incubated in media containing 1 mM IBMX or 5 mM Co^{++}. That a substantial part of the cAMP is located in DA mouse photoreceptors follows from the ability of light to reduce cAMP in DA-DI retinas in media containing 5 mM Co^{++} plus 10 mM glutamate or 1 mM IBMX plus either Co^{++} or 10 mM glutamate or aspartate or 50 μM kainate or 50 μM APB plus 6 mM PDA, agents said to confine the effects of light to photoreceptors. This pool was eliminated in DA-DI retinas if the medium contained either 0.1 μM dopamine (in the presence of 10 μM SCH 23390, a DI blocker), or synthetic D2 agonists, e.g. 1 μM LY171555 (an ergoline), 1 μM (+)N-0437 (an aminotetralin) or 100 μM (+)-3-PPP (a phenylpropylpiperidine). Similar results were observed if cAMP had been further increased by high K^+ in Cl^- free medium. The agonists had no action on the cAMP of LA-LI retinas in IBMX medium. Agonist actions were reversed by spiperone (in the presence of ketanserin) but not by substituted benzamides.

171.13

DIFFERENTIAL INCORPORATION OF DOCOSAHEXAENOYL (22:6)-PHOSPHOLIPIDS IN ROD AND CONE PHOTORECEPTOR CELLS OF THE FROG RETINA. N.G. Bazan, W.C. Gordon,* B.D. Shivers and E.B. Rodriguez de Turco,* LSU Eye Center and Neuroscience Center, New Orleans, LA 70112.

Docosahexaenoic acid (22:6) is avidly taken up by photoreceptors, cells that continually renew their membranes. When ^{3}H-22:6 is delivered to the retina by way of the circulatory system, the outer segments soon acquire an overall diffuse label. A highly labeled region at the base of the outer segment also forms, and gradually accumulates until this dense label reaches the photoreceptor tip about 28 d later (*J. Neurosci., July, 1990*). When ^{3}H-leucine (protein marker) is used, a dark band corresponding to newly synthesized disc membranes forms. This marker also migrates to the receptor tip, arriving in about 28 d. Measurements of migration rates of the 22:6 dense region and the leucine-labeled opsin suggest that these 2 membrane components are linked. However, isolation and counting of outer segment protein reveal no activity, demonstrating that the association is noncovalent. This suggests a functional role for 22:6 in the disc membrane in the region adjacent to the opsin molecule. This autoradiographic and biochemical study investigated the uptake pattern of 22:6 by isolated retinas over very short time periods. Some 22:6 diffusely labeled the outer segments within 15-30 min, however inner segments only began to label in the ER- and Golgi-rich region of the myoid after 30 min. 22:6 accumulated and migrated distally into the ellipsoid region just below the region of disc morphogenesis of the outer segment, where it continued to collect. The basal region of the outer segment began to label after 6-10 h incubation. Grain counts of these regions in autoradiographs revealed that the 435-rods accumulated 2-4 times as much 22:6 as the 502-rods, and cone cells only diffusely labeled. This differential uptake suggests either that photoreceptor types have different metabolic rates, or that the 22:6-containing phospholipid-visual pigment association differs for each type of opsin. NIH grant EY04428.

171.14

APOE-MEDIATED DELIVERY OF DOCOSAHEXAENOYL-PHOSPHOLIPIDS INTO NEURAL RETINA AND PHOTORECEPTOR CELLS (PRC). F. Cai,* M.P. Mims,*▲ and E.B. Rodriguez de Turco,* D. Webster and N.G. Bazan, LSU Eye Center and Neuroscience Center, New Orleans, LA 70112; ▲Department of Medicine, Baylor College of Medicine, Houston, TX.

ApoE is a plasma apolipoprotein synthesized and secreted by the liver and extrahepatic tissues, including the nervous system. It participates in receptor-mediated transport and delivery of cholesterol and other lipids between cells. We have evaluated the involvement of ApoE in the transport and transfer of lipids containing 22:6 fatty acids to PRC and retinal cells using retinas from 10-day-old mouse pups as a model of CNS. At this point of development, PRC are actively building new membranes enriched in 22:6. By incubating retinas with ^{125}I-ApoE/DMPC in the presence or absence of unlabeled ApoE/DMPC, we found specific binding and internalization of ^{125}I-ApoE/DMPC by developing retinal photoreceptor cells. Further studies indicated that binding and internalization were saturable, and that unlabeled ApoE/DMPC specifically competed for binding and internalization. ^{3}H-22:6 labeled phospholipids were assembled into the ApoE/DMPC disk and incubated with the retina. After isolation of retinal photoreceptor cells, lipids were extracted and the uptake of labeled phospholipids determined by scintillation counting. Results indicated that 22:6 was also specifically bound and internalized by retinal photoreceptor cells when combined with ApoE. The ApoE-receptor-mediated uptake of phospholipids and/or cholesterol may be an important mechanism for support of photoreceptor cell membrane biogenesis and synaptogenesis. Supported by NIH grant EY04428.

171.15

VISININ: A NOVEL CALCIUM BINDING PROTEIN EXPRESSED IN RETINAL CONE CELLS. K.Yamagata*, K.Goto, C-H.Kuo and H.Kondo and N.Miki*. Dept. of Pharmacol., Osaka Univ. Sch. of Med., Osaka 530, Japan.

Visinin (24kDa) is a retinal cone cell-specific protein. Visinin cDNA was isolated from a chick retinal λgt11 cDNA library, using anti-visinin serum. The β-galactosidase-visinin fusion protein was used for purifying epitope-selected antibody. The purified visinin antibody reacted only with a 24 kd protein in retinal cone cells. Visinin mRNA was expressed only in the retinal photoreceptor layer. The nucleotide sequence of the cDNA revealed that visinin has three E-F hand structures and is a Ca^{2+} binding protein. Visinin protein expressed in E.coli exhibited Ca^{2+} binding activity. Visinin was also found in chicken pineal body by immunohistochemistry. Northern blot analysis showed that pineal visinin mRNA was the same as retinal visinin mRNA. When hatched chicks were fed in the light or dark for three days, increased numbers of visinin-positive cells in the pineal body from illuminated chicks were observed. These results suggest that visinin is a photoreceptor-specific Ca^{2+} binding protein and may be involved in phototransduction in retinal and pineal cone-type photoreceptors.

171.16

CALCULATED COLORS OF VERTEBRATE RODS AND CONES: THE HUMAN FOVEA Abner B. Lall,[1]* Steven Park[2] and Richard A. Cone[2] [1]Howard University, Washington DC. 20059 and [2]The Johns Hopkins University, Baltimore, MD. 21218

The colors of most photoreceptors have yet to be seen since their visual pigments bleach too rapidly when viewed in a microscope. We have calculated the colors of vertebrate photoreceptors as they would appear if they did not bleach. Transmission spectra for the pigments were obtained from a computer program based on the polynomial expression of Dawis (1981) for the Ebrey-Honig nomogram. At each wavelength, the transmission for a given axial density value was multiplied by the tristimulus functions for human color vision and the product was integrated to obtain the corresponding tristimulus values. These values were used to construct two nomograms for vitamin A_1 and A_2 pigments. The nomograms are plotted on the C.I.E. 1931 chromaticity chart, and can be used for all vertebrate photoreceptors with visual pigments with max between 425-650 nm, and for axial densities between 0.5-1.5. To test our calculations, we obtained the Munsel color chips that correspond most closely to the calculated chromaticity coordinates for frog rhodopsin rods, and these chips were visually compared to the color of fresh dark-adapted frog retinas (N=6) viewed under a calibrated light source. The colors closely matched, confirming the calculations. Munsel chips corresponding to human rods and cones were then used to create a color montage of the human fovea. These Munsel colors were applied to an enlargement of a detailed micrograph of the human fovea published by Ahnelt, Kolb, and Pflug (1987) to depict, as accurately as possible, the actual colors of the human fovea.

Supported by NIH EY00520

171.17

GAP JUNCTIONS BETWEEN PEDICLES OF MACAQUE FOVEAL CONES. Y. Tsukamoto*, P. Masarachia*, S.J. Schein, and P. Sterling. Hyogo Coll. Med., Hyogo; Harvard Med., Boston; U. Penn Med., Phila. PA 19104

Electrical coupling of cone pedicles by gap junctions is universal in vertebrate retinas. Such junctions have been reported between cones in primate fovea, but this claim has been disputed. We reinvestigated the issue by reconstructing a patch of 80 cone pedicles from electron micrographs of serial sections near 1.5° eccentricity.

A glial curtain isolates each pedicle. However, the curtain occasionally parts, exposing each pedicle at 4 ± 1 sites to its neighbors. Each site bears a desmosome-like, adherent junction (en face, roughly elliptical, ≈ 0.2 μm^2). Sections through the adherent junction (tilted appropriately) show several gap junctions. These vary in size, shape, and in their number/adherent junction. Via such clusters of small gap junctions every pedicle connects, with no selectivity, to at least 2 neighbors. The mean gap junction area/pedicle X the density of connexons (estimated from freeze-fractured material, Raviola and Gilula, '74) suggests on the order of 100 connexons per pedicle. Assuming 100pS/connexon, the total coupling conductance of a pedicle might be 10^4 pS.

Such coupling would tend to degrade spatial acuity but would improve contrast sensitivity to middle spatial frequencies. (EY08124).

171.18

AN UNEXPECTED CONE SPECIALIZATION AROUND THE RIM OF THE HUMAN RETINA. Robert W. Williams, University of Tennessee, School of Medicine, Memphis, TN 38163

Retinas from 5 adult humans were flattened in glycerin and studied at high magnification using video-enhanced Nomarski optics. It is hard to get good images from the edge of the retina. Pigment often obscures the mosaic. Furthermore, the retina is often not flat at the ora serrata and oil immersion objectives typical do not have enough working distance to focus on the photoreceptor mosaic. My solution was to transilluminate the retina with a bright monochromatic light source and use a 63x 1.25 NA Zeiss fluorite objective with a working distance of 500 μm. High contrast images of inner segments were quantified using a video overlay system at a focal plane just beyond the outer limiting membrane (Wikler et al. '90; Curcio et al. '90).

Cone inner segments at the extreme periphery have conventional morphology and can be clearly identified and easily distinguished from rods at all sites. In each retina I found a thin belt at the extreme periphery that is heavily populated by cones. This cone-enriched belt varies from 50 to 500 μm in width and is found around the entire rim, including the temporal quadrant. Cone densities increase rapidly and rod densities decrease rapidly moving outward within the belt. This gradient is as precipitous as that at the foveal rim, but of course, opposite in direction. At the edge of the retina many fields are almost entirely covered by a tight mosaic of cones (greater than 90% cone coverage). Here cone densities peak between 10,000 and 15,000/mm^2. This is 3 to 5 times higher than cone densities 1 mm from the edge of the retina. Conversely, rod densities plummet at the edge, dropping from more than 20,000 to about 5,000/mm^2.

The cone-enriched retinal rim may function as an alert field under conditions of bright illumination.

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171.19

Monkey Cone ERG: Analysis by PDA, KYN, CNQX, APB, ASP, Co.
P A Sieving, K Murayama, F Naarendorp. U Mich. Ann Arbor.

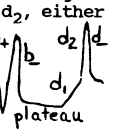
Light-adapted Rhesus ERGs were evoked by 200 ms flashes on 3.3 log Td rod saturating backgrounds. ON- and OFF-pathway agents were studied by intravitreal injections.

APB suppressed the Bwave, and the normally negative "sustained plateau" became more negative. The normal Dwave, at stimulus off, has a positive face, with a slow phase (which we called d_1) and a second phase with steeper slope (d_2), followed by the negative-going face (d_-). APB did not change d_1, but d_2 was larger and d_- smaller.

PDA, KYN and CNQX doubled the Bwave positive face (b_+), but the Bwave negative face (b_-) was nearly absent because the sustained plateau was elevated far above baseline; d_1 remained, but d_2 was gone; d_- amplitude was unchanged.

ASP & Co^{++} abolished the Bwave; the sustained plateau stayed negative; d_1 remained, but d_2 and d_- were missing.

This suggests that the Dwave reflects both cone and post-photoreceptor activity; and both Bwave and Dwave are controlled by both hyper- and depolarizing cells. In this scheme, the cones contribute the light-adapted Awave and d_1 (initial part of Dwave only); depolarizing cells give b_+ and d_-; hyperpolarizing cells give b_- and d_2, either directly or via control of depolarizing cells; and the sustained plateau comes from cones plus the sustained net contribution of hyper- and depolarizing cells.



R01-EY06094 and Retinitis Pigmentosa Fndn, Baltimore, MD.

171.20

CIRCADIAN ROD OUTER SEGMENT DISC SHEDDING IN RABBITS IS ASSOCIATED WITH DECREASED PHOTORECEPTOR SENSITIVITY, AS MEASURED IN THE ISOLATED A-WAVE OF THE ELECTRORETINOGRAM.
M.P. White,D.M. Impelman, and P.A. Hock, Ophthalmology Sect. Veterans Admin. Medical Ctr, Palo Alto, CA 94304

In rabbits a decrease in retinal sensitivity, measured in the b-wave of the AC-ERG, is temporally correlated with the increase in phagosomes in retinal pigment epithelium that defines circadian rod outer segment disc shedding (White, et al,Vision Res.27:357,1987). If shedding of photopigment-containing membrane by photoreceptor cells causes the observed decrease in retinal b-wave sensitivity, it is predicted that the a-wave of the ERG, the component produced directly by the photoreceptor cells, will also decrease in amplitude. Normally, the onset of the positive-going b-wave masks the full expression of the negative-going a-wave. However, by pharmacologically removing the postsynaptic input to the ERG with sodium aspartate, the a-wave can be observed in isolation. We measured isolated scotopic a-waves in 44 albino and pigmented rabbit retinas after intravitreal sodium aspartate (60-150uM). A decrease in a-wave amplitude reliably occurs at the same time as the decrement previously measured in the b-wave, showing that rod sensitivity decreases at approximately 30 min after the usual time of light onset in photoentrained animals. In constant dark the a-wave decrease phase advances, also resembling b-wave measurements. These data fit a model of disc shedding based on removal of photosensitive membrane.

171.21

RETINAL ARTERY OCCLUSION FOLLOWING INTRANASAL INJECTION OF CORTICOSTEROID AND SUBSEQUENT EFFECTS ON THE PATTERN ERG.
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The Pattern Electroretinogram (PERG) is thought to reflect the electrical activity of the inner retina to patterned stimuli. We report two cases of optic atrophy following intranasal injection of methylprednisone into the left turbinate and subsequent effects on the PERG. Intranasal injection of corticosteroids is given as a treatment of chronic sinusitis. Vascular anastomoses between the nasal and retinal circulations are present as branches of the ophthalmic artery reach the anterior and posterior ethmoidal arteries which supply the nasal mucosa. Corticosteroids injected intranasally under pressure can make their way into the ethmoidal artery, and by retrograde flow, to the ophthalmic artery and occlude retinal artery. As a result of emboli of injected material, visual complications, such as blurring or permanent loss of vision have been reported. A reduction in the amplitude of the pattern ERG was found in both of these patients, indicating damage to the inner retinal layers(e.g., ganglion cells) either from the extensive retinal edema at the time of occlusion ,or from a retrograde degeneration of optic nerve fibers.

171.22

ELECTRORETINOGRAM OF GERBIL AFTER TRANSIENT ISCHEMIA
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Mongolian gerbils (Meriones Unguiculatus) were selected by Delbarre method (Delbarre, G., Stroke, 19:26,1988). Adult gerbils were used. Left carotid was ligated during 30 min. Sensitive and non sensitive ischemic gerbils were used for study electroretinogram (E.R.G.). E.R.G. was recorded with a silver electrode placed against the left cornea of anesthetized animals (sodium pentobarbital, 60 mg.kg-1 I.P.). The flash stimulus (intensity:3 Joule, 1 flash per sec.) triggered the averager. E.R.G. was measured before ligation, 5 min. after ligation and again 5 min., 1, 2 and 24 hours after release of the clip. Two waves were studied : the a negative wave (photopic vision) and the most important positive b wave (scotopic vision). 5 min after ligation, the b wave disappeared in sensitive gerbil while this wave persisted in non sensitive gerbil. After reperfusion, latencies and amplitudes of sensitive gerbils were significantly decreased for b wave. Latencies and amplitudes of the a wave were significantly decreased only in sensitive gerbil 5 min after ligation.

It is known that a wave has a high resistance to anoxia caused by circulatory blocage while b wave is abolished by clamping the retinal circulation (Brown, K.T.,NATURE, 193:958, 1962).

These results show that gerbil is a suitable model to study drugs protecting neural retina in ischemia.

171.23

ACCUMULATION OF AMINO-ACIDS AND HYDROXYL FREE RADICALS IN BRAIN AND RETINA OF GERBIL AFTER TRANSIENT ISCHEMIA.
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Mongolian gerbils (Meriones Unguiculatus) have been widely accepted as model of cerebral ischemia because of its incomplete circle of WILLIS (40 to 60 %). Delbarre et al. (Delbarre, G., Stroke, 19:126, 1988) selected sensitive gerbils by examination of the fundus. Two groups of adult gerbils were used. One control group was sham operated and in the second ischemic group, left carotid was ligated during 30 min. Only sensitive ischemic gerbils were used for this study. 60 min. after release of the clip, left brain and left retina were removed and amino-acids (glutamate, aspartate and GABA. XU, X, J. Liquid Chromotog., 9: 2253, 1986) and hydroxyl free radicals (Floyd, R.A., J. Free Radic. Biol. & Med., 2:13, 1986) were determined using HPLC method.

In ischemic brain and retina, amino-acids ($>$122 %) and free radicals ($>$385 %) were significantly increased. These results show that simultaneous determination of amino-acids and hydroxyl free radicals is a good index to study cerebral ischemia after reperfusion.

172.1

SYNERGISTIC ACTIONS OF SPINAL DELTA BUT NOT MU OPIOID AGONIST WITH SEROTONIN.

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It has been demonstrated that intrathecally administered opioids and serotonin have antinociceptive effects. This study is part of a series of experiments aimed at determining optimum drug combinations that produce maximal deppression of noxiously evoked activity at the level of the spinal cord. The present study examined interactions between mu and delta opiate subtypes and serotonin.

This protocol was approved by the Yale Animal Care and Use Committe. Noxious activity evoked by radiant heat was recorded from single discriminated wide dynamic range (WDR) neurons in decerebrate, spinally transected cats. Following baseline determination, 1ug of DAGO(mu selective opioid agonist) or 30ug of DPDPE(delta selective opioid agonist) was combined with 250ug of serotonin and neuronal activity was recorded for 30 minutes. At 31 minutes 0.1mg of naloxone was administered intravenously. The dose of each drug administered alone intrathecally produced no suppression of WDR neuron activity evoked by radiant heating (51°C for 8sec). Although the combination of DAGO and serotonin produced no significant suppression of noxiously evoked activity, DPDPE and serotonin produced significant suppression(to 72.8±8.0%(mean±S.E.) of control values($p<0.05$). 0.1mg of intravenously administered naloxone reversed the suppression produced by the DPDPE-serotonin combination.

The results of the present study suggest that combinations of serotonin with delta selective opiate agonists may be more effective in suppressing noxiously evoked activity than combinations with mu selective opiates.

(This study was supported by NIH Grant NS-09871.)

172.2

COMPARISON OF THE ANTINOCICEPTIVE AND MOTOR EFFECTS OF INTRATHECAL OPIOID AGONISTS IN THE RAT.

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The purpose of this study was to compare the antinociceptive and motor effects produced by the intrathecal (IT) administration of selective mu- (DAMGO; 0.5 ng - 5 ug) delta- (DPDPE; 0.5 ng - 5 ug), and kappa- (U50,488H; 5 ng - 50 ug) agonists. IT catheters were inserted one week prior to the experiments. The Randall Selitto paw-withdrawal test was used to measure changes in mechanical nociceptive threshold. The rat's motor coordination was tested using an accelerating Rota-Rod treadmill (Ugo Basile). The effect of each of the agonists on nociceptive threshold and motor coordination was calculated as a percentage change from baseline threshold. Each agonist produced a statistically significant dose-dependent increase in nociceptive threshold as measured by one-way ANOVA. At the two highest doses tested (500 ng and 5 ug), the selective mu-agonist, DAMGO, produced marked decreases in motor coordination. In contrast, lower doses of DAMGO and the selective kappa- (U50,488H) agonist, over the full range of doses tested, produced smaller decreases in motor coordination. The results suggest that motor side effects need to be considered when interpreting the results of analgesic tests that are dependent on a normally functioning motor system.

172.3

MODULATION OF NOCICEPTIVE AND 'COLD' INPUTS IN THE MEDULLARY DORSAL HORN: THE ROLE OF KAPPA OPIOID RECEPTORS.

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It has previously been reported (Mokha,S.S., Soc. Neurosci. Abs., 15, 372p) that dynorphin, an endogenous ligand for the kappa opioid receptor, produces a widespread influence on somatosensory information from the face. The present study investigated the effects of U-50,488H, highly selective agonist for the kappa opioid receptor, on the responses of neurones in the superficial and deeper layers of the dorsal horn of the medulla (trigeminal nucleus caudalis). Extracellular single unit recordings were made in rats anesthetized with halothane. Nociceptive neurones were activated by thermal stimuli. The effects of intravenously administered U-50,488H (1-2 mg/kg) were tested on the thermal stimuli evoked responses of nociceptive neurones and on the activity of cold receptive neurones. The responses of selectively nocireceptive neurones in the superficial laminae of the dorsal horn were reduced by U-50,488H. However, the responses of multireceptive neurones in the deeper dorsal horn were either reduced or enhanced. A similar action was observed on the activity of cold receptive neurones. These actions of U-50,488H were similar to those observed previously with dynorphin. It is concluded that kappa opioid receptors play an important role in modulating somatosensory information from the face.

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172.4

EPIDURAL AND INTRATHECAL ADMINISTRATION OF SUFENTANIL, ALFENTANIL AND MORPHINE IN THE DOG; A COMPARISON OF ANALGESIC EFFECTS AND THE DEVELOPMENT OF TOLERANCE.

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Sufentanil (Suf), alfentanil (Alf) and morphine (Mor) are *mu* receptor agonists with different receptor affinities, intrinsic efficacies, and lipid solubilities. There is clinical interest in the anilinopiperidines because of their higher liposolubility, and a decreased risk of delayed respiratory depression secondary to bulk redistribution in the CSF. The purpose of this study was to evaluate the analgesic effects and the development of tolerance to these drugs following 14 day EP or 28 day IT administration. Beagles (~10 kg) were implanted with EP and IT catheters (PE-50) under halothane anesthesia. The nociceptive response was quantified by measuring the response latency to a thermal probe (62.5° C) applied to thoracic and lumbar areas of the back. EP Suf (50, 100 µg) and Alf (80, 800 µg) as well as IT Suf (25, 50 µg) and Alf (40, 400 µg) produced a spinally induced analgesic response which was rapid in onset (< 5min) but reliably shorter in duration when compared to EP Mor (1, 10 mg) and IT Mor (0.5, 5 mg). In all cases the analgesic response was dose-related and produced 100% MPE at the higher doses. The development of tolerance was quantified by comparing the extent and the duration of the analgesic effect produced on Day 1 with that observed on the final day (Day-F) of the study. Results are expressed as a ratio of the AUC on Day-1/ AUC on Day-F, where AUC = %MPE vs Time. The magnitude of tolerance observed for a given agent was similar regardless of the route of administration. Mor produced the greatest degree of tolerance over time with a ratio of 25. Alf and Suf demonstrated significantly less tolerance over time with ratios of 2 and 3 respectively. Such differences in the magnitude of tolerance may result from different drug kinetics or possible differences in drug pharmacodynamics. These results suggest that although the duration of action of Alf and Suf is significantly shorter than that of Mor, the more liposoluble and receptor specific anilinopiperidines produce a slower tolerance development which is of significance in the treatment of opioid-sensitive chronic pain.

172.5

INTRATHECAL DOSE RESPONSE CURVES FOR MORPHINE (MOR) AND SUFENTANIL (SUF): ROLE OF DRUG EFFICACY IN THE RIGHT SHIFTS PRODUCED BY INCREASING STIMULUS INTENSITY.

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Agonists with high efficacy by definition are able to produce their effects by occupying a small fraction of the available receptors while low efficacy agonists must show a higher fractional receptor occupancy (FRO). If the intensity of the stimulus is increased, it follows that a larger number of the available receptors will require occupancy to produce a given effect. It can be shown that for any required increase in effect, agents with high FRO's will show a greater right shift than agents with low FRO's. We have shown in studies with the irreversible mu antagonist B-funaltrexamine that the degree of shift in the respective intrathecal dose response curves is: MOR>>SUF, suggesting that relative to SUF, MOR is a lower efficacy agonist. To assess the prediction made above that lower efficacy agonists may show a greater shift in their dose response curves for a given increase in required effect, dose response curves for intrathecal MOR and SUF were assessed in rats with chronic lumbar intrathecal catheters using the 48°C, 52.5°C and 60°C hot plate. Both agents showed a rightward shift in their respective dose response curves with increasing stimulus intensity.

HP Temperature (C):	49°	52.5°	60°
IT MOR:	0.6 (1)^	3.9 (7.1)*	58 (x37)*#
IT DAGO:	(1)	0.4 (3.6)*	0.7 (5.6)

ED50 ug (ED50 ratio); * P<0.05 vs 49C; #P<0.05 vs DAGO

There was a reduction in slope for MOR but not for SUF on the 60°C HP. These data with SUF and MOR indicate that these two 2 mu agonists differ in efficacy and that at high stimulus intensities, morphine displays this by showing a greater shift in its dose response curve and behaving as a partial agonist (DA02110).

172.6

MORPHINE MICROINJECTED INTO THE MEDULLARY DORSAL HORN PRODUCES NALOXONE-REVERSIBLE FACIAL SCRATCHING IN THE MONKEY.

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A frequently observed side effect of spinal opiates in humans is itch and scratching, especially on the face. We examined the effects on facial scratching behavior of morphine sulfate microinjected unilaterally into the medullary dorsal horn (MDH) of a rhesus monkey. The monkey was seated in a primate chair and the number of scratches in five-min blocks were recorded by two raters independently. The location of the scratches in the trigeminal nerve divisions were determined. Morphine (1.0, 2.5, 5.0 µg/0.2µl saline) produced a dose-dependent increase in scratching behavior. Scratching increased on both sides of the face and occurred about twice as frequently on the side of the face ipsilateral to the injection. Increases in scratching were observed 10-15 min postinjection with a peak (M = 253.0 facial scratches/5 min) occurring at 70 min. Scratching increased in all three trigeminal areas, with the greatest increases observed in the ophthalmic and maxillary areas. Naloxone (0.5 and 2.0 mg/kg; IM) administered 50 min after morphine (5.0 µg) completely reversed the effects of morphine on facial scratching behavior. These data demonstrate that MDH-administered morphine can produce a dose-dependent, receptor-specific increase in facial-scratching behavior in the monkey.

172.7

CROSS-DESENSITIZATION BETWEEN MORPHINE AND NOREPINEPHRINE ON THE INHIBITION OF SUBSTANCE P RELEASE FROM RAT SPINAL CORD SLICES. M.R. VASKO, P.M. DULBERGER* AND M.N. PAYNE*, Departments of Pharmacology and Toxicology and Anesthesia, Indiana Univ. School of Medicine, Indianapolis, IN 46202.

Since morphine (M) and norepinephrine (NE) inhibit the release of substance P (SP) from sensory neurons, we studied whether these inhibitory effects could desensitize with chronic exposure of the spinal cord to M.

Rats were implanted with thecal cannulae attached to Alzet miniosmotic pumps containing either saline or 20 nmol M. The pumps delivered lul/hr of solution for 7 days. On day 7 after implantation, rats were decapitated and SP release from a segment of the dorsal lumbosacral spinal cord was measured using an in vitro perfusion technique. Release of SP was evoked by exposing the tissue to 50mM KCl in the presence or absence of drug.

Exposure of dorsal spinal cord tissue from control rats to 50mM KCl produces an increase in the release of SP from a basal level of 0.16±0.04 to 0.78±0.11 pg/mg/min. In tissues from rats implanted with pumps containing saline, exposure of cord slices to M or NE (10^{-5}M) results in a significant inhibition in the stimulated release of SP. In contrast, evoked release of SP is not significantly inhibited by either M or NE (10-5M) in cord slices from rats exposed to M for seven days. Thus, desensitization develops to both M and NE-induced inhibition of SP from the dorsal spinal cord after chronic exposure of the spinal cord to the opioid. (Supported by NS 21697)

172.8

DIFFERENTIAL DOSE-DEPENDENT EFFECTS OF MORPHINE ON THE VENTRAL ROOT POTENTIAL. R.A. Jaffe, M.D., Ph.D., Dept. of Anesthesia, Stanford Univ. Sch. of Med., Stanford, CA 94305.

The ventral root potential (VRP) evoked by stimulation of the corresponding ipsilateral dorsal root (DR) typically consists of three distinct components: two early low-threshold components representing A-fiber mediated activity in monosynaptic and in polysynaptic pathways respectively, and a late high-threshold component representing C-fiber mediated activity related to nociception. An objective of this study was to examine the VRP as a measure of nociceptive processing in the spinal cord. Adult male SD rats were decerebrated under inhalational anesthesia, and a laminectomy extending from T13 thru L5 was performed. Ipsilateral L5-6 dorsal and ventral roots were isolated for subsequent stimulation and recording with suction electrodes. Rats were mounted in a spinal frame, paralyzed with pancuronium and mechanically ventilated. End-tidal CO_2 and rectal temperature were maintained in a normal physiologic range. A pool was formed directly above the exposed spinal cord by removing overlying agar used to stabilize the preparation. All drugs were dissolved in physiologic saline bathing the exposed spinal cord. Analysis of the VRP evoked by DR stimulation suprathreshold for C-fibers revealed that morphine sulfate (MS) 15uM or 150uM eliminated the C-fiber component of the VRP within 30-45 minutes. The C-VRP gradually returned to control levels over a 2-4 hour period following removal of the MS containing medium. This C-VRP effect was completely reversible by naloxone. The early components of the VRP were minimally affected at 15uM MS but clearly enhanced at 150uM MS. This augmentation of A-fiber evoked VR activity may be related to previous observations of MS-induced increased activity in some dorsal horn neurons and with the behavioral observations suggesting hyperalgesia with large intrathecal MS doses. Supported in part by NIH BRSG RR05353.

172.9

CHOLECYSTOKININ OCTAPEPTIDE ATTENUATES MORPHINE-INDUCED INHIBITION OF C-FIBER EVOKED DISCHARGES OF SPINAL CORD NOCICEPTIVE NEURONS. D.E. Kellstein[1], D.D. Price[2], and D.J. Mayer[1]. Depts. of [1]Physiology and [2]Anesthesiology, Medical College of Virginia, Richmond, VA 23298.

Previous studies suggest that cholecystokinin octapeptide (CCK), a putative CNS neurotransmitter or neuromodulator, may function as a selective antagonist of opioid-induced analgesia. Using extracellular electrophysiological recording, the present study investigated the effects of CCK and the CCK antagonist lorglumide (LGM) on 1) A- and C-fiber evoked firing of dorsal horn nociceptive neurons, and 2) the inhibition of C-evoked firing induced by morphine sulfate (MS).

Male Sprague-Dawley rats (450-520 g) were anesthetized with urethane (1.3 g/kg). A laminectomy was performed at spinal segments L4 and L5, and the dura was retracted. Single unit recordings were made from wide dynamic range and nociceptive specific neurons of the dorsal horn (laminae 2 to 7) during controlled repetitive electrical stimulation of the ipsilateral hindpaw. Stimulation consisted of a train of six pulses (3 mA; supramaximal for C-fiber activation) delivered at 2 sec intervals. Following determination of control responses, CCK (6.4 pmol to 20 nmol) or LGM (0.145 to 145 pmol) was superfused onto the spinal cord in a volume of 10 µl, and evoked discharges were quantified at 1, 5, and 10 min after each dose. Eleven min after the last dose of either drug, MS (4, 20, and 100 nmol) was applied and evoked discharges were again counted. Neither CCK nor LGM administered alone significantly altered either spontaneous, A-evoked, or C-evoked firing. CCK pretreatment, however, significantly attenuated, whereas LGM pretreatment significantly enhanced, MS-induced inhibition of C-evoked firing. Consistent with the results of behavioral studies, these findings suggest that CCK is not a neurotransmitter or neuromodulator of nociception, but rather an antagonist of opioid-induced antinociception. Supported by PHS awards NS 24009-1A2, DA 00576, and FIDIA.

172.10

THE EFFECTS OF SULFATED CHOLECYSTOKININ OCTAPEPTIDE ON ANTINOCICEPTION INDUCED BY DIFFERENT OPIOID AGONISTS. Leon F. Tseng and Keith A. Collins Department of Pharmacology and Toxicology, Medical College of Wisconsin and Research Service, C. J. Zablocki VA Medical Center, Milwaukee, WI 53226

The effects of sulfated cholecystokinin octapeptide (CCK8s) given intrathecally (i.t.) or intracerebroventricularly (i.c.v.) on inhibitions of the tail-flick (TF) and hot-plate (HP) responses induced by epsilon (β-endorphin), mu (morphine and DAMGO) and delta (DPDPE) opioid agonists given i.t. or i.c.v. were studied in male ICR mice. CCK8s (1 ng) given i.t. effectively antagonized TF inhibition induced by β-endorphin (2 µg) and DPDPE (10 µg) but not morphine (4 µg) and DAMGO (0.02 µg). However, CCK8s given i.t. did not affect HP inhibition induced by any of the opioid agonists given i.c.v.. CCK8s (0.2-40 ng) in combination with β-endorphin (2 µg) or morphine (4 µg) given i.c.v. did not affect β-endorphin- or morphine-induced TF and HP inhibitions. Intrathecal administration of CCK8s (1 ng) significantly attenuated the TF inhibition induced by i.t. β-endorphin (0.5-1 µg) and DPDPE (5 µg) but not morphine (0.5-1 µg), DAMGO (5 ng) , norepinephrine (5 ng) or serotonin (16 µg). The HP inhibitions induced by i.t. administration of these agonists were not affect by i.t. CCK8s. The results indicate that CCK8s selectively attenuates the TF inhibition by inhibiting the descending pain control system activated by β-endorphin and DPDPE but not morphine and DAMGO given supraspinally (Supported by NIDA Grant DA 03811).

PAIN MODULATION: PHARMACOLOGY I

173.1

THE ROLE OF VAGAL AFFERENTS IN ANTINOCICEPTION PRODUCED BY IV MORPHINE. A. Randich, C. L. Thurston, P. S. Ludwig*, M. R. Timmerman*, and G. F. Gebhart. Departments of Psychology and Pharmacology, The University of Iowa, Iowa City, IA 52242.

This experiment evaluated the role of cervical vagal afferents in the antinociception produced by IV administration of morphine sulphate. Rats were maintained in a lightly-anesthetized state with pentobarbital sodium. Control rats (N=8/group) received IV bolus administration of 0.1, 0.5, 1.0, or 2.5 mg/kg of morphine sulphate followed by tail-flick test trials at 0.16-, 1-, 2-, 3-, 4-, 5-, 10-, 15-, and 20-min. Significant inhibition of the nociceptive tail-flick reflex was observed in the 0.5, 1.0, and 2.5 mg/kg conditions. Experimental rats (N=8/group) receiving prior bilateral resection of the cervical vagi showed significantly less antinociception across all test trials in the 0.5 and 1.0 mg/kg conditions and during the initial test trials in the 2.5 mg/kg condition. Intact rats (N=8/group) receiving prior IV administration of 5.0 mg/kg of naloxone methobromide and tested in either the 0.5 or 2.5 mg/kg conditions showed significant attenuation of antinociception statistically equivalent to that produced by bilateral cervical vagotomy. The antinociception produced by 0.5 mg/kg of IV morphine was significantly attenuated by intrathecal administration of 1 ug of naloxone, but not by IV administration of 1 ug of naloxone. However, intrathecal administration of 1 ug of naloxone also attenuated cardiovascular responses produced by IV morphine suggesting the possibility of rostrad spread of the receptor antagonist. Supported by NS24958.

173.2

ANALGESIC EFFECTS OF INTRANIGRAL INJECTION OF SELECTIVE OPIOID AGONISTS. A.A. Baumeister, Psychology Department, Louisiana State University, Baton Rouge, LA 70803.

Intranigral morphine suppresses pain-related behavior on the tail flick and hot plate tests (Baumeister et al., Brain Research, in press). However, the nigra is less sensitive to this effect of morphine than other sites. A possible explanation for such differences in potency is that analgesic effects in different sites are mediated by different receptor subtypes. To determine the types of nigral opioid receptors involved in analgesia, studies with selective agonists were conducted. As can be seen from the table below, among the most selective agonists (i.e., DAGO, DPDPE, & U50-488H) only DAGO produced analgesia, implicating the mu receptor. DTLET, a mixed delta/mu agonist, also had potent analgesic effects, suggesting that a mu/delta receptor complex within the nigra may mediate analgesia.

Minimum Effective Intranigral Dose (nM/0.5 µl)

Agonist	Preferred Receptor	Tail Flick	Hot Plate
Morphine	mu	10.0	15.0
DAGO	mu	0.3	0.1
DTLET	delta	1.0	1.0
DPDPE	delta	No Effect	No Effect
U50-488H	kappa	No Effect	No Effect

173.3

INTERACTION OF TRICYCLIC ANTIDEPRESSANTS WITH MORPHINE ON C FIBRE-EVOKED ACTIVITY IN RAT THALAMUS. I.Jurna, R. Hecht* and T.L. Yaksh. Dept. of Pharmacology, University of the Saarland, D-6650, Homburg/Saar, FRG and Dept. of Anesth., University of California, San Diego, La Jolla, CA 92093

Activity evoked in the rat thalamus (VDM) evoked by afferent C fibre stimulation was examined when amitriptyline (AMI), imipramine (IMI), and desipramine (DES) were given alone or in combination with morphine. IMI and DES dose-dependently reduced C fibre-evoked activity in the thalamus (ED50: 3.1 mg/kg and 4.1 mg/kg). AMI was ineffective at doses of 10 and 20 mg/kg. At low doses it significantly depressed the activity; the dose-response curve was bell-shaped, 1 and 5 mg/kg being less effective than 2.5 mg/kg. Morphine produced a depression that was significant only 5 min. after the injections. When morphine was given in combination with IMI 2.5 mg/kg on DES 2.5 mg/kg, the depression of evoked activity was enhanced in magnitude and duration beyond the effects caused by each drug alone. A combination of AMI 10 mg/kg with morphine resulted in a long-lasting depression of evoked activity, while combinations of lower doses of AMI with morphine showed reduced depression. The facilitating effect was observed in unanesthetized rats. Small doses (1 mg/kg on 52°C hot plate) of DES and IMI will produce small effects (2x baseline) in unanesthetized rats. Administered concurrently, there is a 5.1 and 7.2 fold shift in the morphine dose response curves. Thus, certain TCAs tested, depressed nociceptive activity in the thalamus and enhanced the antireflexive effects of morphine.

173.4

INHIBITION OF MORPHINE ANALGESIA BY ETHYLKETOCYCLAZOCINE PRETREATMENT IN RAT PERIAQUEDUCTAL GRAY AND LOCUS COERULEUS. R.J. Bodnar, D. Paul and G.W. Pasternak. Dept. of Psychology, Queens Col., CUNY, Flushing, NY 11367 and Dept. of Pharmacology, Memorial Sloan-Kettering Cancer Ctr., NY, NY 10021.

Analagesia elicited by morphine following microinjection into the periaqueductal gray (PAG) and locus coeruleus (LC) in rats appears dependent upon the mu_1 opioid receptor subtype. The present study examined the analgesic properties of ethylketocyclazocine (EKC) and U50488H in the PAG and LC alone and in conjunction with morphine. Neither EKC nor U50488H (30 ug) produced analgesia in PAG or LC loci which supported morphine analgesia. EKC (50 ug, ICV) produced analgesia which was reversed by the mu_1 antagonist, naloxonazine. Coadministration of EKC, but not U50488H with morphine reduced morphine analgesia in the PAG and LC in a dose-dependent manner.Coadministration of EKC into both the PAG and LC produced naloxonazine-reversible analgesia. The data indicate that the actions of EKC in inhibiting morphine analgesia in the PAG and LC are probably due to weak mu_1 agonist actions and not due to antagonistic interactions between mu and kappa receptors.

173.5

POTENTIATION OF OPIOID ANTINOCICEPTION FOLLOWING COADMINISTRATION OF AN INHIBITOR OF ENDOPEPTIDASE 24.15. B. Kest, M. Orlowski*, C.J. Molineaux and R.J. Bodnar. Dept. of Psychology, Queens Col., CUNY, NY, NY 11367 and Dept. of Pharmacology, Mt. Sinai Sch. of Med., NY, NY.

The duration of opioid antinociception is limited by peptidase degradation. Endopeptidase(EP) 24.15 and EP24.11 respectively degrade pro-dynorphin and pro-enkephalin derived peptides. Inhibitors of either EP24.15 or 24.11 produce naloxone-reversible antinociception after 5-7 h; EP 24.11 inhibitors potentiate pro-enkephalin antinociception. The present study compared EP24.15 and 24.11 inhibitors to produce or augment antinociception in rats following central co-administrationwith either dynorphin A(1-8:DYN), metenkephalin (Arg-Gly-Leu:MERGL) or opioid-mediated swims (OMS) on the tail-flick and jump tests. Neither DYN nor either EP inhibitor altered pain thresholds, whereas MERGL and OMS were antinociceptive. The EP24.15 inhibitor produced naloxone reversible antinociception when paired with DYN, and potentiated both MERGL (33%) and OMS (29%) antinociception. In contrast, the EP24.11 inhibitor produced smaller and less consistent effects. Endopeptidase 24.15 appears to exert a modulatory role upon opioid antinociception.

173.6

SITE-SPECIFIC POTENTIATIONS AND REDUCTIONS IN OPIATE AND NONOPIOID ANALGESIA FOLLOWING MESENCEPHALIC MICROINJECTIONS OF THYROTROPIN RELEASING HORMONE. J.A. Robertson and R.J. Bodnar. Dept. of Psychology, Queens Col., CUNY, Flushing, NY 11367.

Thyrotropin-releasing hormone (TRH) modulates opioid and nonopioid analgesia as a function of route; ventricular TRH potentiates nonopioid footshock and continuous cold water swim (CCWS: 2°C, 3.5 min) analgesia. Ventricular and systemic TRH failed to alter morphine (MOR) analgesia but intrathecal TRH produces a U-shaped function. The present study mapped mesencephalic loci associated with pain inhibition to determine whether central MOR and CCWS analgesia were differentially modulated by microinjections (33 g cannula) of TRH as measured by tail-flick latencies and jump thresholds in rats. TRH (10 ug) transiently (5-15 min) produced analgesia but failed to exert effects thereafter. TRH given 20 min prior to CCWS reduced analgesia (48-60%) in III cranial nerve placements, and increased analgesia (23-34%) in dorsal raphe nucleus (DRN) placements. MOR analgesia (1-2.5 ug) was more potent in DRN as compared to III N placements. However the same TRH regimen potentiated MOR analgesia following coadministration into the III N (58-435%) and the DRN (15-36%).

173.7

A-3508: A μ_1 OPIATE AGONIST/μ_2 ANTAGONIST? C.A. Taylor, J.R. Bagley*, S. Harris*, G. Lysko*, R. Lozito*, J. Green*, P. Lloyd*, E. Messineo*, S. Bielen*, T.P. Jerussi, and M.H. Ossipov. Anaquest/BOC Health Care, Murray Hill, NJ 07974, USA.

A-3508 [N-(2-pyrazinyl)-N-(1-phenethyl-4-piperidinyl)-2-furamide] is a novel opioid analgesic with a unique pharmacologic profile. Its IC_{50} in guinea pig brain homogenates is 27 nM against [H^3]-DAGO, 262 nM against [H^3]-DPDPE, and >10 μM against EKC. Antinociception was measured in the rat tail flick (TF) and hot plate tests. Respiratory (PaO_2 and $PaCO_2$) and cardiovascular parameters (arterial pressure and heart rate) were measured in conscious, freely moving rats. GI motility was measured in mice by the charcoal meal test. A-3508 produced dose-dependent antinociception after i.v. (TF ED_{50} = 0.078 mg/kg) and i.t. (TF ED_{50} = 8 μg) dosing. Naloxone reversed the antinociceptive effect of a-3508. Doses of up to 24 times the lowest maximally effective analgesic dose of A-3508 produced no significant changes in $PaCO_2$ or PaO_2 in rats. A-3508 reversed fentanyl-induced respiratory depression in rats, reduced morphine-induced inhibition of GI transit and also precipitated withdrawal in morphine tolerant mice, whereas the antinociceptive effect of combinations of A-3508 with fentanyl or morphine was additive. This pharmacologic profile is consistent with an agonistic activity at the proposed μ_1 and antagonism at the μ_2 opiate receptor.

173.8

PHARMACOLOGICAL CHARACTERIZATION OF THE MU-SELECTIVE DRUG, TRIMU-5. L.A. Tive, D. Paul, G.A. Gacel, B.P. Roques & G.W. Pasternak, Cotzias Laboratory of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Depts. of Neurology and Pharmacology, Cornell University Medical Center, 1275 York Avenue, New York, NY, 10021, U.S.A., and U266 INSERM, UA 498 CNRS, Laboratoire de Chimie Organique, Faculte de Pharmacie, 4 av. de l'Observatoire, 75006 Paris, FRANCE.

In binding studies, the novel opiate Tyr-D-Ala-Gly-NH-CH_2-CH_2-$CH(CH_3)_2$ (TRIMU-5) is 200-fold more potent when competing for mu sites than for delta or kappa sites. Accordingly, we assessed the analgesic activity of TRIMU-5 using the tail-flick assay. In rats, intracerebroventricular (i.c.v.) injections of TRIMU-5 produced naloxone- and naloxonazine-sensitive analgesia at doses as low as 10 ug implying a mu_1 mechanism of action. However, TRIMU-5 injected into the periaqueductal gray (PAG) did not produce analgesia, and blocked morphine analgesia when both drugs were given to the PAG suggesting that it is only a partial mu_1 agonist. In mice, TRIMU-5 injected intrathecally was 100-fold more potent than when injected i.c.v. TRIMU-5 analgesia following i.c.v. injection was naloxonazine-sensitive (mu_1) whereas i.t. was not (mu_2). Therefore, the present results suggest that TRIMU-5 is a potent mu_2 agonist and a partial agonist at mu_1 receptors.

173.9

EFFECTS OF LEU-ENKEPHALIN MICROPRESSURE APPLICATION ONTO RAT NUCLEUS SUBMEDIUS NEURONS. **J.A. Coffield and V. Miletic. Dept. Comp. Biosci., University of Wisconsin, Madison, WI, 53706.**

Animals were anesthetized with pentobarbital (60mg/kg i.p.) and maintained with ketamine (40mg/kg i.p.). Extracellular recordings were used to examine the effects of leu-enkephalin (50mM), applied with micropressure (10-12psi), on the spontaneous firing rate of 20 SM neurons. Thirteen of these units had cutaneous receptive fields (RFs), and could be classified as low-threshold mechanoreceptive (LTM), nociceptive-specific (NS), or wide-dynamic-range (WDR). The following table summarizes the results.

Type	No.	Response to L-enkephalin			
		Inhibited	Excited	Biphasic	No Effect
LTM	3	1	1	-	1
NS	5	2	1	2	-
WDR	5	4	1	-	-
Total	13	7	3	2	1

Enkephalin effects appear correlated with RF properties. Units with confined RFs responded to the opioid with an inhibition in their spontaneous firing. Neurons with complex RFs (whole body or multiple sites) demonstrated complex responses (biphasic or excitation). Naloxone (50mM, 10-12psi) reversed the enkephalin effects in some, but not all cases.

These data suggest that submedius neurons respond to enkephalin differently depending on their physiologic type (nociceptive vs. non-nociceptive) and RF characteristics. (Supported by NIH NS26850).

173.10

DEVELOPMENT OF FORMALIN PAIN AND MORPHINE AND AMPHETAMINE ANALGESIA IN INFANT RATS. F. V. Abbott and E. Guy, Sch. of Nursing & Dept. Psychiatry, McGill Univ., Montreal, Canada.

In rats opioid-induced suppression of defensive reflex responses to noxious stimulation matures at 7-14 days of age but little is known about the development of responses to injury. The present study investigated the maturation of the behavioral response to formalin-induced pain and the analgesic effects of morphine and amphetamine. Formalin was injected sc into the ventral surface of a rear paw of 1 to 20 day old pups. One day old pups responded to 10 ul of 1% formalin by elevating the paw, flexing the limb and occasionally making oral contact to lick the paw, as do adults. General activity was increased by pain and was characterized by swimming movements. As pups matured, the response to 1% formalin decreased so that by 20 days it 2.5% formalin was necessary to obtain a pain response reliably. Morphine (1-2 mg/kg) decreased pain and activity levels at all ages. Pentobarbital (10 mg/kg) decreased activity without decreasing pain. Amphetamine (0.5 - 2 mg/kg) reduced pain and increased activity in pups 10 days and older. The data indicate that the behavioral responses to pain in injured tissue are present from birth. The analgesic effects of morphine involve two mechanisms, one that is present at birth and independant of dopamine systems and a later maturing one that is dependant on dopamine.

173.11

MORPHINE BLOCKS THE INCREASED GLUCOSE METABOLISM IN THE DORSAL RAPHE AND LATERAL HABENULA CAUSED BY NOCICEPTIVE BRAIN STIMULATION. D. Huston-Lyons†, L.J. Porrino§, and C. Kornetsky†. †Boston Univ. Sch. of Med., Boston, MA, § NINCDS, Bethesda, MD.

The present study investigated the effects of morphine (4 mg/kg sc) on local cerebral metabolic rates for glucose ($LCMR_{glu}$) in animals working to escape nociceptive electrical brain stimulation to the mesencephalic reticular formation (MRF). In this supraspinal pain model, morphine as well as other opiate analgesics decrease the sensitivity of animals to this stimulation. Results of this experiment indicate that MRF stimulation alone increased $LCMR_{glu}$ in a number of structures associated with pain such as the periaqueductal gray and raphe nuclei, as well as limbic system structures, e.g., the habenula and ventral tegmental area. Although morphine alone had no effect in any of these structures, it reversed the effect of stimulation in the dorsal raphe and lateral habenula. These data suggest that, although nociceptive MRF stimulation activates various sites, these two structures play an important role in morphine-induced antinociception. (Supported by NIDA grant DA02326 and Research Scientist Award DA00099 to CK).

173.12

COMPARISON OF VOCALIZATION AND TAIL FLICK THRESHOLDS FOLLOWING MORPHINE TREATMENT IN THE RAT: ASSESSMENT OF SENSORY AND PERFORMANCE VARIABLES. G.S. Borszcz and C.A. Pelletier*. Department of Psychology, Dartmouth College, Hanover, NH 03755.

The relative hypoalgesic influence of morphine on tail flick and vocalization thresholds in the rat was examined. Responses were generated by applying graded electric current to the tail. Vocalizations were recorded during the shock epoch (VDS) and for the 1 sec interval following shock termination (VAD). Performance (i.e., latency and amplitude) of all three responses was recorded in order to determine whether increases in response thresholds were confounded by motor deficits.

A dose-response analysis (1-16 mg/kg) of systemic morphine treatment revealed that thresholds of VAD were most readily increased, followed by those of VDS and tail flick. Analysis of performance revealed that morphine treatment more readily disrupted VAD, followed by VDS and tail flick. Nevertheless, across the dose range (1-7 mg/kg) of morphine that did not disrupt performance of any of the responses, the order of susceptibility to increases in response thresholds remained VAD, VDS and tail flick. Results are discussed in terms of the relative influence of systemic morphine on response organized at different levels of the neural axis.

173.13

OPIOIDS BUT NOT INDOMETHACIN OR ASPIRIN ABOLISH RESPONSES TO NOXIOUS COLORECTAL DISTENSION. V. Kumar*, T.J.Ness and G.F.Gebhart. Depts. Anesthes. and Pharmacol., College of Medicine, Univ. Iowa, Iowa City, IA 52242

Male Sprague-Dawley rats were surgically implanted with chronic femoral venous and arterial catheters. Three or more days later experiments were performed in awake, unrestrained rats using colorectal distension (CRD, 7 cm balloon; 0-80 mm Hg) as a noxious visceral stimulus. The effects of i.v. normal saline, morphine (1-16 mg/kg), fentanyl (5-20 μg/kg), alfentanyl (50-200 μg/kg) or sufentanyl (1-8 μg/kg) or i.p. vehicle, indomethacin (1-10 mg/kg) or aspirin (1-10 mg/kg) on the cardiovascular (pressor/tachycardia) and visceromotor (abdominal contraction) responses to CRD were examined.

Opioids produced a dose-dependent, naloxone-reversible inhibition of the cardiovascular and visceromotor responses to CRD in a rank order of potency identical to their clinical analgetic potency (sufentanyl > fentanyl > alfentanyl > morphine). At the highest doses of opioids, all responses to CRD were abolished. Indomethacin and aspirin when administered alone had little effect on the visceromotor or cardiovascular responses to CRD, but significantly potentiated the effects of morphine.

The effects of opioids, indomethacin and aspirin on this model of visceral pain is similar to their clinical effects, supporting use of this model for examining the effects of analgesics and their interactions with other agents.

174.1

PEPTIDE AND GLUTAMATE NMDA RECEPTORS MEDIATE A NOCICEPTIVE REFLEX IN NEONATAL RAT SPINAL CORD. S. J. Woodley and J. J. Kendig, Department of Anesthesia, Stanford University School of Medicine, Stanford, CA 94305-5117.

Substance P[1] and glutamate[2] actions have separately been implicated in the generation of a slow ventral root potential (slow VRP) in neonatal rat spinal cord, which has been linked to nociception. We report that the slow VRP is dependent on both neurotransmitters. Spinal cords were isolated from 1-5 day old Sprague-Dawley rats and superfused with oxygenated artificial cerebrospinal fluid (ACSF) at 27-28°C, pH 7.3. Slow VRP's of 10-40 s duration were evoked by electrically stimulating a lumbar dorsal root and recorded at the corresponding ipsilateral ventral root. The NMDA receptor antagonist APV (5-20 μM) and the substance P antagonist spantide (10-20 μM) both reversibly depressed the slow VRP; spantide and APV applied together nearly abolished it. Spantide-sensitive and APV-sensitive components overlapped, although an APV-sensitive component was more prominent earlier. A slow VRP could be elicited by brief (0.1-1.0 s) applications of either substance P (2-20 μM) or NMDA (10 μM). Both excitatory amino acids, acting on an NMDA receptor, and substance P thus appear to be involved in generating this nociceptive reflex.

1. Acta Physiol.Scand. 116:119, 1982
2. Br.J.Pharmacol. 88:269P, 1986

174.2

EFFECTS OF STIMULATION AND LESIONS OF PRIMARY AFFERENTS ON DORSAL HORN TRANSMITTERS. G. Li Volsi, R.J. Weinberg and A. Rustioni. Dept. of Cell Biology and Anatomy, University of North Carolina, Chapel Hill, NC 27599.

Rats were perfused after 20'-30' of repetitive sciatic nerve stimulation. L4 and L5 spinal segments were removed and immunocytochemically processed for three dorsal horn transmitters: substance P (SP), glutamate (Glu) and GABA. Staining in superficial laminae was analyzed densitometrically with a video microscopic system. SP was reduced, on the average, by 6.5% from control values after stimulation at C fiber strength, whereas Glu levels increased 11% after C fiber stimulation. Stimulation of A-α fibers had no effect on staining for these substances, but increased GABA staining by 5%. Fourteen days after sciatic nerve ligation or lumbar dorsal rhizotomy, similar decreases were seen in SP (11%-13%) and similar increases in Glu (9%-14%) and GABA (2%-7%). SP reduction, expected on the basis of previous work, presumably results from its depletion in terminals. Staining for Glu was generally more variable than for SP and its higher density on the experimental side was observed in both neuropil and cells. Since Glu increased with all experimental paradigms, including rhizotomy, it is unlikely that the effects are related to metabolic changes. Further experiments are underway to establish the mechanisms by which reciprocal effects on SP and Glu are elicited by these three different experimental manipulations.

174.3

OPIOID AND EXCITATORY AMINO ACID ACTIVITY IN THE DORSAL HORN IN VITRO. D. S. K. Magnuson and A. H. Dickenson*. Department. of Pharmacology, University College London, U.K. WC1E 6BT

In order to compare the responses of substantia gelatinosa (SG) neurones with those of deeper dorsal horn neurones we have made intracellular and extracellular recordings in sagittal slices of spinal cord from 21 day old rats. Electrical stimulation of the dorsal root zone elicits depolarizations and spiking activity lasting several hundred msec. These responses can be separated into early and late phases on the basis of stimulus threshold and the effects of elevated Mg2+ (which selectively reduces the late phase) and kynurenate (which reduces both phases). Responses of SG and deeper dorsal horn neurones were similar in their sensitivity to Mg2+ and kynurenate.

Intracellular recordings made from SG neurones show that the μ opioid agonist morphine, and the GABA antagonist bicuculline cause depolarizations and increases in membrane input resistance, as does a high Mg2+/low Ca2+ superfusate (which blocks synaptic transmission). In contrast, GABA and the opioid antagonist naloxone cause decreases in the membrane input resistance of most SG neurones. This action of naloxone is absent when synaptic activity is blocked. These data suggest that in sagittal slices of spinal cord the activity of SG neurones may be under the control of a GABAergic interneurone which is in turn sensitive to both exogenous and endogenous opioids. Opioid enhanced responses may therefore involve a GABAergic disinhibitory mechanism present in the substantia gelatinosa, similar to that found in the hippocampus.

174.4

EFFECTS OF EXCITATORY AMINO ACID AGONISTS AND ANTAGONISTS ON NOCICEPTIVE AND NON-NOCICEPTIVE RESPONSES OF DORSAL HORN NEURONES IN THE CAT SPINAL CORD; AN EXTRACELLULAR AND INTRACELLULAR STUDY *IN VIVO*. V. Radhakrishnan, Y. De Koninck and J.L. Henry, Depts. of Physiology, Anaesthesia Research and Psychiatry, McGill Univ., Montréal, Qué. H3G 1Y6.

We investigated the effects of several excitatory amino acid agonists and antagonists on the responses of dorsal horn neurone responses to noxious and non-noxious cutaneous stimulation in cats anaesthetized with α-chloralose. Extracellular recording and iontophoresis were done using multibarrelled micropipettes. Single micropipettes (KCl, KCH_3SO_4) glued to multibarrelled micropipettes were used for intracellular recording and extracellular iontophoresis. Iontophoretic barrels were filled with: glutamate (1M), quisqualic acid (2.5mM), domoic acid (1mM), N-acetyl-Asp-Glu (NAAG; 10mM), kynurenic acid (1mM), 2-amino-phosphonovalerate (APV; 1mM). Hair stimulation-induced EPSPs were reversibly and repeatably blocked by iontophoretic application of kynurenic acid in two cells. Iontophoretic application of APV and kynurenic acid as well as i.v. administration of ketamine (5 mg/kg) blocked the response to non-noxious thermal stimulation, but, with 4 cells, failed to alter the noxious thermal response. Quisqualate and glutamate potentiated the afterdischarge upon noxious mechanical stimulation (n=5); these cells were unresponsive to domoic acid. Domoic acid produced a prolonged discharge in two non-nociceptive neurones. NAAG had no effect on any cell (n=13). Supported by the Canadian MRC and the NIH. YDK was funded by the FRSQ.

174.5

Application of Whole-Cell Patch Clamp Techniques to Dorsal Horn Neurones of the Hemisected Rat Spinal Cord. **S. Alford*, G.L. Collingridge* and R.H. Evans*. (SPON: Brain Research Association). Dept. Pharmacology, School of Medical Sciences, University of Bristol, University Walk, Bristol BS8 1TD UK.**

We have made whole-cell patch clamp recordings (Blanton et al., 1989; J. Neurosci. Methods 30;203) from dorsal horn neurones (DHN) in hemisected spinal cord preparations from 3-5 day rats. DHN patch clamped with pipettes containing KmeSO4 as the principal ionic constituent had impedances ranging from 300-400 Mohms and resting membrane potentials of -60 +/- 1mV sem. Stable, low noise (<1.0 pA rms), recordings were maintained for 2-3 hrs. DHN were characterised by their regenerative response to a 15 to 20mV step and the presence of both spontaneous and dorsal root evoked E- and IPSCs. Spontaneous and later components (>80ms) of dorsal root-evoked EPSCs were sensitive to the NMDA receptor antagonist (+/-) 2-AP5 (10uM). Short latency (to 80 ms) components of dorsal root-evoked EPSCs were resistant to AP5 (400uM). At a holding potential of -60mV unitary monosynaptic EPSCs with amplitudes of approximately 20pA were evoked by stimulation of the dorsal root at near threshold intensity. This demonstrates the suitability of the patch clamp technique for the study of primary afferent input to an intact spinal preparation.

174.6

GLYCINE AND GABA-MEDIATED IPSPS IN RAT SUBSTANTIA GELATINOSA EVOKED BY PRIMARY AFFERENT STIMULATION. T. M. Jessell & M. Yoshimura, Center for Neurobiology and Howard Hughes Medical Institute. Columbia University. New York, NY. 10032

To provide further information on mechanisms involved in the regulation of pain transmission at primary afferent synapses we have examined the properties of inhibitory synaptic responses (ipsps) evoked by A-delta afferent fibers. Intracellular and whole cell voltage clamp recordings were obtained from substantia gelatinosa (s.g) neurons in an adult rat spinal cord slice preparation which retains an attached dorsal root.

The ipsps were divided into two groups, based on their duration and sensitivity to glycine and GABA receptor antagonists. Fast ipsps were observed in more than 60 % of s.g. neurons and had a duration of 30-60 ms and were blocked by glycine receptor antagonist strychnine. Slow ipsps were observed in about 30 % of s.g. neurons, and had a much longer time course (80-300 ms) and were blocked by the $GABA_A$ receptor antagonist bicuculline. A subset of s.g neurons exhibited both fast and slow ipsps. The excitatory amino acid receptor antagonist CNQX blocked afferent-evoked fast and slow ipsps as well as the monosynaptic afferent evoked epsp. Spontaneous ipsps were observed in 50 % of s.g neurons and were blocked by either strychnine and bicuculline but not by CNQX. Afferent evoked ipsps are likely to be di- or polysynaptic. The fast and slow ipsps were reduced in amplitude with membrane hyperpolarization and reversed in polarity at around -75 mV.

These findings provide evidence that A-delta fibers activate local interneurons which release glycine or GABA as transmitters on subset of s.g. neurons. This inhibitory network may play a role in modulating nociceptive transmission in the substantia gelatinosa.

174.7

CHARACTERIZATION OF INHIBITORY MECHANISMS IN RESPONSE TO LOW-THRESHOLD MECHANICAL STIMULATION; AN *IN VIVO* INTRACELLULAR STUDY USING INPUT FROM SINGLE PRIMARY AFFERENTS TO SINGLE DORSAL HORN NEURONES IN THE CAT SPINAL CORD. Y. De Koninck and J.L. Henry. Depts. of Physiology, Anaesthesia Research and Psychiatry McGill Univ., Montréal, Qué. H3G 1Y6.

The present study focuses on inhibitory responses following input from low-threshold afferents to functionally identified single dorsal horn neurones. Extracellular multibarrelled micropipettes and intracellular single micropipettes (KCl or K-CH_3SO_4) were used to record responses in dorsal horn neurones to low threshold stimulation of the receptive field and to intracellular activation of single primary afferents. Cats were anaesthetized with α-chloralose or decerebrated. Spinal segments L_5-L_7 were exposed for recording of dorsal horn neurones and ganglia L_6-S_1 for intracellular recording and stimulation of single DRG neurones. An automatically controlled mechanical stimulator was used for low threshold mechanical stimulation of the receptive field. Single mechanical pulses outside the receptive field of the dorsal horn neurone induced a large bicuculline-sensitive IPSP (0.3-1.0mg/kg i.v.) in some of these neurones. This IPSP was depressed upon repetitive stimulation at interstimulus intervals of less than 2s. A similar IPSP and depression of the second of paired IPSPs was observed upon stimulation of a single hair-afferent arising from the inhibitory receptive field of the dorsal horn neurone. Single hair afferent input from the excitatory receptive field produced excitatory responses; the second of paired EPSPs followed the same type of depression as the 2 IPSPs. The time course of the depression of the second of these pairs of responses matched that of primary afferent depolarization (PAD). Bicuculline attenuated both PAD and the depression of the second EPSP. These results suggest that both inhibitory and excitatory responses from low threshold afferent stimulation are affected similarly by repetitive stimulation and are subject to similar regulatory mechanisms. (Supported by the MRC, YDK is funded by the FRSQ.)

174.8

IONIC MECHANISMS OF THE PROLONGED SYNAPTIC EXCITATION OF RAT SPINAL CORD NEURONS. R. Cerne, G. Gerber*, M. Jiang*, V. Parpura and M. Randic. Dept. of Vet. Physiol. and Pharmacol., Iowa State University, Ames, IA, 50011.

Slow excitatory postsynaptic potential (EPSP) evoked in deep dorsal horn neurons by single or repetitive stimulation of dorsal roots has two components, an initial transient component that requires activation of excitatory amino acid receptors and a late longer-lasting probably peptidergic component (Neurosci. Lett. 106: 220-228). Since the ionic mechanism of the slow EPSP has not been characterized, we used spinal slice preparation and current- and voltage-clamp techniques to examine the effects of: 1) changes in ionic microenvironment and, 2) agents that interfere with influx, release and/or storage of Ca^{2+}. We found that at least two ionic mechanisms are required to explain the generation of the slow EPSP, a conductance decrease of a voltage-dependent K^+ current and a conductance increase possibly to Na^+ and/or Ca^{2+}. Furthermore, amplitude and especially duration of the late component of the slow EPSP was increased after bath-applied caffeine (5-10 μM) or intracellular iontophoresis of BAPTA (20 mM). In some of the caffeine-sensitive cells the slow depolarization was suppressed by $LaCl_3$ (10-100 μM) and nifedipine (1-10 μM). Whereas dantrolene (20 μM) depressed the late depolarizing component, both depression and potentiation was observed with ryanodine (1-100 μM). The initial component of the slow EPSP was not significantly modified by any of the agents used. These results suggest that the increase in Ca^{2+} influx and the changes in intracellular free Ca^{2+} are important during the late phase of the slow depolarizing response of the rat dorsal horn neurons. Supported by NIH and NSF.

174.9

DIFFERENT POPULATIONS OF PARVALBUMIN- AND CALBINDIN-IMMUNOREACTIVE NEURONS CONTAIN GABA AND ACCUMULATE ^{3}H-ASPARTATE IN THE DORSAL HORN OF THE RAT SPINAL CORD M. Antal[1,4], E. Polgar[1], J. Chalmers[2], J.B. Minson[2], I. Llewellyn-Smith[2], C.W. Heizmann[3] and P. Somogyi[4]. [1]Dept.Anat, Univ.Med.School, Debrecen, Hungary; [2]Dept. Med., Flinders Univ., Adelaide, Australia; [3]Dept.Pediat., Univ. Zurich, Switzerland; [4]MRC Anat Neuropharmacol. Unit, Oxford,U.K.

The coexistence of calcium binding proteins (parvalbumin and calbindin-D 28k) with GABA and the uptake of ^{3}H-Asp was studied in laminae I-IV of the dorsal horn of the rat spinal cord. After injecting ^{3}H-D-Asp into the dorsal horn, perikarya selectively accumulating ^{3}H-D-Asp were detected in araldite embedded semithin sections by autoradiography. Consecutive semithin sections were reacted to reveal parvalbumin (PV), calbindin (CaBP) or GABA by postembedding immunocytochemistry.

Nearly 70% of the total population of PV-immunoreactive cells were also GABA-positive in laminae II and III, but only one fourth of them proved to be GABA-immunoreactive in lamina IV. PV was never detected in neurons accumulating ^{3}H-D-Asp.

Most of the cells accumulating ^{3}H-D-Asp were in lamina II where 16% of them displayed CaBP-immunoreactivity. On the other hand, 99% of CaBP-immunoreactive perikarya were GABA-negative. A significant proportion of the GABA-negative but PV-immunoreactive neurons showed CaBP-immunoreactivity as well.

The results show that interneurons using putative inhibitory and excitatory amino acid transmitters differ in their calcium binding proteins.

174.10

ELECTROPHYSIOLOGICAL PROPERTIES OF FROG SPINAL DORSAL HORN NEURONS AND THEIR RESPONSES TO SEROTONIN: AN INTRACELLULAR STUDY IN THE ISOLATED HEMISECTED SPINAL CORD. **H. Tan and V. Miletic. Dept. Comp. Biosci., Sch. Vet. Med., Univ. of Wisconsin, Madison, WI 53706.**

In the present study we have used intracellular recordings to examine the electrophysiological properties of frog (R. Pipiens) dorsal horn neurons and their responses to serotonin (5HT). We found that the average resting membrane potential and amplitude of action potentials were 63.2±1.8 mV and 57.5±6.3 mV (mean±SD, n=30) respectively. The membrane input resistance ranged from 49 to 240 MΩ (83.7±5.4, n=9). Spontaneous EPSPs and IPSPs were also observed. About 39% of tested neurons (14/36) exhibited spontaneous action potentials at rest. The majority of tested cells (11/14) fired single spikes, while a few (3/14) exhibited burst discharges. Stimulation of dorsal roots with single shocks elicited postsynaptic potentials with different latencies and amplitudes in all of the 17 tested neurons, suggesting the activation of different sizes of afferent fibers. The current-voltage relationship of a few tested cells was found to be linear.

Bath application of 5HT (10-50 μM) increased the excitability and caused membrane depolarizations in 50% of tested neurons (7/14). 5HT also inhibited 4/14 dorsal horn cells by hyperpolarizing the membrane and suppressing the spontaneous activity. Biphasic response to 5HT were seen in 2/14 cells.

These data indicate that the basic membrane properties of frog dorsal horn neurons are comparable with those in mammalian spinal cord slice preparations, and the multiple effects of 5HT may be mediated through different 5HT receptor subtypes. (Supported by NIH NS21278).

174.11

CAFFEINE BLOCKS THE ADRENAL-MEDIATED, NALOXONE-REVERSIBLE INCREASE IN REACTION TIME IN THE TAIL-FLICK TEST FOLLOWING INTRATHECAL ADMINISTRATION OF SUBSTANCE P AT THE LOWER THORACIC SPINAL LEVEL IN THE RAT. K. Yashpal and J.L. Henry, Depts. of Physiol. and Psychiat., McGill Univ., Montréal, Qué.

We have previously shown that intrathecal administration of substance P (SP) in the awake Sprague Dawley rat increases reaction time in the tail-flick test and this increase is blocked by adrenalectomy and by naloxone but not by phentolamine or by morphine antagonists which do not cross the blood-brain barrier (Cridland & Henry, Neuroscience 26: 243, 1988); the suggestion was made that SP activated sympathetic neurons to the adrenal medullae, caused the release of an opioid into the blood and this opioid acts in the central nervous system to depress the tail flick reflex. As adenosine has been proposed to mediate the spinal antinociceptive effects of morphine in the tail-flick test (Jurna, N.S. Arch Pharmacol. 327:23, 1984), the present study was done to determine whether adenosine might also mediate the antinociceptive effects of the adrenal opioid. In control rats, pretreated with 0.5 mL saline i.p., intrathecal administration of 6.5 nmols of SP produced an increase in reaction time of 15-20% which lasted 15-20 min (n=12). Pretreatment with 30 mg/kg of the adenosine antagonist, caffeine, had no effect on reaction time; however, subsequent administration of 6.5 nmols of SP decreased reaction time by about 70% and produced behavioural effects suggesting the animals perceived a noxious stimulus. These results indicate that the adrenal-induced analgesia observed earlier may be mediated by a purine mechanism. (Supported by Canadian MRC)

174.12

GALLAMINE RESPONSES OF SPINAL NEURONAL NETWORKS IN CULTURE T.K.Baumann[1] and G.W.Gross[2], [1]Div. of Neurosurgery and Dept. of Pharmacology, OHSU, Portland, OR 97201 and [2]Dept. of Biological Sciences and Center for Network Neuroscience, Univ. of North Texas, Denton, TX 76203

The effects of the neuromuscular blocker gallamine triethiodide (G) were examined in monolayer cultures of fetal mouse spinal cord and DRG neurons grown on glass plates with 64 photoetched microelectrodes. Under control conditions (in conditioned medium), most cultures (7/8) showed spontaneous activity by random bursts of action potentials (APs). The addition of G to the bath (100 μg/ml) disrupted the discharge pattern; it lead to approx. 10 s of high frequency, tonic AP discharge, followed by a gradual decline in firing to near zero. Minutes later, AP discharge gradually returned, in the form of regularly occurring brief bursts, and was maintained for minutes. Lower doses of G (10 μg/ml) had less pronounced effects. Higher doses of G (1 mg/ml) resulted in a more prolonged period of inactivity. This was reversible upon washing with conditioned medium.

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174.13

ANTINOCICEPTIVE AND BLOOD PRESSURE EFFECTS OF INTRATHECAL GUANABENZ AND CLONIDINE.

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Guanabenz Acetate (GA), an alpha-2 agonist, possesses a higher affinity for alpha-2 receptors than clonidine (CL) (Pharmacol Rev 337,1980). GA suppresses opioid withdrawal with less side effects than CL (Drug Intell Clin Pharm 32,1985). GA may have antinociceptive effects like CL. However, intrathecally administered GA has not been extensively evaluated. The present study was undertaken to assess the antinociceptive and blood pressure effects of spinal GA and CL in rats implanted with chronic intrathecal (i.t.) catheters. Following i.t. administration of drugs, antinociceptive effects were assayed using the hot-plate test, and mean arterial pressure (MAP) was measured via femoral artery during halothane (0.6 %) and oxygen anesthesia. GA (25, 50, 100 μg), CL (5, 10, 25 μg) and yohimbine (10 μg) were administered. GA (100 μg) and CL (25 μg) showed significant elevations of nociceptive threshold. All doses of GA elicited decreases in MAP, but a dose-dependent effect was not observed. CL (25 μg) produced a transient elevation of MAP followed by hypotension, whereas CL (5 and 25 μg) elicited only hypotensive effects. I.t. yohimbine alone caused no significant effects on the resting MAP, and yohimbine pretreatment significantly attenuated the hypotensive effects of GA and CL. Our study demonstrates that i.t. GA has an antinociceptive effect on the hot-plate test, as was shown for CL. However, GA produced different blood pressure effects than CL. Further studies of the antinociceptive actions of GA should be conducted.

174.14

EXPRESSION OF GAD AND GABA BY MOTONEURONS OF THE CHICK SPINAL CORD: IMMUNOCYTOCHEMICAL AND RADIOAUTOGRAPHIC APPROACH.

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Among the phenotypes normally expressed by vertebrate spinal motoneurons, it is well accepted that acetylcholine is the classical excitatory neurotransmitter of the neuromuscular junction.

However, by means of immunocytochemical procedures (according to the peroxidase antiperoxidase method), it has recently been shown that the motoneurons of the chick spinal cord also express a classical inhibitory neurotransmitter: GABA (gamma aminobutyric acid) (Philippe et al., 1990).

In order to establish a better understanding of the presence of GABA in motoneurons and their related neuromuscular junctions, it seems important to determine whether the presence of GABA is due to the action of the biosynthetic enzyme GAD (glutamic acid decarboxylase) or to the result of a high affinity uptake of this inhibitory amino acid.

According to the colocalization of GAD and GABA within chick spinal motoneurons, we could suggest that GABA is synthesized within the perikaryon of these motoneurons. However, these results do not exclude the possibility of a high affinity uptake of GABA by chick spinal motoneurons. (Supported by grants of C.R.M. and F.R.S.Q.)

SOMATIC AND VISCERAL AFFERENTS I

175.1

EXCITATION AND SENSITIZATION OF NOCICEPTORS BY ACID PH IN RAT SKIN, IN VITRO.

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Primary afferent responsiveness to pathologically relevant pH levels was studied in a rat skin-nerve preparation, in vitro. Receptive fields were superfused at the corium side (5 min at 10 min intervals) with CO_2 saturated "synthetic interstitial fluid" (SIF at pH 6.1) and with phosphate buffered SIF at varying pH (7.0-4.3). Mechanosensitive A-beta and A-delta fibers were not excited by such superfusions; in 28 of all nociceptor type C and A-delta fibers (n=84) low frequency ongoing discharge was induced. In contrast, 40% of the mechano-heat sensitive C and A-delta nociceptors (CMH and AMH) showed dose and duration dependent responses with a mean maximum discharge at pH 5.2 and thresholds between pH 6.9 and 6.1. Prolonged application (30 min) evoked non-adapting activity around 5 spikes per s. Phosphate buffered SIF at pH 6.1 was as effective in exciting as CO_2 saturated SIF but showed significantly longer latencies. The carboanhydrase blocker acetazolamide markedly delayed and reduced the CO_2 responses. Prolonged or repeated application of acid pH induced a marked and long lasting decrease of the von Frey thresholds in almost all CMH fibers tested (from 35 to 16 mN on average, p = 0.001 Wilcoxon test). This is remarkable, since previously even an extensive combination of inflammatory mediators had only induced sensitization to heat, but never an increase to mechanical sensitivity in this skin-nerve preparation.

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175.2

CHEMOSENSITIVITY AND SENSITIZATION OF MECHANICALLY INSENSITIVE AFFERENTS IN THE PRIMATE.

K.D. Davis, R.A. Meyer, R.D. Treede, R.H. Cohen and J.N. Campbell. Dept. of Neurosurgery, The Johns Hopkins University, Baltimore, MD 21205.

We recently described a group of A- and C-fiber afferents that are either not sensitive to mechanical stimuli or have very high mechanical thresholds. To explore the role of these mechanically insensitive afferents (MIAs) in the pain and hyperalgesia that accompany injury, we examined the effects of intradermal injection of algesic/inflammatory chemicals.

Standard teased fiber techniques were used to record from 14 A- and 9 C-fiber MIAs in cutaneous nerves of monkeys (Macaca fascicularis). The fiber's presumed receptive field (RF) was located with an electrocutaneous search technique. Sensitivity to mechanical and heat stimuli was determined with calibrated von Frey filaments and a CO_2 laser before and after intradermal injection of a chemical soup into the fiber's RF. The soup consisted of bradykinin, histamine, 5-HT (10^{-8} moles) and PGE1 (10^{-10} moles) in 10μl saline. Nine A- and five C-fiber MIAs responded to the soup either immediately upon injection or after a 1.5-4 min delay. Evoked responses lasted for 3 to >20 min and the peak discharge frequency varied from 1-60 spikes/5s. Six MIAs (3As, 3Cs) became sensitized to mechanical stimuli following soup injection, and in two cases there was an accompanying expansion of RF. Three MIAs were sensitized to heat stimuli.

These data suggest that some MIAs play a role in the pain and/or hyperalgesia induced by certain injuries.
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175.3

PROSTAGLANDIN E_1 SENSITIZES CUTANEOUS NOCICEPTORS IN MONKEY.

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Prostaglandin E (PGE), a product of the cyclo-oxygenase arachidonic acid pathway, is considered to be a mediator of inflammatory pain and hyperalgesia. In this study, we determined the effects of PGE_1 on the response properties of C- and A- fiber nociceptors responsive to mechanical and heat stimuli (CMHs & AMHs). Standard teased-fiber techniques were used to record from single CMHs and AMHs that innervated hairy skin of pentobarbital-anesthetized monkey (Macaca fasicularis). Responses to a sequence of heat stimuli (laser heat stimulator, 39°-49° C) and mechanical thresholds (von Frey probes) were compared before and after PGE_1 (3x 10^{-10} moles in 10 μl) injection into the receptive field of the nociceptors. The injection of PGE_1 resulted in evoked responses in 7 of 12 CMHs and 2 of 4 AMHs. PGE_1-induced sensitization of CMHs was characterized by a decrease in heat threshold (pre= 42.9 ± 0.8, post= 40.8 ± 0.6, $p<0.01$, t-test) and an increase in response to suprathreshold stimuli. The total evoked response of CMHs to the heat sequence increased by 80 ± 30 % after PGE_1 injection ($p<0.01$). Sensitization to heat was also observed in the AMHs studied. The heat threshold decreased from >49° C before PGE_1 injection to 47.5 ± 0.7 after the injection. A decrease in mechanical threshold after PGE_1 injection was observed in 3 of 10 CMHs, and 2 of 4 AMHs tested . The results indicate that PGE_1 sensitizes both cutaneous CMHs and AMHs to heat stimuli. The mechanical threshold was, however, decreased only in a proportion of the nociceptors. These data provide additional evidence for a role of PGE in the hyperalgesia associated with inflammation. (Supported by NS-26363)

175.4

STIMULUS HISTORY AFFECTS THE HEAT RESPONSE OF C-NOCICEPTORS.

D.B.Oakland, R.A.Meyer, and J.N.Campbell, Johns Hopkins University, Baltimore, MD 21205.

To develop a kinetic model which accounts for the heat response properties of C-fiber nociceptors, we studied the effects of stimulus history on responses to heat stimuli. Single C-fiber nociceptors sensitive to mechanical and heat stimuli (CMHs) were studied in the anesthetized monkey (Macaca fasicularis). A Peltier device was used to deliver heat stimuli at interstimulus intervals (ISI) of 10, 30, 60, 120, 300, or 600 s at stimulus rise rates of 1°C/s and 10°C/s. CMHs exhibited a marked suppression of response as the ISI decreased or the intensity of the preceding stimulus increased. This suppression was manifest in three ways: (1) an increase in latency to the first action potential; (2) a decrease in peak discharge frequency; (3) a decrease in total evoked response. The increase in latency was significantly greater for the slow ramps than the fast ramps. This is likely explained by a transient increase in the heat threshold of the receptor. The decrease in peak discharge frequency was significantly greater for the fast ramps. This suggests that the phasic part of the suprathreshold response is more susceptible to suppression. We have recently shown that the apparent heat threshold of a CMH is also dependent on stimulus parameters. These results will be used in a model of the heat response of CMHs that incorporates heat transmission, transduction, action potential initiation and conduction.

175.5

THE APPARENT HEAT THRESHOLD OF A C-FIBER NOCICEPTOR IS DEPENDENT ON STIMULUS PARAMETERS. R.-D.Treede*, D.B.Oakland, R.A.Meyer, and J.N.Campbell, Johns Hopkins Univ., Baltimore, MD 21205.

The skin surface temperature necessary to activate a cutaneous nociceptive receptor is determined not only by the response properties of the neural transducer, but also by the thermal properties and thickness of the overlying skin. To examine the influence of stimulus parameters on heat threshold, we recorded the responses of single C-fiber nociceptors that innervated the hairy skin of anesthetized monkey (Macaca fascicularis). Heat stimuli were delivered by a laser thermal stimulator or a Peltier device. In the first set of experiments, the heat stimulus consisted of a stepped increase in surface temperature. Thresholds were significantly lower when stimulus duration was 30 s as opposed to 1 s. Similarly, thresholds for a 1 s duration stimulus from a 38°C base were significantly lower than those from a 35°C base. In the second set of experiments, the heat stimulus consisted of a linear increase in surface temperature at a rate that ranged from 10 to 0.1°C/s. The heat threshold increased with faster ramp rates. These results indicate that the heat threshold of C-fiber nociceptors depends primarily on the temperature rather than rate of temperature change at the depth of the neural transducer. Differences in threshold as a function of base temperature, stimulus duration, and ramp rate are likely due to the thermal transmission properties of skin.

175.6

CHARACTERIZATION OF ACTIVITY-DEPENDENT MODULATION OF EXCITABILITY IN SINGLE Aβ, Aδ, AND C FIBERS OF RAT SCIATIC NERVE. Hyung-Cheul Shin & Stephen A. Raymond, Anesthesia Research Labs, Brigham & Womens' Hospital, Harvard Medical School, Boston, MA 02115.

The excitability of axon membrane changes after transmission of impulses, making it possible for the axon to serve as a memory element. Theories for the role of axon memory in neuronal information handling rely on properties of activity-dependence in axons of A and C fibers. However, there has been no comprehensive description of activity-dependence for mammalian fibers. Here we report on comparative studies of aftereffects of impulse discharge on the excitability of rat sciatic nerve fibers with resting conduction velocities ranging from 0.5-29 m/s (0.5 Hz).

Across the fiber spectrum there were 4 principal phases: refractoriness, superexcitability (S), hypoexcitability (H1), and depression (H2). After a conditioning impulse (CP), S peaked at 6 ms in Aβ fibers (CV=16-29 m/s) and at 9 ms in the Aδ fibers (CV=2.5-13.0 m/s). Threshold re-crossed the resting level within 25 ms. With more CP's (2 to 4 CP's, 10 ms interval) S diminished, and became briefer in A fibers, and with 8 CP's or more only a "relative" S phase appeared where threshold never descended below the resting level. In C fibers (CV=0.5-2.0 m/s) the S phase was relative even after 1 CP, and appeared only when tonic excitability had been depressed by prior activity. With 1 CP, H1 was observed after S in all A fibers. H1 appeared in only 4 of 9 C fibers, but was more intense in them (Aβ: 10%, Aδ: 15%, C: 30%, 1 CP). H1 peaked earlier in A fibers (Aβ: 48 ms, Aδ: 60 ms) than in C fibers (87 ms), and threshold returned to baseline in ~100 ms in A fibers vs ~200 ms in C fibers. H2 appeared, but with wide variation in degree, in all fibers given repeated bursts of CP's. It generally required more CP's at higher rates to produce significant H2 (>20% rise in threshold) in Aβ fibers.

These results show that excitability in rat fibers depends on impulse activity and that the after-oscillations in threshold differ in phase and magnitude among Aβ, Aδ and C-fibers. They predict fiber-specific patterns of impulse discharge.

175.7

ARE EXCITABILITY AND CONDUCTION PROPERTIES SUFFICIENT TO IDENTIFY MODALITY OF MAMMALIAN CUTANEOUS AFFERENTS? Frederique Popitz-Bergez, Johann G. Thalhammer, G. R. Strichartz and Stephen A. Raymond. Anesthesia Research Laboratories, Brigham & Women's Hospital, Harvard Medical School, Boston, MA. 02115.

Functional identification of cutaneous afferents requires testing the response to natural stimulation of the receptive field as well as determination of the fibre's conduction velocity (CV). Recognizing that resting CV cannot establish modality because of overlap in resting CV among modalities, we have investigated dynamic shifts in excitability and CV during repetitive electrical stimulation of fibres of known modality to learn if such measures could serve as a tool for distinguishing fibre modality even in the absence of the receptive field.

Response latencies to repeated electrical stimulation (1-50 Hz, .2/.5 ms, 20s) were monitored in 18 C fibres, <2m/s (7cold, 11 nociceptors) and 13 Aδ fibres, 4-10 m/s (5 cold, 8 nociceptors) from the sciatic nerve in fully anesthetized rats. As excitability decreased, latency increased in any given fibre. In Aδ and C cold fibres the latency increased initially then stabilized, recovering within a few seconds when stimulation ended. In contrast, the latency in Aδ and C nociceptors increased to a much greater degree than in cold fibres throughout the stimulation period and did not fully recover for up to several minutes afterwards. The magnitude of slowing increased with frequency, but at any given discharge rate the latency changes were tightly clustered among all cold fibres whereas in nociceptors there was a wide range (3-80%). In addition, nociceptors tended to show conduction failure at lower frequencies than the cold fibres.

For the types of fibres studied here, the profile of shift in latency and excitability is a signature of the fibre function. This suggests that measurement of excitability and CV during impulse activity may suffice to characterize the modality of a cutaneous afferent.

175.8

NERVE IMPULSE PATTERNS DEPEND ON ENDOGENOUS OSCILLATIONS IN MEMBRANE EXCITABILITY. Johann G. Thalhammer, Hyung Cheul Shin, Frederique Popitz-Bergez and Stephen A. Raymond. Anesthesia Research, Brigham & Women's Hosp., Harvard Med. Sch., Boston, MA 02115

Intermittent conduction, which is characterized by "on" phases where all impulses conduct or all stimuli succeed and "off" phases where none do, is a common bursting pattern. Here we report a progression in intermittent conduction seen in rat sciatic fibers stimulated at various frequencies with trains of current pulses at intensities ranging from 10-30% above the resting threshold. When the train begins, the alternation between on and off is rapid (0.2-0.5 s), but as the stimulation is sustained the periodicity becomes both slower and more consistent with on and off phases lasting several seconds. Measurements of threshold and conduction latency following bursts of above-threshold conditioning stimuli account for this progression as follows. When stimuli begin, the first discharges each produce superexcitability lasting as long as 50 msec, which favors successive firing. After several spikes, superexcitability declines and a phase of transient hypoexcitability (H1) grows, elevating the threshold by 20-40 % at delays of 20-100 ms after each impulse. This increases the likelihood of failure of a spike. After a failure, H1 decays over several hundred ms yielding a brief off phase where several spikes in sequence are likely to fail. As the intermittent firing proceeds with rapid periodicity for several seconds, the threshold builds up as the "depression" phase (H2) develops. Depending on the intensity of the stimulus pulses it may take 10 or more seconds before the combined effects of depression and H1 are great enough to elevate the threshold above the stimulus to cause a failure. Recovery of the threshold is quick for the H1 component, but as may last as long as a minute for H2, which is consistent with the prolonged off periods that appear when stimulation has been sustained for over a minute.

Rapid intermittent conduction, characteristic of mammalian fibers, may reflect the presence of the H1 phase in these fibers.

175.9

DRG ACTION POTENTIAL DECOMPOSITION DUE TO HIGH FREQUENCY STIMULATION VARIES WITH PERIPHERAL RECEPTOR SUPPLIED. RD Rose. Biological Sciences, Duquesne University, Pgh PA 15282 and Neurobiology & Behavior, SUNY Stony Brook NY 11794

Several spike (AP) components can be resolved via decomposition of APs recorded from DRG somata following high frequency orthodromic stimulation. Brock et al (JP '53) suggested that in motorneurons similar results were indicative of partial AP transmission block occuring at morphological junctures along the conduction path. Ito (Jap JP '57) termed the DRG AP components M, NM, and S for Medulated fiber, Non-Medullated stalk, and Soma. These components can be resolved using high frequency stimulation (Strauss & Duda [Brat lek Listy '82], Harper & Lawson [JP '85]). The present work examines organization of frequency dependent conduction failure in identified DRG neurons.

In lumbosacral DRGs of anesthetized cats, 41 muscle and 32 skin units were examined. Of these 43 followed at frequencies of 1kHz at least briefly (up to 3s) with only minor deterioration in AP configuration that consisted of a slight (<20 mV) decrease in AP amplitude, decreased (or disappearing) afterhyperpolarization amplitude, and increase in baseline AP duration. 30 units failed at frequencies of 40 Hz up. Typically both muscle and skin units with A-alpha/beta conduction velocities followed at frequencies of at least 300 Hz without decomposition. However the range of following frequencies associated with particular afferent types was often broad (from 250 to >1000 Hz for Ias) with no apparent correlation with conduction velocity. APs of units supplying similar peripheral receptors (e.g. Ias, touch domes or high threshold mechanoreceptors [HTMRs]) decomposed in similar and characteristic fashions. For example, in touch dome, HTMR and deep pressure muscle units, spike duration increased due to a delay in the S-component. A concave-up inflexion (termed N2 by Ito) on the rising phase became apparent and then pronounced. N2 however was neither apparent nor pronounced in succesive spikes of muscle stretch of hair afferents. Several other AP characteristics, including augmentation of the shoulder on the repolarizaiton phase, increased baseline AP duration, decreased AP amplitude, etc were also correlated with peripheral receptor type supplied. These data further support the suggestion that DRG neurons innervating different types of peripheral receptors are specialized (physiologically and biochemically) and incorporate different types and/or numbers of ionic channels in their respective membranes (e.g. Rose et al, Neurosci Lett '86).

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175.10

UTERINE PAIN: THE ROLE OF ISCHEMIA AND THE HYPOGASTRIC NERVE. K. J. Berkley, S. Scofield*, and E. Wood*. Dept. of Psychology, Florida St. Univ., Tallahassee, FL 32306-1051.

Sensory fibers in the hypogastric nerve of adult, virgin, anesthetized rats respond to mechanical stimulation of the uterus usually only when the stimuli are noxious. For example, distension of the uterus normally activates fibers only at intensities which produce ischemia (i.e., occlude the blood supply) and which are much greater than those produced by uterine contractions. In order to examine the relation between this neural response and sensation, inflatable balloons were implanted in the uterine horns of rats previously trained to perform an operant escape response to terminate a painful somatic stimulus (tail pinch). The rats' escape responses to tail pinch and to various levels of uterine distension were then assessed before and after bilateral hypogastric neurectomies. Their ability simply to detect different intensities of uterine stimulation was also assessed. Rats escaped and were able to detect uterine stimulation only at distension levels which produced ischemia. Whereas hypogastric neurectomy had no effect on responses to tail pinch, it significantly reduced, and sometimes eliminated both detection and escape responses to uterine distension. These results indicate that, in nulliparous rats, uterine stimulation is consciously appreciated only at levels which produce ischemia and then probably only as pain. In addition, the results suggest that this function is subserved at least in part by the activity of fibers in the hypogastric nerve.

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176.1

SYNAPTIC INTERCONNECTIONS BETWEEN PYRAMIDAL TRACT CELLS AND CORTICOSTRIATAL NEURONS IN RATS. R. L. Cowan and C. J. Wilson. Dept. of Anat. & Neurobiol., U. T. Memphis, Memphis, TN, 38163, USA.

Ipsilateral pyramidal tract (PT) and contralaterally projecting (crossed) corticostriatal neurons were antidromically identified and their synaptic responses to contralateral neostriatal and ipsilateral PT stimulation were examined using intracellular recording *in vivo*. Stimulation of either pathway produced short latency EPSPs in both types of neurons, but these responses overlapped with a much stronger inhibitory response. Intracellular staining revealed that both types of neurons had axon collateral projections to the ipsilateral neostriatum, and extensive intracortical axonal arborizations.

Both types of neurons showed late responses to contralateral striatal stimulation. Late responses to PT stimulation were usually absent in PT neurons, but present in crossed corticostriatal neurons. The late responses to contralateral striatal stimulation and PT stimulation consisted of a prolonged (≈150 ms) period of membrane polarization and an absence of background synaptic noise, followed by a period of depolarization and increased synaptic noise. The latency and duration of the late depolarization were variable. Both components of the delayed response resembled spontaneously occurring but less predictablefluctuations in the membrane potential.

The excitatory EPSPs evoked in corticostriatal neurons and in PT neurons by antidromic activation of their axons suggest that these two kinds of striatal-projecting neurons make direct mutually excitatory synaptic contacts. The powerful IPSPs, which predominate in the response to strong stimulation, indicate the existence of an interneuron pool contacted by the axons of both types of pyramidal cells.

176.2

TWO TYPES OF NEURON AND THEIR SYNAPTIC INPUTS IN RAT NEOSTRIATUM. Z.G. Jiang and R.A. North. Vollum Institute, Oregon Health Sciences Univ., Portland, OR 97201.

Rat brain slices were used for intracellular recording from dorsal striatum neurons (n=338). Two types of cell were distinguished. Principal cells (96%) had very negative resting potentials (-90 mV), low input resistances (40 MΩ), and showed quickly developing (ms) inward rectification (conductance 10 nS at -65 mV, 99 nS at -120 mV). Secondary cells (4%) were less polarized (-60 mV), had higher input resistances (120 MΩ), and hyperpolarization caused a slower increase in conductance with properties of H-current. Intracellular labeling (biocytin-avidin-HRP) showed that principal cells had a medium sized soma (10-18 μm), dendritic trees densely studded with spines and, sometimes, a main axon extending toward globus pallidus. Focal stimulation of the globus pallidus evoked an antidromic action potential in 54 of 85 principal neurons. Stimulation of the corticostriate fibers evoked a depolarizing postsynaptic potential in 78 of 91 principal cells, which was reversibly blocked by CNQX and APV, but unaffected by bicuculline. Focal stimulation within the striatum evoked a synaptic potential that was partially blocked by CNQX and APV, but completely blocked by adding bicuculline and/or picrotoxin. Conclusion: the principal striatal neurons are projection cells receiving glutamate synaptic inputs from cerebral cortex and GABA inputs from intrinsic cells. The minority of cells seem to be interneurons but also receive glutamate and GABA inputs.

176.3

CURRENT TO FREQUENCY TRANSDUCTION IN NEOSTRIATAL NEURONS. J.Bargas, E.Galarraga, M.Cristancho*, J.C.Pineda* and J.Aceves. Depto. de Fisiología, CINVESTAV. I.P.N. Ap. Post. 14-740, México,D.F. 07000, MEXICO.

Intracellular recordings were obtained from *in vitro* brain slice preparations of the rat neostriatum. Intensity-frequency (I-f) and frequency-time (f-t) relationships using direct current injections (>1.5 s) were analyzed. The firing pattern was tonic with relatively little adaptation. Adaptation was present only with the stronger stimuli. I-f overall slopes were: 87±8 (Hz/nA) for initial frequencies and 50±8 (Hz/nA) for final frequencies (n = 14, mean±SEM, 29°C). In many cases the I-f plot presented a greater slope for weaker than for stronger stimuli. I-f relationships were similar for the first and for the last interspike intervals. Most adaptation was reached within the beginning of the stimulus ($\tau \simeq$ 300-600 ms). Cd^{2+}(50, 100 μM) increased the slope of the I-f function, but it did not change the general shape of this relationship. Neurons could still adapt in the presence of Cd^{2+}. Ni^{2+}(100 μM) had no effects on the same parameters. Apamin also (1 μM) increased the slope of the I-f relationship. Supported by CONACyT: P228CCOX-891576 and P228CCOX891559 to E.G. and J.B.

176.4

CARBACHOL DELAYS ACTION POTENTIAL REPOLARIZATION AND DECREASES THE AFTERHYPERPOLARIZATION IN NEOSTRIATAL NEURONS. J.C.Pineda*, E.Galarraga, J.Bargas and J.Aceves. Depto. de Fisiología. CINVESTAV. I.P.N. Ap. Post. 14-740, México, D.F. 07000, MEXICO.

Intracellular recordings were obtained from *in vitro* brain slice preparations of the rat neostriatum. Ionic substitution experiments showed that Cd^{2+} (50, 100 μM), but not Ni^{2+} (100 μM), increased action potential (AP) duration, decreased the repolarizing $\partial V/\partial t$ and reduced both phases (fast and slow) of the afterhyperpolarization (AHP). Apamin (1 μM) only reduced the slow AHP. These results suggest that Ca^{2+}-activated components of the outward current are present in both repolarization and AHP. The Ca^{2+} current involved may be high-voltage activated and Cd-sensitive. Carbachol (10 μM) also reduced the slow AHP and delayed AP repolarization. These results suggest that carbachol might modulate the Ca^{2+} or K^+ conductances involved in the AP and AHP generating mechanisms of these neurons. Supported by CONACyT grants P228CCOX891576 to E.G. and P228CCOX891559 to J.B.

176.5

CONTRIBUTION OF EXCITATORY AMINO ACIDS TO SPONTANEOUS SYNAPTIC ACTIVITY IN CAUDATE NEURONS. C. Cepeda, C. Meier, N.A. Buchwald and P.L. Herrling. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024 (USA) and Sandoz Research Institute, CH-3000, Bern, Switzerland.

The present study assessed spontaneous postsynaptic potentials in caudate (Cd) neurons using in vivo and in vitro preparations. Cd neurons are generally silent. However, in vivo intracellular recordings reveal the presence of a barrage of spontaneous depolarizations (5-15 mV) that rarely produce action potentials. In in vitro recordings spontaneous depolarizations occur infrequently suggesting that they are generated by extrinsic afferents. A major source of excitatory input to the Cd is the cortex. The likely transmitter of this input is an excitatory amino acid. In vitro experiments in rat Cd slices combined iontophoretic application of NMDA with intracellular recordings. Electrode tips were 100-200 μm apart. Ejection of NMDA with low currents (-10 to -30 nA) which were insufficient to induce action potentials, consistently produced a depolarizing shift (about 10 mV). Superimposed on this depolarization a series of smaller depolarizations (1-5 mV) occurred. Higher ejection currents (>-30 nA) produced rapid large depolarizations accompanied by bursts of action potentials. Most of these responses were blocked with the NMDA receptor antagonist (D,L)AP-5. In in vivo experiments iontophoretic application of the NMDA receptor antagonist (D)AP-7 failed to block spontaneous depolarizations. CNQX, a non-NMDA receptor preferring antagonist, and kynurenic acid reduced the frequency of spontaneous synaptic activity but were not able to completely eliminate it. Assuming that at least part of the spontaneous activity is of cortical origin, the in vivo observations imply that Cd spontaneous depolarizations appear to be preferentially mediated through non-NMDA receptors. Supported by USPHS HD 5958.

176.6

INTRACELLULAR RESPONSES OF HUMAN CAUDATE AND NEOCORTICAL NEURONS IN CHILDREN: EFFECTS OF NMDA AND DOPAMINE. N.A. Buchwald, C. Cepeda and M.S. Levine. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

Studies of caudate and cortical neurons were made on brain slices obtained surgically during the course of treatment for intractable pediatric epilepsy. The patients were 9 children (4 mo - 9 yrs of age). 15 neocortical and 6 caudate neurons were studied.

Intracellular recordings were combined with iontophoretic application of excitatory amino acids and dopamine (DA). In all cells, resting membrane potential exceeded -60mV and action potential amplitude exceeded 65 mV. Iontophoretic application of NMDA (-25 to -250 nA) onto neocortical cells induced sustained depolarizations accompanied by continuous firing. In contrast, in caudate cells NMDA induced rhythmic oscillatory depolarizations with bursts of action potentials occurring at the peak of the depolarizations. Each oscillation was followed by a pronounced after-hyperpolarization. Both cortical and caudate cells were blocked by AP-5, a specific NMDA antagonist. Concurrent iontophoretic or bath application of DA did not inhibit NMDA responses but in several neurons in both sites, DA potentiated responses. These experiments indicate that the role of NMDA receptors may differ in human cortical and caudate neurons and that DA is capable of modulating some of these responses. Supported by USPHS Grant HD05958.

176.7

PROPERTIES OF A VOLTAGE-DEPENDENT CHLORIDE CURRENT IN RAT NEOSTRIATAL NEURONS. A. Stefani, D.J. Surmeier and S.T. Kitai, Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, Memphis, TN 38163.

Neostriatal cells manifest an inward rectification commonly attributed to a Ba-sensitive K-current. We tested the hypothesis that a chloride current can contribute to the inward relaxation present at negative potentials using whole-cell voltage-clamp techniques.

Whole cell voltage-clamp recordings were made from embryonically-dissociated neostriatal cultures and acutely-dissociated adult neostriatal neurons using standard techniques at room temperature. The usual composition of the pipette solution was (in mM): KFl 100-120, KCl 0-20, CaCl 1, MgCl 2, EGTA 11, Hepes 10, ATP 2, GTP 0.2. The external solution contained in (mM): 130 NaCl, 2-5 KCl, 2 CaCl2, 1 MgCl2, 10 Na-HEPES, 8-12 glucose, 0.001 TTX, 0.2 CdCl2, 0.2 BaCl2, pH=7.3. A multi-barrel superfusion system allowed fast exchange of the external baths in such a way that Na-isethionate could replace the NaCl solutions.

In response to hyperpolarizing commands, a Ba-insensitive slow inward relaxation was observed. This current was not eliminated by the inclusion of 150 mM TEA-Cl in the bath or 120 mM Cs in the patch pipette, suggesting that chloride, and not potassium, was the primary charge carrier. Although active over a broad range of potentials (-20 mV to -150 mV), the steady-state current-voltage relation displayed a clear inward rectification. At negative potentials, the current activated slowly and did not appear to inactivate. The current was not blocked by external Cd at concentrations sufficient to block all Ca currents. This work was supported by NINDS grants NS 20702 and NS 26473.

176.8

DOPAMINERGIC MODULATION OF VOLTAGE-DEPENDENT POTASSIUM CONDUCTANCES IN RAT NEOSTRIATAL NEURONS. S.T. Kitai, D.J. Surmeier and A. Stefani, Dept. of Anatomy and Neurobiology, College of Medicine, University of Tennessee, Memphis, Memphis, TN 38163.

The dopaminergic innervation of the neostriatum by the substantia nigra is of broad clinical and functional importance. However, the neuromodulatory role of dopamine in the neostriatum is unclear. We have used whole-cell and patch voltage-clamp techniques to examine the effects of dopaminergic agonists on the biophysical properties of potassium conductances in acutely-dissociated and cultured neostriatal neurons.

Adult and juvenile neostriatal neurons were acutely dissociated using a procedure similar to that described by Mody et al. (Neurosci.Lett., 96:70,1989). Embryonic neurons were cultured as previously described (Surmeier et al., Dev. Brain Res., 42:265,1988). Whole-cell and cell-attached patch voltage-clamp studies employed standard techniques to isolate K currents (e.g. Surmeier et al. Neurosci. Lett., 103:331,1989). Dopaminergic agonists and antagonists were applied with a multi-barrel pipette positioned within a few hundred microns of the recorded cell.

Preliminary experiments suggest that dopamine (10-50 μM) reversibly decreases a low-threshold, slowly-inactivating potassium current activated by depolarization. This D-like potassium current exhibits steady-state half-activation and half-inactivation voltages that are 10-30 mV more negative than the A-current. The modulation of this current may underlie dopamine's ability to control first spike latency and discharge frequency.
(Supported by NINDS grants NS 20702 and NS 26473).

176.9

PARTICIPATION OF DOPAMINE RECEPTOR SUBTYPES ON CORTICO-STRIATAL TERMINAL EXCITABILITY. M. Garcia-Munoz, S.J. Young and P.M. Groves. School of Medicine, University of California, San Diego, La Jolla, CA 92093.

We previously found that amphetamine- or stimulation-induced increases in dopamine release decrease the electrical excitability of corticostriatal terminals possibly by activation of presynaptic receptors on the terminals. In this study, we examined the effects of D1 and D2 specific agents on corticostriatal excitability in dopamine depleted rats. Experiments were performed on urethane anesthetised male Sprague-Dawley rats pretreated with alpha-methylparatyrosine (250 mg/Kg, i.p.) and reserpine (2.5mg/Kg, i.p.) 12 and 2 hrs before the recording session. Extracellular action potentials were recorded from the contralateral prefrontal cortex. Bipolar stimulating electrodes for antidromic activation of the cortical terminal field and canulae for local administration of drugs were placed in the contralateral striatum. All drugs were infused in a 300nl volume over 5 min. Excitability was assessed by determining the threshold current required for antidromic activation before and immediately after drug infusion. Local administration of the mixed agonist, apomorphine (10μM) significantly decreased excitability (10.2 ± 1.6%, n=4). The D2 agonist, quinpirole (10μM, n=6) did not affect excitability. The D1 agonist SKF38393 (SKF, 2.5 μM) was also without effect in 5 cases and decreased excitability by 6.9 ± 1 % in one case. However, following simultaneous administration of quinpirole and SKF (Q+SKF, n=22) excitability decreased in 13 cases (17.3 ± 2 %, n=13), increased in 6 of the cells (8.6 ±1%) and on 3 occasions no change occurred. Following a decrease in excitability to SKF+Q, apomorpine (10μM) further reduced excitability (8.7 ± 1%, n=10). The D2 antagonist L-sulpiride (10nM) partially reversed the decrease in excitability to SKF+Q, while the D1 antagonist, SCH 23390 (10μM) reversed this effect in only 4 of 16 cases and was ineffective in 12. These results suggest that dopamine effects on corticostriatal terminal excitability may involve the participation of dopamine receptors which differ from the D1 and D2 subtypes or that, since sulpiride and not SCH 23390 reversed the decrease in excitability to the combined administration of D1 and D2 agonists, excitability changes are mainly mediated by the D2 receptor which requires stimulation of D1 receptors for the full expression of its function. (Partially supported by a grant from NIDA.)

176.10

CALCIUM INFLUENCES ON FIRING PATTERN OF DOPAMINE NEURONS IN THE SUBSTANTIA NIGRA PARS COMPACTA. M.A. Häusser* and W.H. Yung* (SPON: Brain Research Association). University Lab. of Physiology, Parks Rd., OXFORD OX1 3PT, U.K.

We have studied immunohistochemically-identified dopamine neurons in an *in vitro* slice preparation of the guinea-pig substantia nigra pars compacta (SNC), and have found that their electrophysiological properties are strongly influenced by the entry of extracellular Ca and the resting intracellular Ca level. Rythmic oscillations were found to underlie the spontaneous pacemaker firing of these neurons. These oscillations were blocked by extracellular Co or Ni, and their pharmacology and voltage-dependence indicate that a T-type Ca channel plays a necessary role. The oscillations appeared to be related to the low-threshold spike (LTS), which was also Co- and Ni-sensitive. Under voltage clamp, the current underlying the late afterhyperpolarization (AHP) following a fast spike was found to be apamin-sensitive. This late AHP was blocked by Co, which also shortened spike width. Blocking the late AHP caused a large increase in the slope of the frequency-current relation and increased resting firing rate and accommodation. Intracellular EGTA also blocked the late AHP and increased resting firing rate. Prolonged ionophoresis of EGTA allowed the expression of "burst" responses, which were not observed in control neurons. We speculate that neurotransmitters which modulate Ca and Ca-dependent channels or intracellular Ca levels may help regulate the transition between pacemaker and burst firing observed in SNC dopamine neurons in vivo.

176.11

ELECTROPHYSIOLOGICAL ACTIONS OF SOMATOSTATIN ON STRIATAL NEURONS. T.W.J.Watson, Neuroscience Research Group, University of Calgary, Calgary Canada T2N4N1.

Somatostatin (SS) is localized within the medium aspiny striatal interneurons. The physiological role of SS in this brain region is uncertain but reports of the effect of intrastriatal injection of SS in vivo suggest that it exerts a potent influence on striatal function. The mechanism underlying these effects is unknown and may simply represent an indirect action secondary to previously demonstrated SS induced release of dopamine (Chesselet and Reisine, J. Neurosci. 3:232-36). Using the in vitro rat striatal slice we tested the hypothesis that SS exerts direct electrophsiological effects on striatal neurons. Parasagital slices were submerged and constantly perfused with buffered-oxygenated artificial CSF. Intracellular voltage recordings were obtained using conventional techniques and drugs were applied through the bath. At concentrations in the μM range SS caused a 3-10 mV membrane hyperpolarization associated with an increase in membrane conductance in 70% of the cells examined. This was accompanied by a suppression of spontaneous firing and anode break spikes. The hyperpolarization was seen with KCl filled electrodes and persists in the presence of 500 μM $CdCl_2$. Thus SS exerts a direct inhibitory action on striatal neurons which appears to involve activation of a membrane K^+ conductance. This direct action on striatal neurons may explain the effects of SS on striatal function noted in vivo.

176.12

EFFECTS OF ENHANCING GABAergic INPUT ON THE INTRACELLULAR RESPONSES OF STRIATAL NEURONS TO STIMULATION OF CORTICAL AFFERENTS *IN VITRO*. E.S. Nisenbaum, T.W. Berger, and A.A. Grace. *Departments of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.*

We have demonstrated previously using an *in vitro* corticostriatal slice preparation that paired impulse stimulation of cortical afferents elicits a facilitation in probability of striatal cell spike discharge for interstimulus intervals (ISI) of 10-250 ms. When the allosteric receptor agonist, pregnanolone, is used to potentiate the effects of endogenously released GABA, then spike discharge is selectively inhibited in response to short ISIs of 10-30 ms. The present experiments have investigated the synaptic responses which underlie this inhibition of spike discharge.

Intracellular recordings from striatal neurons *in vitro* revealed that paired impulse facilitation of spike discharge in response to ISIs of 10-250 ms was mediated by a facilitation of EPSP amplitude to the second impulse of these ISIs. Pregnanolone (5.0-7.5 μM) selectively inhibited spike discharge and decreased EPSP amplitude to the second impulse of ISIs of 10-30 ms. These inhibitory effects were antagonized by bicuculline (20 μM).

When paired impulse stimulation was applied directly using intracellular current injections, a similar facilitation of spike discharge was found to ISIs of 10-250 ms. However, the magnitude of this facilitation was less than that observed in response to afferent stimulation. Moreover, the facilitatory responses to direct current injection were not altered by pregnanolone.

These results demonstrate that potentiation of GABAergic input *in vitro* selectively inhibits cell discharge to short ISIs, thereby restoring the response pattern characteristic of Type II neurons *in vivo*. These data also indicate that this inhibition results from a shunting of cortically evoked EPSPs by activation of afferent GABAergic neurons. (Support: NS19608, MH00343, MH09717, MH42217, Tourette's Syndrome Association, NARSAD).

176.13

INVOLVEMENT OF CHLORIDE CONDUCTANCES IN THE RESPONSE PATTERNS OF STRIATAL NEURONS TO CORTICAL STIMULATION. S-P. Onn, T.W. Berger & A.A. Grace. Depts. of Behavioral Neurosci.& Psychiatry, Univ. of Pittsburgh, Pgh, PA 15260

We have used intracellular recordings from rat striatal neurons in vivo to characterize the response patterns to paired impulse stimulation of cortical afferents. Two types of responses were observed: 1) 29% (7/24) neurons displayed facilitation to the second stimulus when the interstimulus interval (ISI) was 10-20 ms and inhibition when ISI= 100ms (Type I response pattern) whereas 2) the majority (71%; 17/24) of the recorded cells exhibited facilitation when the ISI was 100 ms (the Type II response pattern). Of the 17 Type II neurons, 7 also showed inhibition when ISI=10-20 ms with the remaining 10 neurons exhibiting facilitation at this interval. Using Lucifer-yellow (in 0.1M LiCl) filled electrodes, similar proportions of Type I and Type II neurons have been observed: 35% (17/48) showed the Type I response pattern and 65% (31/48) exhibited the Type II response pattern. However, of the 31 cells with the Type II response pattern, none showed short-interval inhibition. Since the LY electrodes contained chloride ions, we determined if altering intracellular chloride or changing the membrane potential could account for these differences. Recordings with intracellular KCl-filled microelectrodes to date revealed similar proportions of Type I (25%; 3/12) and Type II (75%;9/12) responses with none of Type II neurons showing short-interval inhibition. This result suggests that the failure to observe the short-interval inhibition in Type II neurons when recording with dye filled electrodes is due to reversal of the electrochemical gradient to chloride ions. The membrane potential does not seem to alter the paired-impulse response pattern. This evidence supports a role for a GABA-mediated chloride conductance in the short-interval inhibition of the Type II response pattern. Support by USPHS NS19608, NS08288, MH42217 & MH00343.

176.14

HALOPERIDOL-INDUCED SYNAPTIC CHANGES IN RAT CAUDATE NUCLEUS ARE PREVENTED BY PRIOR TREATMENT WITH MK-801 OR LESIONING OF THE THALAMUS. C.K. Meshul, A. Janowsky*, D.E. Casey*, and R.K. Stallbaumer*. V.A. Medical Center and Oregon Health Sciences University, Portland, Or. 97201

We have previously shown that 14d treatment with haloperidol (0.5 mg/kg/d) causes a 50% increase in the density of "perforated" synapses within the caudate nucleus (Meshul and Casey, 1989). The nerve terminals undergoing the changes, which have twice the diameter of dopamine terminals, were hypothesized to originate in motor cortex and to release glutamate. Blockade of dopamine receptors by haloperidol may also block the inhibitory pathway from globus pallidus to VA/VL of thalamus. This could activate the thalamocortical and corticostriatal pathways, leading to an increase in the density of perforated synapses. To determine if glutamate receptors are associated with this increased synapse density, the NMDA channel blocker, MK-801 (0.3 mg/kg/d), was given in conjunction with haloperidol to rats for 14d. To test the importance of the thalamocortical pathway in the effect of haloperidol, VA/VL of thalamus was lesioned with kainic acid and 10d later, haloperidol was given for 14d. Both MK-801 treatment and thalamic lesioning prevented the increase in perforated synapses due to haloperidol. In addition, WGA-HRP injections into motor cortex labelled numerous perforated synapses in the caudate nucleus. We conclude that glutamate transmission and VA/VL of thalamus may be important in controlling the haloperidol-induced changes in the density of perforated synapses and some of these synapses originate in motor cortex. Supported by the Dept. of Veteran Affairs and NIMH.

176.15

RESPONSES OF SPINY NEURONS IN RAT STRIATAL GRAFTS TO CORTICAL AND THALAMIC STIMULATION. Z.C. Xu, C.J. Wilson and P.C. Emson Department of Anatomy and Neurobiology, University of Tennessee, Memphis. U.S.A. and AFRC Institute of Animal Physiology and Genetics Research, Cambridge, U.K.

Two months after implantation of striatal primodia into the neostriatum of adult rats, intracellular recording was performed *in vivo* to study the responses of graft neurons to the cortical and thalamic stimulation.

The initial responses of most of the graft neurons to cortical or thalamic stimuli were predominant inhibitory postsynaptic potentials (IPSPs), which could be reversed by intracellular injection of Cl^-. Several minutes after impaling the cell, the amplitude of IPSPs became increasingly smaller. Eventually the IPSPs could not be detected. The same stimuli elicited excitatory postsynaptic potentials (EPSPs). Many EPSPs elicited in grafts were monosynaptic. Polysynaptic EPSPs were also observed in some graft neurons. Unlike the spiny neurons in the intact neostriatum, no long lasting hyperpolarization followed the initial EPSP and no rebound excitation was observed in grafts. Using higher stimulus intensity, a burst was evoked in some graft neurons. The onset and duration of the bursts varied even when the stimulus intensity was constant. The neurons were labeled intracellularly with biocytin. Ninty-five percent of the cells labeled in grafts were spiny neurons.

176.16

ACTIVITY OF NEURONS IN THE STRIATUM DURING VISUALLY-TRIGGERED, SELF-PACED AND MEMORY-GUIDED MODE OF MOVEMENT IN THE MONKEY. M. Kimura, T. Aosaki*, Y. Hu*, and K. Watanabe* Department of Physiology, Jichi Medical School, Minamikawachi-machi, Kawachi-gun, Tochigi 329-04, Japan.

Single neuron activity in the putamen (n=171) and caudate nucleus (n=120) was examined during learned arm movements initiated in 3 different behavioral contexts: A monkey depressed 3 push buttons laid out in a triangular form (side length 9 cm) on a panel in an instructed sequence using its arm in 1)visually-triggered, 2) self-paced and 3) memory-guided modes. The pattern of muscle activity were not significantly different during the 3 modes of movements. Forty-nine out of 68 task-related putamen cells showed movement-related activity, while 38 out of 50 caudate cells showed tonic activity during an instructed delay period or activity related to the reward. In 98 cells in which neuron activity was examined in more than 2 modes, a majority of cells (57% in the putamen, 55% in the caudate nucleus) were selectively activated during either visually-triggered or self-paced or memory-guided mode of movement. These results suggested a role of the striatum in retrieving a learned movement in a particular behavioral context.

176.17

EFFECTS OF SYSTEMIC ETHANOL AND BEHAVIORAL CONTEXT ON MANY-NEURON ACTIVITY IN RAT NEOSTRIATUM AND NUCLEUS ACCUMBENS DURING LONG-TERM ON-OFF TREADMILL LOCOMOTOR BEHAVIOR. R.-S. Lee, B.N. Maddux and D.J. Woodward. *Dept. of Cell Biol. and Neurosci., Univ. of Texas Southwestern Med. Ctr., Dallas, TX 75235.*

The objective of this study was to clarify the effects of ethanol on the firing rate of neurons in neostriatum and nucleus accumbens (NAc) at different stages of the experience of treadmill (TM)-induced locomotion. Long-Evans rats were prepared for chronic recording with 20 microwires (25-60μ), implanted into neostriatum and NAc. Rats were trained to walk on a treadmill (30 sec on/30 sec off). The spontaneous activity of single units (on 2-6 separate wires) in neostriatum and NAc neurons was recorded extracellularly 2-4 hours daily up to 10 sessions for 2-7 weeks from the same microwires. Ethanol (0.8-1.4 g/kg, i.p.) was administered 3-4 times for the same rat (n=8) throughout the recording period. Our results demonstrate that: (1) In simultaneously recorded neostriatal neurons, heterogeneous responses to ethanol occur. The firing rate of some cells was unaffected, increased, or decreased. Furthermore, the firing rate or pattern of neostriatal neuronal activity of TM-trained rats often exhibited abrupt changes over several 30 sec on-off intervals during TM exercise after ethanol: a "mode switch" between contexts of neuronal firing (Lee, et. al., 1989, Soc. Neurosci. Abstr., 15: 286). (2) Preliminary observations demonstrate that the "mode switch" property also exists in the region of NAc of TM-trained rat. Our conclusion is that systemic ethanol causes the emergence of context dependent states which determine the dynamic and phasic responses of neurons in neostriatum and NAc to behavioral and pharmacological challenges. *(Supported by AA-3901, DA-02338, MH44337, AFOSR-90-0146, Biological Humanics Foundation)*

177.1

ACTIVATION HISTORY AND FORCE HYSTERESIS IN HUMAN MUSCLE. C.L. Rice*, F. Furbush*, and B. Bigland-Ritchie, J. B. Pierce Foundation Laboratory, New Haven, CT 06519

Muscle force is generally greater for a given stimulus frequency when preceded by higher, rather than lower rates. Cat motor unit studies suggest this strategy may be used during voluntary contractions (Binder-Macleod & Clamann, J. Neurophysiol. 61:208, 1989). We compared the force-frequency values from adductor pollicis during different patterns of stimulation when stimulus rates were increased continuously from 4 to 80Hz, held for 0.5s, then reduced to 4Hz at the same rate (total duration 6.6s). The forces for rates <60Hz were 5 to 30% greater during the descending compared with the ascending sequence. The largest differences generally occurred at rates <20Hz. However, descending sequence values were similar to those seen when each rate was applied separately (steps). Step values were often slightly lower at rates <15Hz, but increased to match the descending sequence values after pre-potentiation by a brief 50Hz burst. When steady state stimulation was preceded by frequencies initially descending from 50Hz, the force was elevated above the step value, but for only 2-5 pulses. This force increment was similar to that seen when a step train started with only a single short (10ms) interval. Thus, most of the hysteresis seen when frequencies changed continuously can be attributed to a lag in the rate of force development or decay, and post-tetanic potentiation.

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177.2

COMPARISON OF MUSCLE FIBER CONDUCTION VELOCITY AND EMG CHARACTERISTICS BETWEEN THE RIGHT AND LEFT BICEPS BRACHII OF NORMAL SUBJECTS. M. J. Blaschak and K. Keesey*, Dept. of Phys. Ther., Boston Univ., Boston, MA 02215.

Comparisons between the normal and involved limbs of patients with unilateral brain lesions is common, made under the assumption that the limbs were comparable prior to injury. The purpose of this study was to determine if such an assumption is valid regarding muscle fiber conduction velocity and surface EMG characteristics. To date, conduction velocity and EMG characteristics have been recorded from the right and left biceps brachii in 9 normal subjects, with data analysis complete in 4. Muscle fiber conduction velocity was recorded during 5 5-sec. stimulated contractions(stimulated at 20 Hz. and averaged over 1 sec. epochs). Surface EMG was recorded during 2 sec. voluntary constant force isometric contractions at each of 3 trials of 7 different force levels. The EMG was recorded in 500 msec. epochs at a rate of 1024 Hz. Comparisons were then made using matched case t-tests between the right and left conduction velocity and the EMG characteristics of median and mean power frequency, the average rectified value and the RMS value.

Preliminary results from 4 subjects indicate that there are no statistical differences between the two limbs for any of the characteristics, providing initial support that, for these measures, the limbs are comparable.

177.3

NORMALIZED ELECTROMYOGRAPHIC ACTIVITY PATTERNS IN HUMAN EXTENSOR CARPI RADIALIS LONGUS AND FLEXOR CARPI RADIALIS MUSCLES: DIFFERENTIAL AND TOTAL ACTIVITY. S. Wolf, P. Catlin*, R. Segal, A. English, T. McMahon*, T. Craft*, L. Mason*, T. Pianta*, L. Couch*. Dept. Rehabilitation Medicine, Division of Physical Therapy and Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, Georgia.

This study examined the relationship of normalized EMG activity from specific locations in extensor carpi radialis longus (ECRL) and flexor carpi radialis longus (FCR) muscles during twelve movements in an effort to gain insight into the possibility of task-specific muscle partitioning in human subjects. Two sets of bipolar fine wire electrodes were inserted into both the ECRL and FCR muscles of twenty normal individuals (nine men, eleven women). Significant (p .05) differences in normalized EMG between proximal and distal sites in the ECRL were found for most movements with the exception of prehension and unsupported wrist extension. The ECRL increased its activity compared to FCR in unsupported wrist movements. Results indicate: 1) portions of the same muscle can be recruited differentially; 2) these portions of muscles may have distinct functional roles; and 3) the central nervous system may both coactivate and reciprocally inhibit muscles lying about the same joint. To more efficiently reeducate dysfunctional muscle, therapeutic exercise may need to include retraining of muscle portions for particular functional tasks.

177.4

PATTERNS OF ELECTROMYOGRAPHIC ACTIVITY IN THE HUMAN LATERAL GASTROCNEMIUS MUSCLE DURING WEIGHTBEARING AND NON-WEIGHTBEARING TASKS. A. English, S. Wolf, P. Catlin*, R. Segal, C. Richardson-Bond*, L. Coratti*, A. Mast*. Dept. Rehabilitation Medicine, Division of Physical Therapy and Department of Anatomy and Cell Biology, Emory University School of Medicine, Atlanta, GA.

This study compared normalized EMG activity from four previously determined locations within the human lateral gastrocnemius, LG, (N=20), during performance of 5 non-weightbearing and 3 weightbearing tasks to determine if muscle compartmentalization observed in cats might also apply to human muscle.

A similar pattern of normalized EMG activity was found at all recording sites when comparing knee flexion in stance to knee flexion in prone. Each distal location had significantly greater activity compared to the proximal lateral location. Normalized data revealed that plantarflexion in stance consistently exhibited significantly greater amounts of activity at all four locations.

The greatest LG activity was observed across locations during plantarflexion in stance. Activity patterns appeared greater when LG was functioning at one joint or stabilizing the ankle rather than acting about two joints. These data lend support to differential activity within loci of LG during specific tasks. Further explorations into muscle compartments may ultimately produce more anatomically-specific exercise applications.

177.5

FATIGUE EFFECTS ON FORCE AND EMG POTENTIATION. T. Hortobágyi and N.J. Lambert, *Dept. of Sport Sciences/Biology, Univ. of Denver*, Denver, CO 80208

The effects of 4 fatigue protocols on stretch-evoked forces and EMG of the right thigh musculature were evaluated in 12 distance runners (48 mi·wk^{-1}) and 12 power athletes (10 tons·wk^{-1}). Power athletes had 1.33 l larger estimated thigh muscle volume ($p < .05$). On each of 3 days, muscular fatigue was induced with 49 reps of isometric (I, 5-s MVC), shortening (S), and lengthening (L, 0.85 rad·s^{-1}) knee extension and, on the 4th day, with a 1-min stretch-shortening jumping exercise (J). Pre- and post-fatigue, a 1-s ramp stretch at 0.30 rad·s^{-1} was superimposed on a 2-s MVC at 145° knee angle. Peak integrated EMG was monitored on the surface of mm. vastus lateralis and biceps femoris. Potentiation was calculated as the difference between the initial 2-s MVC and its EMG and the ensuing peak stretch force and EMG, respectively. There were no significant changes in the EMG of the m. biceps femoris ($p > .05$). Force and EMG fatigue averaged 39N (5%) and 9 μV·s (6%) in the runners ($p > .05$) and 218N (23%) and 19 μV·s (14%) in the power athletes ($p < .05$). With fatigue, the net change in force potentiation was -27N (-29%, I), -9N (-8%, S), 14N (12%, L), and 7N (8%, J) and the uniform decrease in EMG potentiation averaged 25 μV·s (14%) in the runners. In power athletes, the net change in force potentiation was 30N (18%, I), -36N (-23%, S), 49N (32%, L), and 16N (10%, J) and the uniform decrease in EMG potentiation averaged 38 μV·s (98%, $p < .05$). These data suggest a differential stretch response to fatigue across contraction modes and training history of athletes compared to the prediction of the force feedback hypothesis.

177.6

ELECTRICAL RESPONSE OF HUMAN ANKLE DORSIFLEXORS AND PLANTARFLEXORS DURING FATIGUE. V.Galea and A.J.McComas. Dept. of Biomed. Sci.,McMaster University, Hamilton, ON, Canada L8N 3Z5.

The rate of fatigue in human skeletal muscle depends on the frequency of excitation and on the number of stimuli delivered (Garland, ét.al. J.Appl.Physiol. 362:205-213,1988). We have extended these findings by using a wide range of stimulating frequencies (0 - 30Hz) and by comparing fatigue in Tibialis Anterior (TA;ankle dorsiflexor) and Soleus (SOL;plantarflexor) muscles; the effect of ischemia was also studied, as was recovery from fatigue. Ten subjects (out of a total of fifteen) successfully completed the six experiments, five out of those ten also volunteered for the SOL experiments. At least one week rest was allowed between each fatigueing protocol. As anticipated, stimulation at the highest frequencies induced the greatest change in the M-wave (muscle compound action potential; p<.01), but the decrement in area was always less than that in peak-to-peak amplitude. No significant differences emerged in M-wave changes between TA and SOL. Ischemia accelerated the decline in the M-wave (TA; p<.01). Recovery of the M-wave was limited when tetanic stimulation ceased but progressed rapidly after the circulation was restored. M-wave failure occurred at firing rates not normally associated with neuromuscular junction blockade, implicating propagation failure along the sarcolemmal membrane.

177.7

MEDIAN FREQUENCY OF THE MYOELECTRIC SIGNAL IN ANTERIOR AND POSTERIOR NECK MUSCLES. M. A. SABBAHI, P.P. GOGIA*. Texas Woman's University, School of Physical Therapy, Houston, TX 77030.

Characteristics of median frequency (MF) of myoelectric signal, a measurement of localized muscle fatigue, has been used as objective indicator of muscle function. The purpose of this study was to characterize changes in anterior cervical muscles (ACM) and posterior cervical muscles (PCM) during isometric contractions. Twenty-eight normal subjects between the ages of 22 and 43 (31.7 + 5.7) with normal head/neck posture participated in the study. The subjects were equally divided into two groups. EMG of the ACM and PCM was recorded bilaterally during attempted isometric neck forward bend (FB) and backward (BB) in Group A, and during neck right and left side bend (RSB and LSB) in Group B. Subjects were restrained in sitting position in a specially designed force apparatus and performed constant isometric contractions at 20%, 50%, 80% and 100% of maximum voluntary contractions (MCV) in either FB/BB or RSB/LSB. Frequency spectrum of the EMG signal was analyzed through 0.5 sec. windows and the two parameters measured were initial median frequency (IMF) and the MF slope during sustained isometric 10 sec contraction.

Results showed consistently higher IMF as well as MF slope values for the ACM as compared to PCM in FB/BB AND RSB/LSB. Contrary to PCM, the IMF values increased with increasing force levels in ACM. The MF slope values increased with increasing force levels of contraction, however, the slopes were steeper in ACM. These results indicate that ACM and PCM have different fatigue characteristics i.e., the PCM are more fatigue resistant than ACM.

177.8

EFFECTS OF IMMOBILIZATION ON MOTOR FUNCTION IN A HUMAN HAND MUSCLE. A.J. Fuglevand and R.M. Enoka. Depts. of Exercise & Sport Sciences and Physiology, Univ. of Arizona, Tucson, AZ 85721

Immobilization is known to cause alterations in muscle and in motor neuron properties (Mayer et al., Neurosci., 6:725-729, 1981). Little is known, however, about the functional consequences of these immobilization-induced adaptations on motor function . The purpose of this study was to examine the effect of immobilization on the ability to generate and sustain force in first dorsal interosseus (FDI) muscle. Nine subjects volunteered to have the index finger and thumb of their non-dominant hand immobilized in a cast for three weeks. Each subject participated in five experimental sessions: one prior to immobilization, at one and three weeks of immobilization, and at one and three weeks post-immobilization. In each session, abduction force, FDI surface EMG, and single motor unit action potentials of FDI were recorded while subjects performed several isometric abduction tasks. These included: maximum voluntary contractions (MVCs), slow ramp-and-hold contractions, and a sustained contraction to exhaustion at a target level of 35% of MVC. EMG responses in FDI to supramaximal stimulation of ulnar nerve (M-waves) were recorded prior to and following the fatigue task. Despite the constraint in FDI usage imposed by the cast, MVC force was unchanged following immobilization. Motor unit discharge, however, became more variable with immobilization. Surprisingly, endurance time of the fatigue task was 16% longer and M-wave amplitude after the fatigue test was less reduced in immobilized FDI as compared to the pre-immobilized control values. These findings are consistent with decreased fatiguability observed in cat hindlimb motor units following short-term immobilization (Robinson et al., Muscle & Nerve, in press). Supported by USPHS grants NS 07309 and NS 20544.

177.9

HIGH ENERGY METABOLISM IN SEVERELY ATROPHIC MUSCLE FIBERS IN DERMATOMYOSITIS. S. Sesodia, P.M. Nemeth, R.M. Choksi*, A. Pestronk. Depts. of Neurology, Anatomy and Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

The metabolic changes associated with physical weakness and muscle pathology in dermatomyositis were investigated in individual muscle fibers. Dermatomyositis is characterized histologically by severe perifascicular atrophy of muscle fibers having normal differential staining for three fiber types by myosin ATPase histochemistry. Using highly sensitive microfluorometric enzyme assays, we assessed the activities of 2 energy related enzymes, lactate dehydrogenase (LDH) and adenylokinase (AK), in atrophic and normal-sized fibers dissected from transverse sections of muscle. These 2 enzymes metabolically separate human muscle fibers into 3 types corresponding to myosin ATPase types I, IIA and IIB. We found that the non-atrophic fibers had AK and LDH values in the same range as fibers from control human muscle and that their AK/LDH ratios were appropriate to their histochemical type. The atrophic fibers had increased AK or LDH values depending on fiber type. The AK values of the atrophic type I and IIA fibers were, on average, 2-fold above normal and their LDH values were normal. Atrophic IIB fibers had elevated LDH values and normal AK. In contrast, considerable numbers of muscle fibers from Duchenne muscular dystrophy (DMD) have extremely low energy-generating enzyme levels (Chi et al., Metabolism, 1987). We conclude that, unlike a genetic dystrophy (DMD), fibers from dermatomyositis show a normal, or even high, capacity for energy generation.

CORTEX III

178.1

MOTOR CORTEX OUTPUT AND MOTOR UNIT RECRUITMENT IN MOTOR INITIATION. E.B.Montgomery, S.Sahrman, M.Clare, S.Buchholz*, W.M.Landau. Dept. of Neurol. and Neurosurg. (Neurol.) Wash. Univ. Sch. Med., St. Louis, MO. 63110

Whether motor cortex neuronal output activity codes for the patterns of motor unit recruitment or some less specific command was studied using analyses based upon temporal consistencies between neuronal activity changes and behavioral events within a single task. Output neurons were identified as those whose activity changes preceded their best related behavioral event. 37 neurons related to an isometric ankle force task and 46 neurons to a wrist flexion and extension task were studied. Of 73 occasions where activity changes indicated output functions, 66 (90.4%) were best related to force or movement onset while only 7 (9.6%) were best related to agonist or antagonist EMG changes. There was variability between onset time of agonist EMG and onset of force or movement. There was also variabililty in the amount of EMG during this time period, but there was a precise correlation between the amount of EMG and the timing. These findings indicate that different patterns of motor unit recruitment are put forth to accomplish the same task. Poorly synchronized motor unit patterns are less efficient in producing force or movement onset but are recruited earlier.

178.2

CORTICAL NEURAL NETWORK FOR SENSORIMOTOR INFORMATION PROCESSING. **Jean Requin, Alexa Riehle, John Seal,** Cognitive Neuroscience Unit, CNRS-LNF1, 31 ch. J. Aiguier, 13402 Marseille Cedex 9, France.

Single neuron activity was recorded in the primary motor, premotor and superior parietal cortex of the monkey during the performance of simple, choice and precued-response reaction time tasks. The results obtained can be similarly described in terms of three neuronal populations differentiated on the basis of the temporal relationship between changes in neuronal activity and behavioral events: the first population modified its activity with respect to the sensory cue (*"input neuron"*), the second with respect to the motor act (*"output neuron"*), and the third with respect to both these events (*"interfacing neuron"*). The observation that these three populations were found, although with different proportions, in several cortical areas suggests that they may represent a basic modular mechanism of cortical functioning by which the informational content of sensory cues is processed and used to shape adapted behavioral responses. The results provide a basis for our view that the plan for a sensorimotor act is formulated within a large neuronal network of basic modular units that spans the macroanatomical divisions of the neocortex. To elaborate this model further we need: 1) to determine whether all the neurons within a module are members of either a single class whose role in the module is more probabilistic than discrete or, as suggested here, members of several distinct classes are each characterized by their specific role within the module; 2) to describe the functional cooperation among neurons in a module by deciphering their interconnectivity; 3) to discover whether the differences in function of the cortical areas involved in motor planning are qualitative or quantitative; and, 4) to understand how the cognitive processes underlying sensorimotor information processing are implemented, whether continuous or in sequence, by the proposed functional organization of the neocortex. (Supported by ONR grant N00014-89-J1557)

178.3

NEURONAL PREPARATORY ACTIVITY: PREDICTIVE VALUE FOR PERFORMANCE SPEED. Alexa Riehle, Jean Requin, Cognitive Neuroscience Unit, CNRS-LNF1, 31 ch. J. Aiguier, 13402 Marseille Cedex 9, France.

In the preparation paradigm, a trial consists of the successive presentation of two signals. The second signal (S2) identifies the response to be made; the first signal (S1) may provide complete, partial, or no prior information about parameters of that response. Depending on the information in S1, the subject may prepare completely, partially, or not at all for the upcoming response movement. The reaction time (RT) for that response will decrease as a function of the degree of preparation. Preparatory mechanisms are elucidated by an analysis of the changes in neuronal activity occurring during the preparatory S1-S2 interval. Our behavioral task consisted of a wrist flexion and extension to targets which varied in two directions (left/right) and two distances (near/far). We recorded the activity of 499 neurons in the primary motor (MI) cortex and the premotor (PM) cortex of three macaque monkeys. Significant ($p < 0.05$) trial-by-trial correlations were obtained between RT and the neuronal discharge frequency during the S1-S2 interval for 38.2% (178/466) of the task-related neurons in at least one condition of prior information: when direction was precued, 34.8% of the neurons (162/466) had their activity correlated with RT; when extent, and no parameter, was precued, this number dropped to 10.7% (50/466). The correlation was significantly ($t = 7.9$, $p < 0.001$) stronger in conditions of information about direction than in conditions of information about extent and no information. No significant difference in correlation was found between MI and PM (chi-square = 2.07, $p > 0.1$), but significant differences (chi-square = 24.76, $p < 0.001$) were found between purely preparation-related neurons (16/52, 30.8%), preparation- and execution-related neurons (116/236, 49%), and purely execution-related neurons (46/178, 25.8%). Correlations were negative when S1-S2 activity increased, and positive when activity decreased. (Supported by ONR grant N00014-89-J1557)

178.4

CORTICAL MECHANISMS OF STIMULUS-RESPONSE ASSOCIATION IN THE MONKEY. J. Seal, T. Hasbroucq*, I. Mouret* and S. Kornblum*[1]. Cognitive Neuroscience Unit, C.N.R.S., 31 ch. J. Aiguier, 13402 Marseille Cedex 9, France and [1]Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720, U.S.A.

The aim of this study was to investigate the mechanisms by which the sensory response to a cue for movement becomes linked to the motor activity necessary for the execution of that movement. In a first series of experiments, a Rhesus monkey was trained in a choice reaction time task in which the stimulus was a brief vibration delivered to either the fingers or the thumb and the response was a press with the stimulated digit/digits. Single neuron recordings were made in S1, and areas 5, 6 and 4. The hand region of S1 was localized using N.M.R. imaging, which accounts for the high percentage of task-related activity observed (101/116 neurons). Trial by trial data analysis showed a temporal ordering of the changes in neuronal activity that was: S1 < A5 < A6 < A4. The same sequential organization was observed when we constructed a sensory to motor gradient based on the degree to which a change in neuronal activity was related to the stimulus and the movement. Within-burst analysis showed that A6 neurons displayed several changes in activity, some related to stimulus onset, others to movement parameters. However, all these changes occurred prior to the onset of movement which suggests that such neurons may be involved in the mechanism of associating context-related, sensory input with motor commands. We conclude that the cortical areas studied may contain a neuronal mechanism for sensory to motor transformation. (Supported by O.N.R. grant N00014-89-J1557)

178.5

PREPARATORY ACTIVITY IN AREA 7A RELATED TO MOVEMENT DIRECTION OR EXTENT. William A. MacKay and Alexa Riehle. Dept. of Physiology, University of Toronto, Toronto M5S 1A8, Canada and CNRS-LNF1, 13402 Marseille, France.

Cortical area 7a is known to be involved in directing arm movements to targets in extrapersonal space. The hypothesis that it may code information about direction and extent of an intended reach to a visual target was tested in one cynomolgus monkey trained to reach to two sequentially occurring target positions displayed on a touch sensitive videomonitor. The right hand was held at one of four target positions while the second target was indicated as an open square 11 or 22 cm to the right or left. After 1 s (preparatory period) the square filled in and the monkey then reached toward it. For one movement direction, movements of the same extent could be started from two positions. Of 160 neurons recorded in the left area 7a, 39 changed their activity during the preparatory period in relation to movement direction and 15 in relation to movement extent regardless of starting position (including 9 which were related to both direction and extent). Activity changes were usually phasic in response to appearance of the second target (latency 50-100 ms) but some were sustained over the 1 s delay. Only 6/39 direction-related cells preferred leftward targets to rightward, and 12/15 extent-related cells preferred small movements to large. For 5 of these extent cells, the specificity for small movements was maintained in both directions. The results suggest that some area 7a neurons code visual signals in terms of arm movement parameters and that parameters such as direction and extent are partially specified by independent mechanisms. *(Supported by MRC of Canada and ONR grant NOOO14-89-J1557.)*

178.6

CHANGES IN FORELIMB MOTOR REPRESENTATION IN RODENT CORTEX INDUCED BY PASSIVE MOVEMENTS. D.R. Humphrey, X.Q. Qiu*, P. Clavel* and D.L. O'Donoghue. *Lab. Neurophysiology, Emory Sch. Medicine, Atlanta, GA 30322.*

To test the hypothesis that motor cortical representations are shaped by sensory experience, intracortical microstimulation (ICMS) was used to map the forelimb representation in the motor cortex of Ketamine-sedated adult rats, before and after 90-120 min of passive movements (100/min) of the contralateral forelimb (elbow flexion-extension, N=10), the vibrissae (N=5), the forelimb and vibrissae (N=4), the hindlimb (N=4), or after no movement (N=8). ICMS (0.2 msec cathodal pulses, 330 pps, 400 msec train) was delivered at a depth of 1.7 mm (in lamina V), using glass micropipettes (3.6 M NaCl) and a map-grid spacing of 0.25 mm.

Consistent expansion of the forelimb zone was seen after passive movements about the elbow. No such increase in the forelimb zone occurred in the other movement conditions. However, a slight shrinkage occurred after vibrissae movements, due to expansion of the adjacent vibrissae zone. We interpret this movement-induced expansion to be due to changes in the synaptic strengths of afferent pathways which converge onto forelimb motor control cells at the periphery of the zone, produced by the period of sustained sensory input and now more effectively excited by ICMS. (Supported by NIH Grant NS 20146 to DRH).

178.7

NMDA ANTAGONIST (MK-801) BLOCKS PLASTICITY OF MOTOR CORTEX MAPS INDUCED BY PASSIVE LIMB MOVEMENT. X.Q. Qiu*, D.L. O'Donoghue and D.R. Humphrey, *Lab. Neurophysiology, Emory Sch. Med., Atlanta, GA 30322.*

Two hours of forelimb movement in the ketamine-sedated rat can induce expansion of the motor cortical area from which forelimb movements are evoked with intracortical microstimulation (ICMS). We believe that such expansion results from input-induced changes in the excitability of extant synapses. In this study, we tested the contributions of N-methyl-D-aspartate (NMDA) mediated activities to such putative changes in synaptic efficiency. Two ICMS mappings of the motor cortex forelimb-vibrissae border zone were performed in each of 13 ketamine-sedated rats: one before and one after two hours of passive flexion-extension movements of the contralateral elbow. During these movements, the exposed cortex was perfused with either artificial cerebrospinal fluid (CSF) (N=5 animals), CSF containing 3 uM concentration of MK-801 (N=3), or CSF with 300 uM MK-801 (N=5). Expansions of the forelimb zone occurred in the first two groups. In contrast, significant *shrinkage* of the forelimb zone occurred in the 300 uM MK-801 group. The shrinkage was not accompanied by an increase in the range of threshold currents, indicating that it was not due to a general decrease in cortical excitability. The results are therefore consistent with the hypothesis posed (Supported by NIH Grant NS 20146 to DRH).

178.8

MOTOR CORTEX REPRESENTATION PATTERNS REORGANIZE WITH MAINTAINED FORELIMB POSITION. J. N. Sanes, J. Wang, R. Kim*, and J. P. Donoghue, Center for Neural Science, Brown University, Providence, RI 02912

In previous experiments, we demonstrated that output representation patterns of the primary motor cortex (MI) are flexible. After a motor nerve injury in adult rats, forelimb (FL) EMG can be evoked with intracortical electrical stimulation in regions that before the nerve injury were unrelated to the FL. This effect may occur within 45 min of the nerve transection and endures for at least four months.

To test whether changes in afferent input reorganize MI output, we studied MI in 5 ketamine anesthetized rats with intracortical electrical stimulation while recording biceps and wrist extensor EMG. After initial mapping of the MI FL-vibrissa (VIB) border, a linear array of 4 electrodes (250 μm separation) was placed across the two zones. The FL contralateral to the stimulated MI was first maintained in an elbow-flexed, wrist-extended position (EfWe) and then in an elbow-extended, wrist-flexed position (EeWf), each for 30-150 min. EfWe shortens and EeWf lengthens muscles commonly activated by MI stimulation. The sample data set included biceps and wrist extensor EMG evoked from each of 20 stimulating electrodes (total of 40 data points).

In EfWe, MI stimulation evoked weak FL EMG only from the most lateral electrodes and none from the medial electrodes. Immediately after FL repositioning to EeWf, the size of evoked EMG increased for 9 MI samples (position effect). After an additional 15-150 min of EeWf maintenance, FL EMG appeared for 12 samples within the VIB area where no EMG was previously evoked (reorganization effect) and evoked EMG increased in amplitude for 3 samples (strengthening effect).

These results indicate that maintenance of limb position reveals *new* or *strengthens* existing synaptic relationships between MI and FL motor neurons. Further, changes in FL position can alter MI output immediately. Thus, the effect of an MI output volley on FL motoneurons, in rats, is position dependent. This suggests that limb position feedback influences MI map organization and that the amount of cortex controlling a set of muscles is regulated by competitive dynamic processes.

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178.9

MICROSTIMULATION OF MOTOR CORTEX IN MONKEYS: A FORCE FIELD ANALYSIS. D. Minciacchi, F. A. Mussa-Ivaldi*, S. F. Giszter and E. Bizzi. Dept. Brain and Cognitive Sciences, M. I. T., Cambridge, MA 02139.

We have examined the relationship between focal activation of cells in the motor cortex and the consequent multi-joint motor output. Recent work by Georgopoulos and coworkers based on single recordings in trained alert monkeys have suggested that individual cortical neurons encode for directions of hand movements, independently of the initial arm configuration. According to this interpretation, focal stimulation of a cortical site should result in the same end-point response regardless of the initial arm location. We addressed this by monitoring the total forces produced at the level of the wrist by focal cortical microstimulation in untrained tranquilized monkeys. Animals sat in a primate chair with the arm supported in the horizontal plane and the wrist attached to a six-axes force transducer. A stimulating electrode was inserted in the arm-related area of the precentral motor cortex. We placed the wrist at different workspace locations in the horizontal plane while keeping the electrode in one cortical site. For each arm location, we measured the force vectors elicited at the wrist by stimulation of this single cortical site (stimulation parameters: 7-15 μA, 100-150 msec, 50-150 Hz). Our data show that: (a) the force elicited by the stimulation of a cortical site varied in magnitude and direction at different workspace locations, and (b) for most stimulation sites, the pattern of wrist forces converged to a single equilibrium point. If we assume that the motor cortex of untrained tranquilized animals is functionally equivalent to the cortex of the trained alert preparation, then our results are not consistent with the hypothesis that neurons in the motor cortex encode an invariant direction of hand movement.

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

178.10

DIRECTION AND VELOCITY CODING IN MOTOR CORTICAL CELLS. Andrew B. Schwartz and Bryan J. Anderson. Barrow Neurological Inst., 350 W. Thomas Rd., Phoenix, AZ 85013

Unitary activity from cells located in the proximal-arm area of primary motor cortex was recorded as monkeys performed two drawing tasks. The animal initially moved its finger in eight radial directions from a center start position after a cell was isolated. Sinusoids of different amplitudes and spatial frequencies were then traced on the same touch-screen. Regression analysis of the data from the first task showed that many cells possessed discharge rates that were correlated to the direction of movement ($r^2 > .7$). Each sinusoid was divided into 100 parts and each cell's activity was represented as a vector based on that cell's preferred direction (fixed) and its rate of discharge in that part of the sinusoidal trajectory. These vectors were summed across all the directionally-tuned cells in the population (n= 238). This resulted in 100 "population" vectors for each sinusoidal trajectory. The direction of each population vector matched that in the corresponding part of the trajectory with a time difference of about 150 msec. In addition, the length of each population vector closely matched the speed of that part of the trajectory. Regression analysis of each cell's activity shows that although it is poorly correlated to speed, the relation is non-random. The relation between speed and discharge is more clear when data form the entire population is pooled. This suggests that different information may be weakly represented in individual discharge patterns and only evident when considering large populations. Supported by NIH- NS26375.

178.11

COMPARISON OF NEURAL SIGNALS AND ARM TRAJECTORY DURING DRAWING MOVEMENTS. James L. Adams and Andrew B. Schwartz. Barrow Neurological Institute, 350 W. Thomas Road, Phoenix, AZ 85013.

Our prior research has shown that the population of proximal arm segment motor cortical neurons codes for direction of fingertip movement. The current study examines the motion of the limbs controlled by the proximal arm muscles during performance of the same and additional drawing tasks.

Two Rhesus monkeys (Macaca mulatta) were used in this study. Fingertip positions were recorded while each monkey traced figures with its index finger on a computer touch screen. Instantaneous wrist, elbow, and shoulder positions were captured via an OPTOTRAK system with four infrared emitting diodes affixed to a two-piece sleeve strapped to the monkey's arm and forearm. The OPTOTRAK data show the wrist trajectory resembles the fingertip trajectory. The elbow and upper arm trajectories are much less similar to those of the distal joints.

The population model is based on the activity of cells identified by passive manipulation of the shoulder and elbow. Population vectors were generated from vector sums of firing rates and preferred directions of more than 200 such cells active during drawing movements. The trajectory model derived from these data closely resembled that of the distal (wrist and finger) trajectories.

Supported by NIH grant NS26375.

178.12

CORTICAL NEURONAL ACTIVITY RECORDED IN A DELAY TASK THAT DISSOCIATES LOCATION OF CUE STIMULUS AND MOVEMENT ENDPOINT. J.F. Kalaska and D.J. Crammond. CRSN, Département de physiologie, Université de Montréal, Montréal, Canada, H3C 3J7.

Monkeys were trained to make arm movements to 8 peripheral targets arranged in a circle around a central starting position. In Direct-Delay (DD) trials, a green LED (CUE stimulus) at a peripheral target signalled intended movements to be made directly toward the stimulus location. In Reverse-Delay (RD) trials a yellow LED at a peripheral target signalled intended movements to be made in the opposite direction away from the stimulus location. The monkey withheld its response during the delay period (mean 2 sec) until a red LED (GO signal) was illuminated. DD and RD trials were randomly interspersed. When the RD delay period was short the monkey often erroneously moved in the direction of the yellow LED when the GO signal appeared. Such directional errors were rarely observed in DD trials. This suggests that a period of time is required after the CUE signal is illuminated in RD trials to correctly make the dissociation between stimulus location and the intended movement direction.

All cells recorded in this task in both premotor area 6 and posterior parietal area 5 were directionally tuned during the delay period of DD trials. The initial delay period response to the CUE stimulus in RD trials usually resembled that to CUE stimuli in the same location in DD trials. The RD delay period activity then gradually changed over several hundred msec to resemble that recorded in DD trials for movements intended in the opposite direction. Thus the initial cortical response to a CUE stimulus in RD trials resembled that for the motor response of highest stimulus-response compatibility (move to the stimulus) and may be the neuronal correlate of the directional errors seen in RD trials with short delay periods. Further neuronal processing was required in RD trials before neuronal correlates of the appropriate motor response were observed in both cortical areas. (Supported by MRC Group Grant in neurological sciences)

178.13

MOTOR AND PREMOTOR CORTEX ACTIVITY DURING ARM MOVEMENT SEQUENCES IN MONKEY. I. RESPONSE PROFILES. J.K. Marcario and R.E. Kettner. Dept. Psych, Prog. Neural Science, Indiana University, Bloomington, IN 47405.

Single unit activity was recorded from the motor and premotor cortices of a rhesus monkey performing a movement-sequence delay task. The monkey initiated each trial by depressing a central button when a small LED was lit. Two of four target lights indicating the sequence for subsequent forelimb movement were then randomly illuminated for 500 ms each, followed by a variable delay period (2.5-3.5 s) which terminated when the LED dimmed. The monkey then pressed the previously illuminated target buttons in the proper sequence to obtain a liquid reward. Fixation was maintained for the final 2.0 s of the delay period before the movement sequence was initiated. This task allows the separation of initiation, sensory, delay, and movement responses. At this point 253 single units from both hemispheres have been analyzed. PST histograms were constructed for each neuron and the types of response profiles were categorized on the basis of activity in the four main periods of the task. Plots of response rates in one period versus another were used in the analysis of response categories. Often quite different response profiles were observed within the same penetration for both motor and premotor areas. Further, both similar and different responses could be observed from nearby neurons including pairs of neurons simultaneously recorded from the same electrode tip. One of the most striking findings was that of complementary (e.g. motor/sensory-delay) responses in simultaneously recorded unit pairs. The diversity of response types observed suggests some complexity in local processing. Possibilities include: the activation of different muscle groups; the integration of sensory, mnemonic, and motor activity; interactions between interneurons and output neurons; and combinations of the above. (Supported by NSF grant BNS-8919867)

178.14

MOTOR AND PREMOTOR CORTEX ACTIVITY DURING ARM MOVEMENT SEQUENCES IN MONKEY. II. DIFFERENTIAL RESPONSES. R.E. Kettner and J.K. Marcario. Dept. Psych, Prog. Neural Science, Indiana University, Bloomington, IN 47405.

Variation in single-unit activity was studied during the 12 different movements in the movement-sequence task described in the first abstract. Statistically significant differences were determined using ANOVA tests and, if present, regression techniques were used to determine whether there were systematic variations in response rate relative to either sensory or movement direction. Delay period activity was analyzed relative to both sensory and motor events. Initial results indicate that most units show differential responses during at least one of the initial (0%), sensory (16%), delay (18%), or movement (59%) periods although some neurons had statistically similar response profiles across trial types. Sensory related differences were 3 times more common in anterior penetrations. Differences during one light did not generally predict differences to the other. During the final 2 s of the delay period both eye and arm position were stable. This final period was divided into two 1 s intervals for analysis. Delay period differences were twice as common in anterior penetrations and were also more common during the final delay interval suggesting a similarity to motor set responses that have been reported elsewhere. Movement period analyses were based upon intervals corresponding to the separate task movements. Differences during movement were present in both anterior and posterior penetrations but were slightly more common in posterior penetrations. Significant regression fits generally followed the above trends. These results suggest that information about specific movements may be encoded in the firing patterns of both motor and premotor neurons during the sensory and delay periods of this task as well as during movement. (Supported by NSF grant BNS-8919867)

178.15

EFFICIENCY OF CORTICO-MOTONEURONAL (CM) FACILITATION OF MONKEY HAND MUSCLES R.N. Lemon, K.M. Bennett* and D. Flament. Anatomy Dept., Cambridge University, England.

There is good evidence that the post-spike facilitation (PSF) of EMG activity in hand muscles produced by monkey corticospinal cells is mediated by the monosynaptic CM pathway. Factors which might influence the efficiency of the CM synapse were studied in 3 M. nemestrina monkeys performing a precision grip. CM cells were identified by spike-triggered averaging of EMG and by cross-correlation with motor units. 1) **CM cell firing pattern.** At movement onset, short-interspike intervals (<20ms) produced large PSF, due to temporal facilitation and summation of post-spike effects. But long intervals (>40ms) were also effective, especially during precise maintenance of grip force. 2) **Level of EMG activity** did not appear to influence the amplitude of PSF relative to background EMG. Some CM cells produced similar facilitation of both the lowest threshold motor units (active at grip forces <0.4N) and of higher threshold units. 3) **Task.** One monkey performed two tasks: a precision pincer grip and a rotation task, both involving thumb and index finger. Intrinsic hand muscles were active during both tasks. For 15 CM cells we found dramatic differences in the amplitude of PSF produced by the same CM cell during the two tasks. These changes may be related to the pattern of activity required in the different target muscles, which could be varied by using CM cells with different muscle fields. Supported by MRC and Action Research.

178.16

ANTICIPATED AND LONG-LATENCY REFLEX RESPONSES TO PERTURBATION OF PREHENSION IN MOTOR CORTEX. Allan M. Smith, Nathalie Picard and Claude Dugas, C.R.S.N., Université de Montréal, Québec.

Extracellular unit activity was recorded from the hand representation area of the primary motor cortex of a macaca fascicularis trained to lift and hold an object within a narrow position window for one second. Neural activity related to the task recorded during control, unperturbed trial-blocks was compared to the activity evoked during trials when a predictable perturbation was applied during the holding phase. Two types of grip force responses were observed; a long latency increase that occurred within 100 ms of the stimulus and anticipatory response which appeared prior to the onset of the perturbation. The slip of the object induced by the perturbation was accompanied by a long-latency reflex response in 50% of the cells tested. The latency of the cortical responses ranged from 20 to 105 ms (x= 47.8 ± 21.8). The wide range of observed latencies presumably reflected the diversity of input pathways to motor cortex. The proportion of cells sensitive to the perturbation did not vary significantly between those receiving cutaneous input (63%) tested by stroking the skin and proprioceptive afferents (60%) assessed by passive joint manipulation. Furthermore, no significant difference existed between the mean latency of neurons with cutaneous (x= 45.0 ms ± 20.2) or proprioceptive (x= 45.3 ms ± 25.4) receptive fields, indicating that both types of afferents participate equally in long-loop servo control mechanisms. Twelve of the 124 neurons tested (10%) showed an anticipatory increase in discharge frequency prior to the perturbation. In addition, a significantly higher proportion of cerebellar cortical neurons tested in the same manner demonstrated anticipatory responses (29/104, 27%, $p < .001$). However, the incidence of reflex responses to the perturbation and the mean latencies were equal. This may represent an important difference in the respective contribution of motor cortex and cerebellum to the control of prehension. Supported by the MRC and NSERC of Canada and le Fonds FCAR du Québec.

178.17

MODULATION OF MOTOR CORTICAL ACTIVITY BY THE WEIGHT AND TEXTURE OF A GRASPED OBJECT. Nathalie Picard, Claude Dugas and Allan M. Smith, C.R.S.N., Université de Montréal, Québec, Canada.

A macaca fascicularis was trained to grasp, lift and hold an object of varying weight and texture within a narrow position window. Weights of 15, 65 and 115 g and textures of smooth metal, fine and coarse grain sandpaper were used. The monkey scaled the grip and lifting forces appropriately for the object weight and texture. Unit activity was recorded in the hand representation area of the primary motor cortex, where low threshold intracortical microstimulation (< 30 μA) elicited discrete wrist or digit movements. Receptive fields of the neurons were defined by stroking the skin and passive joint manipulation. In contrast to cerebellar cortex, a greater proportion of motor cortical cells had cutaneous receptive fields and stronger responses to object texture. 93 cells related to the task were tested with weights and 47 of them with more than one texture. 45% of the cells showed a significant activity modulation with texture and 24% with weight. Of the proprioceptive units, 40% were modulated by weight and 33% were modulated by texture. In contrast the units with cutaneous receptive fields were more responsive to texture (58%) than to weight (11%). Alternatively, considering cells modulated by weight, 71% had proprioceptive receptive fields compared to only 25% which had cutaneous fields. Likewise, 74% of cells modulated by object texture had cutaneous fields compared to only 29% with proprioceptive fields. The responses to object texture occurred during dynamic grasping after contact with the object, but *before* either the force peak or the point at which texture causes a divergence in the rate of grip force application. Cutaneous afferents provide the motor cortex with information about friction and slip needed to adjust the grip force and proprioceptive afferents carry information about the loads transported by hand muscles. The weight and texture of a grasped object appear to be encoded by separate but partially overlapping neural populations receiving proprioceptive and cutaneous input. Supported by MRC and NSERC of Canada and le Fonds FCAR du Québec.

178.18

ROLE OF MOTOR CORTEX IN ACCURACY AND COORDINATION OF PREHENSION IN THE CAT. J.H. Martin, O. Priceman*, & C. Ghez. Ctr. for Neurobiol & Behav, Columbia Univ & NYS Psych Inst, New York, NY 10032.

The present study compares the effects of reversible lesion of different portions of the forelimb representation in the motor cortex on prehension. Cats reached to a baited target well, whose position could be changed, to grasp a morsel of food. Pre- and postcruciate areas of the distal forelimb representation were inactivated by microinjection of muscimol. Changes in limb kinematics due to the inactivation were assessed before and up to 30 min after drug injection. Drug spread was monitored autoradiographically.

Prior to injection, prehension and subsequent food retrieval from the target well was accomplished in a coordinated sequence of response components. Following injections in the precruciate distal forelimb representation, aiming of responses to the target became inaccurate. Movements showed consistent directional biases due to defective control of both proximal and distal forelimb segments. Multiple attempts were required to place the paw in the target well and corrections were erratic. Grasping, supination, and limb retraction, required for food retrieval, became ineffective after injections due to impaired coordination of response fragments rather than to their loss. Animals failed to use somatic sensory information for the fine control of paw movements and often neglected food sucessfully grasped. Injections in the postcruciate distal forelimb representation produced signs of limb weakness but not incoordination.

Our results suggest that the distal limb representation of cat motor cortex plays a role in the spatial and temporal coordination of distal and proximal muscles in multijoint movements. The presence of coordination defects and impaired accuracy following inactivation of the pre- but not the postcruciate region supports the hypothesis that there is a dual functional representation of the forelimb in cat motor cortex. (Supported by NS 19205)

178.19

ACTIVATION OF MOTOR CRANIAL NERVES BY ELECTRICAL AND MAGNETIC STIMULATION OF THE MOTOR CORTEX IN THE DOG. S.S.Haghighi, and S.A. Estrem*. Divisions of Neurosurgery and Otolaryngology, Univ. of Missouri. Sch. of Med., Columbia, MO 65202.

Transcranial electrical and magnetic stimulation are now widely used for the functional assessment of the corticospinal tract in man. Central delay time (CD) has been estimated for activation of limb muscles by subtracting the peripheral conduction time, estimated by stimulation of the ventral roots of the cervical spine, and recording peripherally from the same muscle group. In the present investigation, we used surface electrical stimulation of the motor cortex to produce evoked compound muscle action potential (CMAP) from ipsilateral and contralateral muscles innervated by motor cranial nerves (5,7,10,11,12). Monopolar electrical stimulation of the nerves at the cerebello-pontine angle (CPA) yielded CMAP activation of ipsilateral corresponding muscles. These latencies when subtracted from those obtained by direct cortical stimulation established CD for activation of the nerves. Our preliminary data with single pulse magnetic stimulation at high stimulation strengths (>80%) revealed ipsilateral CMAP with onset latencies similar to the direct nerve stimulation at the CPA for all innervated muscles suggesting intracisternal activation of cranial nerves with magnetic stimulation.

178.20

PREMOTOR CORTICAL REPRESENTATION OF MOVEMENT DIRECTION. R. Caminiti, P.B. Johnson, C. Galli*, S. Ferraina*, Y. Burnod* and A. Urbano*. Inst. of Physiology, Univ. of Rome, Italy, and Inst. of Neurosciences, Univ. Paris VI, France.

The activity of 156 cells was recorded in area 6 while monkeys made arm movements of similar directions within different parts of space. In this premotor area, 152/156 (97.4%) cells were directional. As movements traveling along parallel paths were made across the work-space, the preferred directions (PDs) of these cells shifted their orientation in a way which followed the orientation of the arm in space. This suggests that premotor cortical neurons combine information relative to both direction of movement and arm orientation in space. This invariance between cell PD and arm orientation has been observed also in the primary motor cortex. Neuronal movement population vectors, differently from individual cells upon which they are based, did not change their spatial orientation across the work-space, suggesting that they remain good predictors of movement direction regardless of the region of space where movements are made. These results are similar to those obtained in the motor cortex. The use of common mechanisms may facilitate the transformation of information between these areas and suggests a coding of arm movement direction within a coordinate system centered on the shoulder joint.

179.1

THE DISTRIBUTION OF PALLIDOTHALAMIC AND NIGROTHALAMIC PROJECTIONS IN THE DOG. B.A. Hannah and S.T. Sakai, Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

Based on our previous autoradiographic (ARG) tracing studies, the pallidothalamic and nigrothalamic projections appear to distribute to partially overlapping regions of the ventral thalamus in the dog. The purpose of the present study was to determine the extent of anatomical convergence of the pallidothalamic and nigrothalamic projections utilizing a double labeling paradigm. Tritiated amino acid injections were made into the entopeduncular nucleus (EP) at the same time, lectin conjugated horseradish peroxidase injections (WGA-HRP) were made into the substantia nigra pars reticulata (SNr) in the anesthetized dog. The tissue was processed for both ARG and HRP histochemistry using tetramethyl benzidine as the chromogen. The pallidothalamic ARG label was observed in the ventral anterior (VA), ventral medial (VM), parafascicular (Pf) and lateral habenula (LHb) nuclei whereas nigrothalamic HRP label was primarily observed in VA, VM, mediodorsal (MD) nucleus and Pf. The anterograde HRP label was observed in close apposition to the ARG silver grains within portions of VA and in VM and Pf. Although these latter observations suggest that a portion of thalamus may receive converging afferents originating from EP and SNr while other thalamic regions appear to receive separate and parallel inputs from these basal ganglia sources, the possibility of anatomical convergence within the canine thalamus awaits electron microscopic verification.
(Supported by NIH Grant 18551).

179.2

RETROGRADE AND ANTEROGRADE TRANSNEURONAL TRANSPORT OF HSV1 IN THE PRIMATE MOTOR SYSTEM. M.C.Zemanick and P.L.Strick, V.A. Med. Ctr. and Depts. of Neurosurg. and Physiol., SUNY-HSC @ Syracuse, Syr., NY, 13210.

We have examined the transneuronal transport of 2 strains of Herpes Simplex Virus, Type 1 (HSV1) after their injection into the 'arm area' of the primary motor cortex of cebus monkeys (Cebus apella). The McIntyre-B strain was transported transneuronally in the **retrograde** direction. Injections of this virus strain resulted in labeled neurons at sites known to **project** to the arm area of the primary motor cortex (e.g., ventrolateral thalamus, nucleus basalis). In addition, 'second order' neurons were labeled in the deep cerebellar nuclei (dentate and interpositus) and in the globus pallidus (internal segment). These observations support recent suggestions that the arm area of the primary motor cortex is a target of output from both the cerebellum and basal ganglia. In contrast, the H129 strain was transported transneuronally in the **anterograde** direction. Injections of this virus strain resulted in labeled neurons at sites known to **receive** input from the arm area of the primary motor cortex (e.g., putamen, pontine nuclei, spinal cord). In addition, 'third order' neurons were labeled in the globus pallidus (largely external segment) and cerebellar cortex (Granule and Golgi cells). Taken together, these results suggest that the direction of transneuronal transport of HSV1 is strain dependent. We plan to exploit these strain differences to examine cerebellar and basal ganglia loops with cerebral cortex.
Support: VA Med. Res. Serv.; USPHS 2957, 24328, 870501.

179.3

ORIGIN OF THALAMIC INPUTS TO THE 'HAND' REPRESENTATION IN THE PRIMARY MOTOR CORTEX. J.W. Holsapple, J.B. Preston and P.L. Strick, VA Med. Ctr. and Depts. of Neurosurg. & Physiol., SUNY-HSC @ Syracuse, Syr., NY, 13210.

Recently there has been considerable controversy about the origin of thalamic input to the primary motor cortex. There is general agreement that the nucleus ventralis posterior lateralis pars oralis (VPLo), a target of cerebellar efferents, has substantial projections to the arm area of the primary motor cortex. The extent of projections from the nucleus ventralis lateralis pars oralis (VLo), a target of pallidal efferents, has been unclear. To begin to resolve this controversy we made tracer injections into the forelimb representation of the primary motor cortex of macaques (Macaca nemestrina). When injection sites were placed in the central sulcus, at sites where stimulation evoked hand movements, over 60% of the input from the ventrolateral thalamus originated from VLo. In contrast, when injection sites were placed on the crest of the precentral gyrus over 65% of the input originated from VPLo. These observations indicate that both the basal ganglia and the cerebellum 'directly' influence the forelimb area of primary motor cortex. Furthermore, our observations suggest that the basal ganglia has a significant input to the hand representation in the central sulcus.
Support: VA Med. Res. Serv.; USPHS 2957, 24328, 843902.

179.4

ALTERED SOMATOSENSORY RESPONSE PROPERTIES OF NEURONS IN THE "MOTOR" THALAMUS OF MPTP TREATED PARKINSONIAN MONKEYS. J.L. Vitek, J. Ashe, M.R. DeLong, G.E. Alexander, Dept. of Neurology, The Johns Hopkins Hospital, Baltimore, MD., USA.

Previous studies in monkeys rendered parkinsonian by injections of the neurotoxin MPTP have found that neurons in the globus pallidus pars interna (GPi) show increased sensitivity and decreased specificity of somatosensory responses. The present study was carried out to determine whether similar alterations in neuronal response properties are seen in the thalamic subnucleus VLo (ventralis lateralis pars oralis) which receives direct projections from GPi. African Green monkeys were conditioned to permit detailed sensorimotor examinations including passive joint rotation, muscle palpation, tactile stimulation and the elicitation of active movements of the limbs and oral-facial structures. Using the same microelectrode to map the sensorimotor response properties of VLo neurons both before and after MPTP treatment, it was possible to keep the resulting sets of physiologic maps in precise register. Treatment with MPTP (0.5mg/kg/day x 3 days, i.m.) resulted in severe parkinsonian signs, including bradykinesia, akinesia, rigidity, tremor and postural instability. Prior to MPTP treatment the great majority of VLo neurons responded only to active movement; those few which did respond to somatosensory stimulation did so only to muscle palpation or passive movements about a single joint of the contralateral limb. After systemic MPTP treatment, neuronal somatosensory responses in VLo were far more frequent, more pronounced, and less specific. It was now common to find extremely broad somatosensory fields extending over multiple joints and frequently involving more than one limb. Even ipsilateral responses, which were never seen under normal conditions, were now seen with some regularity. Altered sensorimotor integration in the basal ganglia-thalamocortical "motor" circuit may play a significant role in the pathogenesis of parkinsonian signs.

179.5

EVIDENCE FOR SOMATOTOPIC ORGANIZATION WITHIN THALAMIC NUCLEUS VLo (VENTRALIS LATERALIS PARS ORALIS) J.Ashe, J.L.Vitek, M.R.DeLong, G.E.Alexander Dept. of Neurology, The Johns Hopkins Hospital, Baltimore,MD.,21205,USA

The thalamic nucleus (VLo) which receives direct output from globus pallidus is part of the basal ganglia "motor" circuit. While there has been direct demonstration of somatotopic organization at the cortical, striatal, and pallidal stages of the circuit, a somatotopy within VLo has merely been inferred on the basis of the strong topographic nature of the pallidal-thalamic projection. In an effort to determine directly whether VLo is somatotopically organized we did the folowing study. African Green monkeys were conditioned to permit a detailed sensorimotor exam that included passive joint rotation, muscle palpation, tactile stimulation, and the elicitation of active movements of the limbs and orofacial structures. Electrode penetrations were made in parasagittal planes by an anterior oblique approach and neuronal activity was sampled throughout each penetration. We demonstrated clear evidence for a somatotopic arrangement within VLo in the mediolateral plane, with the arm more medial and the leg lateral. These data show that a strict somatotopic organization is maintained throughout the "motor" circuit. Following induction of experimental parkinsonism with MPTP, the somatotopy within VLo was difficult to discern. This appeared to be the result of substantial changes in the somatosensory response properties of VLo neurons (see companion poster:Vitek el al.)

179.6

POSTSPIKE FACILITATION (PSpF) OF EMG ACTIVITY FROM NEURONS IN PRIMATE MOTOR THALAMUS.
K. Mewes and P.D. Cheney. Dept. of Physiol. and R.L. Smith Research Center, University of Kansas Medical Ctr. , Kansas City, KS 66103.

In previous work we showed that spike-triggered averaging of EMG activity in the awake monkey can reveal the target muscles of corticospinal and rubrospinal neurons. Onset latency data coupled with the fact that multiple synapses should diminish cross-correlation peaks, suggest that most postspike facilitations (PSpFs) are mediated by monosynaptic linkages to motoneurons. However, Kasser and Cheney (*J. Neurophysiol.* 53: 959, 1985) showed that postspike suppression (PSpS) is also readily detectable with spike-triggered averaging. PSpS is longer in latency and weaker than PSpF - consistent with mediation by a minimal disynaptic linkage. The purpose of this study, therefore, was to test the extent to which spike-triggered averaging of EMG activity might be effective in detecting non-monosynaptic excitatory linkages to motoneurons. Neurons in motor thalamus were selected for study because they are known to make monosynaptic connections with fast and slow conducting corticospinal neurons; that is, the minimum linkage to motoneurons is disynaptic (Deschenes, M, et al., *Neurosci.* 7: 2149, 1982). Of 116 neurons recorded in motor thalamus, 12 neurons (10%) showed significant PSpF of rectified EMG activity. The magnitude of thalamic PSpF expressed as %-increase above baseline activity was 4.6±3.2. This compares to 7.0±6.6 for CM and 4.1±1.95 for rubromotoneuronal (RM) cells. The mean onset latency of thalamic PSpF was 6.3±2.1 ms. This compares to 6.3±1.6 ms for CM cells and 5.6±1.9 ms for RM cells. Stimulus-triggered averages computed at the sites of thalamo-motor cells produced poststimulus facilitation in the same muscles showing PSpF from the recorded cell at that site. We conclude that spike-triggered averaging of EMG activity is not limited to the detection of monosynaptic connections. The results of this study show that the method can be effective in identifying less direct synaptic linkages to motoneurons and this will extend its application to the analysis of more central brain structures involved in the control of movement. Supported by NIH grant NS25646.

179.7

PLASTICITY IN THE THALAMUS AFTER LESIONS IN THE BASAL GANGLIA. T. De Boom,* K. Kultas-Ilinsky and I. Ilinsky. Department of Anatomy, University of Iowa College of Medicine, Iowa City, IA 52242

We have demonstrated recently (Kultas-Ilinsky et al., 1990, Brain Res., 511:197-208) that unilateral elimination of the substantia nigra pars reticularis (SNr) and entopeduncular nucleus (EPN - the feline homologue of the medial globus pallidus) induces a several fold increase in the number of [^{3}H]-muscimol binding sites in the area of overlap of nigro- and pallidothalamic projections in the cat thalamus (VA). This increase develops gradually over a period of time (1 year) and may be associated with lesion-induced remodeling of GABAergic systems in the thalamus. In this study we used quantitative morphometry to analyze ultrastructural changes in the same thalamic region after similar lesions at short-term (4 days) and long-term (1 year) survival times with the goal of identification of anatomical substrate of neurochemical changes detected earlier. The results demonstrate that combined SNr and EPN lesions induce synaptic reorganization in the VA that involves proximal parts of dendritic arbors of thalamocortical projection neurons. At 1 year post-lesion, the remarkable changes at secondary dendrites include a 4-fold decrease in the density of large size boutons with symmetric contacts; i.e., typical terminals of basal ganglia afferents, and a 4-fold increase in the number of dendro-dendritic synapses accompanied by an increase in the appositional length of LCN dendrites and the noticeable presence of unusual-looking boutons of unknown origin. Such boutons are also found on primary dendrites, however, in contrast to secondary dendrites, the 3-fold decrease in the density of boutons of basal ganglia origin is not accompanied by increased LCN apposition or an increased number of dendro-dendritic synapses on primary dendrites. The findings suggest a lesion-induced process of synaptic remodeling with involvement of GABAergic local circuit neurons that attempt to replace inhibitory basal ganglia input on secondary dendrites of projection neurons. Supported by NS R0119280.

179.8

AN ANALYSIS OF TRANSIENT STRIATOTHALAMIC PROJECTION IN THE RAT. T. Kono, M. Takada and S. T. Kitai. Department of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, TN 38163.

These studies further characterize the striatothalamic (ST) projection reported to exist only during early postnatal period (Hattori et al., Dev. Brain Res. in press). Its development was examined by injecting Fluoro-gold (FG) into the thalamus at various developmental stages (E17, 19, P0, P3, P6) and animals were perfused one day after each injection. Results indicated ST projection emerges by E17 and disappears by P7. Bromodeoxyuridine immunocytochemistry combined with FG tracing revealed that ST cells undergo the final mitosis as ealy as E15. Immunocytochemical analyses on P0 injection/P1 sacrifice showed that ST cells express GABA and leu-enkephalin but not substance P, and are organized in a discrete patch-like manner. A retrograde double labeling using True blue and Diamidino yellow also demonstrated that some of ST cells issue axon collaterals to the substantia nigra. Their distribution also matches well with tyrosine hydroxylase- and substance P-immunoreactive zones in the striatum. We infer from these data that transient ST projection might play a crucial role in the compartmentalization in the striatum.

The retrograde studies of ST cells after short term (P0/P1 and P6/P7) and long term (P0/P7) survivals showed that ST cells are no longer present at short term survival of P6/P7 but can still be present though less in number at long term survival of P0/P7. These results indicate that both cell death and collateral retraction are occurring during development of ST projection. (Supported by NIH grants NS 20702 and NS 23886 to S. T. Kitai).

179.9

PARALLEL ARRANGEMENT OF FOREBRAIN CIRCUITS IN THE RAT. 2. VENTRAL STRIATUM, VENTRAL PALLIDUM, AND MEDIODORSAL THALAMIC NUCLEUS. H.J.Groenewegen and H.W.Berendse*. Dept. Anatomy and Embryology, Vrije Universiteit, Amsterdam, The Netherlands.

In the rat a number of parallel systems can be distinguished within the corticostriatal projections from the prefrontal cortex (PFC) to the ventral striatum (VStr) and the projections from the midline (ML) and intralaminar (IL) thalamic nuclei to the PFC and the VStr (see companion abstract: Berendse and Groenewegen). The aim of the present study is to investigate whether the topography in the ventral striato-pallido-thalamic projections is in register with this parallel arrangement. Injections of anterograde (PHA-L) and retrograde (cholera toxin B) tracers were placed in various parts of the VStr and the ventral pallidum (VP). Both the ventral striatopallidal and the ventral pallidothalamic pathways, the latter primarily directed at the mediodorsal thalamic nucleus (MD), are topographically organized. In the reciprocal ventral striatopallidal projections medial-to-lateral and dorsal-to-ventral coordinates are maintained. The present experiments confirm the previously described topograpy in the projections from the VP to the MD (Groenewegen, 1988, Neuroscience 24:379). The point-to-point relationship in the mentioned projections and in the reciprocal MD-PFC connections is such that, in conjunction with the results discussed in the companion abstract, a number of distinct parallel circuits can be composed that each involve different parts of the PFC, the VStr, the VP, and the MD. Moreover, the topographical organization of the thalamostriatal and thalamocortical projections originating from the ML and IL nuclei is in register with these parallel circuits. The present data in the rat validate and add to the previously formulated concept of parallel basal ganglia-thalamocortical loops in the primate (Alexander et al., 1986).
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179.10

PARALLEL ARRANGEMENT OF FOREBRAIN CIRCUITS IN THE RAT. 1. PREFRONTAL CORTEX, MIDLINE AND INTRALAMINAR THALAMIC NUCLEI, AND VENTRAL STRIATUM. H.W.Berendse* and H.J.Groenewegen (SPON: European Neuroscience Association). Dept. Anatomy and Embryology, Vrije Universiteit, Amsterdam, The Netherlands.

On the basis of anatomical and physiological data in the primate, Alexander et al. (Ann.Rev.Neurosci. 9:357, 1986) have distinguished a number of parallel, functionally different basal ganglia-thalamocortical circuits. The objective of the present study is to unravel the forebrain circuitry that involves the limbic lobe-innervated parts of the basal ganglia in the rat. The corticostriatal projections from the prefrontal cortex (PFC) to the ventral striatum (VStr) and the projections from the midline (ML) and intralaminar (IL) thalamic nuclei to the VStr and the PFC were studied by means of anterograde (PHA-L) and retrograde (cholera toxin B) tracing techniques. The projections originating from cytoarchitectonically and presumably functionally different regions of the PFC to the VStr are topographically organized, such that dorsal-to-ventral and medial-to-lateral coordinates are maintained. A similar topography characterizes the ML and IL thalamostriatal projections (Berendse and Groenewegen, 1990, J.Comp.Neurol., in press). Furthermore, each of the ML and IL nuclei projecting to the VStr has a restricted terminal field in the PFC. A comparison of the organization of the different pathways reveals that in the VStr the projections of individual ML and IL nuclei converge with those of their PFC target areas. Thus, a number of parallel systems can be identified that each comprise distinct parts of the PFC, the ML and IL thalamic nuclear complex, and the VStr. In a companion abstract (Groenewegen and Berendse) the organization of the ventral striatopallidofugal pathways is discussed in relation to this parallel arrangement.
Supported by NWO-Program Grant #900-550-053.

179.11

AN ELECTRON MICROSCOPIC STUDY OF RETROGRADELY LABELED PROJECTION NEURONS AND THEIR RELATION TO THE EPENDYMAL LAYER OF THE THALAMIC PARAVENTRICULAR NUCLEUS. G. Balercia*, M. Bentivoglio and L. Kruger. University of Verona, Italy and UCLA Medical Center, Los Angeles, CA.

Neurons of the thalamic paraventricular complex, retrogradely labeled by fluorescent dyes from the amygdala, extend numerous dendrites towards the ventricular surface. In order to determine the relation of these processes to the cerebrospinal fluid, they were labeled retrogradely with horseradish peroxidase (HRP) and visualized by a reaction product suitable for electron microscopy. The glial variants of this region are less diverse than those in the basal III ventricle, revealing a fairly uniform ependymal layer and a few "dark" ependymocytes lacking descending tanycyte processes. The subependymal layer contains scattered typical oligodendrocytes and astrocytes, the latter generally forming a thin boundary between labeled dendrites and ependyma. Subependymal HRP-labeled dendrites containing lipid and lysosome-like dense bodies are often surrounded by membranous whorls of reactive astrocytes. Neurons and their neurites are isolated from the surface by glial and ependymal elements exhibiting a variety of interdigitating and junctional membrane characteristics including sinuous sheet-like astrocyte processes and basal ependymocyte caveolar zones. (Supported by Italian Ministry of Public Education and NIH awards NS-5685 and TW 1530.)

179.12

EFFECT OF THALAMIC MICROINJECTIONS OF APOMORPHINE AND MUSCIMOL ON THE ACOUSTIC STARTLE STARTLE RESPONSE AND OTHER BEHAVIORS. **K.A. Young[1], P.K. Randall[2] and R.E. Wilcox[2].** [1]Dept. of Pharmacology, Texas A&M College of Medicine, Temple, TX, 76508 and [2]Dept. of Pharmacology, Univ. of Texas, Austin, TX 78712.

The role of DA neurotransmission in the rodent thalamus has not been systematically studied. Rats were administered apomorphine (400 ng), vehicle or muscimol (50 ng) through indwelling cannulae. Catalepsy, sensory asymmetries, and acoustic startle response with prepulse inhibition were measured following drug administration.

Bilateral microinjections of apomorphine (APO) into the ventromedial (VM) thalamus caused a loss of prepulse inhibition of the acoustic startle response, while unilateral APO microinjections into the mediodorsal and posterior thalamus increased the amplitude of startle responses without producing a loss of prepulse inhibition. Loss of prepulse inhibition after APO microinjection was site specific to the VM nucleus and occurred 10-15 minutes postinjection, but not at later testing periods (30-35, 50-55, 70-75 min). Counterbalanced injections of vehicle produced no effect on startle or other behaviors. The profound catalepsy produced by VM microinjections of muscimol did not interfere with the expression of normal startle behavior. These findings are consistent with the observed pattern of prepulse inhibition loss after systemic APO administration. Thus, our data suggest a role for thalamic DA neurotransmission in the modulation of the acoustic startle response.

180.1

CONVERGENCE OF PRELIMBIC AND MOTOR CORTICAL INFORMATION IN THE GLOBUS PALLIDUS AND SUBTHALAMUS OF RATS. Lawrence J. Ryan and Kevin B. Clark, Dept. Psychology, Oregon State University, Corvallis, OR 97331-5303.

Do projections from the neostriatal striosomes and matrix converge? Since both matrix and striosomes possess neurons that project to the globus pallidus, information from these systems may converge directly there or indirectly via pallidal-subthalamic-pallidal connections. Our evidence suggests both types of convergence may occur.

Prelimbic cortex, which projects primarily to striosomes, (PL; Cg3 of Paxinos and Watson, 1986) and frontal agranular cortex (FRI and FRII, primarily to matrix) were stimulated with bipolar stainless steel electrodes (0.3 and 0.7 mA, 0.2 ms monopolar pulses) while single neurons were recorded in globus pallidus (GP) or subthalamus (ST). GP neurons responded with various patterns of inhibition and excitation of firing after cortical stimulation: 38.5% (15 of 39) responded to FRI, 77.2% (44/59) to FRII, and 29.3% (22/75) to PL. Convergence: 19.0% (4/21) for FRI-FRII, 5.1 % (2/39) for FRI-PL, and 26.3% (15/57) for FRII-PL. 9.5% (2/21) responded to all three.

Subthalamic lesion (kainic acid) 1) increased the duration of induced inhibition (e.g. to FRI: 18.24 vs 49.9ms, $t(24)=2.59$, $p<0.02$), and 2) generally reduced excitatory responses, completely eliminating all late (13+ms) excitation not preceded by inhibition. These late responses may reflect reciprocal GP-ST interactions since ST cells show inhibition and excitation that follows GP excitation and inhibition by several ms. GP firing rate was not reduced by ST lesion (24.95 vs 21.71 Hz, $t(105)=1.33$, ns). Antidromically identified (from GP) ST neurons (N=29) responded to FRI (65.5%, 19 of 29), to FRII (62.1%) and to PL (10.3%). Responses to PL were always slow (>22ms). The responses revealed convergence from the cortex: FRI-FRII (44.8%), FRI-PL (10.3%), FRII-PL (10.3%), and all three (10.3%). Thus information from the striosomes and matrix may converge in the globus pallidus and in the subthalamus.

This research was supported by grant MH 45341 (to LJR) from the N.I.M.H.

180.2

MOTOR EFFECTS FOLLOWING ELECTRICAL STIMULATION OF THE GLOBUS PALLIDUS IN RATS. Y.E. Anagnostakis*, E. Miliaressis and C. Spyraki, Lab. of Pharmacology, Medical School, University of Crete, 71409 Iraklion, Greece.

Although the globus pallidus is believed to play an important role in the expression of motor behaviors, a systematic mapping investigation of this area has not yet been performed in the rat. In this study we present a summary of behavioral reactions obtained by electrical intrapallidal stimulation using moveable electrodes (Kinetrods, model SME-01, Ottawa) in 11 freely moving rats. The electrode was lowered by single steps of 0.2 mm, from the dorsal to the ventral pallidus, at lateral coordinates varying from 2.4 to 3.2 mm from the midline. Each brain site was stimulated with single trains (0.4 sec duration) of cathodal rectangular pulses of fixed intensity (200 µA) and width (0.1 msec). The effects of the stimulation were noted at each brain site for pulse frequencies varying from 5 to 50 pulses/train. The following reactions were observed, depending on the vertical and lateral coordinates: head turning, forelimb retraction, vibrissae movement and behavioral inhibition. Directional reactions were always contralateral to the stimulated side.

180.3

THE STRIATO-PALLIDO-STRIATE PATHWAY IN THE RAT BRAIN. DEMONSTRATION OF RECIPROCAL MONOSYNAPTIC CONNECTIONS. M.N. Williams* and R.L.M. Faull.
Department of Anatomy, University of Auckland, New Zealand.

Procedures utilizing combined light microscopic (LM) and electron microscopic (EM) techniques were used to demonstrate direct monosynaptic connections between neuronal pathways in the basal ganglia of the rat brain. The horseradish peroxidase technique was used singly or in combination with the anterograde axonal degeneration technique to investigate the synaptic organization of the pathways interconnecting the striatum and globus pallidus. Small (5-10 nl) injections of 2% HRP-cholera toxin or 5% HRP-lectin dissolved in 1% kainic acid (a neurocytotoxin known to destroy neurons and spare fibres of passage), were injected into the right striatum or globus pallidus. Two days later the animals were killed by transcardiac perfusion of fixative. Serial sections through the striatum and globus pallidus were processed for HRP cytochemistry and embedded in Epon. Blocks taken from regions containing labelled striatal or pallidal neurons were reembedded, thin sectioned and examined in the electron microscope. The results showed that after combined injections of neurocytotoxin and HRP-lectin into the striatum, degenerating boutons in the globus pallidus formed synapses on the soma and dendrites of HRP-labelled pallidostriate neurons. Also, after injections of HRP-cholera toxin into the globus pallidus, HRP-labelled boutons in the striatum made synaptic contact with small spinous HRP-labelled dendrites. These findings demonstrate the existence of two monosynaptic links: one in the globus pallidus between striatopallidal axon terminals and pallidostriate neurons and the other in the striatum between pallidostriate axon terminals and striatopallidal neurons.

180.4

EFFECT OF SUBTHALAMIC NUCLEUS ABLATION ON GLOBUS PALLIDAL CELLS AGAINST KAINIC ACID NEUROTOXICITY. M. Takada, T. Kono and S. T. Kitai.
Department of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, TN 38163.

Recent anatomical and physiological evidence has indicated that subthalamic nucleus (STN) cells are excitatory to their targets and glutamate is considered to be a likely neurotransmitter involved. We have tested this hypothesis by examining the kainic acid (KA)-induced cell loss in the globus pallidus (GP) after STN ablation.

Adult male albino rats (Wistar, 250-300 g) were used. STN lesions were made by injecting KA (5 nmol) unilaterally into the nucleus. One, four, or twelve weeks later, the animals received KA (5 nmol) injections bilaterally into the GP. After a survival of 3-4 days, formalin-fixed brains were stained with cresyl violet. On the control side where the STN was intact, GP cells were almost completely depleted with KA. In contrast, KA-induced GP cell loss was limited to only 20% (i.e. 80% spared) on the side where the STN was lesioned. These findings were consistently observed regardless of time intervals between STN and GP lesioning. On the other hand, decortication resulted in 60% (i.e. 40% spared) of GP cell loss. A combination of STN lesioning and decortication did not change the KA-induced GP cell loss with STN lesioning alone. These observations were interpreted to indicate that glutamatergic inputs to the GP are derived predominantly from the STN and cortical afferents to the GP share glutamatergic receptors with STN afferents.
(Supported by NIH grants NS 20702 and NS 23886 to S. T. Kitai)

180.5

EVIDENCE FOR A PROJECTION FROM THE GLOBUS PALLIDUS TO THE ENTOPEDUNCULAR NUCLEUS IN THE RAT. A.E. Kincaid, S.W. Newman, A.B. Young and J.B. Penney Jr., Dept. of Anatomy and Cell Biology and Dept. of Neurology, University of Michigan, Ann Arbor MI 48109.

The entopeduncular nucleus (EP) of the rat is considered to be one of the major sources of outflow from the basal ganglia. It projects to the ventroanterior and ventrolateral complex and the parafascicular nuclei of the thalamus, the habenula and the pedunculopontine tegmental nucleus (PPT). Afferents to the EP include the striatum (CP), the subthalamic nucleus (STN) and the PPT. We present evidence from both retrograde and anterograde tract tracing studies that the rat globus pallidus (GP) sends a substantial, topographic projection to the EP. Fluoro-gold (FG), a fluorescent retrograde tracer, was iontophoretically injected into the EP of 11 adult Sprague-Dawley rats and *Phaseolus vulgaris*-leucoagglutinin (PHAL), an anterograde tracer, was injected into the GP of 5 Sprague-Dawley adult rats. Following a 4-10 day survival period the animals were transcardially perfused with 4% paraformaldehyde and 40 um coronal and sagittal brain sections were cut on a freezing microtome. The FG sections were mounted, dehydrated, coverslipped with DPX and viewed on a epifluorescence microscope under a UV filter. The PHAL sections were immunohistochemically processed using the Vectastain ABC Elite Kit and viewed under brightfield and darkfield conditions. All FG injection sites that included some part of the EP resulted in retrogradely labeled neurons in GP. PHAL injection sites restricted to GP resulted in anterogradely labeled fibers, boutons en passant and apparent terminals in EP. The projection from GP to EP appears to be topographically organized with dorsal parts of GP projecting to dorsal EP and medial GP projecting to medial EP. This projection may need to be incorporated into models of how the basal ganglia influence behavior. Supported by NIH NS20629 to SWN and NIH NS19613 to ABY and JBP.

180.6

INTRACELLULAR RESPONSES RECORDED IN THE GLOBUS PALLIDUS AFTER STIMULATION OF THE FRONTAL CORTEX (CX), THE NEOSTRIATUM (Str), THE SUBTHALAMIC NUCLEUS (STH) AND THE SUBSTANTIA NIGRA (SN). H. Kita. Dept. of Anatomy and Neurobiology, College of Medicine, Univ. of Tennessee Memphis, Memphis, TN 38163.

Responses of GP neurons to stimulation of the CX, the Str, the STH, and the SN were intracellularly recorded in rats anesthetized with urethane (1.2 g/Kg, i.p.) supplemented with a Ketamine/Zylazine mixture. In some experiments, animals were immobilized by gallamine. Stimulus electrodes were bipolar stainless steel pins separated by 0.8 mm and insulated (on all but their tips) with epoxy. The stimulus current was less than 0.3 mA. Recording glass microelectrodes were filled with 0.5 M KCH_3SO_4 or KCl and 4% HRP in 0.05 M Tris buffer (pH 7.6). Neurons with membrane potential greater than 40 mV and spike amplitude greater than 50 mV were analyzed. Most of the GP neurons recorded were antidromically activated by STH and SN stimulation. Approximately one half of the GP neurons were also antidromically activated by Str stimulation. Results suggest that GP neurons send axons to multiple targets. Stimulation of the CX or Str evoked a sequence of orthodromic responses consisting of initial short duration EPSPs, short duration IPSPs, and late EPSPs. Stimulation of the STH and the SN evoked EPSPs which overlapped IPSPs. All of the IPSPs were decreased in amplitude by intracellular injection of Cl ions and blocked by systemic application of picrotoxin. Chronic decortication was made in some animals 5 days prior to the recording. In these animals, Str stimulation evoked small short latency IPSPs (instead of the initial EPSPs seen in intact animals), second IPSPs, and late EPSPs. The responses of GP neurons to STH and SN stimulation in decorticated rats were very similar to those seen in intact ones. (Supported by NIH grant NS- 25783)

180.7

CONTEXTUALLY DEPENDENT ACTIVITY IN GLOBUS PALLIDUS.
P Brotchie, R. Iansek and M.K. Horne. Department of Clinical Neurophysiology, Alfred Hospital, Prahran, 3181, Australia.

Single cell activity was recorded from the globus pallidus of awake behaving monkeys. Pallidal neurons were found to increase or decrease their background discharge phasically in relation to wrist movements. In approximately half the pallidal neurons which displayed movement related activity, the magnitude of the response was dependent on the contextual setting of the task. If the animal was unaware of the direction of the upcoming motor response, the phasic movement related discharge was diminished in these cells.

Phasic activity was also recorded at the end of the movement task in approximately one third of pallidal neurons. We called this neuronal response "delayed activity" because it occured well after the movement. During the performance of a task containing two ballistic movements in sequence, many cells displayed delayed activity well after the first movement, immediately prior to the cue for the second movement. This activity was often present in a cell, only when the second target movement was expected to occur in a particular direction. Therefore, as with the phasic movement related activity, delayed activity was dependent on the contextual setting of a task for its expression.

We hypothesize that the contextually dependent phasic activity of pallidal cells represents internal cues which assist in the switching between motor programs in sequential tasks.

180.8

ELECTROPHYSIOLOGY OF THE GLOBUS PALLIDUS NEURONS: AN *IN VITRO* STUDY IN GUINEA PIG BRAIN SLICES.
A. Nambu and R. Llinás. Dept. of Physiology and Biophysics, New York Univ. Med. Ctr., New York, NY 10016.

In vivo, globus pallidal (GP) neurons are known to discharge at a high frequency and to change their firing rate in relation to limb and facial movements (DeLong et al, *J. Neurophysiol.* 53: 530, 1985); little is known, however, about the ionic mechanism responsible for this behavior. Intracellular recordings were obtained from GP in brain slices from adult guinea-pig. Two classes of neurons were identified on the basis of their membrane properties. The first type, which includes the majority of neurons recorded, generated a slow rising, all-or-none low threshold response upon direct depolarization from a hyperpolarized membrane potential. This response could trigger a short spike burst and a rebound depolarization that would generate another spike such that the cell could fire at 4-6Hz. The low threshold response was inactivated by membrane depolarizations, was TTX insensitive, and was blocked by $CoCl_2$, suggesting that it is generated by activation of a low threshold calcium conductance (Llinás and Yarom, *J. Physiol.* 315: 549, 1981). Prolonged (300ms) direct depolarization elicited rhythmic repetitive firing which often exhibited a prominent accommodation. These neurons had a fast, transient K^+-dependent current (A-current) and input resistances $\geq$ 50 MΩ. The second type of neuron also demonstrated a fast, transient K^+-current but was characterized by a low input resistance ($\leq$ 50 MΩ), and showed neither low threshold calcium spikes nor accommodation. The 4-6 Hz oscillatory firing seen in the first type of cell may be related to the burst activity which has been reported in the GP of monkeys with Parkinsonism induced by MPTP (Miller and DeLong, *Adv. Beh. Biol.* 32: 415, 1987). Supported by grant 13742 from NINDS and the Naito Foundation.

180.9

BEHAVIORAL AND NEURONAL EFFECTS OF GABA AGONIST AND ANTAGONIST INJECTED LOCALLY IN THE GLOBUS PALLIDUS OF INTACT MONKEYS.
L. Tremblay and M. Filion. Neurobiology Res. Ctr, Laval Univ., Québec, Qué., Canada.

We have shown previously that akinesia and muscular rigidity, induced in monkeys by the neurotoxin MPTP, are correlated with an increased neuronal activity in the internal segment of the globus pallidus (GPi) but with a decrease in the external segment (GPe). Apomorphine suppressed the signs of parkinsonism and induced dyskinesia (repetitive movements of choreic type), which were correlated inversely with abnormally low GPi and high GPe activities. The objective of the present work was to reproduce those behavioral and neuronal alterations in intact monkeys by means of injections of the GABA agonist muscimol and antagonist bicuculline locally within the GP. Bicuculline (30 mmol, 0.5-2 µL) greatly increased the activity of GPe neurons near (0.3-2 mm) the injection site and induced contralaterally localized dyskinesia, with thresholds lowest in the caudal GPe. Simultaneously, in the ipsilateral GPi, neurons with abnormally low activity were surrounded by others with abnormally high activity. Consecutive injections of muscimol (10 mmol, 1 µL) in these area of low GPi activity induced similarly localized dyskinesia, whereas the localizations were different when muscimol was injected in the surrounding area of high GPi activity. The dyskinesia induced by bicuculline in the GPe could be suppressed and replaced by contralateral akinesia following injection of muscimol at the same site. And then, GPi neurons that had been hypoactive during dyskinesia, became hyperactive during akinesia. (Supported by the FRSQ and MRC of Canada).

180.10

IMMUNOHISTOCHEMICAL STUDY OF THE PALLIDAL COMPLEX IN SYMPTOMATIC AND ASYMPTOMATIC MPTP-TREATED MONKEYS, NORMAL HUMANS, AND PARKINSON'S DISEASE PATIENTS.
S. Dacko, M.G. Smith* and J.S. Schneider. Dept. of Neurology, Hahnemann University School of Medicine, Philadelphia, PA. 19102.

The globus pallidus in the normal macaque monkey as well as in the normal human contains many tyrosine hydroxylase (TH) immunoreactive fibers. Met-enkephalin, substance P and serotonin, are also localized to the pallidum, as previously desrcibed by others. The present study examines the extent to which these different neurochemical innervations of the pallidal region might differ between symptomatic and asymptomatic MPTP-treated monkeys and normal humans and Parkinson's disease (PD) patients and whether the pattern of these pallidal innervations differs in monkeys vs. humans. Four monkeys received low dose MPTP over several months, sustained extensive damage to the ventral mesencephalon, had large striatal dopamine depletions, yet remained asymptomatic for a significant motor disorder. These animals had relative sparing of GPi TH immunoreactivity with significant loss of GPe and striatal TH staining. Animals given large doses of MPTP over a short period of time also had large striatal dopamine depletions and loss of mesencephalic dopamine neurons, but were severely parkinsonian. These animals had some loss of GPi TH immunoreactivity but a relative sparing of this innervation compared to the more extensive loss of GPe and striatal TH staining. In contrast, humans with PD had extensive loss of TH immunoreactivity throughout pallidal and striatal regions. The possibility exists that the relative sparing of the dopamine innervation of the GPi in MPTP-exposed monkeys may at least partially contribute to either recovery from the effects of MPTP or the ability to remain motor asymptomatic despite severe damage to the nigrostriatal dopamine system. These results also suggest a major difference in the neuropathology of MPTP-induced parkinsonism in the monkey and PD in man. Changes (or lack thereof) in the other neurochemical systems innervating the pallidal region will also be discussed.

HIPPOCAMPUS AND AMYGDALA: NEUROCYTOLOGY

181.1

SELECTIVE REGULATION OF DYNORPHIN LEVEL IN RAT HIPPOCAMPUS BY GLUCOCORTICOIDS.
P. H. K. Lee, L. Thai, and J. S. Hong. LMIN, NIEHS/NIH, Research Triangle Park, NC 27709.

The hippocampus, a brain region involved in learning, memory and a number of neurological diseases, is a target of adrenal hormones and it may also play a role in the regulation of the endocrine functions of the hypothalamic-pituitary-adrenal axis. In this study, we examined the effects of adrenocorticoids in the regulation of the opioid system in rat hippocampus. Male Fischer-344 rats were either bilateral adrenalectomized or sham-operated under light methoxyflurane anesthesia. Hippocampal dynorphin A(1-8) immunoreactivity (DYN-IR), determined by radioimmunoassay, remained unchanged 24 hr after adrenalectomy (ADX), but it was significantly reduced by 19% 1 week post ADX. A gradual and further reduction of hippocampal DYN-IR was observed in ADX rats, decreased by 43% and 58% at 1- and 2-month post ADX, respectively. Immunohistological study revealed a marked decrease of DYN-IR in the mossy fiber. These decreased DYN-IR were restored by dexamethasone treatment, suggesting glucocorticoids play an important role in modulating the dynorphin level in the hippocampus. No changes in hippocampal Met-enkephalin immunoreactivity were detected in ADX animals, suggesting that glucocorticoids are essential and selectively regulate the dynorphinergic system in rat hippocampus.

181.2

NORADRENERGIC (NA) ENHANCEMENT ON HIPPOCAMPAL SYNAPSES AFTER NEONATAL ADMINISTRATION OF 6-HYDROXYDOPAMINE (6-OHDA) IS DUE TO AN INCREASE OF β2-ADRENERGIC RECEPTOR NUMBER.
H.-M. Hwang, W.-H. Tsai, Y.-P. Lee, and T. H. Chiu*. Depts. of Anatomy & Physiology, National Yang-Ming Medical College, and IBMS Academia Sinica, Taipei, Taiwan, R.O.C.

In order to examine the NA modulation on the hippocampal function, synaptic efficacy was studied on the hippocampal slices of animals with NA depletion. Rats were injected s.c. with mg/kg of 6-OHDA dissolved in 0.9% saline containing 0.04% L-ascorbic acid for 4 days starting from the day of birth, while the control animals received an equal volume/weight of vehicle. Experiments were performed at the postnatal 120-150 days. β2-adrenergic receptor immunohistochemistry revealed an increase of receptor number, while Dopamine-β-hydroxylase immunohistochemistry indicated 90% reduction of NA innervation. Electrophysiology showed a faster decay of potentiation on CA1 pyramidal cells after NE depletion, but greater response to β-adrenergic agonist. Study on expression of β-adrenergic receptors related to NA modulation on hippocampus is in progress.
(supported by NSC79-0412-B010-19 R.O.C.)

181.3

INTRACELLULAR pH REGULATION IN SINGLE MAMMALIAN HIPPOCAMPAL CA1 NEURONS. G. Boyarsky, T.R. Cummins*, G.G. Haddad, and W.F. Boron*, Depts. of Cell. and Molec. Physiol. and Pediatrics, Yale U. Sch. of Med., New Haven, CT 06510.

Intracellular pH (pH_i) was measured in single dissociated rat hippocampal cells (CA1) using a microscope-based fluorimetric technique and the pH-sensitive dye BCECF. In the nominal absence of CO_2/HCO_3^-, two distinct patterns of pH_i regulation were observed. In six "high-pH_i cells", the initial pH_i was 7.47 $\pm$ 0.05 and the cells recovered very rapidly from an NH_4^+-induced acid load (dpH_i/dt of 57.5 $\pm$ 13.3 x 10^{-4} pH/sec at pH_i 7.2). In ten "low-pH_i cells", the initial pH_i was 6.83 $\pm$ 0.06 and the cells recovered very slowly from an NH_4^+-induced acid load (dpH_i/dt of 9.1 $\pm$ 2.2 x 10^{-4} pH/sec at pH_i of 6.4 and 5.5 $\pm$ 2.8 x 10^{-4} pH/sec at pH_i of 6.8). Similar results were obtained for cells isolated from 7-, 11-, 12-, 25-, and 26-day rats. In nine experiments, cells were acidified by removal of Na^+ (replaced by N-methyl-D-glucammonium). In seven of the nine cells, from both "high-pH_i" and "low-pH_i" groups, pH_i spontaneously recovered in the continued absence of Na^+, over a period of ~3 to 15 minutes. The "high-pH_i cells" probably possess a potent acid extrusion mechanism, whereas the "low-pH_i cells" have only a moderate capacity for acid extrusion. The pH_i recovery in the absence of Na^+, which occured in both "high-pH_i" and "low-pH_i" cells, has been observed previously in rabbit S3 proximal tubules and may be due to a H^+-pump. This Na^+-independent mechanism may play an important role in pH_i regulation in neurons, and be a more general phenomenon than previously thought.

181.4

MODULATION OF SYNAPTIC EFFICACY IN FIELD CA1 OF THE RAT HIPPOCAMPUS BY FORSKOLIN.
L. E. Chavez-Noriega and C. F. Stevens. The Salk Institute, Howard Hughes Medical Institute, La Jolla, CA 92037.

An increase in intracellular cyclic AMP levels has been reported to enhance synaptic excitation and reduce the threshold for the induction of long-term potentiation (LTP) in the dentate gyrus and field CA3 of the hippocampus (Stanton and Sarvey, 1985, Brain Res., 361:276; Hopkins and Johnston, 1988, J.Neurophysiol., 59:667).

We have studied the effect of the direct activator of adenylate cyclase, forskolin, on field potentials evoked in area CA1 by stimulation of Schaffer/commissural fibres. Transverse hippocampal slices were prepared and incubated using standard procedures.

Forskolin (10-50 uM) produced a dose-dependent increase in the early field EPSP slope (50 uM: mean +/- SEM: +22 +/- 10%, n=7) and population spike amplitude (+88 +/- 19%). These effects were potentiated in the presence of IBMX, a phosphodiesterase antagonist and adenosine receptor blocker (50 uM; EPSP: +40 +/- 7%; population spike: +142 +/- 37%, n=5). The inactive analogue 1,9 dideoxy forskolin (50 uM, n=3) did not affect the EPSP slope (-3 +/- 3%) nor the PS amplitude (0 +/- 0%).

These results suggest that activation of adenylate cyclase may modulate synaptic efficacy in CA3 - CA1 synapses of the hippocampus.

181.5

IDENTIFICATION OF DYNORPHIN-POSITIVE CELLS IN HIPPOCAMPAL CELL CULTURES. X. He*, P. Lee, R. Tuominen, L. Thai, B.Culver, E. Mar*, W. Zhang* and J. Hong. LMIN, NIEHS/NIH, Research Triangle Park, NC 27709.

The purpose of this study was to examine dentate gyrus (DG) cell cultures as a model system to study dynorphin (DYN) regulation. The hippocampus (HC) was removed from rat pups between 1-12 days of age, the DG was dissected free from HC, and cells from these areas were dissociated and cultured. Localization of DYN and mRNA encoding the peptide were studied using, respectively, immunocytochemical and *in situ* hydridization techniques.

Results from studies of different culture conditions showed that DG provided more DYN^+ cells than the rest of the HC and that these cells were most prominent after 2 wks in culture. Cultures from 7 day old pups yielded more DYN^+ cells than those from younger or older rats. Also, the optimal concentration of corticosterone for supporting DYN^+ cells was 1 pmol, and 2% fetal bovine serum was more effective in maintaining these cells than serum-free media. Ara-C suppressed glial cell growth but also decreased DYN^+ cells. Together these studies demonstrate DYN is localized in cells cultured from DG and that they exhibit mRNA encoding DYN, indicating the ability to synthesize this peptide. It seems reasonable to propose that most of these DYN^+ cells are derived from DG granule cells.

181.6

COMMISSURALLY-PROJECTING HIPPOCAMPAL DENTATE BASKET CELLS ARE A SUBPOPULATION OF GABA NEURONS IMMUNOREACTIVE FOR PARVALBUMIN J.H. Goodman and R.S. Sloviter, Helen Hayes Hosp., W. Haverstraw, NY 10993 and Columbia Univ., New York, NY 10032

Fluorogold (FG) was used to identify commissurally-projecting dentate neurons. Injections (0.05-0.3ul, 4%) were made in the dentate of 15 male Sprague-Dawley rats. Contralateral FG fluorescence was examined in 30µ-thick Vibratome sections. Consistent with previous studies, we noted FG in many hilar neurons and CA3 pyramidal cells. FG was unexpectedly seen in neurons with the morphology of pyramidal-shaped basket cells of the granule cell layer. Each section was photographed and then sequentially stained immunocytochemically for parvalbumin (PV) and GABA. Virtually all FG-labeled basket cells contained PV and GABA. Conversely, many PV cells, particularly those in the upper granule cell and molecular layers, did not exhibit FG fluorescence. Nor did all GABA neurons contain PV. These results identify a previously undescribed subpopulation of GABA-immunoreactive dentate basket cells that have commissural connections. The association between the expression of PV and those dentate basket cells that have commissural projections is intriguing. Supported by NS18201.

181.7

INTRAHIPPOCAMPAL ADMINISTRATION OF NMDA ALTERS OPIOID PEPTIDE AND C-FOS LEVELS IN THE RAT T.E. Helton and J.F.McGinty. Dept. of Anatomy and Cell Biology., East Carolina University School of Medicine, Greenville,NC 27858.

This study examined the effects of N-Methyl-D-aspartate (NMDA) on enkephalin, dynorphin and c-*fos* expression in the hippocampus. Rats were implanted with a 24 g stainless steel guide cannula (AP -5.2, ML -5.6, DV -2.5, <2°) and handled daily for 1-2 wk. All rats received at least 2 saline injections prior to the day of the experiment to habituate them to restraint during injection. This was necessary to reduce non-specific induction of *fos*. On the day of the experiment rats received 0.9% saline or 120 nmols NMDA in 0.5µl into the ventral hippocampus. NMDA treated rats exhibited wet dog shakes, forelimb clonus, hypersalivation and hyperactivity which lasted for 1 to 2 hours after injection. Rats were perfused with buffered 4% paraformaldehyde 1,3,8, or 24 hr after injection. Frozen sections were cut at 50µm and collected for immunocytochemistry. At 1h a significant rise in *fos*-immunoreactivity (IR) in the dentate granule cells (DGC) was observed while pyramidal cells in the CA_3 and CA_1 fields showed little staining. By 3h *fos*-IR in the DGC and the CA_3 and CA_1 pyramidal cells was high. The entorhinal and piriform cortices, amygdala, hypothalamic and thalamic nuclei also exhibited a significant rise in *fos*-IR. At 8h and 24h, *fos*-IR remained significantly elevated in NMDA treated rats vs. control rats. NMDA-induced elevation of *fos* preceded the decrease in opioid peptide IR levels in the hippocampus. Accompaning changes in mRNA levels are being investigated. Supported by DA03982.

181.8

NEUROTOXICITY OF INTRAHIPPOCAMPAL INJECTIONS OF EXCITATORY AMINO ACIDS: PROTECTIVE EFFECTS OF CPP. L.E. Jarrard and B.S. Meldrum, Dept. Psychol., Washington and Lee Univ., Lexington, VA 24450, and Inst. of Psych., London, SE5 8AF.

The pattern of cell loss and neuronal degeneration resulting from focal injections of excitatory amino acids into hippocampus was studied, together with the protection provided by 3-((±)-2-carboxypiperazin-4-yl)propyl-1-phosphonate (CPP). Rats received bilateral injections (.05 - .10 ul) of N-methyl-D-aspartate (NMDA; 60, 40, 20 mM), quisqualate (QUIS; 120 mM), kainic acid (KA; 4.7 mM) or ibotenic acid (IBO; 63 mM) at 13 sites in the hippocampus (see Jarrard, Neurosci. Methods, 29:251, 1989). Half of the animals in each group were given i.p. injections of CPP (20 mg/kg) immediately before the operations, and again after 3 hr. The rats were sacrificed after 7 days, and the brains were stained with cell and silver stains. Within the hippocampus all cell fields including dentate gyrus were destroyed following injections of NMDA and IBO. After QUIS and KA severe damage was limited to hilar and CA3 cells, with partial loss of CA1 cells. CPP injections blocked most of the hippocampal damage due to NMDA and IBO while in QUIS and KA animals the protection was limited to CA1 cells. NMDA and IBO resulted in minimal extrahippocampal damage. QUIS and KA caused extensive secondary damage to olfactory areas, amygdala, ventral neocortex (layers V and VI), and thalamus; this extrahippocampal damage was partially or completely blocked by CPP.

181.9

LOCALIZATION OF SEROTONINERGIC FIBER PLEXUS, 5-HT UPTAKE SITES AND 5-HT_{1A} RECEPTORS IN THE HIPPOCAMPUS OF NORMAL AND PRENATALLY PROTEIN MALNOURISHED RATS. G.J. Blatt, D.L. Rosene, A. Virga, and K.J. Rhodes. Dept. Anat., Boston Univ. Sch. Med., Boston, MA 02118.

Prenatally protein malnourished rats born to dams on a 6% casein diet during pregnancy and then cross-fostered at birth to females on a 25% diet show adult alterations in vigilance state activity, kindling and behavior that suggest hippocampal (HF) dysfunction and neurochemical studies have shown an elevation of brain serotonin. To investigate this an antibody to serotonin (5-HT) was used to assess the density and distribution of 5-HT fiber plexus in the HF in four pairs of normal (25/25) and malnourished (6/25) adult (day 220) rats. In normal rats, 5-HT fiber plexus in the HF was very dense in the molecular layer of the dentate gyrus, stratum oriens and radiatum of CA3 and in stratum moleculare in CA1, prosubiculum and subiculum. In contrast, in malnourished rats, athough there was a similar laminar distribution of 5-HT fibers there was a marked *decrease* in the density in all subfields and levels of the HF, especially in the dentate gyrus. To examine this system further we utilized on-the-slide in vitro ligand binding techniques to assay both 5-HT uptake sites localized to 5-HT afferents using ^{3}H-citalopram and 5-HT_{1A} receptors using ^{3}H-8-OH-DPAT in three pairs of 25/25 and 6/25 rats. In normal rats, the density and distribution of 5-HT uptake sites was generally similar to the pattern demonstrated immunocytochemically. The only difference was in CA1 where there was a moderate density of 5-HT uptake sites where only small caliber lightly stained 5-HT fibers were seen immunocytochemically. In malnourished rats, there was a small decrease in the density of 5-HT uptake sites in all subfields of the HF except in the molecular layer of the dentate gyrus where there was a 20-25% decrease (measured densitometrically). This confirms the immunocytochemical observation but suggests that the effect is greatest in the dentate gyrus. In the same rats 5-HT_{1A} receptors were most dense in the molecular layer of the dentate gyrus, stratum oriens of CA3 and in stratum oriens, radiatum and moleculare of CA1 but there was no difference between the normals and malnourished. "Supported by NIH HD22539".

181.10

INCREASE IN HIPPOCAMPAL NOREPINEPHRINE (NE) AND DOPAMINE (DA) AFTER BLOCKADE OF α_2-ADRENOCEPTORS. K.Xu, K.Frerichs, J.Hallenbeck, G.Feuerstein,[1] J.N.Davis,[2] and A.-L.Sirén, Dept of Neurology, USUHS, Bethesda, MD, [1]Dept of Pharmacology, SmithKline Beecham, King of Prussia, PA, [2]VA Medical Center, Duke University, Durham, NC.

The effect of SK&F 86466 (6-chloro-2,3,4,5-tetrahydro-3-methyl-1-H-3-benzazepine), a potent and selective α_2-adrenoceptor antagonist, was investigated on hippocampal release of NE, DA and serotonin (5-HT) in awake rats (n=17). Microdialysis samples (30-min) were collected and immediately injected (20µl) into an HPLC with electrochemical detection. The basal release of NE was 4±0.4pg. Administration (i.v.) of 5mg/kg and 10mg/kg of SK&F 86466, increased NE two-fold (11±1pg, $p<0.05$) and nine-fold (39±3pg, $p<0.01$), respectively, while the 1mg/kg dose had no effect. DA was not detected at baseline, but after administration of 5mg/kg and 10mg/kg of SK&F 86466, a peak with the retention time of DA was detected (4±0.3pg and 6±1pg, respectively). SK&F 86466 had no effect on 5-HT release (baseline 17±3pg).

The results show that blockade of α_2-adrenoceptors modulates NE and probably DA release in the hippocampus. (Supported by VA and DoD).

181.11

PHYSICAL ACTIVITY EFFECTS ON HIPPOCAMPAL CHOLINERGIC FUNCTION AND SPATIAL MEMORY. D.E. Fordyce and R.P. Farrar. Institute of Neuroscience, The University of Texas at Austin, Austin, TX 78712

In the present study, specific markers of hippocampal cholinergic function, high affinity choline uptake (HACU) and muscarinic quinuclidinylbenzilate (QNB) binding, were shown to be altered by endurance training (6 months of treadmill running, 5 day/week, 30 min/day). HACU and QNB binding were determined in synaptosomes of endurance trained F344 rats and their age matched sedentary controls. Endurance trained rats (trained from 6 months to 12 months of age) showed a reduction in HACU (10.85±0.55 vs. 15.28 pmoles/mg, $p<0.02$) and an increase in QNB binding (4027±155 vs. 3522 fmoles/mg, $p<0.05$) compared to their age-matched sedentary controls. As cholinergic function has been implicated in spatial memory, it was of interest to determine if the cholinergic changes in the hippocampus of run rats would be accompanied by altered performance in spatial memory. Using a modified version of the place learning set procedure described by Whishaw (1985), endurance trained rats showed significantly enhanced performance compared to controls with regard to latency of acquisition (blocks of four trials from fifth to fourteenth day of training, second trials, 4.9±0.25 (N=6) vs. 9.1±0.28 seconds (N=6) and retention (after 4 days of rest from spatial testing, blocks of four, second trial, 4.1 vs. 9.1 seconds).

181.12

ARGININE VASOPRESSIN-INDUCED MOTOR DISTURBANCES: LOCALIZATION OF A SENSITIVE SITE IN THE MEDIAL AMYGDALA. B.J. Willcox, P. Poulin, O.J. Pittman and W.L. Veale. Neuroscience Research Group, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Arginine vasopressin (AVP) can produce motor disturbances when injected intracerebroventricularly (i.c.v.) or when injected into the ventral septal area of the rat brain. These AVP-induced motor disturbances result from an interaction of AVP with the V1-type of AVP receptor and involve a sensitization process, whereby an initial injection of AVP may cause minor motor disturbances such as ataxia, whereas subsequent AVP injections one to four days later cause severe motor disturbances, such as barrel rotations and myoclonic/myotonic convulsions. Since AVP-like immunoreactivity as well as AVP receptors have been shown to exist in the amygdala and since the amygdala is a well known locus for kindling of a variety of motor disturbances including myoclonic/myotonic convulsions, experiments were undertaken to determine if AVP would cause motor disturbances following microinfusion into the amygdala.

Twelve male Sprague-Dawley rats were implanted stereotaxically with bilateral cannulae directed towards the medial amygdala. Following a 7 day recovery period bilateral microinfusion of AVP (40 ng) was given into the medial amygdala on two consecutive days. The severity of motor disturbances was scored (over 10 min) according to a predetermined behavioral code. The results indicated that the initial AVP infusion caused minor motor disturbances while the subsequent infusion 1 day later caused severe motor disturbances including barrel rotations and myoclonic/myotonic convulsions. These results suggest that the amygdala is a sensitive site for AVP-induced motor disturbances and that these motor disturbances undergo a sensitization process after an initial exposure to AVP.

Supported by the M.R.C. of Canada.

181.13

QUANTITATIVE AUTORADIOGRAPHY OF MUSCARINIC BINDING IN HUMAN AMYGDALA. H.R. Brashear, C.A. Levesque, S.S. Wolf* and G.F. Wooten. Department of Neurology, University of Virginia, Charlottesville, VA 22908.

Quantitative autoradiography with [^{3}H]quinuclidinyl benzilate ([^{3}H]QNB) was used to define the subnuclear organization of muscarinic cholinergic receptors in the human amygdala. Series of 10 µm sections at 700 µm intervals through normal human amygdala were incubated with 0.1-10.0 nM concentrations of [^{3}H]QNB. Nonspecific binding in the presence of 1 µM atropine was less than 5% of total. Autoradiographs were compared to adjacent sections stained for acetylcholinesterase and Nissl substance. Muscarinic binding was not uniform and differentiated amygdaloid subnuclei well. Anatomical localization was comparable to binding in the rat. Binding correlated with acetylcholinesterase activity in most regions. Specific binding was highest in the magnocellular basolateral nucleus, followed by basomedial, cortical, central and lateral nuclei, and was lowest in the anterior amygdaloid area. Optimal binding occurred at concentrations of 0.5-1.0 nM, consistent with data on rat and human putamen from this laboratory. These data suggest that muscarinic cholinergic receptors are differentially distributed among subnuclei of the human amygdala.

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181.14

LOCALIZATION OF CORTICOTROPIN-RELEASING FACTOR (CRF)-LIKE IMMUNOREACTIVITY IN MONKEY AMYGDALA. J.L. Bassett and S.L. Foote. Dept. of Psychiatry (M-003) UCSD, La Jolla CA 92093.

Previous investigations of the distribution of CRF-like immunoreactivity in rat brain have reported the presence of this peptide within the amygdala. Most notably, the highest concentrations of both CRF-positive cells and fibers have been observed in the rat central nucleus, consistent with the putative role of this peptide in autonomic and endocrine responses. In addition, moderate to low densities of CRF-positive somata and processes have been noted in other nuclei of the rat amygdala. However, the distribution of CRF-like immunoreactivity in primate amygdala has not been described.

In the present study, sections through the amygdala of non-colchicine treated monkeys (Saimiri sciureus) were processed for immunohistochemistry using a polyclonal antiserum directed against the human form of CRF (donated by J. Rivier & W. Vale). Within the amygdaloid complex, large numbers of CRF-positive cells were seen within the lateral and basal nuclei. Lower numbers of immunoreactive somata also were seen in the nucleus of the lateral olfactory tract, periamygdaloid cortex, anterior cortical and central nuclei. Dense networks of CRF-positive fibers and varicosities were seen within the lateral and central nuclei; moderate densities were evident in the medial nucleus, anterior amygdaloid area, layer Ia of the periamygdaloid cortex, anterior and posterior cortical nuclei. Thus, striking differences are evident between the distributions of CRF-like immunoreactivity in the rat and monkey amygdala.

181.15

CALCIUM BINDING PROTEIN CONTAINING NEURONS OF THE BASOLATERAL AMYGDALA ALSO EXHIBIT GABA AND CYTOCHROME OXIDASE IMMUNOREACTIVITY. A.J. McDonald and K.G. Baimbridge. Dept. of Anatomy, Univ. of South Carolina Sch. of Med., Columbia, S.C. 29208 and Dept. of Physiol., Univ. of British Columbia, Vancouver, Canada VGT 1W5.

The calcium binding proteins parvalbumin (PV) and calbindin (CB) are thought to sequester calcium in neurons with high firing rates. This investigation used two-color immunoperoxidase and adjacent section techniques to characterize PV-ir and CB-ir neurons of the rat basolateral amygdala (BLA) and to determine whether these neurons also contain GABA or cytochrome oxidase (CO) immunoreactivity. Intense PV-ir and CB-ir was observed in nonpyramidal (local circuit) neurons and in small puncta surrounding pyramidal perikarya. The great majority of PV-ir neurons also exhibited CB-ir (comprising about one-half of all CB-ir neurons). Virtually all PV-ir and CB-ir neurons were also GABA-ir (CB-ir neurons comprised more than one-half of all GABA-ir cells). The majority of PV-ir and CB-ir neurons also exhibited high levels of CO-ir. These cells, which may correspond to the bursting neurons noted in electrophysiological studies of BLA, may utilize PV and/or CB to buffer the calcium ion influx associated with high firing rates. Since these active cells appear to be GABAergic local circuit neurons, they may mediate the profound inhibition that characterizes BLA. (NS19733)

181.16

LOCALIZATION OF INSULIN AND INSULIN-LIKE GROWTH FACTOR (IGF) RECEPTORS IN RAT HIPPOCAMPUS FOLLOWING KAINATE AND ISCHEMIC LESIONS. M.G. King, J.E. Franck, and D.G. Baskin. Depts. Biological Structure, Neurological Surgery, and Medicine, University of Washington, Seattle, WA 98195, and V.A. Med. Center, Seattle, WA 98108.

Insulin, IGF-I, and IGF-II binding sites have previously been localized in the hippocampus with quantitative autoradiography (QAR). To test the hypotheses that insulin receptors are on CA1 pyramidal cell dendrites, IGF-I receptors are on CA3 pyramidal cell dendrites, and IGF-II receptors are on CA1 and CA3 pyramidal cell bodies, we examined the effect of hippocampal lesions on the localization of these binding sites with QAR using Hyperfilm ß-max. Frozen sections (20 µm), from Wistar rats which had recovered for 7 days after either kainate (10 min bilateral intraventricular infusion at 0.5 µg/µl saline) or ischemic (4 vessel occlusion) lesions, were incubated in either ^{125}I-insulin, ^{125}I-IGF-I, or ^{125}I-IGF-II at 0.05 nM (16h, 5°C) alone or with 50 nM unlabeled peptide. Autoradiographs were analyzed by computer densitometry. Kainate reduced insulin and IGF-I binding in both the CA3 *s. oriens* and *s. radiatum* by 85% and 50%, respectively, and IGF-II binding in the CA3 *s. pyramidale* by 75%. Kainate also reduced insulin binding in the CA1 *s. oriens* by 30% and *s. radiatum* by 65%. Ischemia reduced insulin binding in the CA1 *s. oriens* by 25% and *s. radiatum* by 60% but did not affect IGF-I binding in either region. These results suggest: (a) insulin receptors are on CA1 pyramidal cell dendrites and possibly Schaffer collaterals of CA3 pyramidal cells; (b) IGF-I receptors are on CA3 pyramidal cell dendrites; and (c) IGF-II receptors are on CA1 and CA3 pyramidal cell bodies. (Supported by NIH Grant NS 24809 and the Veterans Administration.).

181.17

CHARACTERIZATION OF VICIA VILLOSA AGGLUTININ POSITIVE CELLS IN THE HIPPOCAMPAL REGION C. Drake, K. Mulligan, T. Wimpey, A. Hendrickson, and C. Chavkin. Depts of Pharmacology and Biological Structure, University of Washington, Seattle WA 98195.

The lectin from *Vicia villosa* (VVA) has been shown to label a subset of cortical GABAergic cells in several species. We have examined the regional distribution, morphology, and intracellular characteristics of VVA-positive cells in the hippocampal formation. The brains of perfusion-fixed adult male Sprague-Dawley rats were cut coronally or sagittally into 25-micron sections. Visualizing the binding of horseradish peroxidase- or biotin- conjugated VVA with the chromagen diaminobenzidine revealed heavily stained neurons in the subiculum and lightly-stained dispersed neurons in hippocampal strata pyramidale, oriens, and alveus. The morphology of VVA-positive cells was diverse. In the hippocampus proper, vertically-oriented cells predominated in stratum pyramidale, and bipolar and multipolar shapes were most common in stratum alveus and stratum oriens, respectively. The subiculum contained large multi- and bipolar neurons. Consistent with descriptions of VVA binding in other brain areas, VVA labeled the peripheral surface of somata and proximal dendrites in a punctate fashion. Labeling was abolished by preadsorbing HRP- or biotin- conjugated lectin to GalNAc. The extent to which expression of VVA binding sites colocalized with intracellular markers was examined immunohistochemically by double-labeling sections with VVA-HRP or VVA-biotin and antibodies against GABA, parvalbumin, or calbindin. The intensely labeled, relatively concentrated VVA-positive neurons in the subiculum proper were examined in detail. All of the subicular VVA-positive cells contained parvalbumin, none colocalized with calbindin and about 85% were GABA-positive. Taken together, the data from these experiments suggested that these cells are GABAergic interneurons and that VVA might be a useful marker for physiological studies of this population. Accordingly, acutely dissociated subicular neurons were labeled *in vitro* with VVA coupled to small magnetic beads. The viability of labeled dissociated cells was confirmed and identified cells were characterized physiologically using whole-cell voltage clamp recording. The cells exhibited characteristic transient and slowly inactivating potassium conductances. Supported by DA04123.

181.18

SOMATOSTATIN, SUBSTANCE P AND HIPPOCAMPAL INNERVATION OF THE RAT LATERAL SEPTAL AREA "SOMATOSPINY" NEURONS; HIPPOCAMPAL INPUT ONTO SEPTAL SUBSTANCE P-IMMUNOREACTIVE CELLS. R.L. Jakab[1] and C. Leranth[1,2]. Dept. of Obstetrics and Gynecology[1] and Section of Neuroanatomy[2], Yale University, School of Medicine, New Haven, CT. 06510

"Somatospiny" neurons of the lateral septal area (LSA) are targets of hippocampal afferents, and GABA-, monoamine- acetylcholine-, and vasopressin-containing fibers of various origin. The overlapping distribution pattern of the somatospiny neurons and the somatostatin- (SS) and substance P- (SP) immunoreactive (IR) pericellular baskets in the mediolateral part of the LSA suggests further peptidergic afferents of these cells. To test this hypothesis, electron microscopic immunocytochemistry using antisera against SS or SP was performed on rats two days following fimbria-fornix transection. Asymmetric synapses of degenerated hippocampo-septal axon terminals, and symmetric synaptic contacts of SS- or SP-IR boutons were observed on the same somatospiny neurons.

In addition, following fimbria-fornix transection of colchicine treated rats (80µg in 20µl saline), we demonstrated that SP-IR neurons in the lateral border of the medial septum form synaptic contacts with degenerated hippocampo-septal axon terminals. SP-containing neurons of this area were reported to project to the hippocampus (Surlow and Peterson '89). This synaptic arrangement indicates a direct "short circuit", reciprocal interaction between the septum and hippocampus. Supported by NIH grant NS 26068.

181.19

LOCALIZATION OF PRESYNAPTIC CHOLINERGIC MARKERS AND MUSCARINIC RECEPTORS IN THE HIPPOCAMPAL FORMATION OF THE RHESUS MONKEY K.J. Rhodes, D.L. Rosene, and M.B. Moss. Dept. of Anatomy, Boston Univ. Sch. Med., Boston, MA 02118.

The aim of this study was to localize cholinergic afferents and muscarinic receptors to intrinsic neurons or specific populations of afferent axons in the hippocampal formation (HF) of the rhesus monkey. The distribution of presynaptic cholinergic markers, (high affinity choline uptake sites [HACU] and acetylcholinesterase [AChE]), and muscarinic receptors (M1 and M2) was examined in monkeys in which the fornix (n=2) or entorhinal cortex (n=1) were ablated unilaterally to deafferent the hippocampus of basal forebrain and perforant path inputs, respectively, in monkeys in which the hippocampus was bisected unilaterally (n=2) to transect longitudinally directed intrahippocampal association projections, and in monkeys in which discrete (1µl) intrahippocampal injections of ibotenic acid (10µg/µl) were made to destroy intrinsic neurons while sparing afferent fibers (n=5). After 14 to 21 days post lesion the animals were sacrificed, their brains immediately frozen and cut into 15µm thick serial sections. AChE was localized histochemically, and HACU sites, M1 and M2 receptors were localized using *in vitro* ligand binding and autoradiography. Quantitative analysis of the histochemically stained sections and autoradiograms revealed the following major changes: 1) both HACU sites and AChE are associated with cholinergic afferents since the density of both markers was significantly reduced following fornix transection. However, in the dentate gyrus (DG) 80% of the AChE in the inner third of stratum moleculare remained after a fornix transection but only 25% remained after hippocampal bisection, indicating that a significant proportion of this AChE is associated with the dentate gyrus association pathway. 2) M1 sites are located on the somata and dendrites of intrinsic neurons in the DG, CA3, CA2 and CA1 subfields since ibotenic acid lesions in these areas reduced the density of M1 sites; 3) M2 sites are located on CA1 afferents in the subiculum since only ibotenic acid lesions within CA1 reduced the high density of M2 binding in this subfield while other deafferenting lesions and even ibotenic acid injections within the subiculum left it intact. "Supported by NIH 16841, 19416, and 04321"

181.20

THE LAMINAR DISTRIBUTION OF MUSCARINIC AND 5-HT$_{1A}$ RECEPTORS IN THE POSTERIOR PARAHIPPOCAMPAL GYRUS OF THE RHESUS MONKEY. D. L. Rosene, K J. Rhodes, G. J. Blatt, and M. B. Moss. Dept. of Anatomy, Boston Univ. Sch. Med., Boston, MA 02118.

The posterior parahippocampal gyrus (PPHG), comprising cytoarchitectonic areas TH, TL, and TF, forms a critical structural and connectional link between unimodal and multimodal sensory association cortices and the medial temporal lobe limbic system. We examined the distribution of muscarinic cholinergic (M1 and M2) and serotonergic (5-HT$_{1A}$) receptors within each subdivision of the PPHG in five young adult rhesus monkeys. At the time of sacrifice the brains were immediately frozen and cut into 15µm thick serial coronal sections. Muscarinic M1 and M2 receptors were labeled *in vitro* with [^{3}H]-pirenzepine and [^{3}H]-oxotremorine, respectively, and 5-HT$_{1A}$ sites were labeled with [^{3}H]8-OH-DPAT, and all were visualized by autoradiography. The distribution of M1 receptors was similar in all three architectonic subdivisions of the PPHG, with a higher density in supragranular and a lower density in the infragranular layers. However, there was a higher density of M1 sites in TF than in TH or TL. In contrast, the distribution of M2 and 5-HT$_{1A}$ receptors was distinct in each subdivision of the PPHG and the changes corresponded to cytoarchitectural borders. In general, the laminar distribution of M2 and 5-HT$_{1A}$ receptors was less differentiated in the medial proisocortical areas TH and TL than in the lateral isocortical area TF and adjacent inferotemporal cortex (area TE). This trend was particularly obvious in TF where the distribution of M2 sites was clearly multilaminar with a high density in the deep third of layer III, a low density in layer IV, and a high density in layer V. Similarly, from TH to TF there was a clear stepwise change in the distribution 5-HT$_{1A}$ sites, with a steady decrease in the density of these receptors in layers V and VI. Together these findings complement our recent anatomical observations of differential cortical connections of these subdivisions of the PPHG and suggest that there are unique associations between receptor types and particular afferent inputs in each cytoarchitectonic subdivision. "Supported by NIH grants NS16841 and 19416"

182.1

ROLE OF THE AMYGDALA IN STRESS-INDUCED INCREASES IN PLASMA CORTICOSTERONE AND RENIN. T.S. Gray, R. Piechowski*, P. Rittenhouse, and L.Van de Kar, Dept. of Cell Biology, Neurobiology and Anatomy, and Dept. of Pharmacology, Loyola University Stritch Sch. of Med, Maywood, Il. 60153

The effects of amygdaloid lesions upon stress induced increases in plasma corticosterone (CORT) and renin was tested using immobilization or conditioned emotional responses (CER) paradigms. Amygdaloid lesions were made using bilateral injections of ibotenic acid (1.5µg in 0.1µl of solution). Two weeks later, the rats were stressed using immobilization or a CER paradigm.

Rats with lesions in the central amygdala showed attenuated corticosterone responses in both the immobilization and CER tests. Lesions in the central amygdala reduced the renin response in the CER paradigm, but not in the immobilization stress test. Damage to the lateral amygdala or regions adjacent to the amygdala had no effect upon CORT or renin responses to either stress procedures.

Thus, amygdaloid lesions blunt corticosterone and renin responses to CER stress, but only corticosterone levels are attenuated in the immobilization stress test. The data indicate that the amygdala is an important part of the neural circuitry for expression of corticosterone and renin responses to "psychological" stress as measured by the CER paradigm. Supported by ONR N000-14-88-k-0010.

182.2

BEHAVIOR AND TELEMETERED AUTONOMIC RESPONSES TO CLONIDINE, DIAZEPAM AND SOCIAL STRESS. W. Tomatzky and K.A. Miczek. Dept. Psychology, Tufts Univ, MA 02155

Increased cardiovascular and pituitary-adrenal activity as well as defensive and submissive behavior including vocalizations are reactions to threats and attacks by an opponent. We examined how diazepam and clonidine differentially modulate these responses. During a 1-h exposure to the threats by a dominant opponent the heart-rate (HR) and core temperature (T_c) of the experimental intruder rat is monitored by telemetry (Mini-Mitter Dataquest III), and the behavior and ultrasounds (US) were recorded. Neither clonidine (0.01-0.1 mg/kg IP) nor diazepam (1-10 mg/kg IP) affected salient defensive behavior in reaction to the intense initial threats by the resident. Locomotor activity, by contrast, was substantially decreased by both compounds. As the intensity and the variability of the social interaction decreases towards the end of the 1-h threat period neither locomotor nor social behavior were suppressed by diazepam. Clonidine decreases dose-dependently locomotion and also defensive behavior (at 0.1 mg/kg). Neither clonidine nor diazepam decreases rate or duration of 22-30 kHz US. The HR and T_c during the 1 h encounter are elevated above dark cycle mean values (+100 b/min for HR and +1.6 ^{o}C for T_c) and return to baseline within 3 to 4 h in the homecage of the animal. No significant adaptation of the animals T_c response to this relevant stressoris observed over a three month period. During the threat period clonidine decreases the tachycardia and hyperthermia dose-dependently. The increase in T_c, but not the tachycardia, is attenuated by diazepam. The massive autonomic and behavioral reactions to a social stress appear to be modulated differentially by adrenergic and benzodiazepine receptors.

182.3

STIMULATION OF THE BED NUCLEUS OF THE STRIA TERMINALIS PRODUCES STRESS RELATED BEHAVIOR. J.H. Casada and N. Dafny. Dept. Neurobiol. and Anat., The University of Texas Medical School at Houston, TX 77225.

The amygdala is involved with the modulation of both stress-related neuroendocrine function and affective behavior. The pathway by which the amygdala modulates corticosteroid secretion has been suggested to include the bed nucleus of the stria terminalis (BNST). The present experiment was designed to determine if stress related behaviors could also be produced by BNST stimulation and to determine the similarities and differences between the behaviors produced by restraint, BNST stimulation, and a combination of restraint and stimulation. Eight male Sprague Dawley rats (180-250 gm) were anesthetized with 50 mg/kg pentobarbital and permanently implanted with bipolar stimulating electrodes in the BNST and, after 7-8 days of recovery, were given each of the following four treatments on consecutive days: control unhandled, restraint for four hours, BNST stimulation for four hours, and restraint with BNST stimulation for four hours. Eight behavioral measurements were made over the four hours immediately following each treatment using a Digiscan Model RXYZCM(16)DVA animal activity monitor. During the first hour after treatment, all treatments increased horizontal activity, total distance travelled, movement time, low vertical rearings, high vertical rearings, and center time over control values. The effects of BNST stimulation, however, were more prolonged than the effects of stress or the combination of stress and stimulation. Thus, the behavioral effects of BNST stimulation are qualitatively similar to the effects of stress but differ in time course.

182.4

EFFECTS OF β-CARBOLINE (β-CCE) ON DEFENSIVE BEHAVIORS AND STRESS-RELATED HORMONAL CHANGES IN INFANT RHESUS MONKEYS. N.H. Kalin and S.E. Shelton*. VA Hospital and Department of Psychiatry, University of Wisconsin, Madison, WI 53705.

We have established a paradigm in infant rhesus monkeys that elicits specific defensive behaviors in response to changing parameters of threat. Rhesus infants separated from their mothers (A) emit frequent "coos." The presence of a human not engaging the infant in eye contact (NEC) induces freezing; staring (ST) induces barking and hostility. Morphine decreases and naloxone increases the number of coos but has no effect on freezing, barking, and hostility. Diazepam reduces freezing, barking, and hostility but does not affect cooing. To investigate how benzodiazepine systems mediate defensive responses and hormonal changes, we gave the inverse agonist β-CCE (0, 125, 250, 500 µg/kg) to 12 infant monkeys. ST significantly increased cooing, barking, lip smacking, teeth grinding, and hostility toward the intruder. NEC significantly increased freezing. Across all test conditions, β-CCE significantly decreased cooing and self mouthing and increased freezing and orientation toward the intruder. The effects of β-CCE were notable because of the significant test condition × dose interaction characterized by a dose-dependent increase in NEC-induced freezing. These results confirm the anxiogenic effects of β-CCE and support our findings that benzodiazepine systems are important mediators of threat-induced freezing in primates.

182.5

STIMULUS CONTROL OF NEUROENDOCRINE RESPONSES IN A CER PARADIGM. M.A.Hebert*, D.F.Rice*, S.J.Chee, B.N.Bunnell, E.H. Mougey and J.L.Meyerhoff. Dept. of Psychology, Univ. of Georgia, Athens, GA 30602 and Dept. Med. Neurosciences, Walter Reed Army Inst. Research, Washington, DC 20307.

In psychological stress experiments, rats returned to the place where they have been exposed to a physical stressor exhibit a pattern of neuroendocrine responses similar to that produced by the stressor itself. However, it has been difficult to show that this is due to associative processes rather than sensitization. In this study, two groups of nine rats received CER training in which the conditioned stimulus (CS), an illumination change, was paired with 5 sec of 1 ma footshock (US) for 20 trials per day. Seven extinction days, on which the rats received neither the CS nor the US, were randomly interposed among the 13 conditioning days to make the CS discriminable from other situational cues. On the 21st day, the experimental group received only the CS while the other group got another extinction day. Two groups of six rats received noncontingent CS and US presentations on the same schedule as the conditioning groups, another group got only the CS and a sixth group served as a handling control. After the last session, rats were decapitated and plasma was assayed for prolactin, ACTH and corticosterone. Prior exposure to footshock elevated levels of all three hormones, but only prolactin showed evidence of being conditioned to the CS. Results are discussed in terms of the different mechanisms governing the release of these hormones.

182.6

PRENATAL STRESS AFFECTS SENSITIVITY TO DIAZEPAM. L.A. Pohorecky and R. Roberts*. Center of Alcohol Studies, Rutgers University, Piscataway, New Jersey 08855-0969.

We examined the effect of prenatal stress exposure on sensitivity to diazepam. Stress exposure consisted of handling pregnant rats 5 min/day from day 14 to 21 of gestation. Male offspring were tested when 60 days of age in a modified open field apparatus after injection with diazepam (0, 1, 5 mg/kg). The 5 mg/kg dose of diazepam depressed the frequency and duration of crossover, rearing, headpoke and corner activities. Rearing was not affected by the 1 mg/kg dose of diazepam. Defecation was increased by the 1 mg/kg dose, but was decreased by 5 mg/kg of diazepam. Prenagal stress exposure altered responsiveness to diazepam on only crossover activity and defecation. Compared to control, prenatally stressed animals exhibited an increase in crossover at the low dose of diazepam. Also, there were more boli in prenatally stressed rats treated with 1 mg/kg diazepam, while the 5 mg/kg dose of the drug decreased the number of boli. These results indicate that prenatal stress has very specific effects on the sensitivity of adult offspring to diazepam. (Supported by RO1 AA07071.)

182.7

TIME-DEPENDENT EFFECTS OF PRIOR STRESS CAN EITHER INCREASE OR DECREASE SUBSEQUENT RESPONSIVENESS TO HALOPERIDOL. S.M. Antelman,* A.R. Caggiula, D. Kocan*, S.Knopf* and D. Edwards. *Depts. of Psychiatry and Psychology, Univ. of Pittsburgh, Pittsburgh, PA, 15213*

We have shown that prior stress can increase responsiveness to pharmacological and nonpharmacological stressors in a time dependent fashion, a phenomonon that we have termed time-dependent sensitization (TDS). But we also have observed time-dependent decrements in responsiveness. Here we identify one factor that determines which time-dependent effect is manifest. Male rats were subjected to a single exposure to stressors of either higher or lower intensity, as indexed by blood corticosterone levels. No effect was observed on haloperidol (HALO)-induced catalepsy when either stressor preceded HALO by only 1 hour. By contrast, when the interval was 2 weeks, the lower intensity stressor increased HALO-catalepsy whereas the higher intensity stressor decreased the same response. These results demonstrate that the time-dependent process can act on both sensitizing and decrementing phenomena and suggest that the effective intensity of early stressful experiences may be a key in whether an individual is made more vulnerable to or protected from stress in the future. These findings also suggest that individual differences in prior stressful experiences can influence current drug responsiveness in such a way as to contribute to drug variability.

182.8

CHRONIC STRESS IN RATS: 3 DAYS ARE SUFFICIENT TO PRODUCE ADRENOCORTICAL AND BEHAVIORAL ALTERATIONS. R.J.Servatius, B.H.Natelson, S.D.Drastal*, M.T.Bergen*, T.A.Pritzel*, W.N.Tapp, & J.E.Ottenweller*. University of Medicine and Dentistry of New Jersey; Neurobehavioral Unit (127A), V.A.Medical Center, E.Orange, NJ. 07019

Recently we reported on an animal model of chronic stress which exhibited elevated basal corticosterone levels, coupled with behavioral alterations, that persisted for days after the last stress session. To further define the stress parameters which produce the chronic stress state, we compared Sprague-Dawley rats subjected to either 10, 7, 4, or 3 days of tail-shock (2-hr sessions) with nonshocked controls. Shocked rats lost weight in relation to the number of days of stress. Control rats exhibited normal growth rates. Basal corticosterone levels were elevated in shocked rats (4.64 $\pm$ 0.33 μg/dl) compared to nonshocked controls (0.52 $\pm$ 0.10 μg/dl) for three days post-stress. Corticosterone values for the 4 shocked groups were equivalent. On the first day post-stress the latencies to drop from a hanging wire for the chronically stressed rats were equivalent and were significantly longer than controls. Subsequently, all rats exhibited equivalent latencies. The present results are consistent with earlier reports that chronic stress produces visceral arousal that lasts beyond the period of stress, as well as behavioral alterations. Since 3 days of tailshock produced equivalent elevations of basal corticosterone and hanging wire latencies as 10 days, the present results indicate that 3 days of this tail-shock paradigm are sufficient to induce chronic stress. Due to the rapid decrease in hanging wire latencies over days for the chronically stressed rats, we are currently looking for a more robust measure of behavioral disruption. Supported by V.A. Medical Research Funds.

182.9

ALARM PHEROMONE IN THE FORCED SWIMMING TEST. P. Bilitzke and E.L. Abel. Department of Obstetrics and Gynecology and the Fetal Alcohol Research Center, Wayne State Univ., Detroit, MI 48201.

Male rats (2 months old) were tested in the forced swimming paradigm. Immediately after an initial 10 minute test, the animal was placed in a holding cage for 10 minutes and was returned to the cylinder and immobility was again observed. For half the animals, the water was not changed. For the other half, the water was replaced with clean water. Animals that swam in clean water were immobile for 15% of the test period whereas animals placed in the previously swum water were immobile for 0%.

In the next study, animals were tested in clean water versus water containing feces from nonstressed animals. Animals placed back into this feces-contaminated water swam the same amount of time as when placed in clean water whereas animals tested in previously swum water (unchanged) again exhibited almost no immobility.

These data suggest that male rats produce an "alarm pheromone" during the forced swimming test which causes animals to be less immobile (i.e., more frightened).

Supported by grant P50 AA07606.

182.10

EXPOSURE TO INESCAPABLE SHOCK RESULTS IN INCREASED SYNTHESIS OF A SPECIFIC POLYPEPTIDE IN RAT HIPPOCAMPUS. N. Uenishi, T.J. Shors, N.R. Nichols, C.E. Finch, R.F. Thompson. Neurosciences Program, Univ. of Southern Calif., LA CA 90089.

The effect of inescapable and unpredictable stress on protein synthetic patterns was examined in the rat hippocampal slice preparation. One group of rats (n=5) was restrained and exposed to acute tail-shock (1mA, 1 sec, 1/min for 30 min.), while another group served as unstressed controls. Immediately following treatment, hippocampal slices were prepared and equilibrated for 1 hour. Slices were labeled with [35S]methionine for 2 hours and analyzed by 2-dimensional electrophoresis and fluorography. A consistent increase (5/5, 3.9-fold) in a 35kD polypeptide with an isoelectric point of 6.4 was found in stressed rats compared to controls. This 35kD polypeptide may not be the same as glucocorticoid- and stress-responsive glycerol phosphate dehydrogenase (GPDH) since it was found in the 20,000xg precipitate rather than in the supernatant. These results suggest that there is a stress-specific protein synthesized in the hippocampus following exposure to uncontrollable stress. [supported by NIH (AG05142, AG05500, AG07909) and the McKnight Foundation].

182.11

REPEATED EXPOSURE TO A STRESSOR CAN ELICIT HYPER-RESPONSIVENESS OF PLASMA OXYTOCIN IN THE RAT. T.E. Orr*[1], B.N. Bunnell[1], E.H. Mougey, G.P. Chrousos[2], K.T. Kalogeras*[2], E.O. Johnson[2] and J.L. Meyerhoff. Dept. Med. Neurosci., Walter Reed Army Inst. of Research, Wash., D.C. 20307-5100; [1]Dept. of Psychology, Univ. of Georgia, Athens, GA 30602 and [2]Devel. Endo. Br., NICHHD, NIH, Bethesda, MD 20892.

Plasma oxytocin (OXY) was previously shown to increase in response to immobilization (IMO) stress. Using 15 min of tailshock (TS) in male rats as a day 1 priming stressor, we found that TS presented on the second (test) day enhanced plasma OXY response. Rats were decapitated immediately after exposure to stressor or control conditions, and trunk blood was collected, centrifuged at 4°C and the plasma frozen pending RIA. When IMO was used as both the priming and the testing stressor, the OXY response was diminished to the repeat IMO. But, whenever tailshock was administered on day 2, hyperresponsiveness of OXY was elicited, compared to response to single TS. ACTH and corticosterone (CS) responses were also enhanced after repeat TS. OXY has been reported to amplify the ACTH response to CRH, and the increase in plasma levels of OXY is one of several mechanisms which might contribute to hyperresponsiveness of ACTH and CS. Reports vary as to whether OXY releases prolactin (PRL). In the present study, PRL response to stressors was not enhanced, despite the hyperresponse of OXY.

182.12

LESIONS OF THE PARAVENTRICULAR NUCLEUS ATTENUATE STRESS-INDUCED INCREASES IN PLASMA OXYTOCIN LEVELS IN THE RAT. J.L. Meyerhoff, E.H. Mougey, G.J. Kant, G.P. Chrousos[1] and K.T. Kalogeras*[1]. Dept. Med. Neurosci., Walter Reed Army Inst. Res., Wash., D.C. 20307-5100 and [1]Devel. Endo. Br., NICHHD, NIH, Bethesda, MD 20892

Oxytocin (OXY) has been demonstrated in neurons in the paraventricular nucleus (PVN) which may project to the external lamina of the median eminence or to the neural lobe of the pituitary. Plasma levels of OXY have been shown to increase in rats exposed to stressors. We have examined the effect of PVN lesions on the stress-induced increases in plasma levels of OXY. Bilateral radiofrequency lesions were placed in the PVN of anesthetized male rats. One week later, lesioned and sham-operated rats were decapitated immediately after exposure to 15 min of unavoidable, 1.6 mA footshock (FS). Trunk blood was collected, centrifuged at 4°C and the plasma frozen pending RIA. Unstressed control groups of lesioned and sham-operated rats were decapitated immediately upon removal from home cages. FS elicited a nearly fivefold increase in plasma OXY. This response was attenuated by 40% in the PVN-lesioned rats. Stress-induced increases in ACTH and prolactin (PRL) were attenuated 60% in the lesion group. OXY has been reported to enhance the response of ACTH to CRH. Reports vary on the ability of OXY to release PRL. Effects on circulating OXY levels should be considered when assessing the role of the PVN in neuroendocrine regulation.

182.13

THE INVOLVEMENT OF CENTRAL NORADRENERGIC SYSTEMS AND CORTICOTROPIN-RELEASING FACTOR IN DEFENSIVE-WITHDRAWAL IN RATS. X. M. Yang* & A. J. Dunn Dept. of Pharmacology & Therapeutics, LSU-MC, Shreveport, LA 71130-3932

The role of the central noradrenergic systems and corticotropin-releasing factor (CRF) in modulating defensive withdrawal (Takahashi, et al., *Behav. Neurosci.* 3:648, 1989) was studied in rats. When rats were unfamiliar with apparatus, i.p. administration of clonidine (0.03 mg/kg), *l*-propranolol (2.5 mg/kg), prazosin (0.1 mg/kg) or chlordiazepoxide (CDP, 5 mg/kg) each significantly decreased the latency to emerge from a small chamber and the proportion of time spent in the chamber (TIC). When rats were familiar with the apparatus, prior restraint for 20 min significantly increased the latency and TIC. Prazosin, clonidine, CDP, or *l*-propranolol reversed the restraint-induced defensive withdrawal. CRF (10-100 ng, i.c.v.) dose-dependently induced defensive withdrawal in rats familiar with the apparatus. *dl*-Propranolol (5 mg/kg) or CDP blocked the CRF-induced changes in the latency and the TIC; but clonidine and prazosin had no statistically significant effects on these measures. Phenylephrine (PE, 25-200 ng, i.c.v.) dose-dependently induced defensive withdrawal. This effect of PE (200 ng) was significantly antagonized by prazosin or the CRF antagonist, ahCRF (25 or 50 µg i.c.v.), but not by CDP. Our results suggest that the hyperactivity of central noradrenergic systems in the novel environment stimulates the release of CRF, which in turn induces defensive withdrawal in rats.
[This research was supported by NINCDS 27283]

182.14

MICROINJECTIONS OF CGRP WITHIN THE MEDULLARY DORSAL HORN: EFFECTS ON ADRENAL AND AUTONOMIC FUNCTION AND INTERACTION WITH SUBSTANCE P. D.A.Bereiter and A.P.Benetti* Sect. of Neurobiol. & Dept. of Surgery, Brown Univ./R.I.Hospital, Providence, RI 02903.

The role of putative neuropeptides within trigeminal subnucleus caudalis (Vc) in mediating the reflex autonomic responses that often accompany nociception was assessed in chloralose-anesthetized cats. CGRP (2-5pmol in 40-100nl CSF) was injected alone or coincidently with Substance P (SP, 13pmol) via micropipettes into different laminae of Vc. CGRP injections into laminae I-II evoked prompt dose-related increases in the adrenal secretion of catecholamines (CA, $P<0.01$), adrenal blood flow ($P<0.05$), plasma ACTH ($P<0.01$), mean arterial pressure (MAP, $P<0.05$) and heart rate (HR, $P<0.001$), whereas injections into deeper laminae of Vc had no consistent effects. These data were similar qualitatively to those seen previously after injections of SP alone (Brain Res 490: 307,1989). To determine if CGRP and SP interact within Vc to control adrenal and autonomic function, subthreshold doses of CGRP (3pmol) and SP (13pmol) were injected simultaneously into Vc. The adrenal secretion of CA, adrenal blood flow or plasma ACTH was not affected regardless of the laminar site of injection, whereas coincident injections of CGRP and SP into laminae I-II or V-VI evoked increases MAP ($P<0.01$) and in HR ($P<0.01$) that could not have been predicted from the response to either injection alone. Summary: CGRP and SP act separately within laminae I-II of Vc to evoke dose-related increases in the adrenal secretion of CA, in adrenal blood flow, and in plasma ACTH. In contrast, CGRP and SP interact functionally within laminae I-II and V-VI of Vc in control of MAP and of HR. Thus, the co-release of CGRP and SP within the laminae of Vc that process nociceptive input likely interacts to alter specific reflex functions rather than having a generalized effect on the adrenal and autonomic responses often associated with nociception. Supported by NIH grant NS26137.

DRUGS OF ABUSE, ALCOHOL III

183.1

EFFECT OF HIGH DOSE ETHANOL ON THE EEG OF ALCOHOL-PREFERRING (P) AND -NONPREFERRING (NP) RATS. S. Morzorati, L. Lumeng* and T.-K. Li*. Depts. Psych. & Med., Psych. Res. & Regenstrief Insts., Indiana Univ. Sch. Med. & VAMC, Indianapolis, IN 46202.

We have shown that P rats are behaviorally less affected than NP rats by high dose ethanol. In the present study, we examined if high dose ethanol differentially affects the EEG of P and NP rats. Adult male rats were implanted with EEG electrodes in the frontal cortex and dorsal hippocampus. Ethanol (3g/kg) was infused intragastrically via an indwelling cannula. The EEG was subjected to power spectral analysis and the spectra were sorted according to behavior (non-REM and REM sleep, awake/immobile and moving). Prior to ethanol, P and NP rats differed with respect to hippocampal theta peak frequency during REM sleep. After ethanol, both P and NP rats exhibited (a) a decrease in hippocampal and cortical spectral power, especially in higher frequencies, during all behaviors; (b) a predominant theta frequency in the hippocampal signal during awake immobility; (c) a lowering of the hippocampal theta peak frequency during REM sleep. Thus, high dose ethanol is a depressant in P and NP rats. The mechanisms which generate hippocampal theta may be different in the 2 groups; however, high dose ethanol has a similar effect on these mechanisms. The neuronal substrates that produce a differential behavioral sensitivity to ethanol are not reflected in the cortical and hippocampal EEG.

183.2

OPERANT RESPONSE SUPPRESSION BY THE $5HT_2$ AGONIST DOI IN ALCOHOL-PREFERRING (P) AND -NONPREFERRING (NP) RATS. J.M. Murphy, E. Engleman, S. Burns*, W.J. McBride, & J.N. Hingtgen. Psychololgy Dept., Purdue Sch. Science; Psychiatric Research & Regenstrief Insts., Depts. Biochem. & Psychiatry, Indiana Univ. Sch. Medicine & VAMC, Indianapolis, IN 46205.

P and NP rats differ in several facets of CNS serotonin (5HT) systems (McBride et al., Alcohol 7:199, 1990). To test for a functional difference in $5HT_2$ receptors, P and NP rats were compared in an operant task for the suppression of responding following IP injection of the $5HT_2$ agonist DOI. Adult male P (n=9) and NP (n=7) rats were kept at 80% of their free-feeding body weights and trained to bar press for sweetened milk on a VI 1 min reinforcement schedule in three 90 min sessions each week. After stable responding was established, one weekly session served as baseline after IP injections of saline. In the next session, the rats were injected with DOI (0.125, 0.25 or 0.5 mg/kg). The baseline responses/session of the P rats were significantly higher than for NP rats ($p<0.03$; 521±66 vs 292±52). Response suppression (min) following DOI was significantly longer for the P than the NP rats at the 0.5 mg/kg dose ($p<0.007$; 46±12 vs 2±1 min). Compared with NP rats, the P rats are behaviorally more active in this task and may have enhanced functional sensitivity of $5HT_2$ receptors. (AA03243 & AA07611)

183.3

DENSITIES OF OPIOID RECEPTORS IN THE CNS OF ALCOHOL-PREFERRING (P) AND -NONPREFERRING (NP) LINES OF RATS. W.J. McBride, E. Chernet*, X.-M. Guan, L. Lumeng*, T.-K. Li*. Psychiatric Res. & Regenstrief Insts., Indiana Univ. Sch. Med. & VAMC, Indianapolis, IN 46202.

The densities of delta and mu opioid receptors in the CNS of alcohol-naive, adult male P and NP rats (n=3 each) were determined with quantitative autoradiography. Coronal cryostat sections (20 um) were prepared from frozen brains, mounted onto subbed slides and dried. Sections were then incubated with (a) 5 nM [^{3}H]-D-Ala-D-Leu enkephalin (DADL) in the presence of 1 uM D-AlaMe-PheGlycol enkephalin (DAGO) to label delta sites; or (b) 5 nM [^{3}H] DAGO to label mu sites. Compared with the NP animals, the following differences were found for the P rats: (a) 25-30% lower amounts ($p<0.01$) of delta sites in layers I and II of the frontal cortex (87 ± 4 vs 65 ± fmol/mg prot) and the posterior cingulate cortex (62 ± 5 vs 44 ± 1); (b) 20% lower densities ($p<0.05$) of delta sites in the caudate-putamen (139 ± 10 vs 116 ± 4) and medial nucleus accumbens (ACC; 108 ± 2 vs 88 ± 7); and (c) a 40% higher value ($p< 0.05$) for the mu site in the lateral ACC (510 ± 17 vs 367 ± 3 fmol/mg prot). The data suggest that innate differences exist between the P and NP lines in the delta and mu opioid receptors in the ACC, a limbic region considered to be involved in mediating the actions of various drugs of abuse, including alcohol. (AA03243 & AA07611)

183.4

PASSIVE AVOIDANCE BEHAVIOR IN ALCOHOL-PREFERRING AND -NONPREFERRING LINES OF RATS. G.J. Gatto, J.M. Murphy, R.B. Stewart*, W.J. McBride,. Inst. Psychiat. Res., Med. Neurobiol. Prog., Indiana Univ. Sch. Med., & Psychol. Dept., Purdue Sch. Science, Indianapolis, IN 46202.

Alcohol-preferring (P) and -nonpreferring (NP) lines of rats were compared on acquisition and retention of a one-trial passive-avoidance task. Adult male rats were randomly divided into 4 groups (n=9/group/line). One group/line received a 3-sec. 0.25 mA shock after stepping off a platform to a grid floor; the other two groups were given a 0.5 mA shock. There were no difrences between the lines in acquisition step-down latencies, but emotional reactivity may be less in P rats as indicated by almost no fecal boli compared with a mean of nearly two for NP rats ($p<0.05$). When retested on the platform 24 hr later, there was no significant difference in median step-down retention latencies between the P and NP groups trained at the 0.25 mA shock. However, for groups trained at 0.5 mA, P rats stayed on the platform significantly longer than NP rats ($p<0.05$; 532 vs 87 sec) and tended to be more active while on the platform (photobeam crossings/min; $p<0.05$, one-tail). Relative to NP rats, the P rats show greater retention of this passive avoidance task. Factors that could account for this difference include learning abilities, shock sensitivity and emotionality. (AA03243, AA07462 & AA07611)

183.5

EFFECTS OF ETHANOL ON ELICITATION AND RETENTION OF UNCONDITIONED AND CONDITIONED HEART RATE RESPONSES IN THE DEVELOPING RAT. J. A. Saiers*[1], B. A. Campbell[1] and D. J. Knapp*[2]. [1]Dept. Psych., Princeton Univ., Princeton, NJ 08544; [2]Ctr. Alcohol Studies, Rutgers Univ., New Brunswick, NJ 08903

The effects of ethanol on elicitation and retention of unconditioned and conditioned heart rate (HR) responses to an 80 dB auditory stimulus in preweanling, periadolescent, and adult rats were assessed in this research. In Experiment 1, ethanol dose-dependently reduced the magnitude of the (unconditioned) heart rate orienting response (HR OR) equally in all 3 ages. In the second experiment, periadolescent animals exhibited long-term retention of habituation of the orienting response even though alcohol prevented initial elicitation of the cardiac OR. In contrast, adult rats in which initial elicitation of the OR was prevented by ethanol did not exhibit long-term retention of habituation of the OR. These results indicate that periadolescent rats are less susceptible than adult rats to ethanol-induced impairment in nonassociative learning.

Experiments 3 and 4 investigated the effect of ethanol on acquisition of a conditioned bradycardic heart rate response, established by pairing the 80 dB tone with a burst of white noise, in the developing animal. Ethanol dose-dependently impaired both acquisition and retention of the conditioned HR response. Ethanol did not affect acquisition or retention of the conditioned HR response differentially as a function of age, a result that contrasts with ethanol's effects on nonassociative learning.

Previous data collected by the present investigators indicate that periadolescent animals are less susceptible to ethanol-induced motor impairment than either preweanling or young adult animals. These motor data complement results obtained in Experiment 2, in which periadolescents, but not adults, were demonstrated to be more resistant to ethanol-induced impairment in nonassociative learning.

183.6

SENSITIVITY OF HOT AND COLD MICE TO THE HYPOTHERMIC EFFECT OF ETHANOL, SEDATIVE-HYPNOTICS AND TRANSMITTER SPECIFIC DRUGS. D.J. Feller and J.C. Crabbe. Veterans Administration Medical Center and Oregon Health Sciences University, Portland, OR 97201.

Mouse lines are being genetically selected which are sensitive (COLD) and resistant (HOT) to ethanol induced hypothermia. HOT/COLD mice from selected generations 7-9 (S_{7-9}) and 11-12 (S_{11-12}) were tested for their sensitivity to ethanol, longer chain alcohols and other hypnotics. Mice were administered 4 doses of each drug, and body temperatures measured using a rectal probe. There were dose-dependent decreases in temperatures after all drug treatments. COLD mice were more sensitive than HOT mice. The ratio of ethanol's $ED_{-2}{}^{o}C$ value for HOT versus COLD mice from S_{7-9} was 1.58. This ratio was 48% higher in mice from S_{11-12} compared to S_{7-9}. We also observed an increase in the ratios for the other alcohols in S_{11-12} mice. The increased line difference in hypothermic response after continued selection was also found for the sedative drugs, diazepam and methyprylon. While HOT/COLD mice from S_{7-9} were equally sensitive to the hypothermic effect of hydralazine, a peripheral vasodilator, after 5 more generations COLD mice were more sensitive than HOT mice. Among the neurotransmitter specific drugs tested, only morphine and levorphanol, μ-opiate agonists, were more potent at lowering the body temperature of COLD mice compared to HOT mice. These data demonstrate that with further genetic selection the HOT/COLD lines have continued to diverge in response to ethanol and some hypnotics. This may be partially due to changes in vascular mechanisms. Finally, the opiate data suggest that opiate and ethanol sensitivity may share some common genetic basis. These studies were supported by Grants AA05828, AA06243, AA06498, NIDA Contract 271-87-8120 and AA06548.

183.7

BODY TEMPERATURE AND ETHANOL TOLERANCE IN C57, LS AND SS MICE. D.A. Finn, B.L. Jones*, L.S. Kobayashi*, P.J. Syapin and R.L. Alkana, Univ. So. Calif. Sch. Pharm., L.A., CA 90033.

Acute sensitivity to ethanol-induced loss of righting reflex (LORR) increases in SS and C57 mice and decreases in LS mice, as body temperature during intoxication is increased. Adaptation based theories of tolerance predict that the degree of tolerance development is proportional to the magnitude of the disturbance. The present study tested the hypothesis that increasing body temperature during intoxication should increase tolerance to LORR in SS and C57 mice and decrease tolerance to LORR in LS mice. Drug-naive C57, LS and SS mice were injected with a hypnotic dose of ethanol and exposed to either 22 or 34°C for 6 hours for 7 days. On day 8 the mice were injected with ethanol and exposed to 22°C. LORR was measured on days 1 and 8. The results suggest that a greater degree of tolerance to LORR developed in the 34°C versus the 22°C exposed C57 mice, but not in LS mice. Although tolerance to LORR developed in the 22°C exposed SS mice, the 34°C environment was lethal to all SS mice. Studies are underway to investigate the role of ethanol concentration and other body temperatures in the development of tolerance to LORR. Overall, the results in C57 and LS mice suggest that differences exist in the effects of body temperature manipulation on acute and chronic sensitivity to ethanol. (NIAAA grant R01 AA05234)

183.8

BEHAVIORAL AND NEUROANATOMICAL EFFECTS OF DAILY PEAK BEC RESULTING FROM EARLY POSTNATAL EXPOSURE TO ETHANOL P. L. Greene, J. L. Diaz-Granados & A. Amsel. Department of Psychology and Institute for Neuroscience, University of Texas, Austin, Texas, 78712]

Electrolytic hippocampal lesions at 10-11 days of age (P10-11) significantly retard the acquisition of patterned (single) alternation (PA) with 60-s but not 8-, 15- or 30-s intertrial interval (ITI) in rat pups tested on P17-18 (Lobaugh et al., Behav. Neurosci., 103:1159, 1989). Chronic prenatal exposure to ethanol (EtOH) does not disrupt PA in infant rats tested at P17-18 with either 8- (Wigal et al., Behav. Neurosci., 102:43, 1988) or 60-s ITI (Greene et al., unpublished). We examined the effects on PA at P17-18 of postnatal exposure to EtOH with high (H) and low (L) peak blood ethanol concentration (BEC). Artificially-reared pups in the H condition were fed a 10.2% EtOH-adulterated diet (control pups received an isocaloric control diet) on 4 consecutive feedings in a 4-h period each day and unadulterated diet for the remaining 20 feedings. L pups received a 1.7% EtOH-adulterated diet on all 24 feedings. H pups at P17-18 were impaired in PA relative to L and control pups with a 60- but not a 30-s ITI. This result mirrors the earlier lesion results. Neuroanatomical measures were significantly affected in Group H relative to Group L and controls. That H exposure produces more severe neurological damage than L exposure to comparable daily doses of ethanol confirms the findings of other investigators (Bonthius et al., Alc. 5:209, 1988). Supported by NIAAA grant AA07052.

183.9

Ro15-4513 Antagonizes Ethanol-Induced Stimulant Effects on Measures of Exploration in Rats. T.O.Moore,H.L.June,M.J. Lewis. Howard Univ., Washington, DC 20059.

Several reports have implicated GABA-BDZ mechanisms in mediating ethanol (E) effects in both locomotor activity and exploration. The effect of Ro15-4513 (RO), an imidazobenzodiazepine inverse agonist, on E-induced changes in locomotor activity varies depending on the type of activity observed. It has been found that RO blocks E-induced stimulation of holeboard activity in mice (Lister,R.G., Pharm.Bio.Behav., 28:75, 1987). The present study further investigated BDZ compounds in E-effects on holeboard activity in rats. RO and Ro15-1788 (BDZ antagonist) alone and in combination with low doses of E were examined. RO but not Ro15-1788 attenuated E-induced increases in exploration. Ro15-1788 blocked RO's attenuation of E-induced exploration. RO alone did not effect holeboard activity; however, Ro15-1788 alone produced substantial increase in exploratory behavior. These data suggest that GABA-BDZ mechanisms play a significant role in E stimulation of holeboard activity. Moreover, they suggest further that Ro15-1788 may possess intrinsic behavioral effects of its own.

183.10

Failure of Ro15-4513 to Alter Ethanol-Induced CTA in Rats. H.L.June,T.O.Moore,K.R.Domague,M.J.Lewis. Howard Univ., Wahsington, DC 20059.

Previous research (Amit,Z., Pharm.Bio.Behav.,42:26, 1989) suggests that Ro15-4513 (RO), an inverse benzodiazepine agonist attenuates an ethanol-induced conditioned taste aversion (CTA). To further investigate this possibility, the present study examined the effects of RO (3 mg/kg) on rats fluid consumption in both a traditional and a preexposure CTA paradigm. In an attempt to obtain maximal preexposure and unconditioned stimulus effects, 2 g/kg of ethanol (20%v/v) was used in the present study. As previously reported (Cannon, et al., Anim.Behav.Proces. 41:26, 1975) animals given ethanol following 30 min access to saccharin established moderate aversions. RO failed to alter the aversion established under pretreatment or nonpretreatment conditions. These results suggest that GABA-BDZ mechanisms do not mediate an ethanol-induced CTA.

(Supported in part by NIAAA grants AA06263 and RR08016)

183.11

DOES FLUMAZENIL ANTAGONIZE OR POTENTIATE THE BEHAVIORAL EFFECTS OF ETHANOL IN SQUIRREL MONKEYS?

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Compounds acting at the Benzodiazepine receptor (BZR) are useful tools to determine the mechanism of action for some of the behavioral and physiological effects of ethanol (ETOH). However, the currently available BZR "antagonists", flumazenil and ZK 93426, possess intrinsic activities more similar to weak agonists and inverse agonists than pure neutral antagonists. Quantitative ethological studies in socially housed primates are particularly sensitive for detection of subtle drug effects and distinct changes in the behavioral repertoire. During the breeding season, socially housed dominant male squirrel monkeys show an increase in agonistic behavior. Low doses of ETOH (0.1, 0.3 g/kg) further enhanced aggressive behavior in dominant male monkeys. Higher ETOH doses (1.0, 1.5 g/kg) reduced these behaviors, and produce marked motor incoordination. In a similar manner as ZK 93426, pretreatment with flumazenil (10 mg/kg) effectively reduced the pro-aggressive effects of ETOH, but flumazenil potentiated the sedative and motor-incoordinating effects. Subordinate males were threatened and attacked more frequently when administered flumazenil and ETOH, when compared to ETOH alone. These effects may be due to the mild agonistic properties of flumazenil. When administered alone, flumazenil, but not ZK 93426, increased the durations of foraging and feeding. It appears flumazenil pretreatment shifts the ETOH dose response curve to the right as evidenced by increased ETOH-induced staggering and duration of inactivity. The "antagonism " of the pro-aggressive effects of ETOH by flumazenil are more likely a potentiation towards the aggression-reducing properties of ETOH.

183.12

HYPERBARIC EXPOSURE ANTAGONIZES ETHANOL-INDUCED DEPRESSION OF LOCOMOTOR ACTIVITY IN MICE: DOSE RESPONSE.

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Previous studies found that exposure to 12 atmospheres absolute (ATA) helium-oxygen (heliox) antagonized the acute and chronic behavioral effects of ethanol in mice. The present study characterized the effects of hyperbaric exposure on the dose-response curve for ethanol-induced depression of locomotor activity. Drug-naive, male C57BL/6 mice were injected with saline, 1.5, 2.0, 2.5 or 3.0 g/kg ethanol i.p., and were exposed to 1 ATA air, 1 ATA heliox or 12 ATA heliox at temperatures which offset the hypothermic effects of ethanol and helium. Locomotor activity was measured 15 to 45 minutes post-injection. Preliminary analysis indicates that ethanol caused a significant, dose-dependent reduction in activity beginning at 2.0 g/kg. Exposure to 12 ATA heliox completely antagonized ethanol's depressant effect on activity at 2.0 and 2.5 g/kg and partially blocked its effect at 3.0 g/kg. Activity in mice given 1.5 g/kg ethanol was not significantly affected in 1 ATA air, but was significantly activated in 12 ATA heliox. These results suggest that pressure shifts the ethanol dose response curve to the right and that pressure may unmask an activating effect of low dose ethanol on C57 mice. (NIAAA grant AA03972)

183.13

COMBINED EFFECTS OF ETHANOL AND MK 801 ON LOCOMOTOR ACTIVITY IN RATS.

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Some of the actions of ethanol have been suggested to involve an interaction with the NMDA receptor complex. For instance, the non-competitive NMDA antagonist MK 801 has been found to potentiate the anticonvulsant effects of ethanol in rats. However, little data is available regarding the interaction of ethanol and MK 801 using other behavioral measures. The present study examined the effects of these two compounds on locomotor activity in rats. Sixteen rats were treated with saline only, MK 801 plus saline, saline plus ethanol, and MK 801 plus ethanol. Locomotor activity was quantified by placing rats in automated photocell activity cages for a period of 12 hours overnight. Ethanol (10%) was administered i.p. at the dose of 0.75 g/kg, and MK 801 s.c. at the dose of 0.1 mg/kg. Ethanol was found to reduce locomotor activity during the first three hours ($F=13.5$, $df=1$, $p<.002$), while MK 801 increased it ($F=17.4$, $df=1$, $p<.001$). Ethanol and MK 801 appeared to antagonize each others effects during this first three hour period. Three to six hours post-drug, ethanol was observed to augment ($F=6.35$ $df=1$, $p<.02$), and MK 801 to reduce ($F=13.5$, $df=1$, $p<.002$) locomotor activity, but ethanol did not antagonize MK 801's effect during this time period. These results demonstrate that ethanol and MK 801 have opposite effects on locomotor activity in contrast to what has been observed for other behavioral parameters (supported by NIAAA 06059).

183.14

MK801 AND ETHANOL, EFFECTS ON THE EEG AND ERPS IN RATS.

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Recent neurophysiological data have suggested an interaction of ethanol with the glutamate-NMDA receptor complex. For instance, low levels of alcohol have been found to inhibit the ion current activated by NMDA in in-vitro preparations. The present study extends these paradigms in order to evaluate the electrophysiological effects of ethanol and the nonspecific NMDA receptor antagonist, MK801 in awake, behaving rats. Twenty Wistar rats were stereotaxically implanted with electrodes aimed at dorsal hippocampus (DHPC), amygdala (AMYG), nucleus accumbens (NAC), and frontal cortex (CTX). Rats received the following drug regimes: saline SC, 0.01 and 0.1 mg/kg MK801; EtOH, 0.75 g/kg IP; 0.75 kg EtOH plus 0.01 mg/kg MK801; 0.75 g/kg EtOH plus 0.10 mg/kg MK801. At least two weeks elapsed between drug doses. Five minutes of EEG was collected and event related potentials (ERPs) recorded in response to an auditory "oddball" paradigm between 30 and 40 minutes post drug. Spectral analysis revealed that MK801 (0.1 m/kg) produced significant increases in low frequency EEG components at all sites (1-6 Hz) and decreases in higher frequencies (16-32 Hz) Ethanol (0.75 g/kg) produced decreases in power in all frequency bands. Whereas the combined administration of EtOH and MK801 produced antagonistic EEG effects at low frequencies and additive effects in the higher frequency ranges. Evaluation of ERPs revealed that MK801 (0.1 mg/kg) produced significant decreases in the amplitude of the N1 and P2 components in cortex, decreases in the P1 and N2 in NAC and decreases in the P3 components in DHPC and AMYG. Ethanol was also found to produce decreases in the N1 in cortex and P3 in AMYG. The combined effects of MK801 and ethanol on ERPs were primarily additive. These studies suggest the antagonism of the NMDA receptor may produce some effects similar to ethanol, especially in response to sensory events. (Supported by AA 00098, 05069)

MONAMINES AND BEHAVIOR III

184.1

ROLE OF D_1 AND D_2 SUBTYPES OF DOPAMINE RECEPTORS IN NOVELTY-INDUCED PLACE PREFERENCE.

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Previous research has shown that the rewarding effect of novelty is blocked by the dopamine antagonist haloperidol (Bardo et al., Pharmacol. Biochem. Behav. 1989, 32:683-689). The present experiment examined the ability of the D_1 antagonist SCH-23390 and the D_2 antagonist sulpiride to block the rewarding effect of novelty. Adult male rats were exposed to one distinct environment for 30 min per day on eight consecutive days. On the ninth day, rats were injected with either SCH-23390 (0, 0.3, 0.1 or 0.3 mg/kg) or sulpiride (0, 25, 30, 50, 100 or 200 mg/kg). Thirty min after injection, rats were allowed free-choice access to the familiar environment and a novel environment for 15 min.

As expected, saline-injected control rats spent significantly more time in the novel environment than in the familiar environment. This novelty-induced place preference was blocked by SCH-23390 in a dose-dependent manner. However, sulpiride was without effect except for the highest dose tested (200 mg/kg). These results suggest that the rewarding effect of novelty involves primarily a D_1 receptor system.

(Supported by USPHS grant DA 05312.)

184.2

STRIATAL D1 AND D2 INTERACTIONS IN THE CONTROL OF SENSORIMOTOR BEHAVIOR IN RATS DEPLETED OF DOPAMINE AS NEONATES.

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Rats depleted of dopamine (DA) as neonates are spared from the sensorimotor deficits seen in comparably damaged adults. Recent studies suggest that striatal DA neurons mediate the expression of these behaviors in adults depleted as neonates. Our studies directly tested this hypothesis by determining the sensorimotor effects of intrastriatal injections of DA antagonists in these animals. Male rats received intraventricular injections of 6-HDA (100 µg) or its vehicle on postnatal Day 3. Adult animals were tested for akinesia, catalepsy, and somatosensory neglect immediately before and after intrastriatal injections (.5 µl) of the D1 antagonist SCH 23390 (1 µg), the D2 antagonist clebopride (2 µg), or both drugs. Vehicle-treated controls exhibited sensorimotor deficits after injections of D1, D2, or D1 + D2 antagonists. DA depleted animals were insensitive to the individual antagonists, but exhibited sensorimotor impairments after combined D1 and D2 blockade. Thus, activity within striatal DA neurons is necessary for sensorimotor function in normal animals and rats depleted of DA as neonates. The data also suggest lesion-induced differences in striatal D1 and D2 control of sensorimotor function.

184.3

COMPARISON OF THE EFFECTS OF SELF-STIMULATION ON DOPAMINE RELEASE AND METABOLISM IN THE RAT MEDIAL FRONTAL CORTEX, NUCLEUS ACCUMBENS AND STRIATUM STUDIED BY IN VIVO MICRODIALYSIS D. Nakahara*[1], N. Ozaki*[2], K. Yoshida[3] and T. Nagatsu*[4]. Departments. of Psychology[1], Psychiatry[2] and Biochemistry[4], Nagoya University, Nagoya 461, Japan and Department of Psychology[3], Fukui Medical School, Fukui 910-11, Japan.

Changes in dopamine (DA) release and its metabolism in the dopaminergic terminal regions, the medial frontal cortex (MFC), nucleus accumbens (NAC), and striatum (STR), were measured by a microdialysis during self-stimulation (SS) of the medial forebrain bundle (MFB) in rats pretreated with the dopamine (DA) uptake inhibitor, nomifensine (1 mg/kg, i.p.). SS of the MFB caused increased releases of DA in the MFC, NAC and STR. Despite blockade of DA uptake by nomifensine, the extracellular concentrations of the main DA metabolites, 3, 4-dihydroxyphenylacetic acid (DOPAC) and homovanillic acid (HVA), were also increased in these brain regions. However, there were clear regional differences in the neurochemical changes with the STR showing smaller extracellular levels of DA, DOPAC and HVA than either the MFC or the NAC. The results indicate that SS of the MFB mainly activates the mesocortical-mesolimbic DA systems, thereby increases in release and uptake as well as in intraneuronal metabolism of DA being produced in the dopaminergic terminal regions, the MFC and NAC.

184.4

NUCLEUS ACCUMBENS DOPAMINE AND ANTICIPATORY BEHAVIOR. G.H. Jones, D.B. Neill, and J.B. Justice, Jr. Departments of Chemistry and Psychology, Emory University, Atlanta, GA 30322.

Food-deprived rats placed on a scheduled food delivery regimen develop an anticipatory locomotor response which has been suggested to be due to the action of incentive stimuli. The proposed involvement of mesolimbic dopamine in incentive motivational processes and locomotor activity conditioned to drug presentation implicates this pathway in a general anticipatory state. This experiment examined the role of nucleus accumbens (NACC) dopamine in locomotor activity conditioned to food presentation using intracerebral microdialysis.

Rats were housed permanently in individual activity cages and their locomotor activity monitored daily between 09:00 and 17:00. Subjects were gradually reduced to 85% of their free-feeding weight and maintained at this reduced weight for the duration of the experiment. All rats were fed daily at 14:00. For one group of animals a stimulus light was illuminated for the 30 min period prior to feeding (CUED). For another group of rats the light was turned on for a 30 min period which varied daily on a pseudo-random schedule (NON-CUED). Thus, for this latter group of animals there were no external cues for food delivery.

Both groups of rats demonstrated a marked anticipatory response, with locomotor activity increasing prior to food presentation. This response developed to a much greater extent in the NON-CUED group. Extracellular dopamine levels in the NACC, measured by microdialysis, increased in the NON-CUED group prior to feeding. Furthermore, this dopaminergic response did not correlate with the absolute level of locomotor activity.

These results indicate the involvement of mesolimbic dopamine in anticipatory behavior.

184.5

ENHANCEMENT OF CONDITIONED REINFORCEMENT BY DOPAMINE-DEPENDENT MECHANISMS OF VENTRAL STRIATUM:INTERACTION WITH CHOLECYSTOKININ AND GLUTAMATE. B.J. Everitt, L. Burns*, G. Wolterink*, J. Le Noury*, J. Wolterink* & T.W. Robbins. Depts. of Anatomy and Experimental Psychology, Univ. of Cambridge, Cambridge CB2 3EB, U.K.

These studies examined the modulation of the enhancement of conditioned reinforcement (CR) with intra-accumbens d-amphetamine by simultaneous application of either the co-existing neuropeptide in dopamine neurons, cholecystokinin (CCK8 sulphated), or agonists and antagonists at excitatory amino-acid receptors, in order to modulate limbic and cortical glutamatergic inputs to the ventral striatum. Rats were trained to associate a light/noise compound stimulus with reward and the efficacy of the resulting conditioned reinforcer was tested using an acquisition of new response procedure. Intra-accumbens CCK dose-dependently (1,3 and 10ng/1 ul) increased responding with CR, both by itself and in combination with intra-accumbens d-amphetamine (5ug/1ul). In contrast, quisqualate (0.3nmol/1ul) failed to affect responding with CR, either alone or with amphetamine, and NMDA (0.3 nmol/1ul) had only decremental effects. These results are discussed in terms of the regulation of mesolimbic DA neuron activity in reward-related situations and functional interactions between the limbic system and the ventral striatum.

184.6

EATING BEHAVIOR IS ACCOMPANIED BY DOPAMINE RELEASE IN RAT MEDIAL STRIATUM. G.M. Rose[1] and A. Gratton[2]. [1]Medical Research Service, VAMC & Dept. of Pharmacology, UCHSC, Denver, CO 80262 USA and [2]Douglas Hospital Research Center, McGill University, Montreal, Québec, Canada H4H 1R3

We recently began an investigation of the behavioral correlates of dopamine function in rat caudate nucleus, using in vivo electrochemical methods. Chronoamperometric recordings were made using a 2-electrode system (IVEC-5, Medical Systems Corp.) which was modified so that a miniaturized headstage amplifier plugged directly into a connector cemented on the rat's skull. The reference electrode consisted of a chlorided silver wire; the working electrode was a glass-insulated bundle of 3 carbon fibers (O.D. ≈ 100 μ). Working electrodes were coated with Nafion and calibrated before being lowered into the caudate nucleus using a small microdrive.

The animals were observed during a number of natural behaviors. Of these, only eating and, under certain circumstances, sniffing, evoked a reliable increase in the electrochemical signal. The amplitude of eating-induced changes varied from 50->500 nM, and was proportional to the duration of the eating episode. Measurements of reverse current indicated that the signals consisted of a mixture of dopamine and DOPAC. Eating related signals occurred in response to a variety of food items; by contrast, drinking either plain or sugared water produced no reliable changes. In addition, gross or fine motor movements were not accompanied by reliable changes in signal amplitudes. (Supported by NIMH Grant P50 MH442212-01, the VAMRS, and NSERC of Canada.)

184.7

HIGH-SPEED CHRONOAMPEROMETRIC MONITORING OF STRESS-ELICITED DOPAMINE RELEASE IN FREELY MOVING RATS. A. Gratton and M. D. Doherty, Douglas Hosp. Res. Ctr, McGill Univ., Montréal, CANADA, H4H 1R3.

The effects of two stressors, tail pinch and immobilization, on dopamine (DA) release were studied in the nucleus accumbens, the striatum and the medial prefrontal cortex of freely-moving animals using high-speed chronoamperometry in combination with moveable electrochemical electrode assemblies. Male rats were each fitted with a stainless steel pedestal onto which could be attached a microdrive containing an electrochemical electrode assembly made of 3 glass-insulated, Nafion-coated, 30 um diameter carbon fibers. Electrochemical recordings were obtained by applying to the electrochemical electrode, at a rate of 5 Hz, a +0.5V pulse relative to a Ag/AgCl reference electrode. The microdrive allowed several sites in the same animal to be tested for the effects of tail pinch and immobilization. Tail pinch stress was reliably associated with gradual increases in oxidation current in all three regions studied while immobilization stress produced similar but far less consistent results. The magnitude of the reduction current associated with the stress effects suggested that DA as well as DOPAC were the main contributors to the electrochemical signals. Typically, tail pinch-elicited signals reached peak amplitude (100-500 nM) 2 to 5 min after stimulus onset and decayed over a period of 5-20 min following stimulus offset. While the magnitude of the signals varied with recording site, these variations were not correlated with any obvious anatomical division. Furthermore, there was a tendency for the signals at the initial recording site to increase in size with repeated daily application of tail pinch stress. The present study provides a fine grain temporal characterization of stress effects on DA release in the freely moving animal and suggests that tail pinch can activate limbic, striatal and cortical dopaminergic systems. Supported by the MRC of Canada

184.8

SEXUALLY RELEVANT STIMULI ACTIVATE THE MESOLIMBIC DOPAMINE SYSTEM: MEASUREMENT USING HIGH SPEED CHRONOAMPEROMMETRY IN FREELY MOVING ANIMALS.

J. B. Mitchell and A. Gratton, Douglas Hosp. Res. Ctr., McGill Univ., Montréal, Québec, H4H 1R3 CANADA.

Evidence from a variety of sources suggests that common neural mechanisms may underlie at least some aspects of all appetitively motivated behaviors. Exploration, feeding, drinking and sexual behaviors can all be elicited or facilitated by manipulations that activate the mesolimbic dopamine (DA) system. In order to assess the effects of sexually relevant stimuli, without copulation itself, on activity within the mesolimbic DA system, sexually experienced male rats were exposed to bedding from cages that housed conspecific males, ovariectomized females, or estradiol- progesterone-primed females. Electrochemical recordings were obtained using a Nafion-coated carbon fiber electrode lowered, using a microdrive, to three different sites within the ventral striatum and nucleus Accumbens (nAcc). At each site, the male rat was exposed to the three different stimuli. Electrochemical measurements were obtained by applying to the electrochemical electrode a +0.5 V pulse, relative to a Ag/AgCl reference electrode at a rate of 5 Hz. Olfactory stimuli associated with sexually receptive females elicited an increase in the oxidative current at all three sites; the largest increase, however, was obtained from the nAcc. Bedding from cages that housed other males elicited a small increase in oxidative current, but was consistently less potent than the bedding from the cages of sexually receptive female rats. Exposure to bedding from cages that housed ovariectomized, untreated females did not reliably increase oxidative current at any site tested. The reversibility of the oxidation current indicated that DA and DOPAC were the primary contributors to the electrochemical signal. Preliminary data suggest that opioid receptor blockade attenuates this activation. In conclusion, the present data indicate that sexually relevant stimuli potently activate the mesolimbic DA system, without the occurrence of copulation.

Supported by NSERC of Canada.

184.9

INDIVIDUAL DIFFERENCES IN THE BEHAVIORAL EFFECTS OF STRESSORS ATTRIBUTABLE TO LATERALIZED DIFFERENCES IN MESOCORTICAL DOPAMINE SYSTEMS. *J.N. Carlson, R.W. Keller, S.D. Glick.* Dept. of Pharmacology & Toxicology, Albany Medical College, Albany, NY 12208

Rats exposed to uncontrollable (YOK) but not controllable (ESC) footshock develop a transient behavioral deficit upon later shock escape testing. We have previously shown that exposure to YOK footshock stressors induces a change in the direction of rotational behavior and that effects of lack of stressor control are associated with changes in dopamine (DA) activity on the right side of the mesocortical DA system. It was thus of interest to assess possible differences among rats of differing lateral bias in their response to lack of stressor control. Male Zivic-Miller Sprague-Dawley rats were subjected in matched pairs to ESC or YOK footshock and were tested for footshock escape behavior on the next day. Left and right biased YOK rats differed in their escape performance; while right sided rats showed a large deficit, left biased rats performed better than comparable ESC animals. Similar effects of varying magnitude were seen in rats from a different stock (Taconic) and in rats from a different strain (Long-Evans). Neurochemical findings implicated lateralized differences in the mesocortical DA system as a substrate for these differences. These observed differences may have relevance for accounts of human depression that are based upon differences in cerebral laterality. (Supported by ES 04032)

184.10

PERIODIC FOOD PRESENTATION INCREASES EXTRACELLULAR DOPAMINE AND METABOLITES IN NUCLEUS ACCUMBENS DIALYSIS PERFUSATES. L.D. McCullough, D. Singer*, and J. D. Salamone. Dept. of Psychology, Univ. of CT, Storrs, CT 06269-1020.

Dopamine (DA) systems have been implicated in motor functions that are important for responding to motivationally-relevant stimuli. In vivo microdialysis was used to measure DA and its metabolites, homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC), in unanesthetized rats subjected to periodic food presentation (PFP). Four different groups were studied: high rate of PFP (1 45 mg food pellet per 45 s), low rate of PFP (1 pellet per 4 min), mass feeding (15-18 g food), and unfed food-deprived controls. Although feeding on large numbers of pellets generated little locomotor activity, PFP induced substantial increases in locomotion comparable to those produced by low doses of amphetamine. PFP also produced large increases (20-50%) in DA release and metabolism in the nucleus accumbens. These data are consistent with the idea that the nucleus accumbens is involved in both locomotion and the response to motivationally-relevant stimuli.

184.11

DOSE AND TIME RESPONSE ANALYSIS OF APOMORPHINE EFFECT ON PREPULSE INHIBITION OF ACOUSTIC STARTLE. R.E. Wilcox[1], P.K. Randall[1] and K.A. Young[2]. [1]Dept. of Pharmacology, Univ. of Texas, Austin, TX 78712 and [2]Dept. of Pharmacology, Texas A&M College of Medicine, Temple, TX, 76508.

Previous studies have suggested a dopaminergic modulation of the acoustic startle response. Acoustic startle responses of rats during a short (25 min) or extended (85 min) session were observed after systemic injections of the DA agonist apomorphine (APO). Using a 73 dB tone as a prestimulus to 100 dB white noise (PP trials), and 100 dB white noise alone (WN trials), we found that IP injections of APO preferentially elevated PP means to levels statistically indistinguishable from WN means. This result confirms that APO disrupts prepulse inhibition of the acoustic startle response under appropriate conditions.

During the extended session, 5 min blocks of trials were interspersed between 20 min blocks when no stimuli were presented. This pattern of presentation produced increased responses in vehicle controls consistent with the concept of sensitization. Sensitization was not observed in rats injected with a high (3.2 mg/kg) dose of APO. After an initial amplitude elevation compared to controls during the first 25 minutes, amplitudes of WN and PP startle responses for the high dose group decreased to levels lower than controls 65 and 85 minutes postinjection. WN trial amplitudes were not significantly different from PP trials during the 85 min session for this group. These results suggest that besides disrupting prepulse inhibition, APO also interferes with sensitization of the acoustic startle response. Since prepulse inhibition is disrupted in schizophrenics, APO-induced loss of prepulse inhibition in rats may represent a model of sensorimotor deficits of schizophrenia.

184.12

CROSS-SENSITIZATION BETWEEN THE LOCOMOTOR-ACTIVATING EFFECTS OF THE DOPAMINE (DA) D2 AGONISTS BROMOCRIPTINE AND QUINPIROLE. D.C. Hoffman and R.A. Wise.. Center for Studies in Behavioral Neurobiology, Dept. Psychol., Concordia U., Montreal, Canada.

We recently discovered that the locomotor-activating effects of the dopamine (DA) D2 receptor agonist bromocriptine show environment-specific sensitization. To further explore this effect, tests for cross-sensitization between bromocriptine and another D2 receptor agonist were conducted. Three groups of Long-Evans rats were used. The Paired group received 5.0 mg/kg bromocriptine prior to placement in an activity box for 3 h; following the session, vehicle was administered. The Unpaired group was given vehicle prior to testing and bromocriptine in the home cage following the session. The Vehicle group received vehicle injections in the activity box and home cage. Following eight alternate-day pairing sessions, each group was tested with 0.1 mg/kg quinpirole on six separate occasions. When tested with quinpirole, the Paired group showed significantly more activity than the Unpaired and Vehicle groups on the first test day. Thus, prior history with bromocriptine in the activity box altered the behavioral response to quinpirole, indicating the development of environment-specific cross-sensitization. This suggests that a common synaptic or intraneuronal modification may underlie the development of drug-induced behavioral sensitization.

184.13

STRIATAL DOPAMINE D_2 RECEPTOR ASYMMETRY IN A TRANSGENIC CIRCLING MOUSE. K.J. Miller, L.W. Fitzgerald, M. Teitler, S.D. Glick, A.K. Ratty[a], and K.W. Gross[a]*. Dept. of Pharmacology, Albany Medical College, Albany, NY and [a]Dept. of Molecular & Cellular Biology, Roswell Park Cancer Inst., Buffalo, NY.

We recently described the discovery of transgenically-derived circling mice whose pooled striata showed elevated dopamine (DA) D_2 receptors without changes in the levels of DA and its metabolites (Ratty et al., Neurosci. Abstr.,1990). Since circling behavior has been associated with asymmetric striatal DA function, we assayed D_2 binding in homogenates of individual striata using ^{3}H-N-methyl-spiperone (1.0 nM). The % asymmetry between left and right striata of homozygous circling mice averaged 47% while the % asymmetry in heterozygous normal mice averaged 17%. Similar asymmetries were also observed using receptor autoradiography with ^{125}I-IBZM (0.5 nM); the enhanced asymmetry in the circling mice was observed at several anterior-posterior levels of the striatum and was more apparent laterally than medially. We also studied the development of several behaviors in the these mice. Circling mice displayed a characteristic hyperactivity at postnatal day 16 which persisted though day 20. Exaggerated circling behavior by the mutant was present by day 14. These mice also showed less grooming and almost no rearing compared to normal mice. Relationships between the D_2 receptor asymmetries and the observed behavioral abnormalities are currently being investigated.

184.14

GUSTATORY NEOPHOBIA AND PERFORMANCE ON A LEARNED TASK ARE AFFECTED BY HIPPOCAMPAL SYMPATHETIC INGROWTH. V. Ayyagari and L.E. Harrell. Departments of Neurology and Psychology, Veterans Administration Medical Center and University of Alabama, Birmingham, AL. 35294.

After lesions of the medial septum, hippocampal NE levels increase as cholinergic input to the hippocampus is replaced by sympathetic ingrowth (HSI) from the superior cervical ganglion. Certain age related changes in inhibitory avoidance have been attributed to changes in brain NE. To assess whether increased NE levels due to HSI produce behavioral changes, we looked at gustatory neophobia, a passive avoidance task and open field activity in 33 adult male rats. Animals underwent 1 of 4 surgical procedures: sham surgery (CON), MS lesions (MS), Ganglionectomy (Gx), or MS+Gx. Four weeks after surgery, rats were trained on passive avoidance, and tested 24 hours later for retention. Open field activity (5 min) was recorded and 23-hr water/food deprived rats were given a choice of either tap water or 0.1% saccharin solution. Preliminary results indicated that MS animals (increased brain NE) preferred saccharin to water, as did Gx, while CON and MSGx rats showed gustatory neophobia. MS animals were more active on open field and took longer to acquire and did not retain the inhibitory avoidance task. There were no differences in learning, retention or activity in the other groups. While the results from the drinking experiments support the NE hypothesis of age related changes, the results from the memory task need further exploration.

184.15

EFFECTS OF SOME ADRENOCEPTOR-SELECTIVE DRUGS ON AUDIOGENIC SEIZURE ACTIVITY IN DBA MICE. C.E.Lints, B.W.Santi* and D.Capruso*. Department of Psychology, Northern Illinois University, DeKalb, IL 60115.

Vehicle control and drugged (i.p.) DBA mice were exposed to a white noise stimulus of 110 dB (SPL) at their age of peak audiogenic seizure (AGS) susceptibility. This intensity stimulus produced intermediate levels of AGS incidence and latency from which either increases or decreases in AGS severity could be measured. The alpha-1 and beta-1 antagonists, corynanthine and metoprolol, respectively, produced significant dose-dependent protection from AGS. The nonselective beta agonist, isoproterenol, produced dose-dependent proconvulsant effects. The alpha-1 agonist, methoxamine, was without effect by itself (possibly due to poor CNS penetration), but potentiated the proconvulsant effects of a subthreshold dose of isoproterenol. Subthreshold doses of corynanthine plus metoprolol interacted to produce complete protection from AGS activity, as did a maximally protective dose of metoprolol followed by a proconvulsant dose of isoproterenol.

These results are consistent with the midbrain adrenoceptor hypothesis of AGS activity in DBA mice, which posits that excess proconvulsant alpha-1 and beta-1 adrenoceptors in auditory centers of the DBA midbrain modulate seizure severity in these animals.

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184.16

THE EFFECTS OF NORADRENERGIC AGONISTS ON THE DRL 72-S SCHEDULE. R. Dunn, D. Jolly and L. Seiden. Dept. of Pharmacological and Physiological Sciences, Univ. of Chicago, Chicago, IL 60637.

Antidepressants, including the norepinephrine reuptake inhibitors desipramine and protriptyline, increase the reinforcement rate and decrease the response rate of rats performing on a differential-reinforcement-of-low rate 72-second (DRL 72-S) operant schedule (Seiden et al, Psychopharmacol. **86**:55, 1985). The present work examined further the effects of noradrenergic agents on this antidepressant screen. Maprotiline, a tetracyclic antidepressant, and nisoxetine, a non-tricyclic norepinephrine reuptake inhibitor, produced antidepressant-like effects on the DRL 72-S schedule. Neither the α-1 agonist phenylephrine nor the α-2 agonist clonidine produced the same effects as antidepressants. However, isoproterenol, a β agonist, and the β-2 agonists salbutamol and clenbuterol did elicit antidepressant-like effects. Thus, β receptors mediate the noradrenergic component of antidepressant-like effects on the DRL 72-S schedule. This research was supported by PHS MH-11191; RSA MH-10562 (L.Seiden).

184.17

NA NEURONS ARE REQUIRED FOR POSTSYNAPTIC α_2-ADRENOCEPTOR DOWN-REGULATION BY DMI, BUT NOT ECS. D.J. HEAL AND W.R. BUCKETT. (SPON: Brain Research Association) Boots Pharmaceuticals Research Dept, Nottingham NG2 3AA, UK.

While intact noradrenaline (NA) neurones are essential for the ß-adrenoceptor desensitization by desipramine (DMI), this is not so for the electroconvulsive shock (ECS) effect. Recently, we reported that DMI and ECS down-regulated postsynaptic α_2-adrenoceptors assessed by clonidine mydriasis (Heal, D.J. et al Br. J. Pharm., 99, 74P, 1990). We have now studied the effect of NA lesioning on these responses. Zimeldine (5 mg/kg)-treated C57/Bl/6 mice were given DSP-4 (100 mg/kg) twice, 7 days apart. Lesioning was initially confirmed by abolition of methamphetamine (0.5 mg/kg) mydriasis; ultimately by HPLC-ECD. We gave DMI (10 mg/kg) for 14 days; ECS (200 V, 2s) to anaesthetised mice 5 times over 10 days. Clonidine (0.1 mg/kg) mydriasis was measured 24h later. DMI and ECS decreased clonidine mydriasis by 32% ($P<0.001$) and 41% ($P<0.001$), respectively. DSP-4 abolished mydriasis to methamphetamine (98%, $P<0.001$), but did not alter the response to clonidine. The lesion decreased brain NA by 64% ($P<0.001$), but did not alter 5-HT or dopamine levels. DSP-4 abolished the effect of DMI on mydriasis, but did not prevent the attenuation by ECS (24%, $P<0.05$). Hence, like ß-adrenoceptor down-regulation, the ECS effect is independent of an NA input, whereas this is essential for the action of DMI.

184.18

EFFECTS OF VAGOTOMY ON HIPPOCAMPAL LESION-INDUCED BEHAVIOR AFTER PERIPHERAL INTERVENTION: OPEN FIELD ACTIVITY AND NOVELTY RESPONSE. D.L. Maier and R.L. Isaacson. Dept of Psychology, SUNY, Binghamton, NY 13901.

Peripheral pharmacologic interventions attenuate hippocampal lesion-induced deficits in both spatially based behavioral tasks and open field locomotion (Maier, Ryan, and Isaacson, 1990). This report examines the possible role of visceral afferents in the vagus nerve on behavioral alterations produced by peripheral sympathetic intervention in the open field (OF) and response to novelty. Male Long-Evans rats received a bilateral subdiaphragmatic vagotomy or sham surgery. These groups were divided into groups receiving bilateral hippocampal lesions (H) or sham operations. Subsequently, drug treatments consisted of repeated peripheral administration of bretylium (5 mg/kg) for seven days, and norepinephrine (4 µg/kg, ip) or saline vehicle before each test. Locomotor activity and response to novelty were tested sequentially starting 35 days after surgery. In the novelty test, a fourth novel arm was added to a T-maze, making it into a cross-maze. All rats had previous repeated experience with the T-maze. The locomotor hyperactivity of H rats was reduced by prior vagotomy ($p < .001$). There was no drug treatment effect. The results of novelty testing indicated that all groups entered the "novel arm" more frequently than the three familiar arms ($p < .01$), although the sham-vagotomized, saline-treated H group did this least and entered all maze arms more frequently than any other group ($p \leq .01$). The triple interaction of lesion, treatment and vagotomy measured in time spent in the novel arm ($p < .01$) will be interpreted and discussed with relevance to possible beneficial effects of pharmacologic intervention found in specific surgical groups.

184.19

EARLY POSTNATAL HANDLING OR MATERNAL SEPARATION IN RATS: EFFECTS ON BEHAVIOR AND BRAIN BETA-ADRENOCEPTORS. L.A. Hilakivi-Clarke, J. Turkka*, R.G. Lister and M. Linnoila. Lab. of Clin. Stud., NIAAA, Bethesda, MD 20892.

This study investigated the effects of early postnatal handling or temporary maternal isolation on various behaviors and beta-adrenoceptor binding. From the 5th to the 20th postnatal days, male Wistar rat pups were either 1) handled daily for 3 min, 2) isolated from their nursing mother for 1 hr daily, 3) non-handled but housed with handled or isolated rat pups, or 4) non-handled and housed in cages which were left undisturbed. Eight to sixteen weeks later, it was found that the time spent immobile in Porsolt's swim test was shortened ($p<.01$) in the handled rats, as compared with all other groups. Voluntary alcohol consumption, assessed using a procedure in which the rats were allowed a choice between tap water and a 5% (v/v) alcohol solution, was also reduced in the handled rats ($p<.01$). No differences in the measure of anxiety - food consumed in a novel environment - or the time spent in social, aggressive and defensive behaviors in the resident-intruder paradigm, were noted. Neither did the density or affinity of beta-adrenoceptors in the frontal cortex or hippocampus differ significantly between the groups. The results indicate that short-lasting maternal separation does not cause sustained effects on a number of behaviors in the rat. Early postnatal handling, however, leads to shortened immobility in the swim test and reduced voluntary alcohol consumption.

184.20

d-AMPHETAMINE EFFECTS ON LANGUAGE AND MOTOR BEHAVIORS IN A CHRONIC STROKE PATIENT. R. Homan, J. Panksepp, J. McSweeny*, P. Badia, E. Borroughs*, L. Chapman* and R. Conner*. Medical College of Ohio at Toledo, Toledo, OH 43699 and Bowling Green State Univ., Bowling Green, OH.

Presently no established pharmacological maneuvers exist which can facilitate recovery in long-term stroke patients. Because of promising preclinical data, we evaluated the effects of 15 mg oral d-amphetamine (DEA) and placebo using a single-subject double-blind ABAB design, followed by a chronic phase of 6 consecutive daily doses of15 mg DEA or placebo. A standard test series evaluated motor, speech and cerebral functions. The subject, a 53 year-old male was hemiplegic with transcortical motor aphasia 1.5 years after an emergency left internal carotid endarterectomy . SPECT scans demonstrated markedly diminished blood flow in frontal and parietal areas of the left cortex.

Within 5 hrs following drug administration, the subject underwent 5 testing stages: saggital gait analysis, a simulated driving test, an abbreviated neuropsychological battery, language evaluation, and a performance assessment battery accompanied by EEG/ERP analysis at frontal central and posterior sites on each hemisphere. The largest improvements following DEA were observed on the simulated driving test, measures of language performance (including naming, morphemes per second, onset pauses, pauses/utterance). DEA also increased cerebral blood flow, signal detection on a continuous performance task, with decrerases in both errors of commission and omission. The power in the EEG was shifted away from slow potentials in the afflicted hemisphere. There were speech, motor, and IQ improvements as a result of testing, with the best performance being achieved at the end of the chronic DEA phase. Benefits were not sustained, but they were of sufficient magnitude that a therapeutic phase will be conducted (using a titration schedule of DEA) to determine whether improvements can be consolidated into a permanent store.

185.1

CALORIC RESTRICTION PREVENTS AGE-ASSOCIATED IMPAIRMENTS IN LEARNING OF A MAZE STRATEGY BY AUTOIMMUNE MICE.

E.N. Ahanotu, H. Lal, P. L. Prather, and M.J. Forster, Dept. of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, Texas 76107-2690

When present in the MRL/MpJ-+ background , the "accelerator "gene *lpr* (lymphoproliferation) results in rapid development of autoimmune disease and death by 8 months of age. Concurrently, these mice (MRL/MpJ-*lpr*) show evidence of rapid cognitive decline (Forster et al., *Behav. Neural Biol.*, 49:139, 1988). In this experiment we tested the prediction that cognitive decline associated with autoimmunity would be retarded by caloric restriction, an experimental procedure known to decelerate immunologic consequences of *"lpr"*. MRL/MpJ-*lpr* and MRL-MpJ-+ mice were maintained on either *ad libitum* feeding or 40% caloric restriction beginning at weaning and were subsequently compared for learning and memory according to a delayed reversal paradigm (Lal et al., *Soc. Neurosci Abstr.*, 16 [this volume]) when 2.5, 3.5, or 6.5 months old. In order to differentiate short- and long-term effects of the diet conditions, half of the mice under each diet condition were switched to the opposite condition 5 weeks before testing at 6.5 months. The 6.5-month-old MRL/MpJ-*lpr* mice learned the maze reversal strategy more slowly and showed more rapid forgetting when compared with younger MRL/MpJ-*lpr* mice. Caloric restriction completely prevented the age-associated decline in ability to acquire the learning strategy in MRL/MpJ-*lpr*, but was only partially effective in preventing decline of memory capacity. These results suggest that the gene "*lpr*" leads to acceleration of age-associated learning and memory dysfunctions, whereas restriction of calories acts in an opposite fashion. Analysis of the neurological functions influenced by caloric restriction and the *lpr* gene might reveal important neuroimmunologic processes involved in autoimmune-associated cognitive decline. [Supported by NIH grant AG06182 (MJF)].

185.2

MEMORY SCORES IN MIDDLE-AGED RATS PREDICT LATER DEFICITS IN MEMORY, PARADOXICAL SLEEP AND BLOOD GLUCOSE REGULATION IN OLD AGE. W.S. STONE and P.E. GOLD. Dept. Psychol., U. Virginia, Charlottesville, VA 22903.

Paradoxical sleep measures and blood glucose regulation are significantly correlated with memory in old rats. Here, using a longitudinal design, we determined whether measures of sleep or glucose regulation in middle-aged rats would predict individual differences in memory when the rats were old.

Sleep (3-hr records via cortical electrodes), blood glucose regulation (tail nicks after 500 mg/kg glucose injection, IP), and memory (spontaneous alternation, 20 trials, 1 min ITI) were assessed in male Sprague-Dawley rats every 2-3 months from 12 to 24 months of age. The 10/23 rats which completed the protocol were then trained in an inhibitory avoidance task (1 ma, 1 s FS) and tested for retention 24 hr later.

The major finding of the study was unexpected. Rather than finding biological measures in middle-age (e.g. poor glucose tolerance) which predicted poor memory in old age, memory in middle age predicted deficits in both behavioral (inhibitory avoidance) and biological (glucose tolerance and paradoxical sleep) measures in old age. These findings indicate that behavioral measures of memory may be useful markers of biological aspects of aging. They also provide additional evidence that levels of glucose regulation and paradoxical sleep are correlated with measures of memory. (Supported by AG 07648, ONR N0001489-J-1216 and NRSA AG 05408).

185.3

A DELAYED REVERSAL PARADIGM FOR ASSESSMENT OF AGE-ASSOCIATED DECLINE IN MEMORY CAPACITY OF INDIVIDUAL MICE.

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To estimate age differences in memory capacity or rate of "forgetting", it is often necessary to employ several different groups of a given age, each tested under a different retention parameter. In the current experiment, we tested the effect of age on memory using a paradigm which permitted retesting of the same subject under a variety of retention parameters. Mice aged 8, 14, or 27 months were initially trained for discriminated shock avoidance in a T-maze and, subsequently, the mice received daily "reversal" training in which the correct goal arm on all trials (intertrial interval of 1 minute) for that session was the one opposite that selected on the first trial. All mice eventually developed a reliable strategy of reversing arm choices between the first and second trials on each day. During the memory-testing phase, a delay of 7, 15, 30, 60, or 120 minutes was introduced between the first and second trial, and memory capacity was considered to be inversely proportional to the decline in performance as a function of delay. The delays were presented in an alternating ascending and descending sequence until each delay had been presented four times. The stability of the reversal strategy was verified between each series of 5 delays. Although the 27-month-old mice learned the discrimination and avoidance components more slowly than the younger mice, all mice acquired the reversal strategy within a similar number of sessions. In contrast, time-dependent decay of memory performance (i.e, forgetting) became more rapid as a function of age. These findings indicate that delayed reversal provides an "individual" estimate of memory capacity for mice, which would have a variety of applications in gerontology, pharmacology and toxicology. [Supported by NIH grants AG06182 (MJF) and AG07695 (HL)].

185.4

SENESCENCE ACCELERATED MOUSE (SAM): ANIMAL MODEL FOR AGE-RELATED EMOTIONAL DISORDER AND MEMORY IMPAIRMENT. M. Miyamoto, Y. Kiyota,* M. Nishiyama* and A. Nagaoka* Research and Development Devision, Takeda Chem. Ind., Osaka 532, Japan.

We have demonstrated that a substrain of senescence accelerated mouse (SAM), SAM-P/8 mice showing accelerated aging, exert marked deficits in learning and memory compared to SAM-R/1 mice showing normal aging (Physiol. Behav. 38, 399, 1986). Age-related changes in emotional behavior and in learning ability in P/8 and R/1 mice were studied. P/8 mice showed significant deficits in passive avoidance and water maze tasks compared to R/1 mice, which became obvious with aging. The impairments in the P/8 strain were derived from reduced ability in acquisition and retention. SAM-P/8 mice showed age-related decrease in latency to eat novel food in a neophobia test. In an elevated plus-maze, P/8 mice exhibited apparent increases in the number of entries into open arms and time spent on open arms, implying age-related reduced anxiety in the P/8 strain. Aged R/1 mice, 20 months old, showed low anxiety-like behavior such as was seen in the P/8 strain. These findings indicate that the P/8 strain shows age-related low anxiety-like behavior which may be related to deficits in learning and memory in the strain, and suggest that P/8 mice may be a valid animal model for studying emotional disorders and related memory impairment in dementia.

185.5

ENDURANCE TRAINING IN AGED F-344 RATS: FAILURE TO MODIFY SPATIAL MEMORY, BRAIN AUTOIMMUNITY, AND HUMORAL IMMUNE RESPONSE. M.J. Forster, C. A. Barnes, M. Fleshner, E. N. Ahanotu, M. L. Laudenslager, R. S. Mazzeo, S. F. Maier, and H. Lal. Department of Pharmacology, Texas College of Osteopathic Medicine, Fort Worth, TX 76107; Departments of Psychology and Kinesiology, University of Colorado, Boulder, CO 80309; and Behavioral Immunology Laboratory, University of Colorado Health Sciences Center, Denver CO, 80204.

The current studies addressed the possibility that impaired immune and central nervous functions of old individuals could be modified by physical exercise. After ten weeks of exercise training employing a motorized treadmill (Mazzeo et al., *J. Appl. Physiol.* 57:1369, 1984) old F-344 rats (24-27 months) showed increases in several indicators of physical endurance (e.g., oxygen consumption and reductions in the respiratory exchange ratio). However, exercise failed to improve antibody response to keyhole limpet hemocyanin (Laudenslager et al., *Br. Behav. Immun.* 2:1, 1989) or to alter the frequency of serum antibodies recognizing 64- and 45-kD brain cell membrane antigens (Ahanotu et al., *FASEB J.* 3:A571, 1989). Spatial memory performance on a circular platform task (Barnes, *JCPP* 93:74, 1979) was also unaltered following this exercise training. There was, however, a significant relationship between behavioral performance on the circular platform and the presence of brain-reactive antibodies in the old rats, regardless of training group. Overall, these results suggest that some immunological and neurological functions may be insensitive to modification by endurance training which is initiated relatively late in life. The data also suggest that individual differences in cognitive performance of old rats can be predicted by the presence or absence of brain-reactive antibodies. Although the significance of this relationship is not yet clear, it is possible that these antibodies could play a role in central nervous system changes that lead to compromised memory function in old animals. (Supported by NIH grants AG06182 [MJF] AG03376 [CAB], AG07180 [RSM], ONR 0014-85K0211 and BNS-8808840 [SFM].)

185.6

AGE-RELATED DECREASE IN HIPPOCAMPAL RESPONSIVENESS TO THE GLUTAMATE SYNAPTIC RECEPTOR AGONIST AMPA. G. Rao, C. A. Barnes and B. L. McNaughton. Department of Psychology, University of Colorado, Boulder, CO 80309

We previously reported that CA1, CA3 or FD unit responsiveness to glutamate applied near the soma by iontophoresis was unchanged across a range of ages from 5-28 months in F-344 rats. However, the effects of exogenous glutamate may be extrasynaptic in origin,as they are not blocked by the quisqualate receptor antagonist CNQX. In this study, possible changes in hippocampal *synaptic* function with senescence were assessed using AMPA, a specific and potent quisqualate receptor agonist. Hippocampal slices were prepared from rats at ages 5,9,24, and 27 mo (n=4 per group). In both CA1 and the FD, a stimulating electrode was positioned in the apical synaptic zone, and two ringer-filled recording pipettes were placed on either side about 1 mm apart. Stimulus intensity was set to evoke a field epsp of about 2 mV at each location. After collecting baseline responses at .1 Hz, an AMPA (.1 M) pipette was touched to the slice surface within 100 μm of one recording site, -100 nA (7 sec on, 3 sec off) was applied for 2 minutes, and the AMPA pipette was withdrawn. In all age groups, application of AMPA produced an attenuation of the field epsp within 5 minutes, without significantly affecting the control response 1 mm distant. The magnitude of the AMPA response was substantially reduced in the old animals in CA1, but there was no age effect in FD. These results were confirmed in a second study with 5 mo old (n=3) and 27 mo old (n=4) rats in which AMPA was applied at one recording location and glutamate was applied at the other. In this study also, there was no age-related change in responsiveness to glutamate (see below). We conclude that there is a region specific, age-dependent alteration in postsynaptic sensitivity that is localized to the sites (probably synaptic) which are accessible to exogenous AMPA but not to glutamate. (Supported by AG03376)

Suppression of Field EPSP

	CA1		FD	
	AMPA	GLUT	AMPA	GLUT
5 mos	.25 ± .11	.51 ± .12	.33 ± .10	.58 ± .13
27 mos	.52 ± .10	.49 ± .14	.36 ± .10	.59 ± .12

185.7

QUANTAL ANALYSIS OF PERFORANT PATH TO DENTATE GRANULE CELL SYNAPTIC STRENGTH IN OLD AND YOUNG RATS. T. C. Foster, C. A. Barnes, and B. L. McNaughton. Department of Psychology, University of Colorado, Boulder, CO, 80309-0345.

In rats, a decrease in spatial memory and a loss of perforant path to dentate granule cell synaptic contacts are observed with aging. However, the efficacy of remaining synapses appears to increase, as assessed indirectly by the EPSP to presynaptic fiber potential ratio *in vitro* (Barnes & McNaughton, J.Physiol. 1980). To examine this in more detail, we compared quantal parameters of perforant path synapses in 5 (n=13), 9 (n=14) and 24 (n=13) month old F344 rats using minimal electrical stimulation to activate single perforant path fibers, while recording intracellularly from granule cells (McNaughton, Barnes, and Andersen, J. Neurophysiol. 1981) . The average EPSP amplitudes were related to the product "***m***" (mean number of transmitter quanta released per impulse) and "***q***" (the size of the individual quantal components of the EPSP). These estimates were obtained using both the method of failures and by a noise deconvolution algorithm. A positive correlation ($p < 0.05$) of ***q*** with age was observed. These results suggest that average quantal size in the perforant path may increase over the lifespan and thus account for the apparent increase in efficacy of surviving synapses in old rats. This is particularly interesting in light of the fact that Foster and McNaughton have recently shown that ***q*** also increases after the induction of long-term enhancement (LTE/LTP) in Schaffer-collateral-CA1 synapses. (Supported by NS08585 and AG03376.)

	5mo		9mo		24 mo	
Number of Observations	25		23		26	
Mean EPSP (SEM)	224.6	(23)	210.5	(22)	294.3	(36)
Mean quantal parameter ***m***	1.34	(.10)	1.29	(.16)	1.43	(.15)
Mean quantal parameter ***q***	159.9	(13)	183.9	(19)	243.3	(32)

185.8

ACTIVATION OF TRANSCRIPTION FACTOR GENES IN HIPPOCAMPUS OF CHRONICALLY-PREPARED RATS FOLLOWING INDUCTION OF LONG-TERM SYNAPTIC ENHANCEMENT. C. A. Barnes, B. L. McNaughton, B. Christy*, A. J. Cole, J. M. Baraban, and P. F. Worley. Dept. of Psychology, Univ. Colorado, Boulder, CO 80309; Depts. Neuroscience and Molecular Biology, Johns Hopkins Univ., Baltimore, MD 21205.

Programs of gene activation appear important in long-term cellular responses to transmitters and growth factors. In a previous study, we found that synaptic stimulation of the perforant pathway that elicits LTE/LTP in hippocampus of anesthetized rats *in vivo*, activates *zif/268*, a putative transcription factor (TF) gene, in hippocampal granule cells (Nature 340:474, 1989). With the ultimate goal of studying the time course characteristics in the absence of secondary effects of surgery or anesthesia, we have initiated studies using rats with chronically indwelling electrodes.

After electrode implantation, rats were permitted to recover for a minimum of 2 weeks. In initial studies, we ascertained that chronically-implanted electrodes do not alter levels of *zif/268* mRNA in the hippocampus relative to naive controls (n=4). To examine TF responses to synaptic stimuli, animals received high-frequency stimulation (400 Hz) and were sacrificed 30 or 60 min after the initiation of this stimulation. As observed in anesthetized rats, *zif/268* mRNA is markedly increased in granule cells by high-frequency (HF) stimuli that induce LTE but not afterdischarges (n=8 of 8 preparations). In contrast to findings in anesthetized rats, preliminary data suggest that *jun-B*, but not *c-jun* or *c-fos*, is also reliably induced by stimuli that produce LTE in awake animals. Increases in *zif/268* or *jun-B* mRNA are blocked by the NMDA receptor antagonist MK-801 (1.0 mg/kg). Furthermore, *zif/268*-like immunoreactivity is markedly increased in nuclei of granule cells 30 min following HF stimulation. Our results indicate that the chronic preparation may be useful for studies aimed at understanding the relationship between genomic activation and the long-term maintenance of LTE.

185.9

THE CONTRIBUTION OF PROXIMAL AND DISTAL VISUAL COMPLEXITY TO THE DISCHARGE CORRELATES OF HIPPOCAMPAL "PLACE" CELLS. B. W. Leonard, B. L. McNaughton, C. A. Barnes, and M. Marquis*. Department of Psychology, University of Colorado, Boulder 80309.

To assess the contribution of visual complexity to the direction dependency of location-specific discharge of hippocampal complex-spike cells, we recorded cells in two environments differing only in the number and complexity of visual cues and in which the behavioral demands placed on the animals were identical. Three F-344 rats were trained to retrieve food pellets in a cylindrical apparatus which had a single white card on the wall, subtending 100° of arc. Cells were recorded first in this simple-cue condition. Different CS cells were recorded in the same apparatus with more complex visual cues on the wall. Head direction and spatial location were monitored by tracking two points on the rats' headstage. For analysis, the cylinder floor was divided into 32 locations. For each visit, firing rates were calculated as a function of head direction (8 intervals). These rates were analyzed by multiple regression to assess the relative contributions of location and orientation to the overall firing variance.

For 75 CS cells, the amount of variance explained by location did not differ between the two environments, whereas a reliable ($t_{1,73}$=2.54, p .007) difference was found in the explained variance due to head direction. Thus, place cells are more directionally dependent in visually rich environments. Interestingly, however, the explained variance in either environment was considerably lower than that observed in different experiments in which the recording was carried out on an open radial maze. Thus, while proximal visual complexity contributes to direction-dependency of spatial firing, it also appears that in high-walled environments hippocampal CS cells send a rather unreliable signal to their efferent targets. This may indicate that remote visual cues play a dominant role in controlling hippocampal output. (Supported by AG03376 and NS20331)

185.10

HEAD-DIRECTIONAL AND BEHAVIORAL CORRELATES OF POSTERIOR CINGULATE AND MEDIAL PRESTRIATE CORTEX NEURONS IN FREELY-MOVING RATS. L. L. Chen, B. L. McNaughton, C. A. Barnes, and E. R. Ortiz*. Dept. Psychol., Univ. of Colorado, Boulder, CO 80309

Posterior cingulate and medial prestriate cortex form a bridge between parietal/visual cortex, and postsubiculum (Vogt and Miller,1983; Vastola, 1982). Recently, Taube et al. (1990) have shown that cells in postsubiculum code for head direction, independently of spatial location or behavior. It is thus of interest to analyze the directional and behavioral correlates of cells in regions which may project to postsubiculum in order to elucidate how the head-orientation cells may be constructed from simpler sensory and/or motor input.

To quantify head-directional and behavioral correlates, a dual-LED recording headstage was mounted on the heads of freely-moving rats so that position and head-direction were obtained simultaneously with single unit activity recorded using the stereotrode technique. Data were analyzed in terms of unit firing relationships to head orientation, visual stimuli, and angular and linear motion on the maze.

A substantial number of cells (about 10%) were recorded that showed significant directional firing bias in the standard maze configuration. Most but not all of these cells changed their orientation bias following rotations of the major salient visual stimuli. A few cells were resistant to rotations of visual stimuli, and maintained a directional bias in total darkness. Most cells exhibited at least a general sensitivity to state of motion, with many (over 20%) being selective for particular motions (e.g. right turns). A few cells exhibited an interaction between head orientation and specific motion. These overall behavioral correlates could subserve the expression of purely directional correlates in target structures (McNaughton, these abstracts). (Supported by NS20331.)

185.11

HEAD DIRECTION CELLS IN THE DORSAL PRESUBICULUM INTEGRATE BOTH VISUAL AND ANGULAR VELOCITY INFORMATION. E. J. Markus, B. L. McNaughton, C. A. Barnes, J. C. Green*, and J. Meltzer. Dept. Psych., Univ. Colo., Boulder, CO 80309.

Cells that fire selectively when a rat is facing a certain direction, independent of its location in space, have recently been found in the dorsal presubiculum (Taube et al., J Neurosci. 1990). In the present experiment, over 200 cells in total were recorded in this general region (including a few penetrations in subiculum and retrosplenial cortex), from 4 F-344 rats, using a modified version of the stereotrode method (McNaughton et al., J Neurosci Meth. 1983) that permitted multiple penetrations per hemisphere. Of these, approximately 15% exhibited noticeable unimodal directional selectivity, and were subjected to systematic analysis under two environmental conditions. Directional and angular movement effects were first examined in a large room filled with complex sensory stimuli. Next the same measurements were conducted in a controlled-cue room whose main visual stimulus came from a brightly illuminated white panel on one of the surrounding black curtains. Measurements were repeated in the same room in total darkness, again with the lights on, and again following a 90° shift in the white panel. A final measurement was taken in the original environment. Most of the recorded cells exhibited a lesser degree of directional tuning than reported by Taube et al., an effect which may result either from the present use of albino rats or from differences in recording location. The average range of directional specificity was 101°. About 80% of the cells exhibited a pronounced sensitivity to movement, and about 25% were selective for the direction of angular movement. All cells rotated their directional preferences according to the shift in the wall panel, and in all but 1 cell, directionality was abolished during passive rotation in total darkness. In addition, movement sensitivity and sensitivity to the direction of angular movement was maintained in at least 1 cell recorded in the dark. In a different cell, directional preference was maintained when the animal was placed in a novel box with high walls. When the box was rotated very slowly the directional preference shifted with the box. When the box was rotated rapidly, the direction preference was maintained relative to the laboratory. In addition to cells exhibiting a unimodal directional preference, other cells with complex multimodal directional tuning were also found in this region. Taken together, these data indicate that the "head direction system" is strongly influenced by both visual stimuli and angular velocity information, including input from the vestibular system. (Supported by NSERC of Canada, NS20331, and AG03376.)

185.12

AN "ASSOCIATIVE" NETWORK MODEL FOR THE GENERATION OF HEAD-ORIENTATION SPECIFIC NEURONAL FIRING FROM ANGULAR VELOCITY INFORMATION. B.L. McNaughton, Department of Psychology, University of Colorado, Boulder CO, 80309

Many cells in the rat postsubiculum (dorsal presubiculum) are selectively active over a limited range of the animal's head orientation (**h**), apparently independent of either spatial location or behavior, and, although they can be controlled by specific visual stimuli, they retain orientation tuning (sometimes with directional shifts) when these stimuli are absent (Taube et al. J. Neurosci. 1990). A number of cells in the postsubucular region (Markus et al. these abstracts) as well as its cortical afferent structures (Chen et al. these abstracts, McNaughton et al. Psychobiology. 1989) exhibit sensitivity to head rotation (**h'**) or to the interaction of **h** and **h'**. McNaughton et al. '89 presented a simple associative network model which could recall representations of spatial location using input from a system coding for the interaction of location and movement (both linear and rotational). For example, in that model, the compound input configuration **left turn** in location **A** provided a unique cue for the recall of the representation of location **B**. In the same manner, the **hh'** interaction could be used to "recall" the resulting **h**, e.g., an angular velocity input of 20°/time-step in conjunction with an initial heading angle of 70° can "recall" a head angle representation of 90° in the absence of visual stimuli. This need not be recall in the traditional sense, as the transform is so simple and reliable that the necessary connection weights might be hardwired developmentally with no actual learning. A modifiable input from the visual system could allow superimposed associations between sets of visual inputs, and the corresponding head orientation representations. This would be necessary, for example, to allow the **h** representation to be updated during very slow rotations, or to allow visual stimuli to set the reference direction in familiar places.

It is interesting to note that the same network organization would appear to fulfill some of the requirements of the hypothetical neural "integrator" in the oculomotor/vestibular system, which is presumed to derive eye position signals from eye velocity (Robinson, Science. 1968), but with no actual temporal integration. (Supported by NS20331 and A.E. Sloan Foundation)

185.13

SIMULATION OF PERFORANT-PATH EVOKED SYNAPTIC AND POSTSYNAPTIC FIELD POTENTIALS IN THE DENTATE GYRUS OF THE RAT. W. E. Skaggs and B. L. McNaughton, Department of Psychology, University of Colorado, Boulder CO, 80309

Field potentials are the best if not the only available tool for studying experience-dependent changes in synaptic efficacy in the CNS of conscious mammals. However, interpretation of extracellular fields is difficult because they reflect the summed influences of large pools of cells, and are caused by spatial segregation of inward and outward currents within dendritic trees. We have developed a computational simulation of the fascia dentata (FD) of the rat hippocampus, with the aim of modeling field potentials evoked by stimulation of the perforant path. The ultimate goal of the model is to aid in the interpretation of spontaneous and electrically induced changes in synaptic efficacy and postsynaptic excitability in this system. Our initial goal has been to capture the fundamental dynamics of evoked responses, especially their dependence upon location of recording electrodes, location of afferents on the dendritic trees of granule cells, intensity of stimulation, and repetition of stimulation.

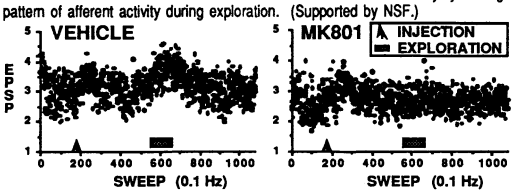
The model simulates FD as a collection of many copies of a small pool of cells, arranged to approximate the curvature of FD in the transverse plane. The pool contains 100 granule cells, and 20 interneurons of two classes, which provide feed-forward and feedback inhibition via fast Cl^- and slow K^+ channels. Each granule cell consists of a spherical soma, an axon initial segment, and a cylindrical dendrite divided into six compartments. Excitatory and inhibitory synapses, modeled as variable conductances within equivalent circuits, are distributed over the compartments. Spiking activity is generated by modified Hodgkin-Huxley Na^+ and K^+ channels.

The simulation reproduces observed field potentials with considerable accuracy. We will discuss several insights the model provides into factors that may underlie specific aspects of the evoked response. Of particular interest has been the need for careful regulation of the timing and extent of feed-forward inhibition in reproducing the detailed behavior of the population spike with stimuli of varying intensity, as well as the possible role of $GABA_B$ mediated dendritic inhibition in shaping the late phase of the population e.p.s.p. (Supported by NSF and the A.E. Sloan Foundation.)

185.14

EXPLORATION-DEPENDENT ENHANCEMENT OF SYNAPTIC RESPONSES IN RAT FASCIA DENTATA IS BLOCKED BY MK801. C. A. Erickson, B. L McNaughton, and C. A. Barnes. Department of Psychology, University of Colorado, Boulder CO 80309.

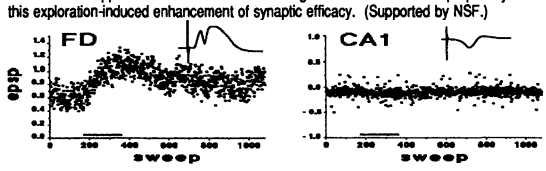
Exploratory behavior results in robust growth of perforant path evoked synaptic responses in fascia dentata (Sharp et al., Psychobiol. 1989; Green et al, J. Neurosci.1990). These changes are due neither to handling nor to low-frequency stimulation, and are related to the integral of the recent history of exploration rather than to instantaneous behavior *per se*. The effects are dissociable from the EEG theta rhythm that accompanies exploration because reversible septal inactivation or septal lesions (which abolished theta) had no effect on the EPSP enhancement. Moreover, forced locomotion on a treadmill did not produce the effect, suggesting that the determining factor was the change in sensory input rather than locomotion *per se* . Blockade of NMDA-type glutamate receptors by the noncompetitive antagonist, MK801, impairs acquisition of spatial memory tasks (Robinson, et al., Psychobiol. 1989) and blocks long-term enhancement (LTE/LTP) of synaptic transmission (Abraham & Mason, Brain Res.1988), a possible physiological substrate for memory. In the present experiment, exploration-dependent changes in the field EPSP were blocked by MK801 (0.3 mg/kg i.p.), suggesting that the exploration-induced increase in synaptic efficacy and artificially-induced LTE may be mediated by the same NMDA receptors. It remains to be determined whether the blockade is due to direct actions of MK801 on the induction mechanism or whether the effect occurs indirectly by altering the pattern of afferent activity during exploration. (Supported by NSF.)



185.15

REGIONAL VARIATION IN THE PROPENSITY FOR EXPLORATION INDUCED SYNAPTIC ENHANCEMENT IN THE RAT HIPPOCAMPUS. J. Meltzer, C. A. Barnes, and B. L. McNaughton, Department of Psychology, University of Colorado, Boulder CO 80309.

An important function of the hippocampal formation is believed to be its involvement in spatial learning. One line of evidence for this possible role in learning is electrically induced enhancement of synaptic transmission (LTE/LTP) in the hippocampus. In addition, exploratory behavior is accompanied by a considerable increase in perforant path-evoked dentate granule cell population synaptic responses (Sharp et al., Psychobiology, 1989). These changes do not result from motor activity *per se*, nor from the accompanying EEG theta activity (Green et al., J. Neuroscience, 1990). This experiment addressed the question of whether similar synaptic plasticity occurs in area CA1 of the hippocampus. Rats were prepared for chronic recordings of perforant path-evoked FD granule cell responses in one hemisphere and CA3 stimulated CA1 responses in the other. After a period of adaptation to the recording situation , the effects of exploration were assessed under two different conditions. In one, the animal ran on a radial 8-arm maze, in either a cue-rich or a cue-poor condition. In the other, the animals explored on an open triangular platform on which food reinforcement could be found. During the exploratory period, there was a gradual growth of the synaptic response in both regions. However, in the three animals studied to date, the change in the CA1 response was less than half that of the simultaneously recorded response in FD. It thus appears that there exist marked regional differences in the propensity for this exploration-induced enhancement of synaptic efficacy. (Supported by NSF.)



185.16

EFFECTS OF MEDIAL SEPTAL INACTIVATION ON MULTIPLE UNIT ACTIVITY IN THE SUBICULUM AND ENTORHINAL CORTEX OF ANESTHETIZED RATS. G. M. Perez, C. A. Barnes, and B. L. McNaughton. Department of Psychology, University of Colorado, Boulder, CO 80309.

Mizumori, et.al., (Brain Res. 1989, J. Neurosci. 1989) showed that reversible inactivation of the medial septum with local anaesthetic reduced the output of CA3 pyramidal cells in both acute (anesthetized) and chronic preparations, while leaving CA1 pyramidal cell output relatively intact. In the awake animals spatial selectivity of CA3 cells as well as spatial memory were disrupted, whereas CA1 cells continued to fire with normal spatial selectivity.

Preservation of normal place specific firing in CA1 following septal inactivation raised the question of why spatial memory impairment was observed. Because the medial septum projects to the output structures of the hippocampus (i.e.,. subiculum and entorhinal cortex), it is possible that the behavioral deficit was due to the blockade of hippocampal output "downstream" of CA1.

To address this hypothesis, preliminary studies involved recording multiple unit activity in hippocampal output structures following reversible septal inactivation with tetracaine (2%) in 7 rats anaesthetized with sodium pentobarbital. Multiple unit activity was recorded simultaneously from the subiculum and entorhinal cortex for a 15 minute baseline period followed by injection of .5 µl of tetracaine into the medial septum. A 30% decrease in multiple unit activity was observed in both the subiculum and entorhinal cortex immediately following septal inactivation and generally recovered within about 20 min. These data suggest that septal activity does maintain the general levels of excitability in these areas. Further studies, however, are needed to resolve how different cell types are affected, and whether these effects occur in the behaving animal. (Supported by an NSF fellowship to GMP, AG03376, and NS20331).

NEUROPEPTIDES AND BEHAVIOR II

186.1

PLASMA PROOPIOMELANOCORTIN PEPTIDES IN AUTISTIC AND SELF-INJURIOUS CHILDREN: RELATIONSHIP TO COGNITIVE AND SOCIAL FUNCTIONING. B.H. Herman, P. Papero*, J. Hartzler*, E. Porter*, I. Chatoor*, J. Egan*, V. Der-Minassian* and A. Arthur-Smith, Brain Res Cen and Dept Psychiat, Children's Natl Med Cen, Wash, DC 20010 and Dept Psychiat, George Wash Univ Sch Med, Wash, DC.

We have proposed that autistic and self-injurious behavior (SIB) individuals may have a dysregulation in proopiomelanocortin (POMC) systems of the HPA axis. Here we measured plasma concentrations of immunoreactive (ir)B-endorphin(B-E, pmol/L), cortisol (nmol/ L) and irACTH(pmol/L) in 10 nonSIB autistic children (Aut Grp) and 7 children with severe SIB(SIB Grp). The relationship between plasma POMC and cognitive functioning as well as social functioning was also evaluated.

Plasma POMC peptide concentrations (M±SEM) were: Aut Grp, irBE(7.17±0.83), cortisol(316±46), and irACTH(7.62±1.19); SIB Grp, irB-E(4.63±0.34), cortisol(419±47), irACTH(5.25± 0.90). (Normal concentrations of irB-E are about 7 pmol/L). In the Aut Grp, no significant differences were found between MR (IQ<70) and NMR subjects for either irB-E or irACTH. In the SIB Grp, 6/7 subjects had IQ's in the MR range.

Thus, these data suggest that the reduced plasma concentrations of irB-E and irACTH of SIB children versus autistic and normal children do not appear to be a function of cognitive differences. Supported by FDA OD, NICHD HD23330 and March of Dimes(BHH).

186.2

EARLY SOCIAL RESTRICTION OF RHESUS MONKEYS ALTERS CHEMOARCHITECTURE IN THE STRIATUM BUT NOT IN THE BED NUCLEUS-AMYGDALA COMPLEX. L.C. Cork, L.J. Martin, M.H. Lewis, and J.P.Gluck*. Neuropathology Lab., Johns Hopkins Univ., Baltimore, MD 21205

Permanent behavioral disturbances (e.g., stereotypic movements and psychosocial anomalies) can result from early isolation rearing of rhesus monkeys (Macaca mulatta). We have shown that compartments of the neostriatal mosaic (patch-matrix) are abnormal in isolation reared monkeys [based on patterns of immunoreactivity for tyrosine hydroxylase (TH), substance P (SP), and leucine-enkephalin (LENK); Martin et al, 1990]. However, it is uncertain whether chemoarchitectonic changes are specific for the striatum or if they are present in other subcortical telencephalic locations. We examined patterns of immunoreactivity for TH, SP, LENK, and somatostatin (SOM) in the striatum and the bed nucleus-amygdala continuum in isolation- and socially-reared monkeys. In isolates, TH-immunoreactive fibers and terminals were lost in striatal matrix; striatal patches (particularly in the caudate) were reduced in neuronal cell bodies and processes immunoreactive for SP and LENK, but not for SOM. In contrast, patterns of immunoreactivity for TH, SP, LENK, and SOM in the bed nucleus-amygdala continuum of isolates and social controls were similar. These results suggest that monoaminergic and some peptidergic systems of the striatum are selectively at risk in infant monkeys reared in social isolation.

186.3

ALPHA-MSH ENHANCED ATTENTION IN A CUED DISCRIMINATION TASK. C.W. SIMPSON AND G.E. RESCH. School of Basic Life Sciences, Univ. Missouri-Kansas City, Kansas City, MO 64108.

Early studies using avoidance and stretch-yawn indices showed that icv or peripheral alpha-melanocyte stimulating hormone (α-MSH) altered attention; however, no brain sites of action were identified. Data in the present work show changes in attention behavior following microinjection of α-MSH into specific brain tissue sites. Changes in attention were described by differential changes between responses similar to attention behavior criteria in the literature. Female Sprague Dawley rats 200-250 gm were implanted with guide tubes in the medial anterior hypothalamus preoptic area (MAHPOA) and trained to an 80 to 85% correct barpress response in a visual cued discrimination task for infra-red heat reinforcement, as previously reported. A significant ($P<.001$) improvement in task performance occurred following the microinjection of α-MSH into previously identified PGE_2-sensitive sites of the MAHPOA. The improvement in performance occurred due to a decrease in error rate following α-MSH. No significant effect was elicited by α-MSH on correct scores in the cued discrimination task. Comparison of barpress rates (strikes/min) show a mean decrease of 54% of incorrect responding vs correct responding following the microinjection of α-MSH. α-MSH elicited responses between 10[-6] and 10[-12] gm but not at lower values tested. Three of 12 animals injected into PGE_2 responsive heat gain sites showed a Tc response but did not show behavioral changes to α-MSH. Nine of 12 animals showed improved task performance correlated with criterion Tc (0.5°C) increases. These data suggest differentiation between site functions. Task performance improved following α-MSH due to a decrease in error rate. The doses of α-MSH eliciting responses were in the nanogram to picogram range. The data also show α-MSH discrimination between two kinds of sites, one showing only a Tc response and the other both Tc and behavior changes. The data show specific MAHPOA sites from which α-MSH elicits changes in both Tc and attention behavior. Supported by AFOSR 87-0297.

186.4

MICROINJECTION OF DYNORPHIN INTO THE HIPPOCAMPUS IMPAIRS SPATIAL LEARNING IN RATS. K.L. McDaniel*, W.R. Mundy and H.A. Tilson. Curriculum in Toxicology, North Carolina State University, Raleigh, NC and Neurotoxicology Division, US Environmental Protection Agency, Research Triangle Park, NC 27711.

The effect of hippocampal dynorphin administration on learning and memory was examined in spatial and nonspatial tasks. Bilateral infusion of dynorphin A(1-8)(DYN; 10 or 20 ug in one ul) into the dorsal hippocampus resulted in a dose-related impairment of spatial working memory in a radial maze win-stay task. Subsequent experiments found that acquisition of a reference memory task in the water maze was impaired by DYN injections (20 ug/ul) in the dorsal, but not ventral hippocampus. This impairment was blocked by naloxone (3 mg/kg). In a nonspatial task, post-training DYN injections (20 ug) in the dorsal hippocampus had no effect on retention of a step-through passive avoidance task. These results suggest that DYN interferes specifically with spatial learning and memory, and that this effect is mediated by opioid receptors in the hippocampus.

186.5

RECOVERY FROM CYSTEAMINE - INDUCED CENTRAL SOMATOSTATIN DEPLETION: RADIAL MAZE ACQUISITION. G.R. Sessions, E. Demetriades*, L.L. Leber* and G.F. Koob. Walter Reed Army Inst. Res., Wash, D.C. 20307, U.S. Air Force Acad., Colorado Springs, CO 80840 and Scripps Clinic & Res. Found., La Jolla, CA 92037.

Cysteamine HCl temporarily depletes brain somatostatin *(Bakhit, et al., 1983, Reg. Peptides, 6:169)* and produces deficits in eight-arm radial maze performance in rats injected after they learn the task *(Sessions, et al., 1989, Neurosci. Abstr., 15:712)*. The present study investigated the acquisition of a radial maze memory task in rats following recovery from cysteamine-induced somatostatin depletions. Groups of animals received four daily ICV injections of cysteamine (250 or 350 μg in 2 μl) or buffered saline, then were allowed 4 to 5 weeks to recover from the effects of the drug before receiving training to obtain food pellets in an eight arm maze. Results showed no differences in latency or error measures during acquisition. Apparent memory deficits associated with acute central somatostatin depletion are not evident in subsequent acquisition of a memory task in recovered animals.

186.6

OXYTOCIN RECEPTOR DISTRIBUTION REFLECTS SPECIES-TYPICAL PATTERNS OF SOCIAL BEHAVIOR. L. E. Shapiro, R. E. Gelhard* and T. R. Insel. LCS, NIMH, Poolesville, MD 20837.

Rodents of the genus *Microtus* are a group of phylogenetically related species that display a diverse range of social and reproductive behaviors. The comparative analysis of such naturally occurring species provides a unique opportunity for the study of brain/behavior relationships. Prairie voles are monogamous, highly social, and intensely parental. In contrast, montane voles have a polygamous mating system, are asocial, and display relatively low levels of parental care. Previous research has indicated that the nonapeptide oxytocin (OT) is involved in the onset of maternal behavior following parturition and may be important for affiliative processes in general. In this study we describe the distribution of OT receptors in these two species and evaluate the role of this peptide in the species-typical expression of social and parental behaviors. The distribution of OT receptors was evaluated using *in vitro* receptor autoradiography with the potent OT receptor antagonist, ^{125}I-d(CH_2)$_5$[Tyr(Me)^2,Thr^4,Tyr-NH_2^9]OVT (^{125}I-OTA). Relative to montane voles, ^{125}I-OTA binding was increased 2-4 fold in several discrete regions of the prairie vole brain, including the lateral bed nucleus of the stria terminalis, anterior cingulate cortex, nucleus accumbens, olfactory tubercle, and putamen. There were no within-species gender differences in any of these brain regions. However, ^{125}I-OTA binding was increased in the lateral septum of female prairie voles relative to male prairie voles; the converse was true for montane voles with males displaying higher binding levels than conspecific females. We have also mapped ^{125}I-OTA binding in two additional species of *Microtus*, the monogamous and highly parental pine vole, and the polygamous and asocial meadow vole. The identical sexually dimorphic pattern of binding observed in the lateral septum of prairie and montane voles was also present in these two species. That is, female pine voles showed intense binding relative to males, with the converse being true for meadow voles. In addition, binding was high in the cingulate cortex of pine voles relative to meadow voles. We are currently evaluating pre- and postpartum binding in prairie and montane voles in an attempt to correlate changes in receptor distribution with changes in behavior.

186.7

OPIOID SUPPRESSION OF DISTRESS ULTRASOUNDS AFTER SOCIAL DEFEAT IN RATS. J.A. Vivian and K.A. Miczek. Dept. of Psychology, Tufts Univ., Medford, MA 02155.

Ultrasounds (US) in adult rats occur in highly significant social situations, such as mating, maternal and agonistic behavior and may serve as communicative signals of affective expressions. In this experiment, US were studied in Long-Evans rats (*Rattus norvegicus*) (1) with three exposures to attacks and threats by a resident opponent while displaying defensive and submissive responses (2) with three exposures to the threat of attack (no physical contact) by a resident opponent. The attack and threat situation consisted of brief agonistic interactions until the intruder rat displayed a submissive posture (crouch, supine) for five s; subsequently, the intruder was exposed for 25 min to threats by the opponent while being protected from physical contact by a wire mesh. Immediately prior to each physical encounter, the intruder was administered morphine or ethylketocyclazocine (EKC); to determine opioid specificity, naltrexone was administered 30 min prior to the first physical encounter followed by morphine. During the physical encounter, intruders received ca. 7 bites in 40 s, prompting frequent US. During the protected encounter, morphine (0-6 mg/kg SC) and EKC (0-1.0 mg/kg SC) dose dependently decreased the rate and duration of US which correlated well with their analgesic effect and was antagonized by naltrexone (0.1 mg/kg IP: 4-6 fold rightward shift). Sonographic analysis revealed no change in modal frequency after administration of either opiate. By itself, naltrexone had no effects on the rate, duration or frequency of US. Morphine and EKC decreased motor activity which was reversed by naltrexone. Naltrexone increased motor activity when administered alone. The potent suppression of US by threatened intruders may reflect opioid actions on affective components of social stress reactions.

186.8

VASOPRESSIN INNERVATION OF LATERAL SEPTUM IN TWO LINES OF MICE SELECTED FOR AGGRESSION. J.C. Compaan[*1], R.M. Buijs[*2], C. Pool[*2], J.M. Koolhaas[*1], A.J.H. de Ruiter[*1], G.A. van Oortmerrsen[*1], B.Bohus[*1], (SPON: European Brain and Behaviour Society), [1]Univ. of Groningen, Dept. of Animal Physiol., P.O.Box 14, 9750 AA Haren, The Netherlands, [2]Netherlands Instit. for Brain Research, Meibergdreef 33, 1105 AZ Amsterdam, The Netherlands.

In the adult rat brain the vasopressinergic (AVP) innervation of the lateral septum (LS) is testosterone (T) dependent. It is unknown, to what extent this system is involved in the differentiation in social behaviour within the male sex. In this study we used male mice (Mus Musculus Domesticus), genetically selected for aggression. Mice with a Short Attack Latency (SAL) possess a higher plasma T level compared to males with a Long Attack Latency (LAL). Neonatally, however, a higher T-production occurs in the non-aggressive LAL males than in SAL males. We found that adult LAL-males posses a higher density of AVP immunoreactive (AVP-i) fibers especially in the posterior part of the LS. This difference is consistent with the differences in neonatal T-production. Neonatally high T perhaps permanently organizes AVP neurons which might be involved in the differentiation in social behaviour of these selection lines.

The investigations were supported by the Foundation for Biological Research (BION), which is subsidized by the Netherlands Organization for Scientific Research.

186.9

STRAIN DIFFERENCES IN THE BEHAVIORAL EFFECTS OF CRF AND AN ANTAGONIST, CRF 9-41 IN MICE. L.H. Conti, D.G. Costello*, L.A. Martin*, M.F. White* and M.E. Abreu. Nova Pharmaceutical Corp. 6200 Freeport Centre, Baltimore, MD 21224.

Corticotropin releasing factor (CRF), a hypothalamic peptide also found in other brain regions, has behavioral effects independent of its pituitary/adrenal effects and acts as an anxiogenic following intracerebroventricular (icv) administration (Dunn and File, Horm Behav, 21, 193). A CRF antagonist, CRF $_{9\text{-}41}$ attenuates behavioral, physiological and immunological effects of CRF and exhibits anxiolytic activity in the fear potentiated startle paradigm (Swederlow et al., Neuropsychopharm, 2, 285).

In the present experiments, the effects of CRF $_{9\text{-}41}$ (25 and 50 ug,icv) were examined in different strains of mice (CF-1 derived, CD and BALB/C, Harlan Sprague Dawley) in the elevated plus-maze anxiolytic test. CRF $_{9\text{-}41}$ significantly increased percent open arm activity in BALB/C mice only. Diazepam (1 - 4 mg/kg, ip) increased open arm activity in all strains.

In a second set of experiments, locomotor activity of each strain was assessed in a 30 min session following icv administration of one of 5 doses of CRF (.1 - 1.0 ug). Locomotor suppression by CRF was seen in all mouse strains. However, CRF was more potent and its effects more sustained in BALB/C mice than in CF-1 or CD mice. Behavioral differences cannot be readily explained on the basis of differential binding of CRF to cortical receptor sites in the three strains.

These data suggest that the BALB/C mouse is more sensitive to the behavioral effects of CRF and its antagonist, CRF $_{9\text{-}41}$, than either the CD or CF-1 derived strain.

186.10

REINFORCING AND MEMORY PROMOTING EFFECTS OF THE NEUROTACHYKININ SUBSTANCE P AND ITS FRAGMENTS. J.P.Huston, R.U.Hasenöhrl*, P.Gerhardt* and P.Krappmann*. Inst. Physiol. Psychology, Univ. Düsseldorf, D-4000 Düsseldorf, F.R.G.

Several studies have shown that the posttrial administration of the neurotachykinin substance P (SP) can promote learning.A theory linking memory and reinforcement explains the action of reinforcers by their strenghtening effects on memory traces. Based on this theory we hypothesized SP to have reinforcing effects as well.For evaluating reinforcing properties of SP in rats, a place preference task was used. SP displayed reinforcing effects where the injection of the peptide had facilitated learning: in the lateral hypothalamus,the n.basalis magnocellularis and in the periphery.Further evidence for a reinforcing action of SP was provided by a self-injection technique,showing that rats self-administered ng-amounts of SP into the ventral neostriatum.
In order to determine the active amino-acid sequence encoding the reinforcing and mnemonic properties of peripherally applied SP, we examined its N-terminal fragment SP1-7 and the C-terminal fragment analogs DIME-C7 and pGlu^6-SP6-11. A different structure-activity relationship was obtained for the effects of the fragments on place conditioning and avoidance learning: while the C-terminal fragment analogs mediated the reinforcing properties, the N-terminus encoded the memory promoting effects of SP. It remains to be determined, whether this behavioral dissociation also occurs when the SP-fragments are injected into the CNS directly.

186.11

D-ALA^2-MET^5-ENKEPHALINAMIDE ATTENUATES STRESSOR INDUCED ALTERATIONS IN INTRACRANIAL SELF-STIMULATION FROM THE DORSOLATERAL VENTRAL TEGMENTUM. Cindy L. Wolfe and Robert M. Zacharko, Department of Psychology, Carleton University, Ottawa, Ontario, Canada.

A decline in responding for previously rewarding intracranial self-stimulation (ICSS) following uncontrollable stressor exposure is evident from a number of mesocorticolimbic sites. It has been suggested that these stressor induced disruptions of ICSS may be mediated by region specific alterations in dopaminergic (DA) activity. Current research has demonstrated that a number of neuropeptides, particularly enkephalin, interact with DA within the ventral tegmental area (VTA), the point of origin of the mesocorticolimbic system. This raises the possibility that enkephalin may play an interactive role with DA in the mediation of stressor related ICSS deficits observed from the VTA. In the present study, uncontrollable footshock induced response deficits in ICSS from the dorsolateral aspects of the VTA. Administration of D-Ala^2-Met^5-Enkephalinamide (DALA) into the lateral ventricles following stressor exposure led to a transient reveral of the footshock induced disruption of ICSS. Administration of DALA prior to stressor exposure blunted the initial impact of footshock on ICSS and attenuated decreases in ICSS both 24 and 168 hours post stressor. These results are discussed with respect to the possible mechanisms by which DALA interacts with DA neurons within the VTA to attenuate the impact of stressor exposure on ICSS.

186.12

CHRONIC STRESS OR ANTIDEPRESSANT TREATMENTS DIFFERENTIALLY REGULATE THE CYCLIC AMP SYSTEM IN RAT LOCUS COERULEUS (LC). R.S. Duman, K.R. Melia, R.Z. Terwilliger*, and E.J. Nestler. Lab. of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale Univ. School of Med., New Haven, CT 06508.

Recent studies have demonstrated that chronic stress or antidepressants differentially regulate neuronal activity and expression of tyrosine hydroxylase (TH) in LC neurons (see Melia et al., this volume). In the present study we extend these studies by examining the effects of stress or antidepressant treatments on the cyclic AMP second messenger system in this brain region.

Levels of both adenylate cyclase (AC) and cyclic AMP-dependent protein kinase (cA-K) were determined in the same samples of LC isolated from rats administered either cold stress (5 D) or imipramine (18 D). Cold stress was found to significantly increase levels of AC and soluble cA-K by 25 to 30 percent; levels of kinase in particulate fractions were not significantly altered. Adminstration of ACTH (7 D) or bilateral adrenalectomy (7 D) had similar results, suggesting that ACTH, not corticosterone, positively regulates the cyclic AMP system in LC. In contrast, chronic imipramine treatment decreased levels of cA-K in soluble fractions of LC; levels of AC were not altered by antidepressant treatment.

These findings demonstrate that 1) the activity of LC neurons, 2) the levels of TH, and 3) the activity of the cyclic AMP system are either up- or down-regulated in parallel by stress or antidepressants, respectively. Moreover, these findings raise the possibility that adaptations of this second messenger system contribute to the long-term effects of stress and the therapeutic actions of antidepressant treatments on LC neuronal function.

186.13

REGULATION OF TYROSINE HYDROXYLASE (TH) IN THE LOCUS COERULEUS (LC) BY CORTICOTROPIN-RELEASING FACTOR (CRF): RELATION TO STRESS AND DEPRESSION. K.R. Melia, E.J. Nestler, J. Haycock, and R.S. Duman, Laboratory of Molecular Psychiatry, Yale University School of Medicine, New Haven, CT 06058.

Electrophysiological, biochemical, and behavioral studies suggest that both the noradrenergic and CRF systems may play a role in stress and depression. Stress increases levels of CRF and TH in LC (see Duman et al., this volume) and increased levels of CRF and norepinephrine have been found in some depressed patients. Moreover, CRF increases LC neuronal activity and CRF antagonists block stress-induced increases in LC firing rates. The present studies investigated the possibility that CRF may mediate the stress-induced changes in TH in the LC.

In the first study, levels of TH were determined by Western blot analysis in animals infused (ICV) for 6 days, twice daily (am,pm), with either CRF (5μg) or vehicle (5μl at 0.5μl/min). Chronic CRF increased TH levels in the LC by 74% relative to chronic vehicle treatment. We next infused either 10μg of a-helical CRF (in 1.0μl) or vehicle unilaterally into the LC twice a day for four days. Five minutes after infusions animals received 10 1.0mA footshock (am) or 1/hr noise stress (pm). On day five, animals were sacrificed and each LC was dissected and analyzed separately. A 58% decrease in TH levels was found in the LC infused with alpha-helical CRF relative to its contralateral control. In contrast, only a 15% decrease was found in TH in the LC infused with vehicle relative to its control. Finally, the ability of antidepressants to modulate stress-induced changes in TH was examined. Administration of imipramine (15mg/kg) or tranylcypromine (7.5mg/kg) for two weeks attenuated the increase in TH in the LC elicited by 5 days of cold stress.

Thus, chronic manipulation of the CRF system modulates the noradrenergic system by actions at CRF receptors nearby or in the LC. Furthermore, the CRF antagonist and chronic antidepressant administration share the ability to decrease stress-induced changes in TH in the LC. As the therapeutic actions of antidepressants may be mediated in part through the regulation of TH, inhibition of CRF might be an effective form of treatment for depression and other stress-related disorders.

186.14

THE EFFECT OF THE CCK ANTAGONISTS L-364,718 and L-365,260 ON THE ACQUISITION OF A DISCRIMINATED ACTIVE AVOIDANCE TASK IN C57BL/6J MICE. J. Rodenhiser and E. Quinton. Lab of Psychobiology, University of Louisville, Louisville, KY. 40292.

The octapeptide fragment of cholecystokinin (CCK-8) seems to modulate a wide range of behaviors, but the data are inconsistent. We have previously shown that CCK facilitates acquisition of active avoidance in a multi-trial discrimination task. The present study seeks to determine whether two CCK antagonists, selective for the CCK_A and CCK_B receptors, affect acquisition in the same task. C57BL/6J mice were given injections of either vehicle control, 200ug/kg of CCK-8s, 120ug/kg L-364,718 or 120ug/kg L-365,260 SC before each daily session (7 trials/session, 8 sessions). The task required the animal to discriminate an illuminated door from among 5 doors, and to exit through that door to escape/avoid shock (.2mA). Attempts to exit through incorrect doors were counted as errors. Contrary to expectation, the two antagonists made fewer errors over sessions than did the CCK or control groups; the L365,260 group made the fewest errors. It seems possible that the high doses of antagonists used in this study may interact with other systems.

186.15

CCK-8 MODULATION OF TOLERANCE TO STRESS-INDUCED ANALGESIA. M.Mims, M. Shuck, J. Prather, and E.E. Quinton. Lab. of Psychobiology, University of Louisville, Louisville, KY 40292.

Research suggests that CCK-8 is a modulator of opioid-induced analgesia. Other research has suggested that CCK-8 may facilitate the development of tolerance to opioid-induced analgesia. The present study was performed to determine whether CCK is involved in the development of tolerance to stress-induced analgesia. Male Sprague-Dawley rats were injected into the PAG with antibodies to CCK-8 (ACCK) five minutes before receiving 90s of footshock at 1 mA on two consequtive days. The animals were tested for analgesia on a hot-plate test two minutes later. On the first day there was a significant increase in analgesia in the ACCK and control groups, with the level of analgesia somewhat greater in the ACCK group. On the second day there was a significant reduction in analgesia in both groups and this reduction was greater in the ACCK group. The data from this study suggests that CCK-8 may not modulate the development of tolerance to stress-induced analgesia. It seems possible that tolerance to morphine-induced analgesia and tolerance to stress-induced analgesia may be mediated through seperate systems.

186.16

INTERACTION OF CCK-8, MORPHINE, AND CCK ANTAGONISTS ON ANALGESIA ON THE HOT-PLATE TEST IN MICE. E.E. Quinton. Lab. of Psychobiology, University of Louisville.

The results of numerous studies employing different methodologies and response systems suggest that CCK modulates pain systems and interacts with morphine. However, the mechanisms underlying the modulation and interaction are unclear. This study seeks to determine whether CCK-induced analgesia is mediated through the CCK_A or CCK_B receptors; and whether antagonists of these receptors would affect morphine induced analgesia in the C57BL/6j mouse. CCK-8s (400µg/kg,SC) was injected 15 min after an injection of L365,260 (120µg/kg, SC), L364,718 (120µg/kg, SC), saline, or proglumide (40mg/kg, SC). The mice were tested on the hot-plate (55° C) 10, 15, 20, 30, 45, 60,75, or 90 min after the CCK injection. All three drugs reduced CCK-induced analgesia with L364,718 and proglumide most effective. The same drugs and CCK-8s were tested with morphine (10µg/kg, SC). Only L364,718 antagonized morphine analgesia, and CCK-8s increased the duration of morphine analgesia. These preliminary data suggest that CCK modulates the pain systems and interacts with morphine through the CCK_A receptors.

NEUROTOXICITY: METALS

187.1

The Effects of Low-Level Lead Exposure During Different Developmental Periods on Growth, Behavioral Activity, and Spatial Discrimination in Male Binghamton Heterogeneous Stock Mice. D.A. Rasile*, P.J. Donovick and R.G. Burright*, Environmental Neuropsychology Laboratory, Dept. of Psychology, SUNY Binghamton, Binghamton, N.Y. 13901.

In the past two decades, numerous experimental studies have established the teratogenic effects of low-level lead exposure on development, behavioral activity and learning. It has also been established from such studies that the effects of such exposure can differ widely in their nature and severity depending, in part, on the developmental stage of the organism when exposed as well as the duration of exposure. The present study addresses such issues and attempts to determine the impact of low-level lead exposure administered during different developmental periods on body weight, running wheel activity, and water maze spatial reversal learning. Four experimental groups are employed: a distilled water control group (WW); a group of mice exposed to lead from gestation to weaning--age 21 days and then switched to distilled water (LW); mice exposed to lead from age of weaning to early adulthood (WL); and mice exposed to lead from gestation and continuing through adulthood (LL).

187.2

EFFECTS OF INORGANIC AND TRIETHYL LEAD AND INORGANIC MERCURY ON THE VOLTAGE ACTIVATED CALCIUM CHANNEL OF *APLYSIA* NEURONS. D. Büsselberg, M.L. Evans, H. Rahmann* and D.O. Carpenter. Wadsworth Labs and School of Public Health, Albany, NY 12201-0509 USA, and Universität Mainz, FRG.

Using conventional two-electrode voltage-clamp techniques we have studied the effects of Pb^{2+}, triethyl lead (TEL) and Hg^{2+} on voltage-activated calcium channels of *Aplysia* neurons and found that all three are potent inhibitors at micromolar concentrations. However the time course of reduction and reversibility of blockade are very different when comparing Pb^{2+} with TEL and Hg^{2+}. With application of Pb^{2+} the calcium current decreases immediately; a steady state is reached within three to seven minutes, depending upon the concentration of Pb^{2+}. The blockade is easily reversed upon washout of Pb^{2+} with a time course similar to that of onset. Perfusion with either TEL or Hg^{2+} resulted only in a small reduction of the current when the substances reached the cell membrane but the decrease continued at about the same speed for the total duration of the application. Upon washing there was no recovery of the response. At the onset of washing the rate of current decline stopped for several minutes, but then the current continued to decline a slower rate in the absence of toxin. Our data suggest that Pb^{2+} acts by a direct and reversible blockade of the calcium channel. In contrast TEL and Hg^{2+} act slowly and irreversibly to block calcium channels at concentrations that do not greatly affect membrane potential or resistance. In spite of the slow time course these substances are probably acting directly on the channel.

187.3

NEUROTOXICITY OF METHYLMERCURY: NEUROTRANSMITTER RELEASE AND GLUTATHIONE STATUS IN THE CEREBELLUM. B.E. Kalisch*, W.J. Racz* and C. Romero-Sierra. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Chronic methylmercury (MeHg) exposure in man and animals is known to selectively damage the cerebellum. Presently, there is no satisfactory explanation for this selective toxicity. Recent studies have shown that MeHg may interfere with neurotransmission. In addition, glutathione (GSH), which protects membranes from oxidative damage, has been shown to decrease following exposure to MeHg. The present study investigates the effects of chronic high levels of MeHg on GSH status and amino acid release from mouse cerebellar tissue and correlates these with the symptoms associated with MeHg intoxication.

Mice were dosed for 6 weeks with 10 mg/kg/week MeHg and subsequently oxidized and reduced GSH levels and amino acid release were measured 24, 48, 72 hours and 7 days after the last treatment. Tissue amino acid content was not significantly different from control in any of the treatment groups. The 35 mM K^+-stimulated release of glutamate was significantly higher in mice exhibiting severe symptoms of toxicity when compared to control at 24 and 48 hours after the last dose of MeHg, but by day 7 had returned to control. K^+-stimulated release of aspartate and gamma-aminobutyric acid (GABA) were not different from control.

GSH content in the MeHg-treated mice was elevated 24 hours after the last dose of the toxicant and paralleled the increase in glutamate release. This increase may be a rebound effect due to GSH depletion. The role of GSH depletion during MeHg intoxication is currently under investigation. (Supported by NSERC)

187.4

DIFFERENTIAL EFFECTS OF MERCURIALS ON SYNAPTOSOMAL PLASMA & MITOCHONDRIAL MEMBRANE POTENTIALS. M.F. Hare and W.D. Atchison. Dept. Pharm./Tox. & Neurosc. Prgm, Mich. State Univ., E. Lansing, MI 48824.

Both methylmercury ($MeHg^+$) and inorganic divalent mercury (Hg^{2+}) alter the flux of ions and small molecules across nerve terminal membranes by mechanisms that may involve membrane depolarization. We examined the effects of $MeHg^+$ and Hg^{2+} on nerve terminal membrane potential in synaptosomes using the potentiometric carbocyanine dye [DiS-C_2(5)]. Compounds known to depolarize synaptosomes (e.g., ouabain, high $[K^+]_e$, and gramicidin) increased dye fluorescence. Depolarization of the mitochondrial membrane by cyanide and oligomycin increased dye fluorescence by 49%. Subsequent addition of valinomycin produced no further fluorescence increase indicating that intrasynaptosomal mitochondria were totally depolarized. Increased fluorescence consequent to a pulse of high K^+ decreased as the resting membrane potential became more positive. There was no significant difference between control and cyanide/oligomycin-treated synaptosomes in this response, which suggests that these agents did not alter the resting plasma membrane potential. Azide substitution for cyanide caused a 64% increase in fluorescence. Azide also depolarized the plasma membrane potential by 18 mV. Five min exposures to either $MeHg^+$ or Hg^{2+} produced concentration-dependent increases in dye fluorescence. Prior depolarization of the mitochondrial membrane reduced the 10 µM $MeHg^+$-induced depolarization by 67% but did not alter the 10 µM Hg^{2+}-induced depolarization. The fluorescence response to a subsequent depolarization was decreased 32% after 10 µM $MeHg^+$ and was abolished totally by 10 µM Hg^{2+}. These effects of $MeHg^+$ and Hg^{2+} were unaltered by tetrodotoxin or Co^{2+}. In addition, $MeHg^+$-induced depolarizations were not altered by removal of Ca^{2+} and Na^+ from the media. In summary, 10 µM $MeHg^+$ depolarized mitochondria and decreased plasma membrane potential by 16 mV. Hg^{2+}, on the other hand, totally abolished the plasma membrane potential with minimal effects on the mitochondrial membrane potential. Supported by NIEHS grant ES03299.

187.5

ACRYLAMIDE OR METHYLMERCURY EXPOSURE INCREASE GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) IN THE PIGEON BRAIN. H. L. EVANS, H.A.N. EL-FAWAL* AND F. R. MOON*. INST. OF ENVIRON. MED., N.Y.U. MED. CTR., N.Y., N.Y. 10016

Acrylamide (ACR) and methylmercury (MeHg) provide models of neurotoxicant induced, central-peripheral axonopathy. It is not known whether these toxicants alter the gliotypic protein GFAP in the CNS. We used the radio immuno assay (RIA) to quantify the astrocyte reaction to ACR or MeHg. Female pigeons were given doses known to cause overt neuropathy [ACR (45 mg/Kg/day for 5 day, p.o.) or MeHg (2.0 mg/Kg/day for 5 day, p.o.)]. Vehicle (H_2O) treated pigeons acted as controls. Brains were immediately removed and snap frozen. On the day of assay the cerebrum (C), cerebellum (CR) and medulla (M) were dissected, weighed and sonicated in SDS and the RIA was performed with GFAP polyclonal antibody and ^{125}I protein A. GFAP was evaluated after 1,3 and 5 weeks of exposure (for ACR) and 1,3,5 and 7 weeks (for MeHg). ACR increased GFAP only at 3 weeks and only in C(136%) and M(150%). GFAP increased in C (150%) at 3 weeks and in the CR (255%) at 3 and 5 weeks of MeHg exposure. These results suggest that the astrocytic response is biphasic, which may indicate initial hypertrophy followed by degeneration. Supported by ES-00260, ES-08945.

187.6

ALTERED LOCOMOTION AND NEUROMUSCULAR FUNCTION IN CHRONIC EXPOSURE TO ACRYLAMIDE (ACR) OR METHYLMERCURY (MeHg) IN THE PIGEON. H.A.N. EL-FAWAL*, F.R. MOON*, S. DANIEL* AND H. L. EVANS. INST. ENVIRON. MED, NYU MED. CTR., N. Y., N.Y. 10016.

ACR and MeHg cause peripheral neuropathy including behavioral deficits. We studied physiological mechanisms in these changes. Pigeons received ACR (45 mg/Kg/day, 5 days/week) or MeHg (2 mg/Kg/day, 5 days/week). Pecking accuracy and gait indicated gradual impairment of locomotion. Early physiological changes occurred in anatomical correlates of the locomotor deficits, the biventer cervicis nerve-muscle (BC) and sciatic-gastrocnemius (SG) muscle preparations in vitro. Strength-duration relationships in response to electrical stimulation of BC indicated an increase in excitability threshold within 1 week of exposure to either ACR or MeHg. Rheobase (volts) and chronaxie (msec) were higher and longer, respectively than controls with ACR. Rheobase and chronaxie following MeHg were higher and shorter, respectively. Responses of both BC and SG to ACh were potentiated beginning 3 weeks of ACR and 1 week of MeHg. Pecking accuracy and gait changed at 3 weeks of ACR exposure and did not change for the duration of MeHg exposure. Eletrophysiological changes precede behavioral deficits with ACR or MeHg exposure. Supported by ES-00260 and ES-08945.

187.7

CORRELATION BETWEEN ALUMINUM DOSE, CLINICOPATHOLOGICAL CHANGES AND NEUROFILAMENT mRNA EXPRESSION IN ALUMINUM NEUROTOXICITY. V.R. Nerurkar*, M.J. Strong, I. Wakayama*, R. Yanagihara* and R.M. Garruto. Laboratory of Central Nervous System Studies, NIH, Bethesda, MD 20892.

We investigated the relationship between the clinico-pathological changes, neurofilament (NF) accumulation and NF subunit protein mRNA expression in 6 week old New Zealand white rabbits 48 hrs after intracisternal inoculation with varying doses of $AlCl_3$ (100, 250, 500, 750 or 1000 ug) or with 0.9% NaCl. Sections of cerebral hemisphere, cerebellum, cervical and lumbar spinal cord were silver stained (Bielchowsky) and immunostained using a monoclonal antibody directed against NF-M and NF-H. Northern blots using ^{32}P-labelled cDNA probes encoding NF-L, NF-M and NF-H subunit protein mRNA were performed for each region.

We observed (i) no clinically differentiating features amongst the 5 dosage groups, (ii) a positive correlation between increasing $AlCl_3$ dosage, topographic localization and extent of neuropathologic changes, (iii) a negative correlation between $AlCl_3$ dose and NF mRNA expression, and (iv) sibship-dependant neuropathological variability. In contrast to previously reported studies, our data support a relationship between the induction of NF inclusions by $AlCl_3$ and alterations in NF subunit protein mRNA expression.

187.8

TOPOGRAPHIC DIFFERENCES IN NEUROPATHOLOGIC CHANGES REFLECT NEURON-SPECIFIC THRESHOLDS OF ALUMINUM TOXICITY. M.J. Strong[a] and R. M. Garruto. Laboratory of Central Nervous System Studies, NIH, Bethesda, MD 20892

We exposed mature dissociated motor neuron-enriched and hippocampal neuron cultures derived from fetal New Zealand white rabbits to 1, 10, 25, 50 or 100 uM $AlCl_3$ in a chemically-defined medium to determine whether the observed disparity in sensitivity between motor and hippocampal neurons to intracisternally inoculated $AlCl_3$ is a neuron-specific phenomenon. Following 14 days of exposure to 1 uM or 10 uM $AlCl_3$, motor neuron-enriched cultures developed perikaryal and neuritic neurofilament inclusions. In cultures exposed to 25 uM or 50 uM $AlCl_3$, the neurons developed inclusions but did not survive beyond 10 days. 100 uM exposure induced cell death within 4 days without the development of inclusions. By contrast, hippocampal neurons exposed to 1, 10, or 25 uM $AlCl_3$ for 14 days did not show morphological changes. Cultures exposed to 50 or 100 uM remained viable but developed perinuclear and proximal neuritic inclusions. We interpret this 10 fold greater threshold sensitivity to aluminum in spinal motor neurons compared to hippocampal neurons as evidence for neuron-specific differences in the regulatory mechanisms of neurofilament metabolism amongst distinct neuronal cell populations, supporting our earlier *in vivo* observations.

[a]Research fellow of the Medical Research Council of Canada

187.9

ACUTE EFFECTS OF ALUMINUM IN THE STRIATUM. **T.I. Lidsky, S.P. Banerjee, H.M. Wisniewski. NYS Inst. for Basic Research, Staten Island, NY.**

Aluminum (Al) is, under certain conditions, neurotoxic and has been implicated in several neurodegenerative diseases (e.g. dialysis dementia). In this context, there has been considerable work concerning the neural effects of long term exposure to Al. There is, however, little known concerning acute effects. The present study was addressed to the latter.

Somatosensory field potentials were recorded in the cortex and striatum of urethane anesthetized rats. Intravenous aluminum chloride (0.125 - 2 mg/kg) transiently attenuated striatal potentials at a latency of about 30 seconds with recovery within several minutes. Striatal potentials from cortical stimulation were similarly affected while somatosensory cortical potentials were not attenuated. Thus, Al effects occurred in the striatum rather than presynaptically.

Ongoing work is investigating the pharmacological bases of these acute Al effects. GABA and glutamate, because of their importance in striatal functioning and the well established influences of Al, are presently the focus of these investigations.

187.10

EFFECT OF ALUMINUM ON RAT BRAIN CHOLINERGIC ENZYMES. J.H. Peng, Z-C. Xu*#, Z-X. Xu*#; J.C. Parker*, E.R. Friedlander*, J-P. Tang*#, S. Melethil*#, Dept. of Pathol., Sch. of Med. and Sch. of Pharmacy#, Univ. of Missouri-Kansas City, Kansas City, MO 64108.

Aluminum (Al) administration has been shown to induce neurofibrillary tangles in brains of experimental animals. However, the role of Al in Alzheimer's disease has not been resolved. Some reports suggest that chronic Al treatment significantly reduces choline acetyltransferase (ChAT) activity, but a negative result has been reported. While Al has been administrated by several different routes, Al has not been shown to cross the blood brain barrier. To gain further insight into Al neurotoxicity, Al was injected via the femoral vein (1 mg/kg) and was elevated in the cerebrospinal fluid, reaching a peak (41.6 ± 21.2 ng/ml) 2 hr following injection. Therefore, the cholinergic neurotoxicity of Al at this stage was further investigated. ChAT and acetylcholinesterase (AChE) activities were measured radiochemically in homogenates prepared from different brain areas. ChAT activities in the basal forebrain, frontal cortex, and hippocampus were reduced 30, 29, and 24%, respectively, while no change was observed in the caudate nuclei. On the other hand, AChE activity was increased 38.5% in caudate nuclei while little change was observed in other brain areas. These data suggest that the Al cholinergic neurotoxicity could be an initial event in neurofibrillary degeneration.

187.11

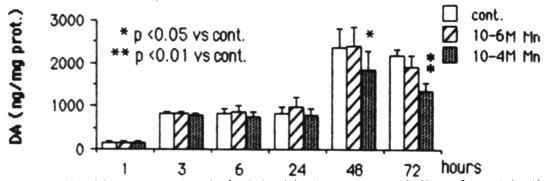
ALUMINUM SILICATE TOXICITY IN PRIMARY NEURONAL CULTURES
E.J. Murphy*, E. Roberts, D.K. Anderson and L.A. Horrocks. The Ohio State University, Department of Physiological Chemistry, Columbus, OH 43210

Aluminum silicates are often found in the plaques of Alzheimer's disease (AD) brains so the toxicity of aluminum silicates towards mixed primary neuronal cultures was examined. Three common aluminum silicate containing clays, erionite, montmorillonite and bentonite, were used. The clays were added in a suspension to the cells at concentration of 0.1 mg/ml. Previous studies with primary cultures of human umbilical vein endothelial (HUVE) cells indicated that this concentration was toxic to the cells. With bentonite, there was some lysis of astrocytes at 5 min of incubation. At 15 min there was a greater degree of lysis of both astrocytes and neurons and following 60 min of incubation the cells were completely lysed. Montmorillonite had the same effect as bentonite although montmorillonite affected primarily astrocytes for the first 15 mins of incubation. After 60 min of incubation there was lysis of astrocytes and to a lesser degree neurons. Erionite had no effect on the cells at any time point. The order of toxicity was bentonite > montmorillonite >> erionite. Unlike the mixed neuronal cultures, lysis of HUVE cells occurred between 6 and 24 h of incubation. This indicates two different lytic mechanisms may be occurring. Supported in part by a grant given to Eugene Roberts by the G. Harold and Lily Y. Mathers Charitable Foundation and by NIH NS-10165.

187.12

PC12 (pheocromocytoma cells) as a model of manganese Dopamine-mediated toxicity. A.Yescovi, A. Nespolo, A. Zaffaroni, M.Clini, A. Colangelo, A. Gritti, and E.A. Parati. Lab Neuropharmacology, Natl. Neurol. Inst. C. Besta, Via Celoria 11, Milan, Italy.

High environmental manganese (Mn) concentrations induce in human an irreversible parkinson-like syndrome. Since an increase in the rate of dopamine (DA) oxidation has been suggested to play a major role in Mn neurotoxicity, we studied the effects of this metal on DA turnover in PC12. We demonstrated that Mn is toxic to PC12, inducing a time dependent decrease in DA intracellular levels,



stricly related to an increase in lactate dehydrogenase activity released in the medium (an index of cell safety). The specifity of the effect was assessed by comparing Mn to equimolar zinc (Zn) concentrations. Briefly: i) Mn induced a complete disappearance of DA in the medium where Zn did not, ii)the DA lack in the medium was due to both an increased oxidation rate and a release blockade where Zn induced an increased release. The Mn-mediated release impairment was not prevented by ascorbic acid alone or in combination with catalase and superoxidedismutase. The data suggest that not only oxidative phenomena occour in Mn neurotoxicity early stages.

187.13

THE COUPLED FREE RADICAL FORMATION IN THE NEUROTOXIC EFFECT OF MANGANESE. A.Y.Sun, D.L.Cowan* and F.F.Ahmad. Dept. Pharmacology and Dept. Physics*, Univ. of Missouri, Columbia, MO 65212.

Manganese poisoning has caused extra pyramidal disorder similar to the behavioral abnormality of Parkinson's disease (PD). Since free radical damage was suggested to be involved in the etiology of PD, we used electron spin resonance (ESR) spectroscopy and spin trapping technique to detect the involvement of free radical in Mn^{++} toxicity. The spin adduct of 1,1-dimethyl pyrrolidine-N-oxide (DMPO) of the hydroxyl radicals were detected when 0.14 mM of Mn^{++} was added to a K-PO_4 buffered system (pH 7.4) containing 0.04% H_2O_2. A Fenton-type reaction similar to that catalyzed by Fe^{++} may be operated in this system. Interestingly, a hydroxyethyl free radical signal was detected when ethanol was added to the above system and the amount of hydroxyethyl free radical was much enhanced if vitamin C was also added into the system. It is possible that the elevated Mn^{++} in basal ganglia may catalyze the free radical formation by coupling to some redox reactions, leading to the symptoms of PD. Since hydroxyethyl free radicals are much longer lived radical species than hydroxyl radical and may cause more damage to the cell, alcohol administration under those conditions may potentiate the harmful effect of Mn^{++} in causing tissue injury and neuronal death. (Supported by AA02054.)

EPILEPSY: HUMAN STUDIES

188.1

MODULATION OF EXTRACELLULAR ASPARTATE LEVEL DURING EPILEPTIFORM EVENTS IN PRIMARY EPILEPTOGENIC AREA OF PATIENTS. K.Q.Do[1], H.G.Wieser-[*2], H.Perschak-[*2] and M.Cuénod[1]. [1]Brain Research Institute, University of Zürich, [2]Department of Neurology, University Hospital, Zürich Switzerland.

Numerous associations have been made between perturbations in inhibitory and/or in excitatory systems and epilepsy. However, the investigation in human epilepsy are generally limited to the biochemical measurement of transmitter either in plasma or CSF or in biopsied or autopsied pathological brain tissue. In the course of presurgical Stereo-EEG evaluation of candidates for epilepsy surgery, we investigated the pattern of the amino acids (aa) efflux in the primary epileptogenic area during spontaneous or electrically induced epileptic discharges. A push-pull cannula was introduced into the lumen of a standard hollow-core multicontact depht electrode placed in the hippocampus. Sterilized Gey's balanced salt solution was perfused (20 ul/min)through the push-pull cannula and one minute fractions were collected over a period of one hour in patients under general anaesthesia. The perfusate was analyzed by on-line o-phtalaldehyde precolumn derivatization HPLC to quantify aa at the femtomole level. A computer-assisted analysis of the background depht-EEG and of spontaneous and electrically provoked epileptiform events were performed and the results were correlated with the biochemical measurements. In three out of four patients, an increase in aspartate level was observed in correlation with the epileptiform EEG events. Their correlation with changes with glutamate concentration was less clear, although there is possibly an elevation related to afterdischarges. Non-transmitter aa levels remained constant and unaffected by the epileptic discharges. These preliminary observations suggest that the excitatory aa aspartate could be involved in pathophysiological mechanisms of human epilepsy and particularly within an epileptic focus.

188.2

ALTERATIONS IN TRH RECEPTORS IN DISCREET REGIONS OF THE HIPPOCAMPUS IN TEMPORAL LOBE EPILEPSY. N. Lexow, J. Phillips, M. Dichter, M. O'Connor, & A. Winokur. Depts. of Psychiatry & Pharmacology, U. of PA, Depts. of Neuology & Neurosurgery, Graduate Hospital, Philadelphia, PA.

The role of thyrotropin-releasing hormone (TRH) in the seizure process has recently received considerable interest. Animal models of limbic seizure phenomena have demonstrated consistent increases (2 to 17 fold) in TRH content in limbic regions as a result of the increased CNS activity associated with seizures (Kubeck et al '84; Walczak et al '83; Kreider et al '90). Moreover, chronic administration of TRH or TRH analogs in rats results in 20-50% reduction in receptor density in the hippocampus, amygdala and hypothalamus (Sharif '87).

Our studies in human postmortem tissue have demonstrated a discreet localization of TRH receptors at points of origin and termination of preferential pathways of seizure propagation. In this study we utilized quantitative autoradiography to examine the density and distribution of TRH receptors in surgically resected hippocampal tissue from 12 patients undergoing temporal lobectomy for intractable seizures. In this tissue the density of binding sites for [3H]MeTRH in the molecular layer of the dentate gyrus was reduced by 48% ($p< 0.0001$) when compared to postmortem tissue. It is unlikely that these alterations are due to cell loss as adjacent stained sections do not demonstrate a loss of dentate gyrus cells. We propose that this dramatic decrease in the density of TRH receptors in the molecular layer of the dentate gyrus is due to a compensatory down-regulation of receptors in response to an excess of neurotransmitter in this region as a result of seizures. These observations suggest a role for TRH in the neurochemical dysfunction of temporal lobe epilepsy.

188.3

ALTERED GABA-A/BENZODIAZEPINE RECEPTOR DISTRIBUTION IN HUMAN TEMPORAL LOBE EPILEPSY. R.W. Olsen, C.R. Houser, A.V. Delgado-Escueta, J.G. Richards and H. Möhler*. Brain Res. Inst., UCLA, Los Angeles, CA 90024; VA Medical Center, W. Los Angeles; Hoffman La-Roche, Basel, Switzerland.

Distribution of the α-subunit of the GABA-A/benzodiazepine receptor (GABA-R) has been determined immunocytochemically in surgical specimens from the hippocampal formation of patients with temporal lobe epilepsy (TLE) using a monoclonal antibody (bd-24) to the receptor complex (Schoch et al., *Nature* 314:168, 1985). In epilepsy specimens, regions of marked cell loss, such of the CA1 field, showed concomitant decreases in the density of GABA-Rs, although isolated neurons remaining within these regions frequently had high densities of receptor labeling on their surfaces. Alterations in GABA-R distributions within the molecular layer of the dentate gyrus were also observed. In control autopsy specimens, dense staining was present throughout the molecular layer with slightly heavier staining in the inner zone. However, in a subpopulation of epilepsy specimens, there was decreased density of labeling in the inner one-third to one half of the molecular layer, with increasing densities in the mid to outer regions of the layer. These changes in receptor distribution may be related to reorganization of other inputs to the molecular layer in severe TLE. Supported by NIH grants NS21908, NS22071 and VA Medical Research Funds.

188.4

SYNAPTIC MORPHOMETRY OF GRANULAR NEURONS IN INTRACTABLE TEMPORAL LOBE EPILEPSY. M.Y. SHEN*, J.H. KIM, P.O. GUIMARAES*, N.C. de LANEROLLE and D. D. SPENCER. Sections of Neuropathology and Neurosurgery, Yale Univ. School of Medicine, New Haven, CT 06510

Granular neurons of the dentate fascia, as part of the trisynaptic hippocampal loop (entorhinal cortex-dentate fascia-CA3-CA1), may play an important role in the pathogenesis of temporal lobe epilepsy. We conducted an ultrastructural morphometric study on the synaptic structures of the stratum granulosum in hippocampi surgically removed from 6 tumor associated epilepsy (TAE) and 8 non-tumor epilepsy (NTE) cases. With a computer assisted scanning device, the following synaptic parameters were investigated in both pre- and postsynaptic profiles of symmetric and asymmetric synapses: the perimeter and area of the synaptic profile, and the length of the synaptic membrane and density. The perimeter and area of the presynaptic terminals were inversely correlated with the granule cell density (mean cell number/mm3) in both asymmetric (R=0.682 and 0.681, respectively) and symmetric (R=0.630 and 0.605, respectively) synapses, but showed no correlation with the length (years) of seizure history. Compared with TAE, NTE showed a statistically significant increase in the perimeter length and area size of presynaptic terminals (p= 0.0126 and 0.0165 respectively for symmetric synapses, and 0.0436 and 0.0438 for asymmetric ones). Excessive neuronal activities may have caused an enlargement of presynaptic terminals in NTE.

188.5

ABNORMAL ASTROCYTE PROCESSES IN THE CEREBRAL CORTEX OF AN EPILEPTIC INFANT. W.A. Schreier, J. de Vellis, H.V. Vinters*, and R.S. Fisher. UCLA School of Medicine, Los Angeles, CA 90024.

Surgical specimens of the cingulate cortex were obtained from a 13 month-old, white male with severe epilepsy. Astrocytes were examined using glial fibrillary acidic protein (GFAP) immunohistochemistry. Reactive astrocyte-like cells were found in layer I of the cortex and in the subcortical white matter. These cells project long thick processes which frequently appear to traverse the entire width of the cortex (greater than 1 mm). Some of these processes are completely straight and smooth, however, most processes have a spiral-like appearance and contain numerous varicosities. Spiraling processes leaving the white matter frequently extend 0.2-0.5 mm toward the cortical surface before giving off right angle branches. We speculate that these cells may be radial glia which have failed to mature properly.

188.6

INTRACELLULAR NEURONAL RESPONSES IN HIPPOCAMPUS AND DENTATE GYRUS OF EPILEPSY PATIENTS STUDIED IN IN VITRO BRAIN SLICES.

L. M. Masukawa, M. Higashima*, D. D. Spencer and M. J. O'Connor*. Depts. of Neurology and Surgery, Graduate Hospital, Philadelphia, PA, 19146 and Sections of Neuroanatomy and Neurosurgery, Yale University Medical School, New Haven, CT, 01610.

Previously, we showed that hippocampal brain slices from temporal lobe epilepsy patients exhibit abnormal field discharges in the dentate gyrus in response to either single or trains of low frequency stimuli presented to the perforant path (Masukawa, L.M. et al, Brain Res., 492:168, 1989). Intracellular recordings from neurons in CA fields and dentate gyrus demonstrate that cells in in vitro brain slices of surgically removed tissue: 1) generate action potentials which are 80-90 mV in amplitude, 2) have resting membrane potentials in excess of -50 mV, 3) have input resistances of at least 30 Mohms at -60 mV, and 4) exhibit repetitive action potentials during depolarizing current steps. Functional inhibition was demonstrated in individual neurons by the presence of ipsps which could interrupt trains of action potentials. Orthodromically driven intracellular responses are correlated with the field responses in the dentate. Such observations indicate that field responses that we recorded previously originated from dentate granule cells. Thus, we suggest that the electrical characteristics of neurons in in vitro hippocampal slices from temporal lobe epilepsy patients are comparable to those in normal rodent tissue under identical slice conditions. Supported by NIH grants NS-23077 to LMM and NS-06208 to DDS.

188.7

IMMATURE HUMAN NEOCORTICAL NEURONS HAVE LOW-THRESHOLD CA^{2+} SPIKES BUT DO NOT GENERATE INTRINSIC BURSTS. J.G. Tasker, N.W. Hoffman, R.S. Fisher, Y.I. Kim, W.J. Peacock*, and F.E. Dudek. Mental Retardation Res. Ctr., UCLA Sch. of Med., Los Angeles, CA 90024.

Pyramidal neurons showing intrinsic bursting properties have been described in infragranular layers of guinea pig and mouse somatosensory cortex (Connors, B.A.,Gutnick, M.J. and Prince, D.A.,J. Neurophysiol. 48:1302,1982; Agmon, A. and Connors, B.A.,Neurosci. Lett. 99:137,1989). It was proposed that bursting neurons were possible pacemaker cells responsible for the synchronization of neocortical neurons during epileptiform bursting (Connors, B.A.,Nature 310:685, 1984). We performed intracellular recordings and biocytin injections in slices of human neocortex removed from children, ages 4 mo - 15 yr, for the treatment of intractable epilepsy. A total of 145 cells were recorded throughout layers II-VI in frontal (n=39), parietal (n=39), temporal (n=53) and occipital lobes (n=14) from 33 patients. Although human neocortical neurons were electrophysiologically similar to neocortical neurons recorded in other species, we never observed intrinsic bursting characteristics of the type described in animal models. Of 39 neurons injected with biocytin, 20 were situated in layers IV (n=5), V (n=9) and VI (n=6). However, evidence of putative low-threshold Ca^{2+} potentials or currents, capable of generating one or two action potentials, was seen in approximately 60% of recorded cells. These potentials were insensitive to tetrodotoxin (1-3 μM, n=2) but were reduced or blocked by a solution containing low [Ca^{2+}] (0.2 mM) and Cd^{2+} (0.25 mM, n=4). Our results suggest that intrinsically bursting neurons are not necessary for the generation of epileptiform activity, but that low-threshold Ca^{2+} spikes may contribute to burst generation in immature human neocortex. Supported by NS 16683.

188.8

INTRACELLULAR RECORDING IN NEOCORTICAL SLICES FROM PATIENTS WITH INTRACTABLE PEDIATRIC EPILEPSY: CHOLINERGIC MODULATION. J.P. Walsh, C. Cepeda, N.A. Buchwald, Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

These experiments assessed the mechanism and pharmacological basis of cholinergic modulation in neurons recorded from neocortical slices from patients with intractable pediatric epilepsy. Thirteen cells were characterized for their response to cholinergic drugs from subjects ranging from 8 months to 13 years of age. All cells were equally responsive to ACh. Each cell that responded also generated low-threshold Ca^{2+} spikes (LTS cells). ACh (10-100μm) produced a depolarization associated with increased input resistance and cell excitability. Two lines of evidence indicated that a reduction of K^+ current produced this response. First, ACh decreased after-hyperpolarizations generated by trains of action potentials. Second, voltage clamp analysis showed that ACh reduced the current elicited by hyperpolarizing steps from depolarizing holding potentials ("m-current"). Separate from these effects, ACh consistently reduced the amplitude of postsynaptic potentials. Combined application of ACh and the nicotinic receptor antagonist, hexamethonium (200μm), had no effect on the ACh response. Application of the muscarinic receptor antagonist, atropine (10μm), completely blocked the ACh effects. All aspects of the ACh response were mimicked by the muscarinic receptor agonist, carbachol (2-10μm). These studies indicate that ACh modulates human neocortical neurons by acting on muscarinic receptors.

189.1

CONSTRUCTION AND TESTING OF AN NSE PROMOTER-MAO-B EXPRESSION VECTOR FOR USE IN CREATING TRANSGENIC MICE THAT OVEREXPRESS MAO-B NEURONALLY. J.K. Andersen, K. Herrup, and X.O. Breakefield. Mol. Neurogenetics Unit, Mass. General Hosp., Charlestown, MA; Dev. Neurobiology Div., E.K. Shriver Ctr., Waltham, MA; Neuroscience Prog., Harvard Med. Sch., Boston, MA., 02115.

Studies are underway to evaluate the effects of abnormally high neuronal MAO-B activity on development and MPTP sensitivity in transgenic mouse lines. Normal humans inherit 50-fold variations in platelet MAO-B activities; this enzyme converts substances like MPTP to neurotoxins, leading to a Parkinsonian syndrome. Individuals with higher levels of MAO-B activity may be more susceptible to the actions of MPTP. An MAO-B cDNA has been generated from human liver mRNA by first strand synthesis, followed by PCR amplification using oligonucleotide primers that encompass the MAO-B coding region, defined previously (Bach et al, 1989). The resulting 1.7 kb fragment was subcloned into a Bluescript (Stratagene) vector for amplification, then into a eukaryotic expression vector containing the neuronal specific enolase (NSE) promoter (kindly provided by Drs. S. Forss-Petter and G. Sutcliffe). This vector construct is being evaluated for its ability to confer high MAO-B activity onto a mouse neuroblasoma cell line with no MAO activity. After MAO-B overexpression is confirmed in cultured cells, the linearized pNSE-MAO-B construct will be injected into early mouse embryos to create transgenics which overexpress MAO-B in neurons. [Funding was provided by NINDS NRSA (JKA) and United Parkinson Fdn. (XOB).]

189.2

DIRECT INFECTION OF RAT C6 GLIOMA CELLS IN THE BRAIN BY GRAFTING OF A RETROVIRUS PACKAGING LINE. M. P. Short, J-K. Lee, B. Choi, A. Malick, X.O. Breakefield and R. Martuza. Mol. Neurogenetic Unit, Neurol. and Neurosurg. Services, Mass. General Hosp., and Neurosci. Prog., Harvard Med. Sch., Boston, MA 02115

Retroviral vectors only integrate into the genome of dividing cells and can thus be used to selectively infect tumor cells in the adult rat brain. Retroviral gene delivery was assessed using the BAG vector (Price et al, PNAS., 84:156,1987) which bears the lacZ gene under the MoMLV LTR promoter-enhancer element, and histochemical staining for beta-galactosidase. Direct injection of this vector (900 cfu) into the adult rat brain, with or without prior inoculation of C6 glioma cells (2 X 10^5cells) resulted in labelling of only a few cells as assessed one week later. When the psi-2 BAG packaging line was grafted into the brain, labelled psi-2 cells could be found up to five-seven days post grafting; however no labelled cells were seen at two weeks post grafting, suggesting that the grafted cells had been rejected and no endogenous cells had integrated released vector. In contrast, when the psi-2 BAG packaging line was grafted into the brain region which had been previously seeded with rat C6 glioma cells (2 X 10^5 cells), extensive labelling of these tumor cells could be demonstrated ten days later. Thus grafting of retrovirus packaging lines into adult brain provides a mean to efficiently infect tumor cells in vivo . Presumably the grafted cells continue to release retroviral particles for an extended period, thus infecting more cells at the stage of division appropriate for viral integration. This approach can be used to selectively deliver "killer" or "suppressor" genes to tumor cells in the brain to curtail their growth.

189.3

SELECTIVE KILLING OF RAT C6 GLIOMA CELLS FOLLOWING RETROVIRUS-MEDIATED TRANSFER OF THE HERPES SIMPLEX VIRUS THYMIDINE KINASE GENE. **Z.D. Ezzedine,* D. Platika, R.L. Martuza & X.O. Breakefield** Molec. Neurogenet. Unit, Neurol. and Neurosurg. Services, Mass. General Hosp. & Neurosci. Prog. , Harvard Med. Sch., Boston, MA 02114 & 02115

The herpes simplex type I virus (HSV-1) thymidine kinase (TK) gene has been inserted into a retroviral vector derived from MoMLV and MoMSV (obtained from M. Rosenberg). The HSV-1-TK gene is under the transcriptional control of the retroviral LTR promoter, upstream from a neomycin resistance gene, itself under the transcriptional control of a Rous sarcoma virus promoter. Vector DNA was transfected into an ecotropic packaging cell line (psi-2), and replication-defective viral particles were produced and used to infect several C6 rat glioma-derived cell lines in culture. Recipient cells included C6-BU1-TK$^-$, a line lacking endogenous thymidine kinase activity, and a derivative of it, C6-BU1-TK$^-$-BAG, expressing bacterial β-galactosidase from a recombinant retrovirus stably integrated into the cell genome following infection with the BAG vector (obtained from C. Cepko). The dose-dependent sensitivity of cells with and without the HSV-1-TK gene to the toxic effects of ganciclovir was evaluated. (The HSV-1-TK enzyme has a greater affinity than its mammalian homolog for this nucleoside analog.) The dose of ganciclovir necessary to kill all cells was found to be approximately tenfold higher for uninfected cells compared to those infected with the HSV-1-TK retrovirus. An *in vivo* model for the selective killing of HSV-1-TK-expressing glioma cells by administration of ganciclovir is being tested in rats . This work paves the way for the use of retroviral vectors to selectively kill tumor cells in the CNS.

189.4

LOCALIZATION OF HPRT mRNA IN THE MAMMALIAN BRAIN BY IN SITU HYBRIDIZATION. H.A. Jinnah, E.J. Hess, M.C. Wilson, F.H. Gage, and T. Friedmann*. Depts. of Neurosciences and Pediatrics, UCSD; Dept. of Neuropharmacology, Reasearch Institute of Scripps Clinic, La Jolla, CA 92093.

Hypoxanthine-guanine phosphoribosyltransferase (HPRT) is a housekeeping enzyme involved in the recycling pathway for the purines. Congenital deficiency of HPRT in humans results in the Lesch-Nyhan syndrome, characterized by choreoathetosis, spasticity, self-injurious behavior, and hyperuricemia. Although the mechanism by which HPRT-deficiency leads to the neurological abnormalities remains unknown, a dysfunction of dopamine systems in the striatum is thought to play a role. In order to determine if the expression of HPRT correlates with known dopaminergic pathways or with the targets of dopaminergic pathways, we have examined the regional distribution of HPRT mRNA by in situ hybridization in the mouse brain. An HPRT-deficient transgenic mouse which does not express the HPRT mRNA served as a negative control. As might be expected for a housekeeping enzyme, HPRT was detected in most brain regions. However, the distribution of HPRT was not homogenous, but varied greatly among different brain regions. The highest levels of HPRT mRNA were observed in locus coeruleus, pons, hypothalamus, and hippocampus. Intermediate levels were detected in cortex, thalamus, substantia nigra, and cerebellum. The lowest levels were observed in the striatum, thalamus, tectum, and most of the brainstem. White matter had non-detectable levels of HPRT mRNA. This distribution suggests no strict correlation with the dopamine system or any other classical neurotransmitter system in the mouse brain. These in situ hybridization results are not consistent with previous biochemical studies indicating that the highest levels of HPRT activity in the primate brain occur in the striatum. This difference may reflect species differences or transport of HPRT protein from the sites of transcription. Studies involving the localization of HPRT mRNA in the primate brain are currently in progress.

189.5

NIEMANN-PICK TYPE C MOUSE- ULTRASTRUCTURAL AND IMMUNOCYTOCHEMICAL STUDIES Y. Higashi*, S. Murayama, C.E. Argoff*, P.G. Pentchev*, K. Suzuki, K. Suzuki, School of Medicine, Univ. of North Carolina, Chapel Hill, NC and Developmental and Metabolic Neurology Branch, NINDS, Bethesda, MD

An inborn cholesterol storage disorder in BALB/c mouse is considered to be an animal model of Niemann-Pick disease type C (Pentchev et al 1986). Neuronal storage, axonal spheroids and myelin abnormalities are prominent neuropathological features. To elucidate relationship of neuronal, axonal and myelin pathologies, ultrastructural and immunocytochemical studies were conducted. Neuronal storage was most prominent in deep cerebral cortex and in the CA3 and CA4 regions of the hippocampus, whereas axonal spheroids were mostly in projection fibers, subcortical relay nuclei and spinal white matter. Spheroids were detected by a monoclonal antibody 03-44 (Sternberger) that recognizes weakly phosphorylated epitope of neurofilament and by DF2, the monoclonal antibody against ubiquitin (Mori, 1987). Degeneration of myelin was observed in the cerebellar white matter, pencil fibers in the striatum and sciatic nerves. Corpus callosum, however, revealed a marked paucity of myelin without apparent myelin degeneration. These morphological differences may reflect regional and/or cellular difference in the cholesterol metabolism in this mutant mouse.

189.6

EXCITATORY AMINO ACID RECEPTORS IN THE SPASTIC HAN-WISTAR RAT: NEUROPHYSIOLOGICAL ANALYSIS USING THE XENOPUS OOCYTE ASSAY. R.W. Cohen, C.D. Hull, A.T. Campagnoni, N.A. Buchwald, and M.S. Levine. M.R.R.C., UCLA, Los Angeles, CA 90024.

A strain of Han-Wistar rat carries an autosomal recessive gene that produces spastic paresis characterized by ataxia, tremor and limb rigidity. Previously, our results suggested that a disturbance in glutamate transmission was found the caudal brain (cerebellum and brain stem) of these rats. The present study utilized the Xenopus oocyte assay to search for the glutamate receptor subtype involved in this brain dysfunction. Brains of unaffected or affected rats were bisected into rostral and caudal halves and mRNA was isolated and injected into oocytes. The oocytes were voltage-clamped at -60 mV and exposed to 1 mM L-glutamate, 500 μM kainate, 200 μM NMDA or 1 mM GABA. Oocytes injected with unaffected-rostral (33.7±5.7 nA; n=6) or affected-rostral (36.3±6.6 nA; n=10) mRNA showed similar current responses to glutamate; oocytes injected with unaffected-caudal (42.8±4.8 nA; n=8) or affected-caudal (103.7±8.5 nA; n=11) brain mRNA had statistically different ($p<0.001$) responses. Values in parentheses are average peak inward current amplitudes ±S.E.M and sample size. The inward current amplitudes were significantly larger in affected-caudal oocytes ($p<0.0001$) when kainate was applied: Unaffected-rostral=46.0±11.5 nA (n=4); affected-rostral=62.2±14.4 nA (n=4); unaffected-caudal=24.0±4.7 nA (n=5); affected-caudal=167.5±10.0 nA (n=11). We verified presence of caudal brain kainate receptors by establishing that the reversal potential for kainate was 0 mV and by applying a kainate antagonist CNQX which blocked 90% of the kainate response. There were no differences in responses of oocytes to NMDA or GABA. The results indicate a genetic disturbance in the kainate excitatory amino acid receptor subtype in the caudal brain of these mutant rats. Supported by USPHS HD05958.

189.7

LOWER HYPERTHERMIC EFFECT OF MK-801 IN SELECTIVELY BRED HYPERCHOLINERGIC THAN IN NORMOCHOLINERGIC RATS. **O.Pucilowski*, W.Danysz*, D.H.Overstreet, A.H.Rezvani*, B.Eichelman and D.H.Janowsky. Ctr. for Alcohol Studies and Dept. of Psychiatry, Univ. of NC, Chapel Hill, NC 27599.**

Flinders Sensitive Line (FSL) of rats has been selectively bred for increased sensitivity to cholinergic agonists. Typically FSL rats react with twice as high temperature drop as the similarly bred Flinders Resistant Line (FRL) not only to the muscarinic agonist oxotremorine but also to other hypothermic agents, e.g. apomorphine, buspirone and ethanol. This suggests that selective breeding altered the activity of many interacting transmitter systems. This study compared the FSL and FRL rats in terms of their hyperthermic response to the PCP receptor agonist MK-801. MK-801 binding characteristics were also compared. After establishing a temperature baseline the rats were injected ip with saline or 0.2 mg/kg of MK-801 and the colonic temperature was measured every 30 min for 240 min. We have found FSL rats to react with a delayed hyperthermia having a significantly lower hyperthermia for the first 120 min. Thereafter the response did not differ in FRL and FSL rats. Both groups also had similar affinity and number of [^{3}H]MK-801 labelled PCP receptors. These findings suggest the genetically developed cholinergic supersensitivity attenuated the secondary mechanisms involved in the PCP receptor-mediated hyperthermic response.

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189.8

SPATIAL LEARNING, PAW PREFERENCE, AND BRAIN ECTOPIAS IN NZB AND BXSB MICE. V. H. Denenberg, G. F. Sherman, G. D. Rosen, and A. M. Galaburda. Biobehavioral Sciences Graduate Degree Program, Univ of CT, Storrs, CT., 06269; and Beth Israel Hospital and Harvard Medical School, Boston, MA 02215

Cortical ectopias have been found in some mouse strains that have autoimmune diseases, but the functional implications of the ectopias are not well understood. We previously found that ectopias and paw preference jointly affected discrimination learning in BXSB mice (Denenberg et al., Neurosci. Abstr., 1988). We now report on the effects of these variables upon spatial learning. Sixty-two NZB and 67 BXSB 6-week old mice were given the Collins paw preference test. At 8 weeks they were tested for water escape, a task requiring the animal to use spatial information to locate a submerged platform. At 12 weeks they were perfused and their brains examined for cortical ectopias. Mice were classified by sex, paw (left/right) and ectopia (yes/no), and their swimming speed over 5 trials was evaluated. Within the NZB strain there was a Paw x Ectopia interaction ($p<.02$). Within the Ectopic groups left pawed mice had the fastest swimming time while right pawed ones were the slowest. The right and left pawed Non-Ectopic mice did not differ. The identical profile was also significant in the BXSB strain except that it was restricted to male mice. In both strains there was a Sex x Trials interaction: both groups of females were slower on Trial 1 and faster thereafter. Thus, cortical ectopias are associated with simple spatial learning for left pawed males of both strains and also left pawed NZB females.

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189.9

LEARNING IN NZB MICE AND THE EFFECTS OF ENVIRONMENTAL ENRICHMENT. L. M. Schrott, N. S. Waters, G. F. Sherman, G. D. Rosen, A. M. Galaburda, and V. H. Denenberg. Biobehavioral Sciences Graduate Program, Univ of CT, Storrs, CT. 06269; and Beth Israel Hospital and Harvard Medical School, Boston, MA 02215

NZB mice are known to perform poorly in active and passive avoidance learning. However, NZBs, as well as BXSB mice, are able to learn a simple spatial water escape task (Denenberg et al., Neurosci. Abstr., 1990). This implies that the NZB does not have a generalized learning deficit. The purpose of this study was to (1) examine NZB learning in a variety of tasks, (2) determine if rearing in an enriched environment facilitated learning, and (3) investigate the effects of cortical ectopias (typically present in 20-40% of NZB mice) upon learning. Twenty male NZB/BINJ mice were placed in enriched environments at 6-7 weeks of age and remained there throughout the course of behavior testing; 18 other male NZBs were group reared in standard laboratory cages. Testing started at 7-8 weeks and included water escape, discrimination learning, Morris maze learning, and two-way active avoidance . They were perfused at 18-19 weeks and their brains examined for ectopias. All mice performed poorly in avoidance learning. They had a weak learning curve in water escape, and learned well in the discrimination apparatus and the Morris maze. The Enriched group learned faster on the latter two tests ($p<.01$ in both cases). In addition, Enriched mice without ectopias had the best percent correct in discrimination learning ($p<.04$). Conclusions: (1) NZBs can effectively learn both spatial and non-spatial associative tasks; therefore, they do not have a generalized learning deficit; (2) learning speed of both normal and mice with ectopias can be improved by Environmental Enrichment; however (3) Enrichment was not able to increase the percent of correct discriminations of mice with ectopias.

Supported, in part by NIH grant HD20806

189.10

Separation of Nature and Nurture in an Animal Model of Affect, L.Fochtmann, E. Edwards, and F.A.Henn. Dept of Psychiatry, SUNY at Stony Brook, NY 11794.

The learned helplessness model of depression and/or anxiety developed by Seligman has proven to be an excellent model for looking at such diverse issues as the pharmacology of anti-depressants and the role of control in shaping behavior. In this model an animal is exposed to random uncontrollable aversive stimuli for a period and then develops a behavioral deficit when subsequently exposed to aversive stimuli. We have developed this model in rodents and using a design with a yoked control have duplicated the finding that the development of escape deficient behavior is dependant on the lack of control over the aversive stimuli, not the stimuli itself. In addition, we have shown that the development of "helpless" behavior is correlated with long term receptor changes in specific brain regions suggesting that psychological inputs can alter brain structure and behavior in parallel. This demonstrates the effect of nurture on behavior. We now report that the susceptibility to develop helpless behavior in rats can be enhanced by selective breeding. We will report on the development of two strains of rats by selective breeding. The first, after twenty generations, is essentially spontaneously helpless while the second is resistant to the development of helplessness. We will also present quantitative autoradiographic assessments of β and 5-HT_{1b} receptor binding in these two strains, as well as several behavioral parameters which distinguish between them.

189.11

SELECTIVE BREEDING FOR DISTINCT STIMULUS FILTERING/ SELECTIVE ATTENTION BEHAVIORAL PHENOTYPES. C. Kilts, R. Scibilia, L. Dunn. Dept. of Psychiatry, Duke Univ. Med. Ctr., Durham, N.C. 27710.

The maintenance of a flexible and efficient information processing strategy represents a primary defect in schizophrenia. We sought to develop via the selective breeding of rats under standardized environmental conditions a homogeneous population of subjects exhibiting an analogous deficit in stimulus filtering/ selective attentional functioning, using a latent inhibition (LI) paradigm as an index of behavioral phenotype. The LI performance of a population of outbred rats exhibited a large variance. The generation of successive filial generations by the selective intra-litter breeding of subjects exhibiting maximal and negligible LI resulted in both F_2 and F_3 progeny which exhibit statistically significant differences in the LI of a conditioned response. These results indicate that behavioral genetics represents a promising means of developing animal models for schizophrenia research.

(Supported by Scottish Rite Foundation)

190.1

AMYLOID PRECURSOR PROTEIN EXPRESSION IN RAT BRAIN. G.S. Huber, J. Löffler*, A. Schuler* and W.E. Haefely. Pharmaceutical Research Department, F.Hoffmann-La Roche Ltd, 4002 Basel, Switzerland.

The deposited B-amyloid protein in senile plaques of Alzheimer's disease presumably derives from one or more precursor proteins (APPs) by aberrant proteolytic processing. A family of three different APPs have been identified so far. We have raised a series of monoclonal antibodies against synthetic peptide domains of APPs in order to differentiate between the individual forms and also to examine their expression in rat brain. We use Western blotting to characterize different molecular forms of APP in soluble and particulate fractions of brain homogenates. We find most of the immunoreactive proteins present in homogenates and soluble brain fractions containing 0.15 M NaCl. In the adult rat brain, the most abundant N-terminal-immunoreactive forms are of M_r 135 kD, 100 kD and 75 kD (an additional 50 kD form is recognized by one of the antibodies). In contrast, the major Kunitz inhibitor containing form in the soluble fraction has a M_r of 75 kD accompanied by less abundant forms of 135 kD, 100 kD and 50 kD. The presence of these proteins in our soluble brain fraction in the absence of detergents suggests a rather loose association of these proteins with cellular membranes.

190.2

THE NEUROTOXIC CARBOXYTERMINAL FRAGMENT OF THE ALZHEIMER AMYLOID PRECURSOR PROTEIN MAY INTERFERE WITH CELL ADHESION. L.R. Dawes, S.F. Fidel, M.R. Kozlowski, A. Spanoyannis*, R.L. Neve. Dept. of Psychobiology, U. of Calif., Irvine, CA 92717 and Bristol-Myers Squibb, Wallingford, CT 06492.

We previously reported that the carboxyterminal 105 amino acids of the Alzheimer amyloid precursor protein (AB1) is toxic specifically to neurons (Science 245: 417, 1989). Addition of conditioned medium (CM) from AB1-transfected cells to primary rat hippocampal cultures leads to death of the neurons in these cultures within 4 days. We demonstrate that there are characteristic morphological features of this AB1 neurotoxicity. Freshly plated E18 hippocampal cultures demonstrate an initial burst of process outgrowth when exposed to AB1 CM. However, after several days the cultures treated with AB1 CM display clumping of neurons and increased fasciculation of their processes. A significant number of neurons have abnormally short processes with enhanced arborization relative to controls. These morphological features suggest that AB1-treated neurons possess a decreased affinity for substrate compared to controls. We hypothesize that AB1 may alter cell surface protein processing and thereby influence neuron-substrate interactions. To determine whether AB1 interacts with a cell surface molecule, we synthesized radiolabeled AB1 in an *in vitro* transcription/translation system, and showed that it binds to the surface of PC12 cells. This binding was partially displaceable by unlabeled AB1.

190.3

QUANTITATIVE CHARACTERIZATION OF THE AMYLOID PROTEIN PRECURSOR (APP) EXPRESSION IN RAT BRAIN PRIMARY CULTURES. P.I.Woloshin, A.Parent, F.P. Eckenstein, Dept. of Cell Biology and Anatomy, Oregon Health Sci. Univ., Portland, OR 97201

The cellular sources of APP in the brain have not been well determined and analyses of the changes in the relative expression levels of the various transcripts has produced conflicting results. We have focussed on a defined cellular population and have quantitatively analyzed the relative levels of expression of the three most abundant APP mRNA species, APP695, 751 and 770, under various conditions. Changes in the relative levels of these transcripts in the brain may be a contributing factor to the deposition of the amyloid peptide, a component of senile plaques. Over 90% pure populations of Type 1 and Type 2 astrocytes, neurons and meningeal fibroblasts were obtained from neonate rats and were grown in a serum-free defined medium. The cells were then stimulated with one of the following factors: basic fibroblast growth factor, platelet derived growth factor, interleukin-1, fetal calf serum or dibutyryl cAMP.

Total RNA was extracted from these cells and analyzed with Polymerase Chain Reaction (PCR), Northern Blot and Solution Hybridization. High specific activity cRNA probes were synthesized which are complementary to a part of the APP 695 coding sequence. For PCR, oligonucleotides were synthesized which flank the Kunitz inhibitor domain and were found to produce different length reaction products depending on which APP transcript is present. Preliminary results indicate that cultured Type 1 astrocytes produce relatively higher levels of APP 751 and APP 770 than whole cortex and that fetal calf serum stimulates the overall level of APP mRNA in Type 1 astrocytes over that found in cells grown serum-free. It is interesting to speculate that these changes may be analogous to the reactive gliosis which occurs in Alzheimer's disease.

Support of this work by a grant from the American Health Assistance Foundation is gratefully acknowledged.

190.4

THE AMYLOID PRECURSOR-RELATED TRANSCRIPT LACKING THE ß/A4 SEQUENCE IS INCREASED IN ALZHEIMER DISEASE BRAIN. G.A. Higgins[1], J. Rogers[2] and R.L. Neve[3]. [1]Gerontology Res. Ctr., NIA/NIH, Baltimore, MD 21224, [2]Inst. for Biogerontology Res., Sun City, AZ 85351, and [3]Univ. Calif., Irvine, CA 92717.

We have used a combination of RNA blotting, RT-PCR, and *in situ* hybridization to study the differential expression of APP-695, APP-751, and APP-770 mRNAs in Alzheimer's disease (AD). We have also examined the expression of the amyloid precursor-related protein (APRP) transcript that encodes a 563 amino acid protein which lacks the ß/A4 protein but contains the Kunitz-type protease inhibitor (KPI) sequence. Six brain regions were examined in AD patients and normal aged controls, using a combination of these methods within the same brains. KPI-containing forms of APP were elevated relative to APP-695 in AD. Dramatic increases in APRP-563 mRNA were observed in the nucleus basalis, parahippocampal gyrus and occipitotemporal cortex in AD, but not in the striatum, primary visual cortex, or hippocampal formation. These studies suggest that a secreted form of APP (APRP-563) may play a role in amyloid pathogenesis.

190.5

PARTIAL CHARACTERIZATION OF RAT cDNA CLONES SIMILAR TO A HUMAN AMYLOID PRECURSOR-RELATED PROTEIN mRNA. C. E. Byrne, C.J. Smith-Maxwell, V.H. Mah, C.A. Sherman, R.L. Neve[2] and G.A. Higgins. Univ. of Rochester Med. Ctr., Rochester, NY 14642; Gerontology Res. Ctr., NIA/NIH, Baltimore, MD 21224; and [2]Univ. California, Irvine CA 92127.

Increased expression of amyloid precursor protein (APP) mRNAs has been associated with amyloid deposition in Alzheimer's disease. Recent work suggests that forms of APP mRNA which contain a Kunitz protease inhibitor (KPI) motif are increased in pathologically-vulnerable brain regions in AD. Another amyloid precursor-related protein (APRP) of approximately 563 amino acids, which lacks the ß/A4 protein sequence, but contains the KPI motif, has also been isolated (de Sauvage, F. and Octave, J-N., Science 245:651-653 (1989)). Preliminary northern analysis using a probe for human APRP-563 shows that several related transcripts of 1-2 kb in length are expressed in the rat CNS. In order to further characterize rat APRP mRNAs, we used RT-PCR to isolate several different rat cDNAs. Partial sequencing shows that several rat brain mRNAs share partial identity with human APRP-563. Further studies are aimed at a more complete characterization of these rodent transcripts.

190.6

DIFFERENTIAL EXPRESSION OF AMYLOID PRECURSOR PROTEIN TRANSCRIPTS IN THE DEVELOPING RAT CNS. C.A. Sherman, S. Koh and G.A. Higgins. Univ. Rochester, Rochester, NY 14642 and Gerontology Res. Ctr., NIA/NIH, Baltimore, MD 21224.

Recent evidence suggests that the amyloid precursor protein (APP) may regulate cell growth. In order to study the contribution of different APP variants on neuronal growth in the developing CNS, we used the reverse transcription - polymerase chain reaction (RT-PCR) to examine differential APP gene expression in postnatal rat brain. Multiple species of APP mRNA were detected in the rat CNS, including APP-695, APP-714, APP-751 and APP-770. To quantify the relative amounts of different APP transcripts at postnatal days (PD) 1, 5, 10, 15, 22, 30 and in the adult, we used competitive RT-PCR analysis (Wang, A.M. *et al*, PNAS, 86:9717, 1989). APP-695 accounts for more than 75% of total APP mRNA at all developmental stages. APP-695 mRNA also showed the greatest variation in abundance during development, reaching a peak of expression at PD 15 - 22, the period of maximal NGF-responsiveness in the rat forebrain. These results suggest the possibility of an interaction between growth factor responsivity and APP gene expression in the developing CNS.

190.7

STUDIES ON THE EXPRESSION AND SUBCELLULAR ORGANIZATION OF THE ALZHEIMER'S AMYLOID PRECURSOR PROTEIN IN GLIA.
L.M.Refolo†,V.L.Friedrich Jr.,D.Casper,M.Blum,F.Berkenbosch and N.K.Robakis.Dept. of Psychiatry,Brookdale Center of Molecular Biology and Fishberg Center for Neurobiology. Mt. Sinai Medical Center,New York,N.Y. 10029.

Neuritic plaques are a histopathological hall mark of Alzheimer's disease.However,the source of the B-peptide comprising the plaque amyloid core remains unknown.Much work has focused on a neuronal source of the B-peptide. We are currently testing the hypothesis that glia are a B-peptide source.Northern and Western analysis of rat primary, cortical glia indicated that BAPP 695 is the predominant form expressed,while KPI forms are not dectable.In contrast the rat glioma C6 expresses high levels of KPI containing BAPP.Immunocytochemical examination of primary glia revealed that BAPP expression was limited to Type I astrocytes and neither oligodendrocytes nor A2B5+ progenitors expressed detectable levels of BAPP.Immunolocalization indicated that the subcellular organization of BAPP was similar to the fibrillar pattern observed for GFAP,suggesting a cytoskeletal association for BAPP.Detergent extraction of C6 cultures futher support the notion of a cytoskeletal association for BAPP.Experiments designed to study "factors" modulating BAPP expression in glia have found that both Il-1 and bFGF increase BAPP several fold,while both 8-bromo cAMP and NGF were found to have no effect.

190.8

CELLS TRANSFECTED WITH AMYLOID PRECURSOR PROTEIN DNA FRAGMENT AB-1 PRODUCE MEDIUM TOXIC TO DIFFERENTIATED PC12 CELLS.
T.L. Martin & E.E. Baetge. Bristol-Myers Squibb Pharm. Res. Inst., CNS Biology Wallingford, Ct. 06492

Amyloid deposits in cortex and hippocampus comprise one of the two pathological hallmarks of Alzheimer's Disease. Amyloid precursor proteins (APP) are present in normal brain and have been shown to be synthesized in neurons and glial cells by alternative splicing of a single gene. We report here on data corroborating the findings of Yankner et al., Science 245: 417-420, 1989 who demonstrated that a portion of the APP gene (AB-1) is toxic to postmitotic neurons and differentiated PC12 cells.

PC12 cells were transfected with an SV40-neo selection/expression vector containing the Bgl II-Xmn I fragment of the human APP gene (AB-1 as defined by Yankner et al.). G418 resistant clones were isolated and RNA extracts probed for expression of the foreign fusion mRNA. The 600 bp Bgl II-Xmn I APP fragment hybridized to two bands, the lower molecular weight form being unique to the AB-1 transfected cell lines. Medium conditioned by AB-1 or control transfected cell lines was tested for toxicity in NGF differentiated PC12 cells. Viability was spectrophotometrically quantitated with the vital dye MTT revealing up to 90% death of cells exposed to AB-1 conditioned medium. Our results indicate that the protein(s) secreted from AB-1 transfected cells is toxic to differentiated PC12 cells. These findings further suggest the possibility that an APP fragment may play a role in the cell death characteristic of the Alzheimer's diseased brain.

190.9

AMYLOID PRECURSOR PROTEIN AND NEURONAL CELL SURVIVAL AS STUDIED BY ANTISENSE TRANSFECTION.
A. LeBlanc,* D. Kovacs, F. Villare, M.Tykocinski, L. Autilio-Gambetti and P. Gambetti. Div. of Neuropathol, CWRU, Cleveland OH 44106.

The function of the amyloid precursor protein (APP) was investigated in the neuroblastoma cell line, LaN-1 using antisense RNA to inhibit translation of all forms of APP. Lipofectin-mediated transfection of LaN-1 was achieved with a replicating episomal vector containing a 1Kb fragment of APP_{695} in antisense orientation. APP mRNA as well as cellular and secreted APP proteins were reduced by 75-90% in antisense amyloid transfected (ASAT) LaN1 relative to that of normal LaN-1. ASAT cells grew slowly compared with normal and RepIII-transfected cells, and exibited a high rate of cell death . These conditions were partially reversed by the addition of normal LaN-1 conditioned media (CM). To determine if the addition of CM increased cell division, ^{3}H-thymidine was added to normal, ASAT and RepIII transfected LaN-1 at 1,2,3 and 4 days of culture in the presence(+) or absence(-) of CM. The percentage of ASAT cells synthesizing DNA was lower (~30%) than normal LaN-1 (~50%) or RepIII transfected LaN-1 (~46%) in CM- and this difference did not change with time. A higher number of normal LaN-1, RepIII-transfected and ASAT cells in CM+ incorporated ^{3}H-thymidine at day 1 of culture but then decreased to the level found in CM- by day 2 and thereafter. Despite the lack of significant difference in DNA synthesis by ASAT cells in CM+ or CM-, at day 4 the cells grown in CM- were less abundant and appeared to undergo degeneration. These results indicate that APP affects cell survival rather than cell proliferation of LaN-1. Supported by NIH Merit Award AG08155 and the Britton Fund.

190.10

A HIGH RESOLUTION ELECTRON MICROSCOPIC STUDY OF ALZHEIMER NEUROFIBRILLARY TANGLES AND SENILE PLAQUE CORE AMYLOID.
K. Iqbal, G.C. Ruben*, H.M. Wisniewski* and J.E. Johnson Jr. Inst. Basic Res., Staten Island, NY, Dept. Biol. Sci., Dartmouth Coll., Hanover, NH, Dept. Neurosci., Stanford Res. Inst., Menlo Park, CA.

Tangles and plaque cores were isolated, using non-denaturing conditions, from frozen autopsied brains of patients with Alzheimer disease, were freeze-dried and vertically replicated (FDVR) and the replicas were examined by electron microscopy. Tangles contained paired helical filaments (PHF) and 2.1±0.2 nm filaments in different proportions. The PHF were generally right-handed helices with a twist period (L) averaging 79.3±5.9 nm and a maximum filament width (W) averaging 14.9±1.0 nm. The regions of minimum width (T) showed three size averages of 2.4±0.3 nm, 4.9±0.6 nm and 9.6±1.4 nm. These measurements are very similar to previous negative stain measurements and when averaged together produce L=74.4±8.0 nm, W=16.2±2.7 nm, and T=5.2±2.6 nm. The average PHF reported from thin sectioned Alzheimer tissue indicate that the PHF widths have been increased by a positive stain deposition of 9.4 nm. The concept of PHF twisting around each other longitudinally is not supported by the FDVR images or by the measurements from negative staining and FDVR. Images of plaque cores showed that amyloid has a more solid appearance than the tangles and was constructed of 6.8-19.5 nm filaments which are unlike any of those found in tangles. Since the term PHF incorrectly describes the filament structure we suggest that a more accurate term would be Alzheimer helical filaments (AHF).

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SYMPOSIUM. BRAIN MODULATION OF SENSORY SIGNALS.
R. B. Barlow, Jr. , Syracuse University, (Chairperson); I. Ari*, University of Chicago; S. M. Highstein, Washington University School of Medicine; R. Dubner, NIH-NIDR; E. Kaplan, Rockefeller University.

Sensory pathways are two-way channels that allow the brain to modulate the sensory information it receives. The brain's output may tune the signals of a sensory organ to specific stimuli, shape sensory signals to current or anticipated environmental conditions, modulate the metabolic activity of a sensory organ, or influence other properties such as neuronal development.

The symposium will address the following questions:
- What controls the brain's output?
- What effect does the brain's output have on sensory signals?
- What cellular mechanisms underlie the brain's modulation of sensory signals?

Answers to such questions are far from complete. However, numerous studies show that efferent modulation of sensory signals is a fundamental aspect of brain function. Clear examples will be presented for the auditory, pain, vestibular and visual systems. Knowledge of the central modulation of sensory signals should provide a better understanding of the functional organization of the brain and of the neural mechanisms underlying behavior.

195.1

EXPRESSION OF C-FOS IN CRH NEURONS OF THE FETAL HYPOTHALAMUS DURING PARTURITION IN THE SHEEP.
G.E. Hoffman, T.J. McDonald *, and P.W. Nathanielsz. Dept. Physiology, Univ. Pittsburgh, School of Med., Pittsburgh, PA 15261 and Laboratory for Pregnancy and Newborn Research, Dept. of Physiology Cornell Univ., Ithaca, NY 14853.

The fetal pituitary adrenal axis plays an important role in parturition. Fetal corticotrophin releasing hormone (CRH) neurons in the paraventricular hypothalamus undergo changes in immunoreactivity (IR) late in gestation suggestive of cellular activation; however, direct evidence for stimulation of CRH neurons at time of parturition is lacking. The present study tested the hypothesis that activation of the fetal hypothalamic CRH system, evidenced by expression of the oncogene product c-fos, accompanies labor in sheep. Monitoring of uterine EMG activity determined the onset of labor. The brains of six fetuses (removed by caesarian section under anesthesia) and five newborn sheep were perfused and stained for c-fos and CRH. c-Fos was detected with an N-terminal directed antibody (Cambridge Research OA 11-821, 1:44,000); an anti-CRH serum (Dr. A.-J. Silverman, 1:50,000) detected comparable numbers of CRH neurons in all animals. Prior to labor, less than 5% of CRH neurons expressed c-fos. At the time uterine contractions were first detected, 70% of CRH neurons expressed c-fos, and c-fos IR persisted until just after birth. c-Fos staining declined rapidly reaching pre-labor levels by 2-3 hrs after birth.

These data are consistent with the hypothesis that fetal CRH neurons are stimulated during labor, and that termination of stimulation probably occurs rapidly after delivery.

Supported by NIH PO1 HD 21350 and RO1 NS 23858.

195.2

c-fos mRNA, FOS AND FOS-RELATED ANTIGENS INDUCED BY HYPERTONIC SALINE AND STRESS: AN IN SITU HYBRIDIZATION AND IMMUNOCYTOCHEMICAL STUDY. Frank R. Sharp and Stephen M. Sagar, Depts. Neurology and Physiology, Univ. Calif. and VA Medical Center, San Francisco, CA. 94121

Hypertonic saline (HS) induced c-*fos* mRNA in magnocellular (PVNm) and parvocellular (PVNp), supraoptic n. (SON), and lamina terminalis (LMT). This occurred within 5', was maximal at 30-60', and disappeared by 180'. Fos protein, detected using a monoclonal antibody, was maximal at 1-2h, and disappeared 4-5h after hypertonic saline.

In contrast, Fos-like immunoreactivity (FLI) was detected in PVNm, PVNp, SON, and LMT at 1h, 4h, 24h, 3d, and 7d following administration of HS. This and other data suggests that FLI at >4h represents FRAs, proteins immunologically and functionally related to Fos.

Induction of c-*fos* mRNA due to "stress" of injections was assessed by comparing isotonic saline (IS) injected subjects to untouched controls. IS induced c-*fos* mRNA in PVNp, hypothalamus, suprachiasmatic n., cingulate cortex, neocortex, ventral lateral septal n., piriform cortex, hippocampal pyramidal neurons, dentate gyrus granule cells, certain thalamic n., bed n. of stria terminalis, the amygdaloid n. and other structures.

Osmotic and stressful stimuli both induce the transient c-*fos* (0-4h) and longer duration *fra* (0-7d) genes which in turn regulate target gene expression.

195.3

ALTERATIONS TO FLUID HOMEOSTASIS IN THE RAT LEADS TO CHANGES IN PEPTIDE GENE EXPRESSION IN DISCRETE REGIONS OF THE LATERAL HYPOTHALAMUS. by Alan G. Watts. NSL, The Salk Institute, La Jolla, CA 92037.

Fluid homeostasis in the mammal is achieved by the complex interaction of cellular, hormonal and behavioral mechanisms. Dehydration initiates a series of responses aimed at restoring normal hydration. A number of studies have shown that forced dehydration or increased ingestion of sodium both lead to increased synthesis of vasopressin and oxytocin within the magnocellular population of the hypothalamic paraventricular (PVH) and supraoptic (SON) nuclei. Additionally, changes in gene expression have also been reported for corticotropin-releasing hormone (CRH) within the PVH and SON after both treatments. In the present study, in situ hybridization (ISH) using a number of different peptide mRNAs was used to investigate possible changes in other regions of the hypothalamus known to be involved in the response to osmotic stimulation.

Male Sprague-Dawley rats (275-400g BW; maintained on a 12:12 light:dark schedule) were given 2.5% saline to drink for 3 days or allowed unrestricted access to water. At the end of the treatment all animals were deeply anesthetized and perfused (between 13.00h and 15.00h) with ice-cold 4% paraformaldehyde in 0.1M borate buffer at pH9.5. After 24h post-fixation 15μ coronal sections through the hypothalamus were cut and mounted on poly-L-lysine coated slides. Sections were pre-hybed, hybridized and post-hybed as previously described (J.Histotech 12:169). Hybridized sections were exposed to Cronex-4 X-ray film for an appropriate period, and then dipped in Kodak NTB-2 emulsion and developed accordingly. cRNA probes for a number of different peptide mRNAs were used including prepro-CRH (ppCRH), prepro-enkephalin A (ppENKA) and prepro-neurotensin/neuromedin N (ppNT/NMN). Examination of the exposed material showed that ppENK and ppNT/NMN mRNAs were found in a number of regions of the hypothalamus including discrete regions of the lateral hypothalamus (LHA). ppCRH could only be found in the medial parvicellular part of the PVH. However, after osmotic stimulation the content of ppNT/NMN mRNA was increased in the LHA, but now ppCRH mRNA could also be seen in the same LHA region that expressed ppNT/NMN. These results suggest that regions of the hypothalamus that provide descending autonomic projections and also may be involved in the control of ingestive behavior, show increased amounts of peptide mRNA after a treatment known to alter behavioral mechanisms, and that alterations in gene expression after osmotic stimulation are not restricted to the hypothalamo-neurohypophysial axis.

195.4

CONTRIBUTION OF ARACHIDONIC ACID METABOLITES TO BASAL AND INTERLEUKIN 1-INDUCED CRF SECRETION. E. Redei, B.J. Branch*, R.E. McGinnis* and A.N. Taylor. Dept. of Psychiatry, Univ. of Pennsylvania, Philadelphia, PA 19104 and Dept. of Anatomy, UCLA, Los Angeles, CA 90024.

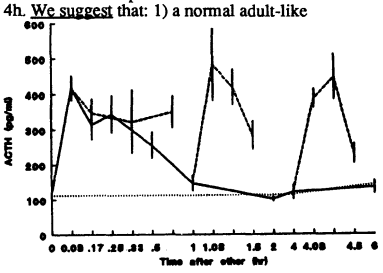
The contribution of arachidonic acid (AA) metabolites to basal and Interleukin 1 beta (IL-1) induced release of CRF was studied using superfusion of hypothalamic blocks from male rats. Inhibition of both lipoxygenase and cyclooxygenase pathways by 500 uM indomethacin (INDO) increased CRF secretion significantly (167.3+35.0 vs. 58.7+3.3 pg/ml CRF). Of the AA metabolites tested only 15-HETE increased in response to INDO (814.1+32.3 vs. 336.1+19.3 pg/ml 15-HETE) with kinetics identical to that found in the CRF response. Nordihydroquaiaretic acid (NDGA, 10 uM), a lipoxygenase inhibitor, produced a moderate CRF response with 4-6 min latency, 15-HETE again showing the same kinetics and percentage increase. Addition of IL-1 (50 U/ml) resulted in marked increases of CRF with simultaneous 15-HETE secretion over basal release (CRF: 93.7+17.6%; 15-HETE: 120.7+25.8%). This response was not altered by NDGA.

In conclusion: INDO, NDGA and IL-1 increased CRF and 15-HETE secretion with a constant ratio despite very different degrees of stimulation by these secretagogues. This suggests that CRF and 15-HETE secretion are intimately connected, possibly consequtive because 15-HETE serves as a second messenger for CRF secretion.

195.5

IMPAIRMENT AND RECOVERY OF THE ACTH RESPONSE TO A SECOND STRESSOR IN 10 DAY-OLD RATS: IS STEROID FAST FEEDBACK INVOLVED? C.-D. Walker*, K. Scribner*, M.J.Meaney and M.F. Dallman*. Dept of Physiology, UCSF, San Francisco, CA 94143 and Dept of Psychiatry, McGill University, Montreal H4H 1R3, Canada.

From days 3 to 14 of age, it is recognized that pituitary-adrenocortical activity is low in rat pups as assessed by a lack of ACTH and corticosterone (B) secretion at specific times after exposure to stress. In this study, we determined the time course of ACTH and B responses to a single or a double 3 min-exposure to ether vapor at 5, 60 and 240 min after the initial stressor in intact or adrenalectomized (ADX, 5d previously) 10 day-old rats. Plasma ACTH levels in intact pups are shown in the figure. In ADX pups no rapid decline in ACTH levels was observed at 10min (cont=1489±222 pg/ml, 5min =2125±203, 10min=2347±323) and baseline values were restored 30min post stress. Further ACTH and B increases after a second stressor were abolished when ether was given 5min after the first exposure but were normal if the 2nd exposure occured at 1h or 4h. We suggest that: 1) a normal adult-like response to one or two stresses is observed in 10 day-old pups if the interval between the two stressors is greater than 5min and 2) depletion of readily releasable ACTH pools or fast B inhibition of ACTH release at 10 min after ether stress might explain the lack of pituitary responses to ether stress described earlier.



195.6

HEMORRHAGE-INDUCED POTENTIATION OF PITUTARY-ADRENOCORTICAL RESPONSES IN AWAKE RATS. K.V. Thrivikraman and P.M. Plotsky. The Clayton Fndn. Laboratories. for Peptide Biol., The Salk Institute, La Jolla, CA 92037.

Potentiation of pituitary-adrenal responsiveness to a second stimulus in the face of glucocorticoid negative feedback occurs in many species. A model of pituitary-adrenocortical potentiation in response to paired hemorrhages (HEM) has been developed in awake, male rats (Sprague-Dawley, 450-500 g). All experiments were performed between 0900-1330 h in preinstrumented (3-4 da) rats habituated to the test chamber. Systemic blood samples (300 µl) were collected at -5, 0, 3, 6, 9, and 18 min with respect to HEM. Volumes were replaced by saline. HEM was performed by removal of 14 ml blood/kgBW (estimated 20% of blood volume) over 3 min via jugular cannula, with-holding blood for 7 min, then reinfusing over 3 min. All rats sustaining blood loss exhibited increases in plasma ACTH (ANOVA, $p<0.01$) and corticosterone (B; $p<0.01$). ACTH returned towards prestimulus levels after retransfusion. In group A rats, HEM1 and HEM2 were separated by 90 min. A significant enhancement of the peak change in ACTH at +9 min was observed in HEM2 vs HEM1 (929±292 vs 730±269 pg/ml), with the integrated response of HEM2 significantly greater (paired t, $p<0.05$) than that of HEM1 (1726±541 vs 1272±439 pg). Prehemorrhage B levels were 16±1 ng/ml (HEM1) and 18±2 ng/ml (HEM2). A significant ($p<0.01$) elevation in B was evident by +9 min during HEM1 (95±36 ng/ml) and HEM2 (129±55 ng/ml); 4 of the 5 rats exhibited larger, significant ($p<0.05$) total increases in B during HEM2 vs HEM1 (386±111 vs 248±83 ng). Rats receiving only 1 HEM episode corresponding to the time of HEM2 (group B) exhibited ACTH secretory responses equivalent to those measured during HEM1 of group A (525±172 vs 730±269 pg/ml; $p>0.05$). These data suggest that pituitary-adrenocortical responsiveness to HEM is potentiated by a prior HEM in awake rats. Underlying mechanisms are currently under investigation.

195.7

ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL EVIDENCE FOR ADRENOCEPTOR SPECIFICITY IN THE HYPOTHALAMIC REGULATION OF ADRENOCORTICAL SECRETION. D. Saphier Department of Pharmacology, LSU Medical Center, Shreveport, LA 71130.

Noradrenergic projections to the hypothalamic paraventricular nucleus (PVN) play a primary role in the regulation of the activity of corticotropin-releasing factor (CRF) neurones. Thus, electrical stimulation of the ventral noradrenergic ascending bundle (VNAB) causes increased adrenocortical secretion. Using rats bearing cannulae implanted above the PVN for administration of adrenergic agonist and antagonist agents, we have demonstrated that these effects are mediated by both α1- and α2-adrenoceptors. In parallel electrophysiological experiments, iontophoretic application of the α1-antagonist ergotamine, prevented the excitation of antidromically identified putative CRF-secreting PVN neurones elicited by low frequency VNAB stimulation. Iontophoretic application of the α1-agonist, l-phenylephrine excited such cells. Responses to application of the α2-agonist, clonidine produced an initial excitation of the majority of cells tested but the α2-antagonist, tolazoline had less clearly defined effects upon responses evoked following VNAB stimulation. There is some evidence for an inhibitory role for ß-adrenoceptors in the regulation of adrenocortical secretion and iontophoresis of the ß-antagonist, propranolol caused almost ubiquitous excitatory responses, supporting the concept of an inhibitory role for ß-adrenoceptors. In addition, application of propranolol was found to block the inhibitory responses of putative CRF-secreting PVN neurones following high frequency stimulation of the VNAB.

195.8

ACUTE AND CHRONIC EFFECTS OF THE 5-HT_2 AGONIST, (±)-1-(2,5-DIMETHOXY-4-BROMOPHENYL)-2-AMINOPROPANE (DOB) ON HPA AXIS ACTIVITY. M.J. Owens, J.C. Ritchie, D.L. Knight and C.B. Nemeroff. Depts. Psychiatry & Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

In vitro studies have suggested that hypothalamic CRF secretion is under stimulatory 5-HT_2 receptor control. We examined the acute and chronic effects of the highly potent and selective 5-HT_2 agonist, DOB, on a number of indices of HPA axis activity in vivo.

DOB dose-dependently increased plasma ACTH and corticosterone concentrations at doses greater than 0.1 mg/kg. Peak effects occurred by 30 minutes and returned to basal values by 4 hours. This was hypothesized to be due to release of hypothalamic CRF because pretreatment with the CRF antagonist, α-helical CRF_{9-41} (0.4 μmol/kg), resulted in a blunted ACTH response to DOB. Median eminence CRF content was also decreased following DOB administration in the prescence of cycloheximide. Seven day administration of DOB via osmotic minipump resulted in down regulation of both anterior pituitary CRF receptors and brain 5-HT_2 receptors. These receptor changes were physiologically significant as challenge doses of DOB or CRF resulted in blunted ACTH responses. Neither acute nor chronic DOB administration altered CRF concentrations in any of 14 extrahypothalamic brain regions studied. These results support a stimulatory role for 5-HT_2 receptors on hypothalamic CRF secretion.

195.9

TIME-COURSE OF ACTION OF THE 5-HT_2 AGONIST, DOB, ON THE DOWN-REGULATION OF CRF AND 5-HT_2 RECEPTORS DURING CHRONIC INFUSION. D.L. Knight, M.J. Owens, J.C. Ritchie and C.B. Nemeroff. Depts. Psychiatry & Pharmacology, Duke Univ. Med. Ctr., Durham, NC 27710.

We have previously found that 7 day infusion of the 5-HT_2 agonist, DOB, resulted in tolerance to the stimulatory actions of the drug on the HPA axis. These rats also exhibit decreased anterior pituitary CRF and cortical 5-HT_2 receptor binding. In order to further examine these drug-induced changes, a series of experiments was performed to scrutinize the time-course of these alterations.

Administration of DOB (0.35 mg/kg/day) via osmotic minipumps resulted in increases in plasma corticosterone which returned to baseline by 48 hours post-implantation. This was apparently associated with desensitization of the 5-HT_2 receptor because high affinity cortical ^{3}H-DOB and hypothalamic ^{125}I-DOI binding was decreased 48 hours post-implantation. Median eminence CRF content was unchanged at all time points; anterior pituitary CRF receptor binding was significantly decreased 7 days post-implantation. These results suggest that tolerance of the HPA axis to the stimulatory actions of DOB, as evidenced by plasma corticosterone, initially results from down regulation of 5-HT_2 receptors. Additionally, by 7 days post-implantation, down-regulation of anterior pituitary CRF receptors may also contribute to the observed tolerance. (Supported by NIMH MH-42088)

195.10

BLUNTED ACTH RESPONSE TO PHYSIOLOGIC STRESS IN DEPRESSED PATIENTS BUT NOT PATIENTS WITH SCHIZOPHRENIA. RG Kathol, TL Gehris*, BT Carroll, SD Samuelson, AF Pitts, WH Meller*, J Carter*. The University of Iowa College of Medicine, Iowa City, IA 52242.

In this study seven hospitalized patients with major depression (MD), five hospitalized patients with schizophrenia (S), and 13 control subjects (C) were administered 0.15 units regular insulin/kg at 1600 hours by intravenous bolus infusion. ACTH, cortisol, and glucose levels were measured intermittently for two hours following infusion. Baseline ACTH, cortisol, and glucose levels were similar in Cs, MDs, and Ss. The mean glucose nadir was 23.6 mg/dl for Cs, 28.4 mg/dl for MDs, and 21.0 mg/dl for Ss (F=1.44; df=2,22; p=.258). The MDs had a blunted ACTH response to hypoglycemia when compared to Cs and Ss (F=3.28; df=12,126; p=.0004). They also had an appropriate decreased cortisol response (F=4.20; df=12,132; p=.0001). This was observed despite the Ss having Carroll Depression Scale scores (23) similar to MDs (30) and significantly different than Cs (1.4) (F=55.2; df=2,22; p=.0001). When MDs (28 mg/dl) were matched with Cs (27 mg/dl) for glucose nadir, significant differences between the two remained (F=4.51; df=6,72; p=.0006).

This study replicates in a new cohort a previously reported comparison of controls and patients with MD by showing blunting of the ACTH response to hypoglycemia and extends the original findings by demonstrating that patients with schizophrenia do not show similar changes. This suggests that patients with MD show different ACTH responses to physiologic stress which are not explained by the negative feedback of baseline ACTH or cortisol. Furthermore, the presence of depressive symptoms alone do not appear to influence the development of a blunted ACTH response but that the underlying state of primary major depression is more important.

195.11

ELEVATED CEREBROSPINAL FLUID CORTICOTROPIN-RELEASING HORMONE AND ARGININE VASOPRESSIN IN DEPRESSED PATIENTS WITH DEXAMETHASONE NON-SUPPRESSION. AF Pitts, RG Kathol, TL Gehris*, SD Samuelson, BT Carroll, WH Meller*, J Carter*, CB Nemeroff, and G Bissette. The University of Iowa College of Medicine, Iowa City, IA 52242.

Dysfunction of the hypothalamic-pititary-adrenal (HPA) axis is one of the best documented findings in major depressive disorder (MDD). Recent evidence, including elevated cerebrospinal fluid (CSF) corticotropin-releasing hormone (CRH), suggest hypersecretion of CRH as a likely cause.

CSF was obtained by lumbar puncture at 1600 hours on 19 inpatients with MDD and 18 healthy normal controls. We found no significant difference in CSF CRH levels between the MDD (mean ± S.D.: 38.59 ± 8.14pg/ml) and control (43.28 ± 9.97pg/ml) groups. Similarly, no significant differences were found on measures of CSF arginine vasopressin (AVP) or CSF oxytocin (OT). When subdivided by dexamethasone suppression test (DST) status, MDD nonsuppressors (MDD-NS) (n=5) had the highest CSF CRH levels (45.92 ± 7.94pg/ml) and the MDD suppressors (MDD-S) (n=14) demonstrated the lowest (35.98 ± 9.50pg/ml) (F=3.51; df=2,33; p=0.04). CSF AVP and CSF OT measurements, though not significantly different, followed the same pattern. There was a significant positive correlation of CSF CRH with CSF AVP (r=0.536; p=.0006) for all subjects.

We conclude that CSF CRH is higher in depressed DST non-suppressors. Furthermore, AVP, which has been documented to be an ACTH cosecretogogue with CRH, also tends to be higher in the same depressive subgroup and may also be hypersecreted in this group. Lastly, a strong positive correlation between CSF CRH and CSF AVP provides support for their conserted action in enhancing ACTH release.

195.12

DYNAMICS OF IN VIVO SECRETION OF ACTH IN MEN AFTER INSULIN-INDUCED HYPOGLYCEMIA. A.Iranmanesh*, G. Lizarralde* and J.D. Veldhuis. Divisions of Endocrinology, VA Medical Center, Salem, VA 24153 and University of Virginia, Charlottesville, VA 22908.

The kinetic responses of the corticotropin axis to insulin-induced hypoglycemia were investigated by deconvolution analysis of plasma concentrations of ACTH measured by IRMA in blood samples collected at 10 min intervals for a period of 3 hrs in 7 normal men after iv administration of insulin. Plasma concentrations of glucose deceased to a mean of 34 ± 3 (SE) mg/dl, 36 ± 2.4 min after insulin. Concomitantly plasma concentrations of ACTH increased from 18 ± 3 (baseline) to 347 ± 66 pg/ml (maximum) occurring at min 54 ± 2. Deconvolution analysis revealed significant increases in the mean rate (5.2 ± 0.9 vs 1.0 ± 0.1 pg/ml/min; p=0.03), maximal rate (29 ± 6 vs 1.5 ± 0.2 pg/ml/min; p=0.0003) and mass (936 ± 170 vs 173 ± 23 pg/ml; p=0.03) of ACTH secreted. Maximal ACTH secretory rate occurred 8 ± 2 min after maximal hypoglycemia. We conclude that acute insulin-induced hypoglycemia causes marked increases in ACTH secretion rates and mass, and consequently its plasma concentration. By coupling, additional measurements of POMC-related products such as B-endorphin with deconvolution analysis, the exact kinetic of coordinate activation of the adrenocorticotropin axis can be evaluated quantitatively in vivo.

196.1

RAPID DIFFUSIVE LINKS IN NEURAL DEVELOPMENT AND FUNCTION. P.R. Montague, J.A. Gally*, and G.M. Edelman. The Neurosciences Institute, 1230 York Ave., New York, NY 10021.

The segregation and refinement of axonal projections in the vertebrate central nervous system depend critically on the correlation of presynaptic firing and postsynaptic depolarization as well as on NMDA receptor activation. To account for these activity-dependent phenomena, we have proposed that a short-lived diffusible signal is released from postsynaptic sites in a calcium-dependent fashion, diffuses to both presynaptic and postsynaptic sites nearby, and affects afferent branching and synaptic stabilization in a restricted volume of neural tissue (PNAS 87, 3547). This diffusive signal labels a small volume of neural tissue as being previously active. Those afferents whose firing is correlated with transiently increased levels of the substance are induced to branch through transient stabilization of their growth cones either by synapse formation or by slowing neurite extension.

We have modeled these mechanisms by simulating the growth of afferent axons into a 3-dimensional region containing neurons with explicit dendritic geometry. Synaptogenesis and axonal branching occur according to the mechanisms hypothesized above. Two kinds of neuroanatomy are considered: 1) regions that have both local and longer range excitatory connections and 2) regions that lack local excitatory connections. Both cases contain inhibitory neurons and their role in shaping the segregation of the terminals has been explored. The simulations show that a rapid diffusive signal, for example nitric oxide (PNAS 87, 3547), can account for known features of axonal segregation in the thalamus and cortex. The overall model demonstrates the sensitive dependence of developmental anatomy on the history of afferent correlations and reveals the effects of diffusive links in a network with fixed anatomy. (Supported by the Neurosciences Research Foundation)

196.2

MODULATION OF PRESYNAPTIC TRANSMITTER RELEASE BY POSTSYNAPTIC DEPOLARIZATION AT DEVELOPMING NEUROMUSCULAR SYNAPSE. Y.J. Lo*, D. Sanes+, and M. Poo, Dept. Biological Sciences, Columbia Univ., N.Y., NY 10021, and +Depts. Otolaryngology and Physiol. & Biophys., NYU Med. Ctr., N.Y., NY 10016.

The development and stability of early nerve connections are affected by the synaptic activity. We have examined the effects of postsynaptic depolarization on the efficacy of synaptic transmission at developing *Xenopus* neuromuscular synapses in culture. Spontaneous synaptic currents were recorded in single innervated muscle cells by whole-cell patch recording method under voltage-clamp condition. The recordings were interrupted for brief (15 to 30 min) episodes of pulsatile current injections (in current-clamp mode) which resulted in repetitive depolarizations of the muscle membrane (70-100 mV amplitude, 5-20 ms duration, 4-8 Hz). In 17 out of 25 cases we found that the frequency of the spontaneous synaptic currents (SSCs) were significantly reduced (range: 21 to 87%). Similar episodes of hyperpolarization did not induce this change. The effect of repetitive depolarizations was significantly diminished in the presence of constant local perfusion of the synaptic site with fresh culture medium, suggesting involvement of diffusible factor(s) in the observed depolarization dependent modulation of presynaptic transmitter release. Since presynaptic neurons in this culture rarely fire action potential spontaneously, the present result is consistent with the notion that postsynaptic activity de-stablizes synapses that are not concurrently releasing transmitter.

196.3

LOCAL APPLICATION OF BASIC FIBROBLAST GROWTH FACTOR INDUCES POSTSYNAPTIC DEVELOPMENT IN MUSCLE CELLS. H. B. Peng and L. P. Baker. Dept. of Cell Biology & Anatomy and Curr. in Neurobiology, Univ. of North Carolina, Chapel Hill, NC 27599.

Peptide growth factors are universally used by cells for signaling proliferation and differentiation. Little is known about their role in synaptic induction. In this study, we examined the effect of basic fibroblast growth factor (bFGF) in signaling post-synaptic differentiation in cultured muscle cells, since previous studies have shown the importance of this protein in myogenesis. To mimic the local cellular interaction at nerve-muscle contact, we applied bFGF to cultured *Xenopus* muscle cells via coated latex beads. After an incubation period of 12-24hr, we observed that acetylcholine receptor (AChR) clusters appeared at the bead-muscle contacts. The size of the clusters conformed to the size of beads applied and their number was proportional to the number of bead-muscle contacts. In addition to cluster induction, a dispersal of preexisting AChR clusters (hot spots) was also elicited by the treatment of bFGF beads. Ultrastructural studies showed that the bFGF bead-induced clusters were associated with membrane infolding and basal lamina. Beads coated with bovine serum albumin or acidic FGF were ineffective in inducing AChR clustering. Heat-denatured or reduced bFGF was also ineffective. Suramin, a polyanionic molecule that interferes with the binding of growth factors to their receptors, abolished bFGF bead-induced AChR clustering. bFGF receptor, similar to many other receptors for growth factors, has an tyrosine kinase domain on its cytoplasmic tail. When the cells were incubated with tyrphostin RG50864, a tyrosine kinase inhibitor, the bFGF bead-induced AChR clustering was reversibly blocked. These results show that local application of bFGF can induce the postsynaptic development in *Xenopus* and this induction may be mediated by tyrosine kinase activity of bFGF receptors. (Supported by NIH grant NS23583 and the Muscular Dystrophy Association)

196.4

Motor neurons contain an mRNA that codes for an agrin-like molecule. C. Magill-Solc, R. H. Scheller & U. J. McMahan. Dept. of Neurobiology and Dept. of Biological Sciences, Stanford University, Stanford, CA 94305.

Several lines of evidence indicate that agrin or a molecule very similar to it mediates the motor neuron-induced aggregation of acetylcholine receptors and acetylcholinesterase at the neuromuscular junction. In the study described here we tested this hypothesis by seeking to determine whether the cell bodies of motor neurons contain an mRNA that encodes agrin-like proteins, which would be expected if motor neurons synthesized such proteins.

^{32}P-labelled RNA probes were generated from a cDNA that codes for an agrin-like protein in the marine ray *D. ommata* and were used to probe Northern blots of poly (A)$^+$ mRNA from various *D. ommata* tissues. The probe hybridized to two prominant mRNA species, 9.5 kb and 7 kb in CNS tissues. A relatively small amount of the 9.5 kb mRNA only was detected in muscle, electric organ and liver. *In situ* hybridizations using ^{35}S-labelled RNA probes demonstrated that the mRNA is concentrated in motor neurons of the *D. ommata* electric lobe and spinal cord, although it appears to be expressed at a low level throughout the CNS.

196.5

AGRIN-LIKE MOLECULES ARE INVOLVED IN THE MOTOR NEURON-INDUCED AGGREGATION OF AChR'S AT DEVELOPING NEUROMUSCULAR JUNCTIONS. Noreen E. Reist and U.J. McMahan. Dept. of Neurobiology, Stanford University, Stanford, CA 94305.

According to the agrin hypothesis, agrin, or a molecule similar to it, is synthesized by motor neurons, transported to the neuromuscular junction and released from nerve terminals into the synaptic cleft, where it directs the formation of acetylcholine receptor (AChR) aggregates on developing muscle fibers. In order to test the involvement of agrin in directing the formation of this postsynaptic specialization, we generated polyclonal antisera against purified agrin from *Torpedo* electric organ extracts and tested the sera for the ability to inhibit the formation of neurally-induced AChR aggregates on cultured myotubes. Dissociated chick motor neurons grown in culture with chick myotubes normally induce a dramatic increase in the number of AChR-aggregates on the myotube surface at or near the point of contact with neurites. IgG's purified from anti-agrin serum markedly inhibited the motor neuron-induced aggregation of AChR's in cocultures of chick motor neurons and myotubes, while IgG's purified from the preimmune serum had little effect. This is the most direct evidence to date that agrin plays a role in the formation of the postsynaptic apparatus at the neuromuscular junction.

196.6

AGRIN INDUCES THE REDISTRIBUTION OF ACh RECEPTORS AND Na CHANNELS ON ADULT SKELETAL MUSCLE FIBERS IN CULTURE. M.T. Lupa* and J.H. Caldwell. Department of Cellular and Structural Biology, Univ of Colorado, Denver, CO 80262.

It has been shown that acetylcholine receptors (AChR) and Na channels (NaCh) are both highly concentrated at the neuromuscular junction (NMJ). The nerve is believed to influence the formation of the AChR patch, possibly through the action of agrin, which is a basal lamina protein that induces the formation of AChR clusters on myotubes in vitro. We have applied agrin (kindly supplied by J. Fallon) to adult muscle fibers in culture in order to test whether agrin can play a role in the formation of NaCh clusters on muscle fibers. Enzymatically-dissociated adult rodent FDB muscle fibers were used because these have a higher density of NaCh than embryonic myotubes. Rhodamine-a-bungarotoxin was used to visualize AChR patches, and the loose patch voltage-clamp was employed to map NaCh densities on the muscle fiber. Agrin increased the number of AChR clusters about 4X within 24 hr on 5-10 d denervated muscle fibers. 55% of the AChR aggregates had NaCh densities more than twice the average extra-cluster density, a figure similar to naturally-occurring AChR clusters. Some NaCh 'hot spots' were found in areas devoid of AChR clusters, both in the presence and absence of agrin. We conclude from these results that 1) agrin can induce new clusters of AChR and NaCh on adult muscle fibers in culture, and 2) though often co-localized, the distributions of AChR and NaCh are not closely co-regulated.

196.7

THE BINDING OF TORPEDO AGRIN TO THE MUSCLE CELL SURFACE IS CALCIUM DEPENDENT, J. R. Fallon and E. Lieth, Worcester Fdn. for Exper. Biol., Shrewsbury, MA. 01545

Agrin is likely to play a central role in directing the differentiation the postsynaptic apparatus during development and regeneration of the neuromuscular junction. Our current studies are aimed at characterizing the muscle cell surface receptor for the agrin molecule. The binding of Torpedo agrin to the muscle cell surface detected by immunofluorescence microscopy is saturable and does not require the presence of serum or embryo extract. When myotubes are incubated with agrin at 37°C, agrin binding sites become concentrated at the induced AChR clusters. This redistribution of agrin binding sites is unaffected by 5mM Na azide or 100μg/ml cycloheximide. No binding of agrin to the myotube surface is detected when Ca^{2+} is omitted from the incubation medium or specifically chelated with EGTA. Mg^{2+} or Mn^{2+} are not required for binding nor do they substitute for Ca^{2+}. The omission of Ca^{2+} does not irreversibly alter either the ligand or receptor since pre-incubation of either the cells or the agrin with EGTA did not affect their subsequent binding in the restored presence of Ca^{2+}. Moreover, even after overnight incubation at 37°C, Torpedo agrin can be dissociated from its binding site by brief incubation in EGTA. These results indicate that both the formation and maintenance of agrin-receptor complexes is dependent upon the presence of extracellular Ca^{2+}.

196.8

LOSS OF SYNAPTIC SITES DURING COMPETITIVE SYNAPSE ELIMINATION IS BOTH RAPID AND SALTATORY. R.J. Balice-Gordon & J.W. Lichtman, Dept. Anatomy & Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

We are studying the competitive interactions between motor axons vying for capture of the same muscle fibers by following neuromuscular junctions innervated by two axons over time in the sternomastoid muscle of living mice. At transiently multiply innervated junctions, loss of ACh receptors precedes loss of overlying motor nerve terminals and retraction of the parent motor axon. Studies in which we labeled the two competing axons converging at the same junctions with different lipophilic dyes (see Chua et al., this volume) suggest a surprising stepwise elimination of terminals belonging to one axon. Consistent with this is the observation that although each axon initially occupies roughly equal synaptic territories, only 1-2 very small regions per junction lose motor nerve terminals (and ACh receptors) at any one time. Moreover, because fluorescent alpha bungarotoxin labeled junctions containing faint ACh receptor regions (in the process of being eliminated) are infrequently observed, we calculate that any one synaptic site may be eliminated in as few as 2-3 hours. Observations of multiply innervated junctions in living animals confirm that the loss of synaptic sites is indeed rapid. Because each axon occupies roughly 6-8 synaptic sites, the rapid loss of each site means that all of the synaptic sites occupied by the retracting axon may require a total of 18 hours to be eliminated. At some junctions, however, multiple innervation persists for several days even though synaptic sites are being lost, suggesting that these 18 hours might not be contiguous. We are studying the possibility that the rapid loss of an individual synaptic site may be followed by a period of relative quiescence before the next site is eliminated. These studies suggest that synaptic competition may occur locally, site-by-site, rather than on an all-or-none basis.

196.9

AN *IN VITRO* ANALYSIS OF THE ONSET OF SYNAPTIC TRANSMISSION BETWEEN CHICK AUTONOMIC NEURONS. James J. Walker and Richard I. Hume. Department of Biology , University of Michigan, Ann Arbor, MI 48109.

In the developing neuromuscular system there is an almost instantaneous onset of synaptic transmission following growth cone-muscle contact, due to the high degree of maturity of both the pre- and postsynaptic elements. We were interested in whether synaptogenesis occurred over a comparable time course in a neural system. We have examined the initial interactions between developing chick preganglionic sympathetic neurons and their target ganglion cells *in vitro*. Preganglionic neurons were retrogradely labeled with DiI, dissociated into single cells and plated on a laminin substrate. Several hours later sympathetic ganglion cells were manipulated onto the growth cones or neurites of the established preganglionic neurons. To test for synaptic function we monitored the spontaneous and evoked whole cell currents in the ganglion cells. Our major finding is that within the first two hours of recording, no synaptic currents were detected in ganglion cells in contact with preganglionic neurons. This failure to observe synaptic interactions was not a consequence of the recording methods, since when similar methods were used to examine the interaction between ciliary ganglion neurons and spherical myoballs, synaptic interactions were commonly observed within minutes of contact.

Further experiments explored why no synaptic currents were detected. Immaturity of the postsynaptic neurons was indicated by the observation that the responses of sympathetic ganglion neurons to application of 50 μM acetylcholine (ACh) were very small. The average ACh receptor density was estimated to be less than 1 receptor per μm^2. Immaturity of the presynaptic cell was indicated by the observation that no synaptic currents were recorded from myoballs placed onto preganglionic growth cones. Since evoked ACh release from ciliary ganglion neuron growth cones was readily detected by myoballs, it seems likely that preganglionic neurons release very little transmitter. In conclusion, the absence of functional transmission at newly formed contacts between these developing autonomic neurons was due to immaturity in both the pre- and postsynaptic cells. Thus there are striking differences in the rate of synapse formation between this system and the developing neuromuscular system.

196.10

CELL INTERACTIONS REGULATE DENDRITIC MORPHOLOGY AND RESPONSES TO NEUROTRANSMITTERS IN EMBRYONIC CHICK PREGANGLIONIC NEURONS *IN VITRO*. B. Clendening and R. I. Hume. Dept. of Biology, University of Michigan, Ann Arbor, MI 48109.

We studied the influence of non-neuronal cells and interneurons on the morphological development of chick sympathetic preganglionic neurons (SPN) and on the responsiveness of these neurons to the neurotransmitters, GABA, glycine and glutamate. SPN were retrogradely labeled with the fluorescent dyes, diI and diO, and then separated from spinal cord non-neuronal cells and interneurons by fluorescence-activated cell sorting. SPN were grown in culture either alone, or in co-culture with non-neuronal cells, with interneurons, or with both of these cell types (control cultures). The responsiveness of SPN to neurotransmitters was assessed by whole cell recording, while cell morphology was assessed after intracellular staining with 6-carboxyfluorescein.

Cell size and morphology were affected by non-neuronal cells. In the absence of non-neuronal cells, SPN had smaller cell bodies and fewer major processes, whether or not interneurons were present. In contrast, responses to the three neurotransmitters were affected by both non-neuronal cells and interneurons but in different ways. In the absence of both non-neuronal cells and interneurons, responses to all three transmitters were much smaller than in control cultures. Responses to glutamate were most profoundly affected. The addition of non-neuronal cells or interneurons alone had no significant effect on the responses of SPN to GABA. The addition of either non-neuronal cells or interneurons significantly increased the amplitude of SPN responses to glutamate, but the level of responsiveness was still much lower than those of SPN grown in the presence of both cell types. Finally, the addition of either non-neuronal cells or interneurons to SPN cultures brought responses to glycine back to control levels. These experiments demonstrate that although interneurons can have a powerful inductive effect on the responses of SPN to the neurotransmitters, not all of the changes in neurotransmitter responsiveness can be related to the formation of functional synapses.

196.11

TARGET CONTACT REGULATES THE CALCIUM SENSITIVITY OF SECRETORY MACHINERY DURING SYNAPTOGENESIS. M. J. Zoran, R. T. Doyle, and P. G. Haydon, Department of Zoology, Iowa State University, Ames, Iowa 50011

Buccal neuron 19, isolated from the pond snail Helisoma trivolvis and plated into cell culture, requires sustained periods of muscle-specific contact to induce the acquisition of functional excitation-secretion machinery. The contact-dependent transformation of this motoneuron's terminal into a secretory state requires protein synthesis (Doyle et al., 1990; Soc. Neurosci. Abst.). In this study, we determined whether contact regulates synaptogenesis through presynaptic actions involving increased density of calcium currents or the ability of calcium to promote exocytosis.

Giant somatic synapses of B19, with appropriate buccal muscle contact but not novel neuronal contact, develop functional excitation-secretion machinery in cell culture. This preparation provides direct access to secretory membrane for studies of mechanisms underlying synaptogenesis. The types and density of calcium currents in B19 somata cultured alone, in contact with inappropriate neuronal targets, or in contact with appropriate muscle targets were not significantly different. Since calcium current densities were not different between these groups, the contact-dependent induction of secretory capabilities at the nerve terminal of B19 must involve a component of the secretory apparatus distal to the influx of calcium ions. This idea was supported by experimental manipulations of intracellular calcium levels in presynaptic cells with the photolabile calcium cage, DM-nitrophen. Photolytic release of calcium increased presynaptic free calcium levels, as indicated by the co-injected calcium fluorophore Fluo-3, and evoked the release of neurotransmitter at somatic synapses only following sustained periods of appropriate muscle contact.

This work was supported by NIH grant NS24233.

196.12

REQUIREMENT FOR CONTACT-DEPENDENT PROTEIN SYNTHESIS FOR SYNAPTIC SPECIFICITY R.T. Doyle, M.J. Zoran and P.G. Haydon Department of Zoology, Iowa State University, Ames IA 50011.

Cholinergic neuron B5 of *Helisoma* is indiscriminate in synaptogenesis since it readily forms chemical synapses with novel cholinoceptive targets. By contrast, cholinergic neuron B19 is restricted and forms functional chemical connections with appropriate muscle targets only. It has been shown that neuron B5 forms its functional chemical connections with novel target cells within seconds of contact (Haydon and Zoran, *Neuron* 2:1483, 1989). By contrast, functional chemical connections were never detected within the first 30 minutes of specific target (supralateral radula tensor (SLT) muscle) contact with neuron B19 (Zoran et. al. *Dev. Biol.* 138:202,1990).

We determined whether protein synthesis is required following target contact for the formation of both novel and appropriate functional chemical connections. Neurons B5 and B19 were plated into culture where they extended neurites. After 2-3 days, future presynaptic neurons were challanged with target cells in the presence of the protein synthesis inhibitor, anisomycin (20μM).

Indiscriminate neuron B5 reliably formed functional chemical connections in the presence of anisomycin. By contrast selective neuron B19 was unable to form a functional synapse with its appropriate target cell when protein synthesis was inhibited.

Thus, indiscriminate neuron B5 synthesizes presynaptic macherinery before target contact and can form novel chemical connections. Neuron B19, in contrast, requires target specific contact-dependent protein synthesis to complete the functional assembly of its presynaptic machinery.

Supported by NIH grant NS24233.

196.13

ELECTRICAL COUPLING BETWEEN *APLYSIA* SENSORY CELLS IN CULTURE DISRUPTS THE SPATIAL SEGREGATION OF THEIR VARICOSITIES ON A POSTSYNAPTIC TARGET. M. Bank and S. Schacher . Ctr. for Neurobiol. and Behav., Columbia University CPS and NYS Psych. Inst., New York, NY 10032.

Sensory cells of *Aplysia* form chemical synapses with the motor neuron L7 in culture. Sites of synaptic interaction between these cells are typically found at varicosities of sensory cell processes overlying the main axons of L7, since these structures have been shown ultrastructurallly to contain active zones (Glanzman et al., Neuron 3:441, 1989). Using flourescent dye injection and video microscopy, we have investigated a) whether varicosities contributed by two different sensory cells segregate or intermingle on the main axons of L7, and b) how conditions such as electrical coupling between the sensory cells affects this pattern. At early times after plating (day 2), varicosities from different non-electrically coupled sensory cells can be seen to intermingle on the main axons of L7 (6 of 8 cocultures). At later times (day 4), varicosities from different non-electrically coupled sensory cells always segregate to separate areas of the motor axons (7 cocultures). In contrast, sensory cells which are electrically coupled (coupling coefficient of 0.05 to 0.10) do not segregate their varicosities on day 4 (4 of 5 cocultures). These observations suggest that the mature segregated pattern of varicosity distribution from non-electrically coupled sensory cells may arise through a process of rearrangement from an immature intermingled pattern. This process may be disrupted when sensory cells are electrically coupled.

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS IV

197.1

CALCIUM HOMEOSTASIS IN AN IDENTIFIED NEURONAL GROWTH CONE. Vincent Rehder, John R. Jensen, Ping Dou, Stanley B. Kater. Dept. of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523, USA

Calcium homeostasis was investigated in growth cones of identified neurons from the snail *Helisoma trivolvis*. The intracellular calcium concentration ([Ca]i) was experimentally altered by increasing the calcium flux into the cytoplasm, and resulting changes in [Ca]i were measured with the fluorescent calcium-indicator Fura-2 (K-salt). Sustained elevation of calcium influx was achieved by addition of the calcium ionophore 4-bromo A23187. The [Ca]i reached its peak value of 910nM 5min after the application. Most interestingly the growth cones gradually restored their [Ca]i back towards pretreatment levels despite the continuous presence of the ionophore. Two candidate mechanisms for lowering [Ca]i are the mitochondria and the Na/Ca exchanger. We tested their involvement in the restoration process by blocking these mechanisms, singly or in combination, and then compared the restoration in the presence of an increased calcium influx to the unblocked situation.

The mitochondria were rendered dysfunctional by use of FCCP which dissipates the proton gradient across the mitochondrial membranes and prevents calcium reuptake. Subsequently 4-bromo A23187 was applied to continuously increase calcium influx. Under this condition the growth cones were still able to restore their [Ca]i. To test for the possible contribution of the Na/Ca exchanger, this system was blocked by reducing the extracellular [Na]. Upon increased calcium influx growth cones were again able to restore their [Ca]i. However, one could envision a situation where the inactivation of one mechanism could be compensated for by another, and vice versa. When **both** the mitochondria and the Na/Ca exchanger were blocked simultaneously, and growth cones subsequently challenged with increased calcium influx, the ability to control the [Ca]i was lost immediately, and neurons died within minutes. These results demonstrate a powerful calcium homeostatic capability within growth cones, based on an overlapping system of mechanisms controlling [Ca]i.

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197.2

CALCIUM HOMEOSTATIC CAPACITY IS REGULATED BY PATTERNED ELECTRICAL ACTIVITY IN GROWTH CONES OF MOUSE DRG NEURONS. R.D. Fields[1], P.B. Guthrie[2], P.G. Nelson[1], and S.B. Kater[2]. Lab. Developmental Neurobiology, NICHD, NIH, Bethesda MD 20892[1], and Dept. Anatomy and Neurobiology, Colorado State Univ., Fort Collins, CO 80523[2].

We have previously shown that electrical stimulation causes growth cones of several types of neurons to collapse. This collapse is due to an influx of calcium into the growth cone. Interestingly, following prolonged stimulation of mouse DRG neurons (>24 hrs.), motile growth cones are once again observed. This adaptation to electrical stimulation could be due in part to restoration of normal intracellular calcium concentration ($[Ca^{2+}]_i$). We tested this hypothesis directly using dissociated DRG neurons grown in a three-compartment culture chamber.

We stimulated neurons crossing from a side compartment into the center compartment. Calcium levels were monitored with fura-2. Electrical stimulation resulted in a large increase in $[Ca^{2+}]_i$ and growth cone collapse. With continued stimulation calcium levels decreased substantially, but never returned to control levels. Immediately after cessation of chronic (>24 hrs.) stimulation, $[Ca^{2+}]_i$ fell significantly below control levels, suggesting additional calcium removal capacity activated by chronic electrical stimulation. Activity-dependent regulation of calcium homeostatic capacity could have a major influence on neurite outgrowth and interact with a range of other calcium-dependent developmental processes.

197.3

AMITRIPTYLINE TREATMENT DECREASES cAMP LEVEL IN EMBRYONIC CHICK EXPLANTS, AND INTERFERES WITH NEURAL DEVELOPMENT *IN VIVO*. K. L. WONG*, R. C. BRUCH* AND A. I. FARBMAN. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Our laboratory has shown that amitriptyline (AMI), a typical tricyclic antidepressant, has potent effects in inhibiting neurite outgrowth from chick embryonic cerebral and olfactory explants [Brain Res. (1988) 457:281], and that dibutyryl cAMP can partially reverse the inhibitory effects of AMI [Soc. Neurosci. Abstr. (1989) 875]. These data suggest the possibilty that AMI may inhibit neurite outgrowth via the alteration of intracellular cAMP level.

In 8-day chick embryonic cerebral explants exposed to 8 μM of AMI, there is a significant 43% decrease in cAMP level ($t=4.03$, $df=7$, $p<0.005$). Interestingly, there is a significant correlation between the level of cAMP and the degree of neurite outgrowth ($r=0.56$, $df=20$, $p<0.01$).

Furthermore, we have studied the physiological and biochemical effects of AMI on brain development *in vivo*. Six day treatment of AMI (single daily injections of 48-172 μg) results in a significant 14% decrease in the weights of chick forebrains ($t=4.18$, $df=8$, $p<0.005$). The body weights, on the other hand, show a non significant 12% decrease. In addition, immunoblots and densitometric scans have shown a selective decrease in the level of a 93.6 K Da protein (-24%), β-tubulin (-18%), and actin (-20%). Several other proteins are not affected. Both flourescent and spectrophotometric DNA assays have shown that DNA:protein ratio in the AMI-treated and the control groups are the same, thus strongly suggesting that the reduction in forebrain weights may be due to a lower number of developing neurons, as well as an arrest of neurite outgrowth.

197.4

THE IN VITRO FORMATION OF PUTATIVE PEPTIDE RELEASE SITES USING A PHORBOL ESTER AND CYCLIC AMP R.J. Knox, E.A. Quattrocki, J.A. Connor and L.K. Kaczmarek, Dept. Pharmacology, Yale University, New Haven, CT 06510 and Roche Inst. Mol. Biology, Nutley, NJ 07110.

We have investigated the effects of a phorbol ester (TPA) and agents that elevate cAMP levels on morphology and calcium influx in the growth cones of bag cell neurons from *Aplysia* by ratio imaging fluorescence of microinjected fura-2 and by DIC microscopy. In control cells in an arrested state of outgrowth, action potential driven Ca influx in the growth cones occurred in the central regions of the structures but did not appear at the leading edges of the lamellapodia. Bath application of TPA (20 nM), but not inactive analogs, triggered a thickening of the central region of the growth cone and extension of the lamellipodium. Electrically driven calcium influx now occurred at leading edges of the lamellae as well as the more central regions. The change in influx pattern may reflect the rapid recruitment of previously inactive Ca channels into the lamellae (Strong *et al.*, 1987). There was no significant difference in resting Ca levels of the growth cones when effects of increased excitability were nulled out. Cotreatment with cAMP analogs or with forskolin promoted the movement of organelles into the newly extended lamellae but appeared not to alter the redistribution of the voltage gated Ca channel activity. Our findings suggest that activation of protein kinases produces morphological specializations in which secretory granules move to sites where calcium channels have been newly recruited.

197.5

TUBULIN IS PHOSPHORYLATED BY PP60^{C-SRC} IN NERVE GROWTH CONE MEMBRANES. P. Maness and W. T. Matten. Department of Biochemistry, University of North Carolina School of Medicine, Chapel Hill, NC 27599.

pp60^{C-src} is enriched in a subcellular fraction from fetal rat brain containing membranes of the nerve growth cone. Tubulin was the major phosphotyrosine-containing protein in growth cone membranes *in vivo*, as detected by immunoblot with anti-phosphotyrosine (Ptyr) antibodies. Ptyr immunoblots of growth cone membranes showed that α- and ß-tubulin comigrated with the most prominent Ptyr-modified proteins on one-dimensional gels, and that they were highly enriched in the growth cone fraction. α- and ß-Tubulin were the most prominent proteins phosphorylated at tyrosine in *in vitro* protein kinase ractions with growth cone membranes (0.12 mol [^{32}P]-Ptyr/mol tubulin dimer. Ptyr antibodies immunoprecipitated tubulin from growth cone membranes. Tyrosyl residues in a subset of α- and ß-tubulin isoforms were phosphorylated in [^{32}P]-labeled cultures of rat cortical neurons. pp60^{c-src}, either in growth cone membranes or in immune complexes, phosphorylated purified brain tubulin *in vitro*. The consequence of tyrosine phosphorylation was to inhibit polymerization of α-tubulin isoforms into microtubules. These results suggest a model in which tyrosine phosphorylation of tubulin by pp60^{c-src} at the growth cone membrane could regulate neurite extension by inhibiting microtubule assembly and destabilizing local adhesive contacts.

197.6

TURNING RESPONSE OF NERVE GROWTH CONES IN PULSATILE GRADIENTS OF DIBUTYRYL CYCLIC ADENOSINE MONOPHOSPHATE. M. Quillan* and M-m. Poo, Department of Biological Sciences, Columbia University, New York, NY 10027.

The directionality of neurite outgrowth is controlled by subcellular processes which are poorly understood. These processes may involve signal transduction which transmit information from the surrounding environment via second messengers. Cyclic nucleotides are known to serve second messengers in a number of systems. We thus tested directly the effect of membrane permeable cyclic nucleotides in influencing directionality of nerve growth, bypassing the action of the primary extracellular signal. A periodic pulsatile gradient of cyclic nucleotides was created near the growth cone of Xenopus spinal cord neurons in culture by using a picospritzer. In a typical experiment, we used an air pressure of 15 psi, a pulse duration of 4 ms and frequency of 0.5 Hz, and a 20 mM pipette concentration of cyclic nucleotide. The pipette tip (1-2 μm opening) was positioned at 100 μm from the center of the growth cone, at a 45° angle with respect to the direction of neurite extension, and the turning response was determined after 2 hrs in the presence of pulsatile chemical gradient by measuring any change in direction of neurite extension. We found gradients of dibutyryl cyclic adenosine monophosphate (dB-cAMP) caused significant turning of the growth cone toward the source of the chemical (average angle of turning 24.1 ± 4.7° , n=16), while no effect was observed for gradients of sucrose (6.4 ± 7.8° , n=12) and dB-cGMP (2.1 ± 12.4° , n=7). The result is consistent with the notion that concentration gradients of cytoplasmic second messenger, cAMP in particular, may underlie cellular mechanisms involved in the turning of growth cones in response to environmental cues.

197.7

NGF-REGULATED PHOSPHORYLATION OF TYPE III β-TUBULIN DURING NEURITOGENESIS IN RAT PC12 CELLS. J.M. Aletta and L.A. Greene*. Dept. of Pathology, Columbia University, N.Y.C., N.Y. 10032.

Phospho-β-tubulin (PβT) has previously been identified as an NGF-regulated phosphoprotein which undergoes increased phosphorylation in temporal correlation with PC12 cell neurite formation. The present work establishes that this increase occurs on neuronal-specific isotype III β-tubulin, but not on isotypes II or IV. Little, if any, β-tubulin is phosphorylated before NGF-treatment as determined by immunoprecipitation with both isotype-specific and general β-tubulin reagents. PβT is associated predominantly with Ca^{2+}-/cold-sensitive microtubules, but taxol-driven assembly does not produce phosphorylation equivalent to long-term NGF-treatment, nor does it enhance that obtained by NGF. NGF is necessary, but not sufficient, for maximal phosphorylation. Cells grown with NGF for 1-2 w, either in suspension or on a substrate with colchicine, are neurite-less and contain less than 10% of the PβT of control neurite-bearing cultures. When the cells are replated on a substrate or the colchicine is removed, they rapidly extend long processes and attain levels of PβT comparable to those in matched controls. Finally micro-excision experiments revealed that PβT is enriched in neurites relative to that in their corresponding cell bodies (2.9-fold±0.7; n=10), despite comparable total β-tubulin levels in the two regions. The rapid phosphate turnover on neuronal β-tubulin, its association with Ca^{2+}-/cold-sensitive microtubules and its enrichment in neurites suggest some functional role in dynamic events related to polymerized microtubules in the specialized neuritic compartment of the cell.

197.8

GROWTH AND DEGENERATION OF RAT SYMPATHETIC NERVE FIBERS IN COMPARTMENTED CULTURES: EFFECTS OF SPHINGOSINE, PHORBOL ESTER, AND THE MECHANISM OF NGF ACTION. R. B. Campenot, A. Walji* and D. D. Draker*. Dept. of Anatomy and Cell Biology, Faculty of Medicine, Univ. of Alberta, Edmonton, Alta., Canada T6G 2H7.

Local application of 8 or 10 μM sphingosine to distal neurites of NGF-treated rat sympathetic neurons in compartmented cultures caused their degeneration within 24 hrs, but sphingosine applied to cell bodies and/or proximal neurites caused no apparent damage, and the distal neurites continued to grow. Interference with the action of NGF was not the cause since anti-NGF produced no similar effect.

Treatment for 24 hrs with 2 μM of the phorbol ester, TPA, was used to down regulate protein kinase C activity (Matthies et al., J. Neurosci. 7, 1198-1206, 1987; Reinhold & Neet, J. Biol. Chem. 264, 3538-3544, 1989), but this neither destroyed distal neurites nor blocked the effect of sphingosine. Thus, protein kinase C appears not to mediate the degeneration of distal neurites by sphingosine.

Although TPA treatment caused a 50% reduction in the rate of neurite extension, the TPA-resistant component of neurite growth was completely blocked by anti-NGF. Thus, as with PC12 cells (Reinhold & Neet, 1989), protein kinase C does not appear to play an essential role in the mechanism by which NGF promotes neurite growth. The selective degeneration of distal nerve fibers by sphingosine may reflect the existence of a ligand-actuated system, not involving NGF or protein kinase C, that can trigger the degeneration of nerve endings and which may be important in synapse and collateral elimination, synaptic competition, and growth cone collapse.

197.9

ENDOGENOUS FATTY ACIDS OF PHOSPHOLIPIDS IN GROWTH CONES: INCORPORATION AND METABOLISM OF DOCOSAHEXAENOIC ACID (22:6) DURING DEVELOPMENT. **R.E. Martin,* D. Kline and N.G. Bazan, LSU Eye Center and Neuroscience Center, New Orleans, LA 70112.**

Nerve growth cones are the dynamic endings of neurites that are actively growing, regenerating and undergoing plasticity changes. They represent a site of rapid membrane expansion and are the developmental precursors of synapses. Docosahexaenoic acid (22:6), an essential polyunsaturated fatty acid, is enriched in synaptic membranes and photoreceptors. We isolated nerve growth cone particles from mouse cerebrum and examined a) their endogenous fatty acid content as a function of development and b) the metabolism of 22:6 in phospholipids. Our results indicate marked changes in fatty acid composition of growth cones during development. In the homogenates, total nmol fatty acid per μg lipid P remains relatively constant during the first 15 d of life, whereas in growth cones these values decrease. Arachidonic and other fatty acids followed this pattern; however, 22:6 increased in the homogenate and decreased in the cone fraction. Expressed as mole percent fatty acid, 22:6 increased in both the cone fraction and the homogenate. At 5 d after intraperitoneal injection of ^{3}H-22:6 into 5-day-old pups, more than 98% of the label in brain remained as 22:6. The 22:6 was incorporated in phosphatidylethanolamine (PE), phosphatidylcholine (PC) and phosphatidylserine (PS) in all cell fractions. The growth cone fraction was unique because it contained high concentrations of ^{3}H-22:6 in ethanolamine-containing and neutral lipids. This correlated well with endogenous fatty acid content of phospholipids in that most of the 22:6 was seen in PE. Collectively these data indicate that growth cones are sites of active phospholipid metabolism and are different from other subcellular fractions. How these lipids are engaged in cell signaling, pathfinding and synapse formation are questions being addressed. **NIH grant NS23002.**

197.10

CAP50, A CORTICAL CYTOSKELETON-ASSOCIATED PROTEIN OF DEVELOPING TISSUES AND NEURONS WITH PROPERTIES SIMILAR TO THE ONES OF GAP43. **P. Caroni and F. Widmer. Friedrich Miescher Institute, P.O. Box 2543, 4002-Basel, Switzerland.**

The subcellular distribution and expression pattern of GAP43 indicate that this protein might play an important role in axonal growth. The lack of both significant similarities between GAP43 and known proteins and of cell biological correlates of axonal growth has hindered the formulation of testable hypotheses concerning the function of GAP43.

We have searched for proteins with physical properties and subcellular locations similar to those of GAP43 and report here on primary structure, distribution, and aspects of the expression of one such protein, CAP50, a novel cell membrane and cortical cytoskeleton-associated protein of developing tissues and of neurons in the chick and the rat. Properties shared by CAP50 and GAP43 also included deduced amino acid composition, phosphorylation by protein kinase C, approximate abundancy in the developing nervous system, and characteristic immunofluorescence labeling pattern in neurites and in growth cones. A similar patchy subplasmalemmal labeling pattern was also observed for CAP50 in non-neuronal cells. CAP50 was a ubiquitous protein in E2 chick embryos. Subsequently, the protein and its mRNA became restricted to an increasingly narrow range of tissues and cell types. In the nervous system CAP50 levels displayed marked regional and quantitative variations during development. Overall, CAP50 levels were highest and most persistent in the nervous system, where the protein was not restricted to neurons. CAP50 and GAP43 might belong to a class of extremely hydrophilic small components of the subplasmalemmal domain, whose presence is linked to specialized cellular functions.

197.11

PIEZOELECTRIC POLYMER SUBSTRATES ENHANCE MOUSE NEUROBLASTOMA NEURITE OUTGROWTH IN VITRO.
R.F. Valentini, R. Bellamkonda*, E.G. Fine* and P. Aebischer
Section for Artificial Organs, Biomaterials and Cellular Technology, Brown University, Providence, RI 02912

Bioelectric fields may play an important role in neuronal development, plasticity and regeneration. Polymer films of polyvinylidene fluoride (PVDF) may be rendered piezoelectric by electrical poling in a high intensity electric field, which permanently aligns molecular dipoles. Displacement of the oriented dipoles by mechanical stress results in a transient charge on the PVDF surface. No external power source or electrodes are required. Mouse neuroblastoma (Nb2a) cells were cultured directly on transparent films of electrically poled (i.e. piezoelectric) or unpoled (i.e. non-piezoelectric) PVDF. Poled and unpoled PVDF feature identical chemical surfaces as ascertained by electron spectroscopy for chemical analysis. Neurite outgrowth and length in serum-containing or serum-free media was assessed at 24, 48, 72 and 96 hr. Poled PVDF film placed in a standard tissue culture incubator showed a voltage output of 3-4 mV at 1200 Hz, the vibrational frequency of the incubator shelf. Unpoled PVDF showed no output. At all time periods and under both media conditions, a significantly greater percentage of Nb2a cells cultured on poled vs. unpoled PVDF films displayed neurite outgrowth. Nb2a cells grown on poled PVDF also extended longer neurites. These results suggest that biologically relevant electrical fields produced by piezoelectric polymers enhance neuronal differentiation and neurite extension. PVDF and related materials may provide novel techniques to evaluate the influence of bioelectrical fields on neuronal development and regeneration.

DRUGS OF ABUSE: ALCOHOL IV

198.1

CHOLECYSTOKININ OCTAPEPTIDE INCREASES THE EXCITATORY EFFECT OF ETHANOL ON NEURONS OF THE VENTRAL TEGMENTAL AREA RECORDED IN VITRO. Mark S. Brodie, Abbott Laboratories, Abbott Park, IL 60064

Cholecystokinin octapeptide (CCK8) is co-localized with dopamine in some neurons of the ventral tegmental area of Tsai (VTA). Dopamine-containing neurons of the VTA have been implicated in the rewarding properties of drugs of abuse. We have demonstrated in earlier studies that CCK8 potentiates the inhibitory effect of dopamine on VTA firing studied with extracellular recording from putative dopamine-containing neurons in a brain slice preparation (Brodie and Dunwiddie, *Brain Res.* 425:106, 1987). In addition, ethanol has been shown to excite VTA neurons, both *in vivo* (Gessa *et al.*, *Brain Res.* 348:201, 1985) and *in vitro* (Brodie, *et al.*, *Brain Res.* 508:65, 1990). In the present study, the effects of CCK8 on ethanol-induced excitation of putative dopamine neurons of the VTA was examined. Coronal brain slices containing the VTA were prepared from Sprague-Dawley rats (75-150 gm), as previously described. Drugs were applied to the brain slices by addition to the superfusate via calibrated syringe pumps. Extracellular recording of single unit activity was performed using glass microelectrodes (6-10 MΩ, 0.9% NaCl). Only units conforming to characteristics of putative dopamine neurons were studied; these characteristics included slow firing rate (0.5-5 Hz), and long duration action potential (>2.5 msec). In addition, cells selected for study were excited by ethanol in concentrations from 20 to 160 mM; this concentration range is behaviorally relevant for rats. Sixty percent of these cells exhibited a CCK8-induced potentiation of ethanol-induced excitation; the mean enhancement of the ethanol response by 10 nM CCK8 was 59% ± 19%. This enhancement seemed to persist in some recordings for more than one hour after cessation of CCK8 infusion. These data indicate that ethanol-induced excitation of dopaminergic VTA neurons is potentiated by CCK8. This long-duration enhancement of the response to ethanol may provide important information about the action of ethanol on VTA neurons, as well as to the role of CCK8 in dopaminergic neurotransmission in the central nervous system.

198.2

ETHANOL-INDUCED CHANGES IN NEURONAL FUNCTION AND NOREPINEPHRINE SYNAPTIC OVERFLOW IN LOCUS COERULEUS GRAFTS IN OCULO: IN VIVO ELECTROCHEMISTRY AND ELECTROPHYSIOLOGY. M.R. Palmer[1], A.-Ch. Granholm[3] and G.A. Gerhardt[1,2]. Depts. of Pharmacology[1] and Psychiatry[2], Univ. of Colorado Health Sciences Center, Denver, CO 80262 and Dept. of Cell Biology[3], Univ. of Linköping, Sweden.

In the present study, fetal Sprague-Dawley locus coeruleus (LC) brain grafts were transplanted into the anterior eye chamber of adult host rats and allowed to mature 4 - 8 months. High-speed in vivo electrochemical measurements were used in urethane-anesthetized rats to characterize the ethanol-induced alterations of potassium-evoked synaptic overflow from norepinephrine (NE)-containing cells in these brain grafts in oculo. Ethanol, when superfused over LC grafts consistently attenuated potassium-evoked synaptic overflow of NE at doses between 3 - 10 mM. Furthermore, in preliminary experiments, these responses appeared to be augmented by 30 mM ethanol. Electrophysiologically, the neuronal firing rates in LC grafts were depressed by the superfusion of 1 - 30 mM ethanol. Ethanol, when superfused over sequential co-grafts of LC with either hippocampus or cerebellum caused excitations of hippocampal and cerebellar neuronal activity at doses between 1-10 mM. These ethanol-induced excitations in the double grafts could be prevented by depressing the activity of LC graft neurons before and during ethanol exposure with a co-superfusion of 0.5 - 1.0 μM clonidine. Clonidine caused similar excitations of neuronal activity, but was ineffective in single grafts of either hippocampus or cerebellum. Thus, the ethanol-induced excitations in the hippocampal and cerebellar co-grafts appear to be disinhibitions mediated by a reduction in the release of NE from LC nerve terminals in the target grafts caused by ethanol-induced depressions of the LC neurons. (Supported by USPHS grants AA 05915, AA 00102, AG 06434, and AG 00441, and by the Magnus Bergvall Foundation in Sweden. MRP and GAG are supported by Research Scientist Development Awards from ADAMHA and NIH, respectively.

198.3

STRESS RESPONSES IN RAT LINES SELECTED FOR DIFFERENTIAL ETHANOL-INDUCED MOTOR IMPAIRMENT. E.R. Korpi and K. Tuominen*. Res. Labs, Alko Ltd., P.O.B. 350, SF-00101 Helsinki, Finland.

Alcohol non-tolerant (ANT) and alcohol-tolerant (AT) rats, developed by selective outbreeding for high and low sensitivity to ethanol-induced motor impairment on the tilting plane test, were measured for ethanol sensitivity with the tilting plane (2 g/kg IP) and sleep time (3.5 g/kg IP) tests and for non-intoxicated behavior in the plus-maze test. The lines differed on each of these tests. Daily handling of half of the animals for 2 weeks prior to testing had no significant effects in either rat line. Following the behavioral tests, the rats were decapitated after a 10-min swim at 25 °C. This stress increased the adrenal dopamine concentration and the hypothalamic MHPG/norepinephrine ratio more in the AT than ANT rats. Plasma and adrenal corticosterone was also higher in the stressed AT than ANT rats.

The results indicate that the AT rats have more intense stress reactions without ethanol than the ANT rats, which may at least partly account for their apparent insensitivity to ethanol. This difference appears to occur irrespective of any handling habituation.

198.4

REGIONAL CHANGES IN FUNCTIONAL BRAIN ACTIVITY WITH ETHANOL STIMULANT AND DEPRESSANT EFFECTS. M.J.Lewis,L.B.Perry,H.L. June,M.L.Garnett*and L.J.Porrino*. Howard Univ.,Washington, DC 20059 and *NINDS, NIH, MD 20892.

Increases in open field activity and enhanced brain stimulation reward have been found within the first 30 min after intraperitoneal(IP) injection of low doses of ethanol (E). These effects occur during ascending concentrations of the blood alcohol curve(BAC), but not during the descending portion. To determine the brain systems involved in these effects, the quantitative 2-[^{14}C]-deoxyglucose(2-DG) method was used to map the distribution of changes in local rates of cerebral glucose that accompany E administration. Rats were injected with E(0.50g/kg,IP) and then tested in an open field 0-10 or 30-40 min after administration. For measurement of functional brain activity, rats were implanted with arterial and venous catheters on the day of experimentation and injected with 2-DG 10 or 40 min after E. Activation was found 0-10 min and depression or no effect was found at 30-40 min after E injection. Quantitative autoradiography showed increases in functional activity at both 10 and 40 min in the nuc acc, sub nigra, b-l amyg and caudput. Increased functional activity in the olfactory tubercle(OT), however, occurred only at 10 min(ascending BAC). This selectivity suggests that the OT may play a significant role in the activation and reward properties of E.

(Supported in part by NIAAA grants AA06263 and RR08016)

198.5

ELEVATED PLASMA ANGIOTENSIN II: AN EARLY MANIFESTATION OF CHRONIC ALCOHOL INTAKE.
KB Brosnihan, GB Collins*, JL Joyce*, RA Zuti*. Departments of Brain and Vascular Research and Psychiatry. Cleveland Clinic Foundation, Cleveland, OH 44195.

Recent studies have suggested relationships among alcohol intake, the renin-angiotensin system (RAS), and hypertension. We have developed a chronic alcoholic animal model to examine blood pressure and plasma and cerebrospinal fluid (CSF) levels of RAS, vasopressin (AVP), and catecholamines. Conscious, trained dogs (n = 9) were given ethanol (4 g/kg/day, 50% v/v) or saline (n = 5) intragastrically for 4 weeks. Plasma Ang II increased from baseline (21.1 ± 9.7 vs 7.1 ± 1.5 pg/ml, p = 0.05) and PRA tended to increase after one week of ethanol intake. Both plasma Ang II and PRA were significantly correlated with plasma ethanol ($p < 0.0001$) over the treatment period. In contrast, CSF Ang II did not change and was not correlated with ethanol. Likewise, no significant changes in blood pressure, plasma aldosterone, or CSF and plasma catecholamines were found. A significant reduction in plasma AVP was only observed after 4 weeks of treatment. In summary, our study suggests that plasma Ang II may be an early manifestation of alcoholism. In addition, we have demonstrated for the first time that elevations in plasma Ang II are not mirrored in CSF under conditions of chronic ethanol intake. (Supported in part by the Dietz Fund and PepsiCo).

198.6

CLASSICAL GENETIC ANALYSES OF SENSITIVITIES TO ALCOHOL AND NICOTINE IN CROSSES DERIVED FROM LONG-SLEEP AND SHORT-SLEEP MICE. C. M. de Fiebre and A. C. Collins. Institute for Behavioral Genetics, Univ. of Colorado, Boulder, CO 80309.

Alcohol and nicotine, two of the most commonly used drugs by humans, are often used simultaneously. In an attempt to ascertain whether common genes regulate responsiveness to these two drugs, sensitivities to these compounds were assessed in mice derived from a classical (Mendelian) cross of the long-sleep (LS) and short-sleep (SS) mouse lines which were selectively bred for differential "sleep-time" following ethanol. Analysis of the isogenic generations (LS, SS, F1) and segregating generations (F2, F1XLS, F1XSS) revealed that the inheritance of sensitivities to both of these drugs is primarily additive; however, evidence of epistatic interaction was also found. While the segregation patterns for these two drugs were similar, they were not identical. The segregation patterns for sensitivity to nicotine-induced hypothermia and nicotine-induced seizures more closely resembled the segregation pattern for ethanol "sleep-time" than the other nicotine tests. Previous evidence has indicated that these two nicotine responses may be regulated by the sites which bind [^{3}H]nicotine and α-[^{125}I]bungarotoxin, respectively. These data are consistent with the hypothesis that these two drugs may be regulated, in part, by common genes. Furthermore, as a consequence of this common genetic control, these two drugs may act, in part, on nicotinic cholinergic systems.
Supported by AA-06391, DA-00116 and MH-16880.

198.7

MECHANISM FOR INDUCTION OF ALCOHOLISM: A NEW HYPOTHESIS. B. A. McMillen and R. D. Myers, Dept. of Pharmacol., Sch. of Medicine, East Carolina Univ., Greenville, NC 27858.

Formation of alkaloidal products from aldehyde condensation reactions occurs in mammalian brain. Injections of ng amounts of tetrahydropapaveroline (THP) or related alkaloids i.c.v. or into specific brain areas enhance alcohol (EtOH) preference in rats. Increased EtOH consumption persists long after the injections, which suggests a permanent change in neural function. Radio-ligand assays were used to determine THP affinity for several transmitter receptor sites. THP had uM affinity for alpha, beta, D1, D2 and sigma receptors and no affinity for 5HT1a or 5HT2 receptors. It is hypothesized that taste and other aversive aspects of EtOH limit daily intake by normal rats to 1.0 gm/kg/day or less. THP injections produce a stimulus that is not perceived at this low level of drinking, 'primes' the animal to recognize the reward component of EtOH and causes increased consumption of EtOH in order to maintain a desired reinforcing level of formation of THP and related compounds. In an EtOH preferring individual, either increased formation of rewarding alkaloids or decreased aversion to EtOH will result in increased EtOH consumption. Thus, the decreased level of 5HT function in the P line of rats and Type II alcoholics may result in less aversion to EtOH and release EtOH drinking. (DA 04895 and AA 04200)

198.8

BEHAVIORAL MODIFICATIONS INDUCED BY CHRONIC ADMINISTRATION OF ETHANOL IN RATS. F.Drago, A.A. Genazzani, G.M.Fontana, F. Spadaro and M.Grassi . Institute of Pharmacology, University of Catania Medical School.

Chronic administration of ethanol induces changes in social and motor behavior. We studied the possibility that different doses of ethanol exert opposite effects on the behavior of male rats. Ethanol (0.01, 0.25, 6.25 ml/kg/die) was dissolved in the drinking water and made available ad libitum. Control animals received tip water with the same procedure. The total volume of the solution drunk daily was recorded up to 56 days. Results showed that higher doses of ethanol induced the development of tolerance increasing significantly the consumption after 7 days. That remained elevated for 35 days. Interestingly,animals administered with 0.01 ml/kg of ethanol did not develop any tolerance and they showed a decrease in drinking compared to controls. Animals tested in the hot plate showed that chronic administration of ethanol reduced pain sensitivity and the effect was dose-dependent. Chronic administration of ethanol was also followed by an increase in locomotor activity.

ALZHEIMER'S DISEASE: NEUROPATHOLOGY I

199.1

DISTRIBUTION OF NEUROFIBRILLARY TANGLES AND NEURITIC PLAQUES IN THE CEREBRAL CORTEX IN ALZHEIMER'S DISEASE. S.E. Arnold*, J.E. Flory*, B.T. Hyman, A.R. Damasio and G.W. Van Hoesen. Depts. of Neurology and Anatomy, Univ. of Iowa, Iowa City, IA 52242.

The distribution of neurofibrillary tangles (NFTs) and neuritic plaques (NPs) was mapped in 35 cortical areas in 11 cases with dementia and Alzheimer's disease. Whole hemisphere blocks were embedded in polyethylene glycol, sectioned coronally and stained with thioflavin S and thionin. Some were immunolabeled with Alz-50. The density of NFTs and NPs was assessed using a semi-quantitative scale for each of 577 observations. Analysis of variance yielded highly significant differences between types of cortex for NFT densities ($F=44.959$, $p<.0001$). Limbic periallocortex (area 28) was the most densely invested with NFTs, significantly more than any other type of cortex. It was also the most invariantly affected cortical area analyzed. Descending order of density of NFTs was: limbic periallocortex > limbic proisocortex (areas 11, 12, 24, 23, anterior insula, 38, 35) > nonprimary association cortex (areas 32, 46, superior temporal sulcus, 40, 39, posterior parahippocampal cortex, 37, 36) > primary association cortex (areas 7, 18, 19, 22, 21, 20) > agranular cortex (areas 44, 45, 8, 6, 4) > primary sensory cortex (areas 41, 42, 3, 1, 2, 17). NPs were distributed more homogeneously throughout the cortex. There were no differences in the densities of NPs between types of cortex with the exception of limbic periallocortex (area 28) which had significantly fewer NPs than any other cortical area. No significant left-right differences were found. (Supported by: AG 08487, NS 14944, PO NS 19632.)

199.2

LESIONS OF ANTERIOR THALAMUS IN ALZHEIMER'S DISEASE. H.Braak and E.Braak. Dept. Anatomy, J.W. Goethe University, 6000 Frankfurt 70, Fed. Rep. Germany.

Sensitive silver methods for amyloid and neurofibrillary changes were employed to examine the pathological changes of the anterior thalamus in Alzheimer`s disease. Large numbers of amyloid deposits occurred in the antero-ventral, antero-medial and antero-dorsal nucleus while portions of the nuclear grays situated close to the ventricular surface were virtually devoid of amyloid. Neurofibrillary changes were found in all nuclei of the anterior complex. The antero-dorsal nucleus showed the most severe affection with very large numbers of neurofibrillary tangles and neuropil threads. Many ghost tangles were present reflecting a considerable loss of nerve cells. It is concluded that the circumscribed and thoroughgoing changes revealed by the limbic thalamic nuclei hamper the transport of information from the hippocampal formation to the cingulate cortex, in particular to the retrosplenial region. Such impairments of the Papez circuit may contribute to the development of dementia (Supported by the Deutsche Forschungsgemeinschaft).

199.3

A QUANTITATIVE COMPARISON OF ALZHEIMER NEUROPATHOLOGY AS DETECTED BY A128, ALZ-50, TAU-1 AND TAU-2 IMMUNOLABELLING AND BIELSCHOWSKI SILVER STAINING TECHNIQUES. M-C. de Lacoste, D.R. Sparkman, K.S. Pollan*, C.L. White III*, Depts. Cell Biology and Neuroscience and Pathology, U.T. Southwestern Med. Sch., Dallas, TX 75235

The aim of this study was to obtain comparative quantitative data on the relative reliability of various histological techniques for identifying neuritic plaques (NP), neurofibrillary tangles (NFT) and dystrophic neurites (DN) in cases with neuropathologically confirmed Alzheimer disease (AD).

Formalin-fixed tissue blocks of the hippocampal formation were sectioned at 50 µm on a freezing microtome. NP, NFT, and DN were immunolabelled in a series of adjacent sections using the avidin-biotin immunoperoxidase method and four different antisera including the polyclonal A128 antibody to paired helical filaments, monoclonal TAU antibodies TAU-1 and TAU-2, and the ALZ-50 monoclonal antibody. Sections in the same series were processed with a modified Bielschowski silver stain. CARP software (Computer Assisted Reconstruction Package) was utilized for semiautomated counts of NP, NFT and DN in a representative series of sections.

The results suggest that there is significant variation in the total counts of NP, NFT and DN depending on the labelling technique. Quantitative studies of AD-related neuropathology should take into account the variation in the sensitivity of available techniques.

ALZ-50 antiserum was kindly provided by Dr. P. Davies. TAU-1 and TAU-2 antibodies were a gift from Dr. L.I. Binder. Supported by UT ADRC NIH-Ag-08013 (MCL, DRS, CLW) and NIH-HD21711 (MCL).

199.4

OLFACTORY BULB PATHOLOGY IN ALZHEIMER DISEASE. R.G. Struble and H.B. Clark. Depts of Psychiatry, Pathology and Laboratory Medicine. Southern Illinois University School of Medicine and Memorial Medical Center, Springfield IL 62704

Deficits in olfactory function are an early abnormality in Alzheimer disease (AD). However, several previous reports have not found the hallmarks of AD, senile plaques and neurofibrillary tangles, in the main olfactory bulb (MOB) suggesting that the MOB is relatively free of lesions. We have confirmed the general absence of NFT and SP but, in addition, we find marked loss of mitral and tufted neurons in the MOB from AD cases. Further, there is a spectrum of abnormalities of glomeruli. In some cases there is a severe depletion of glomeruli. In other cases there is an apparent 'hypertrophy' of glomeruli, associated with glomerular invasion of the external plexiform layer. These observations suggest that an early manifestation of AD is the loss of mitral and tufted neurons followed by aberrant regrowth of olfactory nerve. These observations also suggest that the loss of mitral and tufted neurons may underlie the olfactory deficit characteristic of AD.

199.5

NASAL EPITHELIUM IN AGED RHESUS MONKEYS DOES NOT SHOW FEATURES OF HUMAN ALZHEIMER'S DISEASE. B.R. Talamo, W.H. Feng*, L. Cork# and J.S. Kauer. Tufts Medical School, Boston, MA 02111; #Johns Hopkins Med. School, Baltimore, MD 21205.

Nasal epithelium taken at autopsy from 13 rhesus monkeys ranging in age from 8 to 30 years was immunohistochemically examined for cytoskeletal components that have been seen in olfactory epithelium of Alzheimer's (AD) patients. Monkeys older than 20 years were mildly impaired behaviorally. All monkeys had extensive areas of sensory olfactory epithelium with MAP 5 (Sigma; D. Asai) immunoreactivity (ir). Receptor cells also showed variable OMP-ir but no neurofilament-ir. Similar to human tissue, monkey sensory epithelium was patchy, interspersed with non-sensory regions. Some areas of the epithelium were invaginated into sac-like structures which contained variable numbers of sensory cells. Occasionally neurons were tau-ir (tau 14, V. Lee); staining in olfactory axon bundles was bright, but heterogeneous, suggesting that only a subset of neurons reacted with this antibody. Unlike human and rat, there were abundant fine beaded fibers in non-sensory epithelium and in the lamina propria. These fibers were MAP5-ir, and sometimes tau-ir, but did not stain for neurofilaments, including peripherin. Other smooth, thick fibers that were ir for neurofilament protein and/or peripherin paralleled the basement membrane and occasionally projected into the epithelium, primarily in non-sensory areas, but these did not resemble the masses of neurites seen in AD cases. These findings suggest that the neuritic accumulation in olfactory epithelium of AD patients (Talamo et al, Nature 337:736,1989) is not a normal concommitant of aging in non-human primates. (Supported by the Pew Charitable Trusts and ADRDA grant IIRG-89-041).

199.6

CONTINUOUS CULTURE OF HUMAN OLFACTORY EPITHELIAL NEUROBLASTS. B.L. Wolozin, T. Sunderland*, B. Zheng*, I. Resau*, R.D. Swerdlow* and H.G. Coon* Lab. of Clinical Science, NIMH and Lab. of Genetics, NCI, Bethesda, MD 20892

The primary sensory neurons of the olfactory epithelium are the only neurons in mammals that have been shown to maintain the capability to regenerate throughout life. Recent reports show that neuroblasts can be grown in culture from explants of neonatal rat olfactory epithelium (Coon et al, PNAS 86:1703-7, 1989). Using similar methods, we have been able to generate cell lines of neuroblasts from explants of adult human olfactory epithelium taken at autopsy. The cells have a robust odorant response, yielding a doubling of basal cAMP in response to odorant challenges in a dose dependent fashion. Using Western blot and immunohistochemical methods, the cells have been shown to exhibit many of the antigens of olfactory neurons *in vivo*, including: neuron specific enolase, tau protein, MAP 2, 68 and 200 KD neurofilaments, neuron specific calcium binding protein, olfactory marker protein and calcitonin gene related peptide. When allowed to grow in a collagen-laminin gel, many of the cells appear to develop into mature olfactory neurons exhibiting short dendrites with apical olfactory vesicles having cilia and basal axons. We are currently growing cells from two individuals who died from Alzheimer's disease and plan to study these cells to determine if any disease related pathophysiology exists. The ability to create cell lines of differentiating neuroblasts from individuals with neuropsychiatric disorders may prove to be very useful in the study of these illnesses.

199.7

DENDRITIC NEURITES ARE HISTOCHEMICALLY AND MORPHOLOGICALLY DISTINCT FROM AXONAL NEURITES IN ALZHEIMER'S DISEASE. A.C. McKee, K.S. Kosik, and N.W. Kowall, Dept. of Neuropathology & Neurology, Harvard Medical School, Boston, MA 02114

The origin of tau immunoreactive (imr) argyrophilic neurites in Alzheimer's disease (AD) is not known. Using monoclonal antibodies against tyrosylated & acetylated tubulin, tau, Alz 50, MAP2, phosphorylated (SMI 31) & non-phosphorylated neurofilament (SMI 32), we compared the hippocampal distribution of these axonal & dendritic markers in normal elderly and AD patients. In AD, granular & spindle shaped Alz 50 imr dystrophic neurites (DN) were found in the white matter, outer dentate molecular layer, & molecular layer & stratum oriens of Ammons horn. Similar Alz 50 imr DN were found in senile plaques (SP). Tau imr DN were filiform fibers often with spike-like projections. This type of neurite was infrequently Alz 50 imr. Their density paralleled that of Alz 50 in Ammon's horn, but they were rare in the dentate molecular layer. Tau imr DN of SPs were both filiform & spheroidal. The axon specific antibody SMI 31 largely retained its axonal location in AD. Numerous spheroids with axonal tails were found in the white matter & within SPs. Small granular DN, similar to those found with Alz 50 were seen in the white matter & stratum oriens. The distribution of tau imr DN in the molecular layer of Ammon's horn was co-extensive with a dense network of tyrosylated tubulin imr distal dendritic branches. This same plexus was imr for the dendrite specific markers, MAP2 & SMI 32. In contrast, acetylated tubulin imr was confined to proximal axons & dendrites. DN were not imr for MAP2, SMI 32, or the tubulins. We show that DN are both axonal & dendritic in origin. Tau antibodies preferentially demonstrate dendritic DN, whereas Alz 50 & SMI 31 DN are largely axonal. Axonal DN have a typical spheroidal & spindled shape; dendritic DN arise in association with unstable forms of tubulin & are filiform. Alz 50, a marker of early neuronal degeneration in AD, clearly demonstrates axonal pathology which may suggest that the initial focus of injury in AD is the axon.

199.8

LAMININ IMMUNOREACTIVITY IS INCREASED IN ALZHEIMER DISEASE HIPPOCAMPUS. N.W. Kowall and A.C. McKee. Dept. of Neurology and Pathology, Mass. General Hospital, Boston MA 02114.

Laminin, a major glycoprotein component of the basal lamina, is a potent promoter of neurite outgrowth and the principal factor produced by cells promoting neurite extension in culture. It allows nerve growth factor (NGF)-dependent cell lines to survive temporarily without NGF and plays a role in regeneration following injury. Laminin was initially thought to be confined to the basement membranes of blood vessels in the central nervous system (CNS). Subsequent studies showed laminin immunoreactivity in non-neuronal cells in the CNS. Recently, laminin mRNA has been localized to neurons in rodent brain and laminin immunoreactive neurons have been found in several forebrain structures of the human brain, including the hippocampus. We used 2 monoclonal anti laminin antibodies to study the distribution of laminin immunoreactivity in normal elderly and Alzheimer's disease (AD) brain. One antibody (MAB 1975, Chemicon) was raised against murine laminin and recognizes a conformational epitope on the B1 and B2 heterodimer and P1 fragment of laminin. This antibody does not inhibit laminin mediated adhesion. The second antibody (LAM-1, ICN) stains basement membranes and smooth muscle in a wide variety of organs in both mouse and human. MAB 1975 stained the basement membrane surrounding blood vessels in both control and AD hippocampus. No cellular or other localized immunoreactivity was visible. Perivascular staining with LAM-1 was present, although weaker, in all cases. In control brains scattered pyramidal neurons and occasional astrocytes were weakly immunoreactive with LAM-1. In AD, neuronal perikarya were more frequently immunopositive in the CA fields and subiculum. Astrocytes were occasionally seen. Senile plaque cores were often laminin immunoreactive. Increased expression of laminin or laminin-like peptides in AD may play a primary role in neuritic proliferation in the neuropil and around senile plaques. Alternatively laminin synthesis may be increased in response to neuronal injury and degeneration in AD.

199.9

ACETYLCHOLINESTERASE, BUTYRYLCHOLINESTERASE AND NON-SPECIFIC ESTERASE OF CEREBRAL CORTEX AND MICROVESSELS IN ALZHEIMER'S DISEASE. R. N. Kalaria and I. G. Grahovac*. Departments of Neurology and Neuroscience, Case Western Reserve University, Cleveland, OH 44106, USA

Previous investigators have shown the consistent presence of acetylcholinesterase (AChE) and butyryl-cholinesterase (BChE) in amyloidotic lesions of Alzheimer's disease (AD). We similarly used histochemical methods to confirm AChE and BChE activities in amyloid deposits and vessels in tissue sections of neocortex and hippocampus from subjects with AD and other related disorders. We also stained for non-specific esterase (NSE) for comparison. Like AChE and BChE, NSE activity was evident in plaques.

It has been suggested by others and us that AChE and BChE bind to the heparan sulphate proteoglycan-type of molecules (HSPG) described to be present in these lesions and in cerebral vessels. Thus, we investigated the activities and inhibitory properties of these enzymes in cerebral microvessels (CMV) and plaque fractions isolated from AD subjects. Activities of both AChE and BChE were significantly decreased in CMV from AD subjects compared to age-matched controls. The inhibition of AChE and BChE by diisopropylfluorophosphate and other specific inhibitors was markedly reduced in AD whereas EDTA and other anti-proteases had no apparent effect. Our results imply that co-localization of ChEs with HSPG-type of molecules may affect their inherent properties. Supported by the ADRDA.

199.10

ANTIBODY IN THE CEREBROSPINAL FLUID OF ALZHEIMER'S DISEASE PATIENTS RECOGNIZES AMOEBOID MICROGLIAL CELLS : ANOTHER LINK TO IMMUNOLOGICAL INVOLVEMENT IN THIS NEURODEGENERATIVE DISEASE. A. Dahlström[1], E.A. Ling*[2], R. Polinsky*[3], A. Wigander*[1], K. Lundmark,*[1] and A. McRae[1]. University of Göteborg, Dept. of Histology, PO 33031, S-400 33 Göteborg Sweden[1], National University of Singapore, Dept of Anatomy [2], NINDS Bethesda, MD, USA[3]

Previous investigations showing the presence of antibodies in cerebrospinal fluid (CSF) from Alzheimer's disease (AD) patients support a pathogenetic role for immunological abberations in this disorder. Paraformaldehyde (PF) fixed 2 weeks old cultures, prepared from dissociated cells from the medial septum of 18 day (E18) fetal rats, were incubated with CSF from 12 AD patients and 22 non-demented healthy controls. The cultures were processed for immunocytochemical observations with the avidin-biotin complex method. Only AD-CSF recognized neuronal like cells and microglia in culture. The study was extended to developing central nervous system (CNS) by using rats ranging in age from E18 through postnatal day 5. After PF fixation frozen brain sections (20μm) were incubated with CSF samples. The AD-CSF antibody recognized diverse morphological forms of amoeboid microglial cells, located mainly in the cavum septum pellucidum and corpus callosum. Electron microscopy revealed that the AD-CSF antibody recognizes specific membrane receptors in the macrophagic microglia. The unexpected recognition of amoeboid microglia by AD-CSF is particularly significant since these cells proliferate in response to nervous system disease and also engulf debris. These results add further support to the concept that inflammation and similar immune mechanisms may contribute to AD pathogenesis.

199.11

MEASUREMENT OF ALZHEIMER'S DISEASE ASSOCIATED PROTEIN (ADAP) WITH A SANDWICH ENZYME IMMUNOASSAY INCORPORATING ALZ-50. G. Bissette[1], W. Smith[1], K. Dole[1], H. Ghanbari[3], B. Miller[2], B. Crain[2] and C.B. Nemeroff[1]. Depts. of Psychiatry and Pathology, Duke Univ. Med. Ctr., Durham, NC and Abbott Labs.[3], North Chicago, IL.

Using a 2 antisera sandwich enzyme-linked immunoassay for Alzheimer's disease (AD) associated protein (ADAP) that employes the Alz-50 antibody but excludes such non-Alzheimer specific antigens such as tau or microtubule-associated protein, we measured ADAP concentrations in micro-punched post-mortem samples of frozen temporal and frontal cortex from 91 cases of histologically confirmed AD compared to 27 non-AD controls with and without a clinical history of dementia. Both temporal and frontal cortex samples from AD patients were significantly higher in ADAP concentration than the non-AD controls. Only 10 of the 91 AD patients had ADAP concentrations below the mean control concentration in both temporal and frontal cortex. ADAP concentration correlated with numbers of both plaques and tangles. Several of the non-AD controls had high levels of ADAP that were shown to represent authentic ADAP antigen by Western blot analysis. This assay has the potential as an adjunct in early diagnosis in biopsy samples and, perhaps with refinement of the assay, in cerebrospinal fluid. (Supported by NIMH MH-40524 and NIA AG-05128.)

199.12

NON-HEME IRON IS INCREASED AND ASCORBATE-STIMULATED LIPID PEROXIDATION IS REDUCED IN POSTMORTEM PREFRONTAL CORTEX IN ALZHEIMER'S DISEASE (AD): A COMPARISON OF FINDINGS IN AD AND NORMAL AGE MATCHED CONTROLS. A.C. Andorn, R.S. Britton*, B.R. Bacon*, M. Franko*, N. Hamazaki* and R.N. Kalaria. Depts. of Psychiatry, Pharmacology and Medicine, LSU Med. Cntr., Shreveport, LA, 71130 and Dept. of Neurology, Case Western Reserve Univ., Cleveland, Ohio 44106.

Some etiologic theories about AD have been based on the hypothesis that lipid peroxidation (LP) occurs to a greater extent in AD brain in vivo than in normal controls. If a difference in LP does occur between AD and age-matched controls, such a difference could be due to differences in the concentrations of total iron, antioxidants, or oxygen radical scavenging enzymes. In the studies reported here, we determined ascorbate-stimulated (0.1 mM) LP and non-heme iron in particulate membrane fragments from AD (diagnosed by NIH consensus criteria), and normal age-matched controls. The membrane preparations and ascorbate-stimulated LP (measured by malondialdehyde [MDA]) were done as previously described (Andorn, et al., Mol. Pharmacol., 33:155, 1988). Total non-heme iron was determined by spectrophotometric assay. A total of 16 AD brains and 8 age-matched controls were used (both studies were not done on all brains).

BRAIN TYPE	MDA (nmoles/mg)	Fe (μg/mg)
ALZHEIMER'S	11.3 ± 2.3*	0.43 ± 0.11**
CONTROL	20.8 ± 6.1	0.31 ± 0.11

Values are mean ± S.D. per mg protein. Significantly different as determined by Student's t-test at p = 0.01 (*) and p = 0.027 (**).

The differences in LP in AD could be due to changes in lipid composition, antioxidant balance and/or increased ante-mortem LP.

EXCITATORY AMINO ACIDS: PHARMACOLOGY II

200.1

BEHAVIOURAL PROPERTIES OF ANTAGONISTS ACTING AT THE GLYCINE MODULATORY SITE ON THE NMDA RECEPTOR/ION CHANNEL COMPLEX. M.D. Tricklebank and K. Saywell. Merck Sharp & Dohme Research Laboratories, Neuroscience Research Centre, Terlings Park, Harlow, Essex CM20 2QR, U.K.

Non-competitive NMDA receptor antagonists are anticonvulsant and induce in rodents a motor syndrome of head weaving and ataxia. We have examined the behavioural effects of antagonists acting via the glycine modulatory site on the NMDA receptor, viz: 5,7-dichlorokynurenic acid (5,7-DCK), (+)-HA-966 and D-cycloserine (D-CS).The results in units of μmol/mouse, icv, are summarised in the table.

	Audiogenic seizures (ED50)	Electrogenic seizures (ED50)	Rotarod (MED)	Head weaving (MED)
5,7-DCK	0.01	0.05	0.04	0.01
(+)-HA-966	0.01	1.00	0.90	0.90
D-CS	0.20	>2.90	>2.90	1.00

All compounds antagonised seizures induced by sound in DBA/2 mice, but D-CS was inactive against electroshock in Swiss Webster mice. Ataxia (measured by rotarod performance in Swiss Webster mice) was induced by 5,7-DCK and (+)-HA-966 at doses similar to those protecting against electroshock. D-CS did not induce ataxia at the doses tested. All compounds induced head weaving, but the peak intensity of the response was never more than 12% of that seen with the non-competitive NMDA receptor antagonist, MK-801 (0.03 μmol/mouse). Thus, glycine receptor antagonists are anticonvulsant, but differ from NMDA ion channel blockers by their reduced propensity to induce head weaving. The greater separation between ataxic and audiogenic anticonvulsant doses of (+)-HA-966 and D-CS may reflect their partial agonist properties.

200.2

SPERMIDINE REVERSES ARCAINE'S INHIBITION OF NMDA-INDUCED HIPPOCAMPAL [^{3}H]NE RELEASE. **A. I. Sacaan and K. M. Johnson. Department of Pharmacology, Univ. of Texas Med. Br., Galveston, TX 77550.**

Arcaine, a putative polyamine antagonist (Reynolds,1990) completely inhibited [^{3}H]NE released from hippocampal slices in the presence of 100μM NMDA. The IC_{50} value was 102μM, a value about 10-fold higher than its K_B calculated from binding studies (see Johnson and Sacaan, this meeting). Arcaine's inhibition was noncompetitive with respect to NMDA. Arcaine (100μM) had no effect on release induced by either 300μM kainic acid or 15mM K^+, suggesting specificity for the NMDA receptor ionophore complex. To address the question of whether arcaine's inhibition was mediated through the polyamine site, we unsuccessfully tried to reverse the effect of 30μM arcaine with 1mM spermidine. When the release experiments were conducted in buffer containing 5% DMSO spermidine had no effect alone at either 1 or 3mM, but was able to completely reverse the inhibitory effect of 100μM arcaine in a concentration-dependent manner. We speculate that DMSO increased membrane permeability, thus allowing spermidine access to its putative intracellular site, resulting in reversal of arcaine's inhibition. Why arcaine was equipotent in physiologic and DMSO containing buffer is unknown. Interestingly, putrescine (a noncompetitive antagonist of spermidine-induced [^{3}H]TCP binding) also inhibited NMDA-induced [^{3}H]NE release in DMSO containing buffer. Unexpectedly, this inhibition was also reversible by spermidine, perhaps suggesting a metabolic conversion of putrescine to a competitive inhibitor. Supporting this possibility is the observation that putrescine can be metabolized in invertebrates to arcaine by two successive transamidination reactions (Robin et al., 1967). These data suggest that polyamines play an important, perhaps obligatory role in the function of the NMDA receptor ionophore complex. Supported by DA-02073.

200.3

THE STIMULATORY, BUT NOT INHIBITORY, EFFECTS OF SPERMIDINE AND MAGNESIUM ON [^{3}H]TCP BINDING TO THE NMDA IONOPHORE ARE MEDIATED BY THE SAME SITE. K. M. Johnson and A. I. Sacaan, Department of Pharmacology, Univ. of Texas Med. Br., Galveston, TX 77550.

Spermidine and magnesium stimulate the binding of [^{3}H]TCP to well washed buffy coat (BC) membranes with EC_{50} values of about 25 and 250μM, respectively. The maximal effect after 2 hrs incubation is about 10-fold for spermidine and 5-fold for magnesium. At higher concentrations, the dose effect curve turns downward, bringing binding back to control with IC_{50} values of 13 and 9mM, respectively. Arcaine (30μM) produced a four-fold, parallel, rightward shift in the concentration-response curve for both spermidine and magnesium, confirming that arcaine is a competitive spermidine antagonist (Reynolds, 1990) and suggesting that magnesium is a partial agonist at this site. This was confirmed by redetermining the spermidine concentration-response in the presence of $MgCl_2$. Magnesium (0.1mM) had little effect while 3mM completely blocked the stimulatory effect of spermidine. The inhibitory effects of spermidine and $MgCl_2$ were studied in the presence and absence of different activators. $MgCl_2$ right shifted the IC_{50} value for spermidine from 13 to 27mM, while spermidine left shifted the IC_{50} value for $MgCl_2$ from 8 to 2.5mM, implying distinct inhibitory sites. This distinction was further amplified by 10μM glutamate which left shifted the IC_{50} value for $MgCl_2$ 2-fold, but shifted that for spermidine 13-fold and reduced its maximal inhibition by about 50%. We speculate that the spermidine site is distinct, but overlaps that for Mg^{+2} and that glutamate induces a conformational change in the NMDA ionophore that uncouples the spermidine site from the inhibitory Mg^{+2} site. On the other hand spermidine and Mg^{+2} stimulate [^{3}H]TCP binding via activation of the same site, which is distinct from the inhibitory site. Supported by DA-02073.

200.4

IN VIVO INTERACTION OF A POLYAMINE WITH THE NMDA RECEPTOR.

L Singh*, R J Oles and G N Woodruff
Parke-Davis Research Unit, Cambridge, England

Polyamines in vitro potentiate the specific binding of radioligands that interact with the ion channel of the NMDA receptor complex. It is not clear whether this interaction would have any physiological consequences in vivo. We have investigated the ability of the polyamine spermidine to modulate NMDLA-induced tonic seizures in mice.

The s.c. administration of NMDLA (25-300 mg/kg) and pentylenetetrazol (PTZ 40-120 mg/kg) dose-dependently induced tonic-seizures. Spermidine administered i.c.v. 15 min before the convulsant dose-dependently (0.5-2.0μmols) potentiated NMDLA-induced seizures, decreasing the dose of NMDLA required to induce seizure activity in 50% of mice (ED_{50}) by more than 3 fold. However, spermidine (2.0μmols, i.c.v.) decreased the PTZ ED_{50} by only 14%.

These results suggest that polyamines may interact with the NMDA receptor in vivo and the polyamine modulatory site under normal circumstances may not be fully activated.

200.5

NMDA-, QUISQUALATE- AND KAINATE-DEPENDENT INCREASES IN CEREBELLAR cGMP IN VIVO; MEDIATION BY NITRIC OXIDE. P.L. Wood. Department of Biological Research, Hoechst-Roussel Pharmaceuticals Inc., Somerville, NJ 08876.

The excitatory amino acid agonists N-methyl-D-aspartate (NMDA), quisqualate and kainate all dose-dependently increased mouse cerebellar cGMP after direct intracerebellar injection, in vivo. These actions were time dependent, with NMDA-dependent responses being selectively antagonized by the noncompetitive NMDA antagonist, dizocilipine and the competitive antagonist of the NMDA-associated glycine site, 1-hydroxy-3-aminopyrrolidone-2 (HA-966). Quisqualate responses were blocked by the nonselective antagonist, 6,7-dinitroquinoxaline-2,3-dione (DNQX).

To examine possible mediation of these excitatory amino acid responses by nitric oxide, an inhibitor of nitric oxide synthase, N-monomethyl-L-arginine, was used. This enzyme inhibitor was found to antagonize the responses of all 3 receptor agonists; with this inhibition being reversed by coadministration of a molar excess of the competitive enzyme substrate, L-arginine. These data are all consistent with modulation of cerebellar cGMP by all 3 major excitatory amino acid receptor subtypes and mediation of these responses by the intercellular messenger molecule, nitric oxide.

200.6

GABA$_B$ RECEPTOR STIMULATION AND EXCITATORY AMINO ACID-INDUCED PHOSPHATIDYLINOSITOL TURNOVER IN RAT CEREBELLUM. Sheryl S. Smith and B.-K. Jin, Dept. of Anatomy, Hahnemann Univ., Phila., PA.

Excitatory amino acid (EAA) effects on turnover of phosphatidylinositol (PI) were assessed under different conditions as a possible mechanism for their observed actions on synaptic development. PI hydrolysis was measured in 160 x 160 μ minces of cerebellar tissue from 7-10 day old female Long-Evans rats using [^{3}H] myo-inositol. Inositol phosphates (IP's) were separated by chloroform/MeOH extraction and anion exchange chromatography, and the PI turnover rate assessed as a percentage of basal under varying conditions. Addition of NMDA (10^{-4} M) enhanced the ability of QUIS (10^{-6} M) to increase PI turnover by 71 ± 3.5% ($P<0.05$) in the presence of the GABA$_B$ agonist baclofen at a dose of 50 μM ($n=10$). A lower dose of baclofen (1 μM) exerted permissive effects on the ability of NMDA to stimulate PI turnover ($n=10$), an effect not diminished by the addition of 500 nM tetrodotoxin. Normally inhibitory to this parameter, NMDA increased IP formation by 47 ± 2.6% above basal levels ($P< 0.01$, a 25 ± 1.2% increase above QUIS-stimulated values) in the presence of the lower dose of baclofen. Under these conditions, combined administration of QUIS and NMDA resulted in levels of PI turnover intermediate between those obtained with either EAA alone. At neither dose of baclofen were effects on basal or QUIS-stimulated PI turnover observed. These effects of the GABA$_B$ agonist were specific for this receptor subtype as stimulation of the GABA$_A$ receptor with isoguvacine (50 μM) did not result in any significant change in EAA agonist stimulated levels of PI turnover. In addition, both phaclofen (10^{-2} M) and APV (5 mM) completely blocked the permissive effect of baclofen on NMDA and QUIS/NMDA induced production of IP's, suggesting that functional GABA$_B$ and NMDA receptors are both required for the observed interaction of these amino acids on stimulation of PI turnover. These results are consistent with the hypothesis that interactions of amino acids may play a role in development of the cerebellum. *(Supported by NS 25809 to SSS.)*

200.7

GABA$_B$ RECEPTOR-MEDIATED INHIBITION OF THE NMDA COMPONENT OF SYNAPTIC TRANSMISSION. R.A.Morrisett, D.D.Mott, H.S.Swartzwelder, D.V.Lewis, and W.A.Wilson. Depts. of Med. (Neurol.), Pharmacol. and Pediatr. (Neurol.), Duke University and the VA Med. Ctrs., Durham, NC 27710.

Recent developments in excitatory amino acid pharmacology have allowed the demonstration of a GABA$_B$-receptor mediated monosynaptic IPSP in the rat hippocampal slice preparation (Davies and Collingridge, *SON Abstr.* 15:529, 1989). This demonstration has led us to hypothesize that this post-synaptic action of GABA$_B$ receptors may promote the voltage-dependent block of the NMDA channel by Mg^{++}.

We now report the direct demonstration of GABA$_B$ receptor-mediated inhibition of the NMDA component of synaptic transmission. In order to prevent complications of other excitatory and inhibitory amino acid receptors, we utilized the non-NMDA receptor antagonist, DNQX (10 uM) and the GABA$_A$ channel antagonist, picrotoxin (10 uM). Under these conditions large, long-lasting NMDA EPSPs were recorded from s. radiatum of area CA_1 upon stimulation of the Schaffer collaterals. NMDA EPSPs were sensitive to competitive and non-competitive NMDA antagonists. When NMDA EPSPs were paired at interstimulus intervals between 100-400 msec, we observed an almost complete block of the second response. The paired pulse inhibition was completely reversed by GABA$_B$ receptor antagonists. Intracellular recordings revealed a late phaclofen-sensitive IPSP which correlated in time with the period of inhibition. Non-NMDA EPSPs showed no such inhibition suggesting that the relevant GABA$_B$ receptors were located post-synaptically. These data suggest that GABA$_B$ receptors may play a significant role in regulating NMDA-dependent processes. (NS #17701, MH #15177-13 and Vet. Admin).

200.8

PD117302 ATTENUATES GLUTAMATE NEUROTOXICITY IN VITRO. M. A. DeCoster, J. C. Hunter[1], J. Hughes[1], and F. C. Tortella. Neuropharmacology Br., Dept. Med. Neurosciences, Walter Reed Army Inst. of Research, Washington, DC 20307-5100 and [1]Parke-Davis Res. Unit, Addenbrookes Hospital Site, Cambridge, CB2 2QB, England.

Anticonvulsant and N-methyl-D-aspartate (NMDA) antagonist effects of the selective kappa opioid PD117302 have recently been described (Tortella et al., Life Sci., 46: 1990). Here we report that PD117302 protects cultured rat neurons from glutamate (glu) toxicity. Fetal neurons from E-14 cortex were cultured for 2 weeks, exposed for 1 hour to 50 um glu in Locke's solution (Locke's) without Mg^{++} and, 20 hours later, the degree of neuronal injury was assessed by measuring lactate dehydrogenase (ldh) release. Cells treated with 50 um glu released 193.8 + 10.9 units/ml of ldh; those treated with Locke's alone released 76.1 + 30.2. Treatment with 25 nm PD117302 or 25 nm MK801 resulted in 32.6% (155.4 + 13.5) or 74.6% (105.9 + 8.0) protection, respectively. Doses of 50 nm PD117302 and MK801 protected against 100 um glu to the extent of 95.9% and 100%, respectively. PD117302 or MK801 alone had no significant effect on ldh release when compared to cells receiving only Locke's. These results indicate that in addition to its anticonvulsant and NMDA antagonist properties in vivo, PD117302 protects cultured rat neurons from glutamate-induced cell death, raising the possibility that this drug, or related compounds, may have therapeutic utility as a neuroprotective agent.

200.9

TRANSIENT DEPRESSION OF THE ELECTROGRAPHIC ACTIVITY OF THE RAT SENSORIMOTOR CORTEX FOLLOWING LOCAL MICROPERFUSION OF N-METHYL-D-ASPARTATE, *IN VIVO*. N. Ludvig, P.K. Mishra, R.L. Burger, S.M. Lasley, J.W. Dailey and P.C. Jobe. Dept. of Basic Sciences, University of Illinois College of Medicine at Peoria, Peoria, IL 61656.

This study was designed to obtain insight into the role of N-Methyl-D-aspartate (NMDA) receptors in the cerebral cortex in physiological conditions. Intracerebral microdialysis was utilized to perfuse NMDA (2 ul/min, 15 min duration) in the sensorimotor cortex of freely moving Sprague-Dawley rats. Before, during and after the drug microperfusions the behavior of the rats was videotaped, and the local electrographic activity was analyzed on-line with a Cadwell 8400 system. The microdialysates were collected for subsequent HPLC analyses.

Control aspartate and glutamate concentrations in the extracellular space were in the range of 0.2-2.0 μM and approximately 60 % of the perfused NMDA entered in the tissue. Dialysis with 1.0 mM NMDA did not cause significant behavioral or electrographic effects. However, 10 mM NMDA induced a marked depression of the electrographic activity. This was reflected in a 74.3+8.1 % decrease of the spectral power of the high frequency EEG bands and the concomitant decrease of EEG amplitude. The depressant effect of NMDA was transient: 2 hours after the drug perfusions the electrographic activity returned to normal. Remarkably, neither behavioral nor electrographic seizures were observed.

These data may indicate that in the cerebral cortex the NMDA receptor-linked excitatory, potentially epileptogenic, effects are controlled by powerful regulatory mechanisms of which activation can lead to depression.

200.10

REDOX PHENOMENA MODULATE FUNCTION OF THE NMDA RECEPTOR, M.D. Majewska, J.A. Bell, and E.D. London, Neuropharmacology Laboratory, NIDA Addiction Research Center, Baltimore, MD 21224.

Redox phenomena play a vital role in the function of proteins, including some receptors (Karlin, A. and Winnik, M., Proc. Natl. Acad. Sci., 60:668, 1968). Ascorbic acid (AA) and glutathione (GSH), are prominent biological reductants, present in the CNS at millimolar concentrations. Previously we showed that AA and GSH inhibit the binding of [^{3}H]glutamate and [^{3}H]thienylcyclohexylpiperidine ([^{3}H]TCP) to the N-methyl-D-aspartate (NMDA) receptor, and block NMDA-gated currents in neurons (Majewska, M.D. et al., Soc. Neurosci. Abstr., 15:1167, 1989). To determine if redox phenomena regulate the function of the NMDA receptor, we subsequently examined interactions of other reductants with this receptor. Hydroquinone (mM) behaved as AA or GSH, inhibiting TCP binding and reversibly reducing NMDA-gated currents in cultured rat cerebral cortical neurons. However, dithiothreitol (10 μM - 1 mM), which cleaves disulfide bonds in protein cystine residues, and which was almost inactive in altering [^{3}H]glutamate and [^{3}H]TCP binding, markedly and reversibly potentiated NMDA-gated currents. Mercaptoethanol had a similar effect. Effects of the reductants were rapidly decreased or abolished by addition of an oxidizing agent. Our data suggest that redox phenomena play a complex role in regulating the function of the NMDA receptor.

200.11

DUAL REGULATION OF KYNURENIC ACID PRODUCTION IN RAT HIPPOCAMPUS IN VIVO. H.-Q. Wu and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

Kynurenic acid (KYNA), an unspecific and competitive excitatory amino acid receptor antagonist, can be synthesized from its immediate bioprecursor L-kynurenine (KYN) in the brain in vitro and in vivo (J. Neurochem., 52: 1629, 1989; Neurosci. Lett., in press). We have now examined the regulation of extracellular KYNA levels in the rat hippocampus by microdialysis in unanesthetized rats. KYN (500 μM) and all drugs were applied through the dialysis probe (2 mm) which was positioned in the dorsal hippocampus. Drugs were applied after 3 hours of KYN perfusion (at steady-state KYNA levels). 300 μM aminooxyacetic acid (AOAA), an inhibitor of kynurenine aminotransferase decreased KYNA levels by 80%. After discontinuation of AOAA, no return to basal levels was seen for at least 3 h. Veratridine (50 μM) caused a KYNA decrease of 50%, and levels recovered completely within 1 hour of discontinuation. KYNA production was doubled one week after injection of quinolinic acid (20 μg/1 μl). The lesion blocked the effect of veratridine but did not prevent the inhibitory action of AOAA. Tetrodotoxin (5 μM) abolished the effect of veratridine but not that of AOAA.

These data suggest that KYNA function in the brain in vivo can be influenced both by a direct block of its biosynthetic enzyme and by neuronal depolarization.

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200.12

PYRAZINAMIDE STIMULATES 3-HYDROXYANTHRANILATE PRODUCTION FROM ANTHRANILATE IN RAT BRAIN AND SPLEEN SLICES. H. Baran and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

Upon systemic administration to rats, the anti-tuberculosis agent pyrazinamide (Pyr) substantially raises serum and urine concentrations of the endogenous excitotoxin quinolinic acid (QUIN). The mechanism of action has been suggested to involve a block of aminocarboxymuconate-semialdehyde decarboxylase, resulting in a dysfunctional glutarate pathway and a concomitant shift towards enhanced QUIN production (BBA, 677:109, 1981). We have now tested the ability of Pyr to affect anthranilate 3-hydroxylase, an enzyme which may be preferentially involved in QUIN production in the brain (Baran and Schwarcz, J. Neurochem., in press).

In the presence of 100 μM anthranilic acid and an inhibitor of 3-hydroxyanthranilic acid (3HANA) degradation, rat tissue slices (base: 1 x 1mm) were incubated in Ringer buffer for 2 h at 37°C. In brain and spleen, 1 mM Pyr stimulated 3HANA production (2.3- and 2.8-fold, respectively). In brain slices, Pyr effects were linear up to 1 mM. Incubation for 5 min yielded 56% of that seen after 2 h. Notably, Pyr was without effect on 3HANA production from 3-hydroxykynurenine in both brain and spleen slices.

Stimulation of anthranilate 3-hydroxylase may be responsible for the effect of Pyr on QUIN production in mammalian cells. (Supported by USPHS grant NS 28236).

200.13

CLOZAPINE ATTENUATES EVENTS MEDIATED BY N-METHYL-D-ASPARTATE (NMDA) RECEPTOR COMPLEX: FUNCTIONAL EVIDENCE FOR AN ADRENERGIC MODULATION OF CEREBELLAR PURKINJE CELL ACTIVITY IN VIVO. T. S. Rao, P. C. Contreras, J. A. Cler, M. R. Emmett, S. J. Mick, S. Iyengar and P. L. Wood. G. D. Searle-Monsanto, St. Louis, MO 63198.

Due to the putative role for dopamine (DA) and noradrenaline (NE) in the etiology of ischemia-induced neuronal cell death, the modulation by clozapine, an atypical antipsychotic agent that interacts with NE receptors of NMDA receptor complex-mediated events were studied by examining its effects on mouse cerebellar cGMP levels. Clozapine decreased basal cerebellar cGMP levels and antagonized harmaline-, methamphetamine (MA)-, pentylenetetrazol (PTZ)- and D-serine-induced cGMP levels with AD_{50} values of 3.9, 2.36, 2.13 and 2.1 mg/kg (ip), but did not affect quisqualate-response. Antagonists of DA (D_2) and serotonin did not reverse the harmaline response. However, WB-4101, an α_1-adrenergic antagonist, reversed harmaline-, D-serine-, PTZ-and MA-induced cGMP levels indicating an adrenergic modulation of the NMDA receptor complex's response. These results indicate an adrenergic modulation of Purkinje cell activity.

201.1

CALCIUM FEEDBACK AND SENSITIVITY REGULATION IN PRIMATE RODS. K. Nakatani, T. Tamura* and K.-W. Yau. Howard Hughes Medical Institute and Dept. Neuroscience, Johns Hopkins School of Medicine, Baltimore, MD 21205.

We and others have previously shown in amphibians that when retinal rods are illuminated there is a net efflux of Ca^{++} from the cells via a Na^{+}-Ca^{++} exchange mechanism. This Ca^{++} efflux leads to a decrease in free calcium in the rod outer segment, which in turn feeds back negatively on the phototransduction process to regulate the light sensitivity of the cells. We now report a similar mechanism in primate rods. Using a suction pipet to record membrane current from an isolated primate rod, we examined the kinetics of the electrogenic Na^{+}-dependent Ca^{++} efflux during illumination after dark-loading the cell with Ca^{++} (by either removing Na^{+} from around the outer segment or exposing it to IBMX). We found that the rate of this efflux, which reflects the internal free Ca^{++} concentration, declined with a time constant of about 100 msec. In separate experiments involving the removal of this Ca^{++} efflux (and hence the negative feedback) with a ONa^{+} - OCa^{++} external solution, the response of a primate rod to a dim flash was found to increase by 2-3 fold, accompanied by an increase in the time-to-peak of the response. These changes can be broadly reproduced by a quantitative model of phototransduction that has the Ca^{++} feedback as one of its features. Finally, the adaptation to background light that we have observed in these cells most likely also arises from the Ca^{++} feedback.

201.2

SIGN INVERSION OF SIGNAL TRANSFER FROM SALAMANDER RODS TO HORIZONTAL CELLS AT LOW EXTERNAL CALCIUM CONCENTRATION. J. Kleinschmidt, Dept. of Ophthalmology, NYU Med. Ctr., New York, NY 10016.

Normal synaptic transmission from rods and cones to horizontal cells (HC's) in the superfused salamander retina persists when the medium is "Ca-free" (no Ca salts added) or when external Ca (Ca_o) is buffered to a level as low as 30 μM but fails rapidly and completely when Ca_o is buffered to 5 μM or less. In the intervening range of buffered free Ca_o (10-25 μM), light-evoked responses of HC's which are normally purely hyperpolarizing become depolarizing. These depolarizing light responses are spectrally univariant and derive exclusively from rods; rods, however, still produce purely hyperpolarizing light responses. Thus in this range of Ca_o, cone input to horizontal cells is abolished whereas rod input persists but inverts its sign. Hyperpolarizing light responses of HC's at normal Ca_o (1 mM) and depolarizing light responses at low Ca_o are fully blocked by 1 mM kynurenate or 50 μM DNQX indicating that both types of response are mediated by activation of glutamate receptors on HC's.

Since at these low levels of Ca_o, Ca_o-dependent tonic vesicular transmitter release in the dark has probably ceased, horizontal cell light responses under these conditions appear to reflect a mode of sign-inverting, rod-exclusive signal transfer with a mechanism different from that of vesicular transmitter release. Reverse mode operation of a plasma membrane glutamate uptake carrier can be ruled out as the mechanism underlying this signal transfer. A remaining possibility is glutamate secretion through a voltage-dependent glutamate transporter present in glutamatergic synaptic vesicles and incorporated continuously into the rod plasma membrane during tonic vesicular transmitter release.

Supported by NIH grant EY05213.

201.3

EVIDENCE FOR ELETROGENIC TRANSPORT OF GABA INTO CONE-DRIVEN HORIZONTAL CELLS OF THE CATFISH RETINA. R. P. Malchow, H. Qian*, and H. Ripps, Depts. Ophthalmology and Anatomy & Cell Biology, Univ. Illinois College of Medicine, Chicago, Il. 60612.

Recently we reported that GABA induces an inward current in rod-driven horizontal cells of the skate retina (Biol. Bul. 177: 324, 1989); the current appeared to result from the electrogenic transport of this compound into the cells. We now find that GABA induces a current with similar properties in cone-driven, but not rod-driven, horizontal cells of the catfish retina.

Current responses from enzymatically-isolated catfish horizontal cells were obtained using the whole-cell voltage-clamp technique. With the membrane held at -70 mV, application of 100 uM GABA to cone-driven horizontal cells elicited an inward current. The magnitude of the current decreased with progressive depolarization, becoming undetectable at +30 to +50 mV, but it was never seen to reverse into an outward current. At 100 uM, neither the GABAa agonist muscimol nor the GABAb agonist (-) baclofen were capable of mimicking the GABA response. The current was not significantly reduced by superfusion of 100 uM picrotoxin, a GABAa antagonist, or by 100 uM of the GABAb antagonist phaclofen. However, replacement of external sodium with either choline or lithium markedly reduced the GABA-induced current.

100 uM GABA had no effect on rod horizontal cells of the catfish. Interestingly, autoradiographic experiments have shown that cone horizontal cells, but not rod horizontal cells, take up tritiated GABA (Lam, D. M. K., Lasater, E. M., and Naka, K.-I. PNAS 75: 6310, 1978). In sum, our results are consistent with the hypothesis that the GABA-induced current in catfish cone horizontal cells reflects the activity of an electrogenic transport system.

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201.4

INHIBITION BY GABA OF DEPOLARIZATION-INDUCED CALCIUM INFLUX IN GOLDFISH RETINAL BIPOLAR NEURONS. Ruth Heidelberger and Gary Matthews, Dept. of Neurobiology, SUNY, Stony Brook, NY 11794-5230.

Fura-2 measurements from retinal bipolar cells have shown that depolarization elicits Ca-influx via voltage-activated Ca-channels (Heidelberger & Matthews, 1990, *Invest. Ophth. Vis. Sci.*, 31, 389). Because bipolar cells receive GABAergic feedback synapses from amacrine cells, we examined whether GABA might affect such depolarization-induced Ca-influx. Goldfish bipolar cells were isolated after papain treatment and were incubated with fura-2AM for monitoring $[Ca]_i$. Increases in $[Ca]_i$ produced by 62.6 mM $[K]_o$ with & without 50 µM GABA were compared. With GABA, the peak increase in $[Ca]_i$ averaged 35 ± 10% of the control response (mean ± s.e.m., n=16). In 50% of the cells, GABA completely abolished the K-induced increase in $[Ca]_i$. Baclofen (50 µM) had no significant effect on Ca-influx (92 ± 2% of control; n=8). In whole-cell current-clamp recordings, 62.6 mM K_o depolarized bipolar cells to -17.5 ± 3.3 mV (mean ± s.e.m.; n=4), which is sufficient to activate Ca-currents (Matthews & Heidelberger, this meeting). When combined with 50 µM GABA, however, 62.6 mM K_o produced only a small depolarization to -43.5 ± 1.8 mV, which is insufficient to activate Ca-current. This is due to a large GABA-activated chloride conductance that is the dominant conductance in the cell (average 3.9-fold increase in G_{in} produced by GABA). A simple interpretation is that GABA affects Ca-influx in Goldfish bipolar cells by activating chloride conductance and clamping membrane potential near the resting level, even in the face of high K_o. This suggests that the GABAergic feedback synapses in the intact retina might have a similar inhibitory action on Ca-influx into bipolar terminals and thus on presynaptic transmitter release.

201.5

A CHROMATIC HORIZONTAL CELL IN THE XENOPUS RETINA: MORPHOLOGY AND SYNAPTIC PHARMACOLOGY. S.L. Stone, P. Witkovsky and M. Schütte*. Dept. of Ophthalmol., NYU Medical Center, New York, NY 10016.

We identified a chromatic type horizontal cell (C-cell) in Xenopus retina by intracellular dye injection with Lucifer Yellow or HRP. Under photopic or mesopic conditions, moderate intensity blue and red light flashes evoked responses which were inverted with respect to each other but of similar kinetics, waveform and latency. Both hyperpolarizing and depolarizing response components showed similar large receptive fields with no center-surround antagonism. The perikaryon, located in the distal INL, emitted 4-7 long tapering processes that ran horizontally for 90-100 µm. Two kinds of terminal dendrites, short and long, extended from the tapering processes towards the photoreceptor terminals.

Superfusing the retina with glycine eliminated the depolarizing light response; the hyperpolarizing waveform evoked by blue light was unaffected. In contrast, GABA had no obvious effect on either component. cis-PDA modified the C-cell light response in two stages; first the depolarizing response disappeared, then the membrane potential hyperpolarized concomitant with a large reduction or elimination of the hyperpolarizing response. In contrast, APB had no obvious effect on either response component, or on the dark membrane potential.

The pharmacological results support the view that the hyperpolarizing C-cell response is mediated by direct synaptic input from a blue-sensitive photoreceptor. The depolarizing response could be mediated by direct input from a red-sensitive cone or via an indirect pathway involving L-type horizontal cells. Supported by EY0 6960 to S.L.S. and EY0 3570 to P.W.

201.6

HORIZONTAL CELLS CONTAIN TWO TYPES OF INHIBITORY AMINO ACID RECEPTORS. T. A. Gilbertson*, S. Borges and M. Wilson. Department of Zoology, University of California, Davis CA 95616

The amphibian retina is thought to contain both glycinergic and GABAergic interplexiform cells (IPCs). To find out if these IPCs modulate the performance of the outer retina, as is the case for the dopaminergic IPCs of teleosts, we have examined the effects of glycine and GABA on isolated horizontal cells (HCs) of the salamander.

Morphologically identified HCs were studied under whole-cell patch clamp conditions. These cells showed large TEA-sensitive outward currents which were activated positive to -30 mV. A minority of the cells (<20%) also displayed an inward current that was TTX-sensitive. Both glycine and GABA activated conductances that had a Nernstian dependence on Cl^- concentration. GABA and glycine responses desensitized at concentrations above 10 µM. The timecourse of desensitization in each case was approximated by a single exponential with a time constant of about 2-2.5 seconds. The glycine response was completely blocked by the competitive antagonist strychnine (2 µM). The GABA-induced current appeared to be mediated entirely by $GABA_A$ receptors as the response was abolished by 10 µM bicuculline methiodide. The response of HCs to bath-applied glycine had an EC_{50} of 28.7 µM and a Hill coefficient of 1.0, while for GABA these values were 42.5 µM and 1.04 respectively. Rapid application of GABA produced a Hill coefficient of 2.1, indicating the presence of faster desensitizing states.

201.7

LOCALIZATION OF IP_3 RECEPTOR IN THE VERTEBRATE RETINA. Y.-W. Peng*[1,2], A. H. Sharp[2], S.H. Snyder[2] and K.-W. Yau[1,2]. Howard Hughes Medical Institute[1] and Dept. Neuroscience[2], Johns Hopkins School of Medicine, Baltimore, MD 21205.

We have carried out immunocytochemical localization of the IP_3 receptor in the retina using a rabbit polyclonal antibody (Sharp & Snyder, in preparation) against the IP_3 receptor protein purified from rat cerebellum. Positive staining was observed in rat retinal sections at the outer and the inner plexiform layers. In addition, the outer and the inner limiting membranes (composed of Muller cell processes) were also stained. Western blotting of retinal proteins from rat showed a band that corresponds in molecular weight to the purified IP_3 receptor from rat cerebellum. Very similar patterns of staining were observed in retinae of primate, salamander, and other species (except for some variability in staining of the outer and inner limiting membranes). Study of dissociated retinal neurons from salamander indicated that the staining at the outer plexiform layer came predominantly from the synaptic terminals of photoreceptors, while that of the inner plexiform layer came from amacrine cell processes and the synaptic terminals of bipolar cells. Horizontal and ganglion cells did not show staining. The specific localization of the IP_3 receptor to synaptic terminals suggests a role of IP_3 in synaptic modulation (e.g., gain control) in the retina. The absence of staining in the photoreceptor outer segment also argues against a role for IP_3 in phototransduction.

201.8

"DAPI-3" CELLS IN RABBIT RETINA: GLYCINE-ACCUMULATING AMACRINE CELLS THAT CO-STRATIFY WITH CHOLINERGIC AMACRINE CELLS. H.M. Young*, G. Elston*, J.F. Dann and D.I.Vaney*. Vision, Touch and Hearing Research Centre, University of Queensland, Queensland 4072, Australia.

In rabbit retina, the fluorescent nuclear dye, 4,6-diamidino-2-phenylindole (DAPI), selectively labels the type b cholinergic (C*b*) amacrines in the ganglion cell layer (GCL) and three types of amacrines in the inner nuclear layer (INL), including the matching population of type a cholinergic (C*a*) amacrines (Tauchi, M. & Masland, R.H. *Proc. R. Soc. Lond.*, B223:101, 1984; Vaney, D.I. *ibid.*, B220:501, 1984). Most of the remaining DAPI-labelled cells in the INL comprise a distinct type of small-field amacrine with fine convoluted dendrites, termed the "DAPI-3" amacrine cell (Vaney, D.I. *Prog. Retinal Res.*, 9:49, 1990). The dendritic morphology of the DAPI-3 cells was revealed by Lucifer Yellow injection under microscopic control. The dendritic-field diameter of the DAPI-3 amacrines ranges from 100 µm on the peak visual streak to 330 µm in far-superior retina, with an estimated two- to three-fold dendritic-field overlap. Although the DAPI-3 cells have previously been characterized as "diffuse" amacrines, examination of photoconverted neurons indicates that the DAPI-3 dendrites are essentially bistratified. Lucifer injection of three overlapping neurons comprising DAPI-3, C*a* and C*b* amacrines showed that the DAPI-3 dendrites costratify with the cholinergic dendrites in strata 2 and 4 of the IPL. In order to determine the transmitter content of the DAPI-3 amacrines, DAPI-labelling was combined with GABA immunohistochemistry and [^{3}H]glycine-uptake autoradiography. Glycine was accumulated *in vitro* by about 30% of the DAPI-labelled cells in the INL, but by none of the DAPI-labelled C*b* amacrines in the GCL. We have previously reported that all of the C*b* amacrines and about 70% of the DAPI-labelled INL cells (presumably the C*a* amacrines) show GABA-like immunoreactivity (Vaney, D.I. & Young, H.M. Brain Res., 438:369, 1988). None of the DAPI-labeled cells showed both [^{3}H]glycine-uptake and GABA-like immunoreactivity. We conclude that the DAPI-3 amacrines correspond to the glycine-accumulating population of DAPI-labelled cells.

201.9

DISTINCT CELL TYPES IN LIVING MACAQUE RETINA DISPLAY AN UNEXPECTED GRANULAR FLUORESCENCE. D.M. Dacey. Dept. of Biological Structure, The Univ. of Washington, Seattle, WA 98195.

When the isolated, superfused retina of the macaque monkey is observed under blue episcopic illumination an unexpected fluorescence is present in distinct subpopulations of inner retinal neurons. This fluorescence appears as a profusion of intense yellow 'granules' in the cytoplasm of the soma and in some cases the proximal dendrites of cells in the ganglion and amacrine cell layers. The size and shape of the granules suggests that the fluorescence may be restricted to lysosomes.

Intracellular injections of horseradish peroxidase made into the fluorescing cells reveals a distinct ganglion cell type and two amacrine cell types. The ganglion cells have large somata (~20 µm diameter) and extremely large, sparsely branched dendritic trees. One amacrine cell type shows spiny, sparsely branched dendrites and gives rise to a distinct axon-like process that projects for mm beyond the dendritic tree. The other amacrine cell type shows a densely branched tree composed of extremely thin, smooth processes that are studded with distinct varicosities.

The extent and the intensity of the granular fluorescence varies from retina to retina. This variability, and its yellow color suggests the possibility that the fluorescent granules somehow result from uptake of serotonin (5-HT) released by small and variable amounts of blood present during the dissection of the retina from the eye. It has recently been shown that intense 5-HT immunoreactivity can be demonstrated in the rabbit by 5-HT-accumulating amacrine cell types after exposure of the retina to blood (Sandell, J.H. and Masland, R.H. *Histochem.*, 92:57,1989). If the yellow fluorescence does result from exposure to blood then it might be possible to enhance and control the effect by incubating the retina in known concentrations of 5-HT.

201.10

A SUBGROUP OF ALPHA GANGLION CELLS IN THE ADULT CAT RETINA EXPRESSES SOMATOSTATIN-LIKE IMMUNOREACTIVITY (SRIF-IR) C.A. White and L.M. Chalupa. Department of Psychology and the Neurobiology and Physiology Graduate Groups, University of California, Davis, CA 95616.

Two types of cells in the ganglion cell layer of the adult cat retina are immunoreactive for somatostatin or a somatostatin-like substance (White et al., *JCN*, 293:134,1990). One of these types is a large cell that resembles the alpha ganglion cell in soma size and shape. The other is a small-to-medium size cell that we have identified on the basis of morphological criteria as a wide-field amacrine cell. Both types are distributed preferentially in the inferior retina. We now provide unequivocal evidence that the large cells are alpha cells. Retinal ganglion cells were labeled by injections of rhodamine-filled microspheres or Fluoro-gold into the superior colliculus and the dorsal lateral geniculate nucleus. The retinas were processed for immunocytochemistry with a mouse monoclonal antibody to somatostatin 14 (Buchan et al., *Histochem.*, 83:175,1985). SRIF-IR large cells were labeled with either retrograde tracer, but the SRIF-IR wide-field amacrine cells were not. The diameter of SRIF-IR large cells ranged from 33-47 µm, comparable to alpha ganglion cells (Boycott and Wässle, *J. Physiol.*, 240:397,1974). Within a local patch of inferior retina, the mosaic of SRIF-IR large cells differed significantly from random and was more regular than that of the overall alpha cell population. Thus, use of a somatostatin antibody has revealed a cytochemically distinct subgroup of alpha cells that are arrayed regularly and preferentially within the inferior hemiretina.
(Supported by NINCDS T32 NS07300 and EY03991)

201.11

DISPLACED GANGLION CELLS OF RABBIT RETINA.
E. V. Famiglietti and J. E. G. Downing. Dept. of Anat. and Lions' Sight Ctr., Univ. of Calgary, Calgary, AB, Can. T2N 4N1.

Displaced ganglion cells (GCs) with cell bodies in the amacrine cell sublayer of the inner nuclear layer (INL) were first identified by Dogiel (1891), and were subsequently characterized in greater detail by Cajal (1893), who identified them in amphibians, reptiles and birds but not in mammals. Common characteristics of these cells are dendritic branching in stratum 1 (or 1-2) of the inner plexiform layer (IPL), cell bodies of relatively large size, and in birds at least (Karten et al.,'77) axonal projections to the accessory optic system (AOS). Subsequently, displaced GC bodies have been demonstrated in the INL of most mammalian retinas by retrograde transport of markers. In rabbit, displaced cell bodies of all sizes have been labelled by retrograde transport from injections in superior colliculus (SC), giving rise to the suggestion that GC displacement is accidental, and not typical of any GC type (Vaney et al.,'81). We have identified a unique type of displaced GC in Golgi preparations of rabbit retina with a small soma in the INL and typically three very long (600-700 µm) unbranched dendrites lying in stratum 1. We have confirmed by retrograde transport of HRP from SC that the predominant displaced GC is in the smallest soma-size group. These cells evidently bear no relationship to the previously characterized directionally selective and AOS-projecting GCs of rabbit retina.
(Supported in part by the AHFMR and MRC of Canada).

201.12

GANGLION CELL SURROUND INHIBITION IS DIVISIVE, NOT LINEAR, IN RABBIT RETINA. DK Merwine[1] & FR Amthor[2]
[1]Optometry, [2]Psychology/NRC, U. Alabama Birmingham 35294

We have shown that the linear difference-of-gaussians model frequently used to describe the center-surround receptive field structure of retinal ganglion cells does not hold in rabbit retina, because the effect of surround stimulation on the center response is *divisive*, not subtractive, causing a decline in the slope, rather than a parallel shift downward, of the center's response versus contrast function. We now report that this result holds not only for directionally selective ganglion cells, but also for most concentric ganglion cells, including brisk-transient, brisk-sustained and sluggish cells. The time course of the surround inhibition appears to be longer for concentric than directionally selective ganglion cells, but still decays markedly by 500 ms. We have also determined that both stimulus onset and offset in the surround inhibit the center response of both ON-center and OFF-center ganglion cells, but mixed sign interactions differ somewhat in dynamics. Our stimulation protocol has also revealed that some previously thought "silent" inhibitory surrounds are actually excitatory, but suppressed by the center mechanism. The overall effect of this surround inhibition is to linearize the center response as a function of contrast and therefore increase the contrast dynamic range.
Supported by EY05070, EY07033(T32) and the Sloan Foundation.

202.1

GENESIS OF INHIBITORY POSTSYNAPTIC POTENTIALS (IPSPs) BY INTERNEURONS IN THE FELINE ANTERIOR THALAMIC (AT) NUCLEI. D. Paré, R. Curró Dossi and M. Steriade, Lab. Neurophysiol., Sch. Med., Univ. Laval, Québec, Canada.

Interneurons can influence relay cells through their presynaptic dendrites (PSDs) and their axon. Prethalamic fibers arising in the mammillary body (MB) end mostly on PSDs while cortical (Cx) terminals usually synapse on parent dendrites. We now provide evidence that this synaptic arrangement has an electrophysiological counterpart in the IPSPs evoked by MB and Cx stimulation (St) in AT cells, known to be devoid of input from the reticular thalamic nucleus.

Three types of MB-evoked IPSPs were distinguished: 1- a "miniature IPSP" (E_R: -75 mV; latency 6 ms); 2- an early IPSP (E_R: -75 mV; latency: 13 ms); and 3- a late IPSP (E_R: from -86 to -100 mV; latency : 55 ms). The first one was evoked selectively by MB St and the other two by MB and Cx St. In decorticated cats, weak MB St elicited only miniature IPSPs. At low rates ($\leq$ 0.5 Hz), MB and Cx St evoked biphasic IPSPs composed of an early and a late phase. While Cx-evoked IPSPs were abolished at high rates ($\geq$ 3 Hz), MB-evoked IPSPs were replaced by a miniature IPSP. At intermediate rates (1-2 Hz), the amplitude of MB- and Cx-evoked IPSPs varied in a stepwise manner. Yet the amplitude of the early and late part of the IPSPs remained highly correlated. These findings suggest that the miniature IPSPs reflect the action of PSDs while the biphasic IPSPs are caused by the firing of a small group of interneurons. The E_R of these IPSPs indicate that the miniature and the early IPSPs are mediated by $GABA_A$ receptors while the late IPSP is mediated by $GABA_B$ receptors. Since weak MB shocks evoked only miniature IPSPs in decorticated cats it is likely that corticothalamic axons provide an excitatory input which allows otherwise subthreshold EPSPs mediated by prethalamic axons to bring the soma of interneurons to the firing threshold. Supported by MRC grant MT-3689.

202.2

CORTICALLY- AND SUBCORTICALLY-EVOKED IPSPs IN ANTERIOR THALAMIC (AT) CELLS ARE AFFECTED DIFFERENTIALLY BY STIMULATION OF A BRAINSTEM CHOLINERGIC NUCLEUS. M. Steriade, R. Curró Dossi and D. Paré, Lab. Neurophysiol., Sch. Med., Univ. Laval, Québec, Canada.

The influence of ACh and brainstem stimulation on thalamic inhibitory processes is a controversial topic. McCormick and Pape (1988) reported that ACh applied in vitro hyperpolarizes morphologically-identified interneurons (Is) of the lateral geniculate nucleus. In view of data demonstrating an ACh-induced enhancement of short-range inhibitory processes, we have suggested that the cholinergic inhibition may affect the soma of Is without depressing the intraglomerular inhibition mediated by presynaptic dendrites (PSDs). The present study was performed to test the hypothesis that brainstem cholinergic afferents affect differentially the inhibitory processes mediated by the axons and PSDs of Is.

We have studied how stimulation of the cholinergic laterodorsal tegmental (LDT) nucleus affects the IPSPs evoked by subcortical (mammillary body, MB) and cortical (Cx) stimulation in 26 intracellularly recorded AT relay cells, devoid of inputs from reticular thalamic nucleus. MB and Cx stimulation evoked a biphasic IPSP in respectively 66% and 100% of the cells. In all cases, Cx-evoked IPSPs were abolished by LDT stimulation. The MB- evoked early IPSP was reduced or abolished by LDT stimulation in 50% of cells, whereas the late IPSP disappeared or underwent great reduction in 100% of cells. In the cells where MB stimulation evoked only miniature IPSPs (37%) LDT stimulation had no effect or increased the size of the IPSPs. Since miniature and biphasic IPSPs are respectively mediated by the PSDs and axons of Is (see Paré et al., this meeting), our findings suggest that brainstem cholinergic stimulation inhibits Is somatically while preserving the inhibitory processes mediated by PSDs. Supported by MRC grant MT-3689.

202.3

SLOW EPSPS ARE MEDIATED BY ACETYLCHOLINE AND NOREPINEPHRINE IN RAT NEOCORTEX. L.S. Benardo, Dept. of Neurology, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

Intracellular recordings were obtained from 90 cells recorded in layers IV-V of rat somatosensory cortex (SmI) slices maintained in vitro. In approximately 90% of cells repetitive stimulation at high stimulus intensity gave rise to a slow excitatory postsynaptic potential (EPSP), often preceded by a slow inhibitory postsynaptic potential (IPSP). The optimal stimulus paradigm was 20 Hz for 100-400 msec (1-8 stimuli). Slow hyperpolarization began with the first stimulus of the train and lasted seconds, followed by slow depolarization, lasting tens of seconds. Slow EPSPs were voltage-dependent, such that depolarization with DC current resulted in larger slow EPSPs. Thus, while slow EPSPs elicited at rest potential (about -70 mV) were often low amplitude and subthreshold for spike generation, those triggered when cells were gradually depolarized from rest were larger, and usually generated bursts or trains of action potentials. The slow IPSP was associated with increased conductance while the slow EPSP was associated with decreased conductance. Slow events resisted blockade of excitatory amino acid and $GABA_A$ receptors. Muscarinic or beta-adrenergic antagonists alone reduced slow EPSPs, but when added in concert reversibly blocked them. Catecholamine reuptake blockers or an acetylcholinesterase inhibitor resulted in more prolonged slow EPSPs. When bath potassium concentration was increased from 3 to 10 mM, both the slow IPSP and EPSP were diminished, implying both processes result from interactions with potassium channels. These experiments suggest that synaptic release of acetylcholine and norepinephrine can be effected in neocortical slices, and provide the first demonstration of slow synaptic events resulting from such transmitter release in the neocortex in vitro. (Supported by CIDA NS01386-01)

202.4

ELEVATION OF INTRACELLULAR Ca INHIBITS GABAERGIC IPSPs IN HIPPOCAMPAL NEURONS. T.A. Pitler & B.E. Alger, Dept. of Physiol., Univ. of MD Sch. Med., Baltimore, MD 21201

GABAergic synapses provide a powerful inhibitory influence on neurons in the CNS, and their depression may be an important disinhibitory mechanism that facilitates epileptic burst potentials and the development of LTP. GABA receptor function can be suppressed by intracellular calcium (Ca_i), but it is not known if IPSPs can be suppressed by Ca_i under physiological conditions. We provide evidence using intracellular recording techniques in the hippocampal slice preparation that GABA IPSPs are potently inhibited by increases in Ca_i. KCl electrodes were used to cause spontaneous IPSPs to be depolarizing and easily observed. These spontaneous depolarizations were blocked by picrotoxin. The IPSP frequency was increased dramatically by carbachol, which provided a sufficient background of activity so that their regulation could be studied.

Following short trains of action potentials induced by intracellular current injection (5-100 at 20 Hz), the number of spontaneous IPSPs decreased. The magnitude and duration of the effect was proportional to the degree of stimulation. Including EGTA or BAPTA in the recording electrode prevented inhibition of spontaneous IPSPs, while bath application of BAY K 8644, which increases Ca influx, enhanced the effect. The duration of the IPSP block was transient, but was not related to the activation of Ca-dependent K conductance since this was blocked by carbachol.

202.5

OPTICAL IMAGING OF BACLOFEN-INDUCED CALCIUM ACCUMULATION IN CA3 PYRAMIDAL CELL DENDRITES IN THE HIPPOCAMPAL SLICE. W.Müller, A.Preuss and J.A.Connor Dept. of Neurosciences, Roche Institute of Molecular Biology, Nutley, NJ 07110

The GABA-derivative baclofen is well known to inhibit hippocampal pyramidal neurons via activation of dendritic, G-protein-linked receptors. It inhibits calcium-currents and activates a potassium current. In the present study we used high resolution microfluorometric imaging of CA3 pyramidal neurons in the guinea pig hippocampal slice to study directly the effects of baclofen on somatic and dendritic calcium levels. CA3 pyramidal neurons were filled with the potassium salt of fura-2 by iontophoretic microinjection. Bath applied baclofen induced a fast rise of dendritic calcium levels, up to 400% of resting levels, followed several 10's of seconds later by a relatively smaller rise in the soma. Dendritic as well as somatic calcium levels recovered to resting levels with washout of baclofen. Our study suggests a messenger role for intracellular calcium increases in a receptor-mediated postsynaptic inhibition.

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202.6

The time course of the NMDA and non-NMDA synaptic currents in hippocampal neurons. Shaul Hestrin, Pankaj Sah* and Roger A. Nicoll. Depts. of Physiology and Pharmacology. U.C.S.F., S.F. CA 94143

We studied the mechanisms that generate the dual-component EPSC recorded from pyramidal neurons using patch-clamp techniques. The NMDA mediated EPSC is much slower than the EPSC mediated by non-NMDA receptors. The slow time course of the NMDA component is surprising given that both receptor types respond to transmitter released from the same nerve terminal. We considered two models to explain the time course of the NMDA component: 1) Effective transmitter concentration rise and fall is slow. 2) Transmitter concentration increase is brief but NMDA channel activation-deactivation is slow.

To test model 1 we considered diffusion and glutamate uptake as mechanisms controling the concentration of transmitter. We found that the time course is highly temperature sensitive ruling out diffusion. The uptake blocker: dihydrokainic acid while greatly effecting the response to exogenous glutamate did not effect the NMDA component time course. This data suggests that diffusion or uptake do not determine the time course of the NMDA component. On the other hand the NMDA channel blockers: MK-801 and ketamine greatly reduced the decay time constant of the NMDA component. Since drugs which reduce channel life time decrease the EPSC time course we suggest that the NMDA mediated EPSC reflects slow channel kinetics rather than slow transmitter clearance.

202.7

WHOLE-CELL RECORDINGS OF PARALLEL-FIBER AND CLIMBING-FIBER EPSCs FROM PURKINJE CELLS IN RAT CEREBELLAR SLICES D. J. Perkel, S. Hestrin, P. Sah* & R. A. Nicoll
Depts. Physiol. and Pharmacol. UCSF, San Francisco, CA 94143-0444.

To investigate the conductances underlying the two different types of excitatory input to Purkinje cells we used whole-cell recording in thin (100-250 μm thick) sagittal slices of adult rat cerebellum. Cells were visualized directly using Nomarski optics, and debris overlying the soma was removed with a large "cleaning" pipette (Edwards et al., 1989, Pflugers Arch. 414:600). Recording pipettes contained (mM): 140 CsF, 10 NaCl, 10 HEPES, 10 EGTA.

Electrical stimulation of the white matter caused an all-or-none excitatory post-synaptic current (EPSC) that displayed paired-pulse depression. Stimulation of the molecular layer, on the other hand, resulted in a graded EPSC that exhibited paired-pulse facilitation. Both EPSCs showed reversal potentials around 0 mV, and were entirely blocked by application of the non-NMDA receptor antagonist CNQX (10 μM). When the cell was voltage clamped at -40 mV and/or bathed in Ringer containing no added Mg^{2+}, neither type of EPSC was affected by the NMDA-receptor antagonist DL-APV (50 μM). We conclude that both types of EPSC are mediated entirely by non-NMDA receptors.

Bath application of NMDA (100 μM) did not evoke an inward current, even in Mg^{2+}-free Ringer or at a holding potential of -40 mV. Superfusion of aspartate (0.1 - 1 mM) caused an inward current, but this was blocked by 10 μM CNQX. Non-NMDA receptors thus appear to mediate all the electrophysiological effects of excitatory amino-acids on Purkinje cells.

202.8

EXCITATORY AMINO ACID RECEPTORS UNDERLYING THE ASSOCIATIONAL/COMMISSURAL AND MOSSY FIBER SYNAPTIC CURRENTS OF CA3 PYRAMIDAL NEURONS. J. S. Isaacson and R. A. Nicoll.
Depts. of Physiology and Pharmacology, Univ. of Cal., San Francisco, CA 94143.

CA3 pyramidal neurons receive two anatomically distinct types of synaptic input that exhibit different forms of long term potentiation (LTP): an associational/commissural (a/c) input that requires NMDA receptor activation for the induction of LTP and mossy fiber (mf) input which does not. NMDA receptor binding is low at the mf synaptic region compared to the a/c synaptic region, however, little more is known concerning the contributions of non-NMDA and NMDA receptors at these synapses.

To address this question, synaptic currents in CA3 pyramidal neurons were studied using the method of "blind" whole-cell recording (Blanton, M. et al., *J. Neurosci. Meth.*, 30: 203, 1989) in thick (500 μm) hippocampal slices from adult guinea pigs. A/c excitatory post synaptic currents (EPSCs) were elicited by a conventional bipolar stimulating electrode placed in stratum radiatum. To eliminate the contribution of contaminating non-mf inputs which can be activated by conventional stimulating electrodes, "pure" mf EPSCs were evoked by the direct stimulation of granule cells with a glutamate-filled iontophoretic electrode positioned in the dentate gyrus.

EPSCs evoked from stimulation of a/c fibers displayed a slow component at depolarizing holding potentials (> -40 mV). The slow component to the EPSC was blocked by the addition of APV to the perfusing medium.

Glutamate-evoked mf responses, presumably reflecting the activation of single fibers, often yielded EPSCs ranging in amplitude from 200 to 1000 pA at a holding potential of -80 mV. These EPSCs also contained a small, slow component at more depolarized membrane potentials which was blocked by APV. A larger APV-sensitive component to mf EPSCs was observed in slices perfused with Mg^{++}-free Ringer.

These results provide evidence that both the a/c and mf inputs to CA3 pyramidal neurons activate NMDA and non-NMDA receptors. The functional role of NMDA receptors at the mf-CA3 synapse remains to be elucidated.

202.9

ARE SPINES AND DENDRITES DYNAMICALLY DISTINCT CALCIUM COMPARTMENTS? Menahem Segal, Peter Guthrie, and Stanley B. Kater. The Program in Neuronal Growth and Development and the Department of Anatomy and Neurobiology, Colorado State University, Fort Collins, CO 80523.

Dendritic spines are likely to be a primary site for neural plasticity. Intracellular calcium is thought to be the prime tool for regulating neural plasticity. Despite extensive interest, little is known about calcium's resting levels and dynamic changes in spines. One major unresolved question is whether spines constitute a functional unit separable from adjacent dendritic shaft. That is, is calcium in the spine ([Ca]s) regulated differently than intradendritic calcium ([Ca]d)?

We have injected CA1 pyramidal neurons in hippocampal slices from adult rats with fura-2 and monitored [Ca]d and [Ca]s with high power objectives in a dual wavelength Ca imaging system. Resting calcium levels in spines and dendrites were not notably different. Calcium was elevated in individual dendrites by irradiating a distant site with a brief pulse of light. Resting [Ca]d (approx. 175 nM) rose to 600 - 2500 nM after irradiation. This increase traveled as a wave from the site of irradiation to the distant site where spine measurements were made. In most (n=20) spines on the irradiated dendrite, there was a parallel rise in [Ca]s.

Notably, in 11 cases, the [Ca]s was lower; as low as 20% of the level in the parent dendrite. In three other cases, [Ca]s actually exceeded [Ca]d by as much as 200%. Clearly, these data are obtained near the limits of current resolving power. Nonetheless, these data strongly suggest that [Ca]i is heterogeneously regulated in spines and parent dendrites of the same neuron. (supported by Alzheimer's Association)

202.10

INTRACELLULAR ATP AND PHOSPHOCREATINE IN CORTEX AFTER NMDA, QUISQUALATE AND KAINIC ACID : NMR SPECTROSCOPY ON NEONATAL RAT BRAIN SLICES. J. Champagnat*, G. Fortin*, T. Jacquin*, B. Gillet*, J.C. Beloeil* and M. Denavit-Saubié
Laboratoire de Physiologie Nerveuse & Institut de Chimie des Substances Naturelles, CNRS, 91198 Gif-sur-Yvette, France.

^{31}P- and ^{1}H- nuclear magnetic resonance spectroscopy of 3-10 day-old rat cortical slices were used to monitor evolution of intracellular ATP, phosphocreatine (Pcr), lactate and pH following 0.5-1 min application of excitatory amino acids agonists. Pcr was dephosphorylated during agonist-induced neuronal excitation and afterward fully rephosphorylated within 15-100 min, a duration related to the amount of agonist applied (threshold agonist concentration : 1μM). Reversion was abolished by mitochondrial impairement (atractyloside 50 μM, 5 min) or suppression of glucose supply. In the presence of glucose (10 mM), agonists raised lactate level and decreased pH reversibly. Effects were reproducible and sensitive to selective pharmacological blockers of neuronal membrane receptors. Dose/response analysis revealed situations in which [ATP]i was reversibly decreased and provided evidence for two different desensitizing mechanisms : one preventing change of [ATP]i following quisqualate, another one reducing NMDA effect on Pcr after decrease of [ATP]i. Neither mechanism was found after kainate.

202.11

CAN SOMATIC SHUNT CONDUCTANCE BE MEASURED BY PEELING EXPONENTIALS? C.J. Wilson, Dept. of Anat. and Neurobiol., U.T. Memphis, Memphis, TN, 38163, USA.

The uniform membrane neuron model originally proposed for the motoneuron by Rall, and by Jack and Redman can be thought of as a mapping function that allows extraction of the physiologically meaningful values, dendritic length and dendritic dominance, from the less meaningful measured values, the equalizing time constants and their coefficients. As such, it is very well-behaved, that is, errors of measurement of the time constants and coefficients make roughly commensurate errors in the estimates of dendritic length and dendritic dominance. Viewing the somatic shunt model as a mapping function yielded a very different result. The surface generated by the function in the space defined by the first two equalizing time constants and their coefficients was very complicated. Although is was not multivalued, it folded back and approached itself in several places. It was most complex when the dendritic length was less than one length constant. Adding the third time constant and its coefficient helped only a little.

As a result, small errors in measurement of time constants and coefficients can produce large and unexpected errors in the estimate of dendritic length and dendritic dominance when using the somatic shunt model to extract these parameters. With some values of time constants and coefficients, the uniform and somatic shunt models both fit the data reasonably well, but with very different values for dendritic length and dendritic dominance. These results suggest that measurement of somatic shunt conductance, and even the determination of which model best fits neurophysiological data, should be approached with caution.

202.12

INHIBITORY INTERNEURONS MAY HELP SYNCHRONIZE FIRING OF POSTSYNAPTIC CELLS. W.W. Lytton and T.J. Sejnowski. Salk Institute, La Jolla, Ca 92037.

Phase locking within groups of neurons is a ubiquitous phenomenon in the central nervous system. This synchronization may reflect the operation of a central oscillator that owes its periodicity to the intrinsic properties of individual cells or it may be a resonance property of small neuronal networks. We studied a model of a cortical pyramidal cell that had Hodgkin-Huxley type sodium and potassium channels. Frequency entrainment was seen to a rapid train of brief, strong (20-100 nS) compound inhibitory postsynaptic potentials (IPSP) onto proximal apical dendritic shaft, soma or axon initial segment. The IPSP in this case modulated postsynaptic cell behaviour by either increasing or decreasing the rate of firing. Facilitation by the IPSP was caused by activation of sodium channels and turning off potassium channels, both reducing the threshold to firing. Using an intermediate sized IPSP (60 nS), entrainment to a 40 Hz input frequency occured when the initial rate of firing of the postsynaptic cell was between 32 and 47 Hz. The phase relation between the inhibitory cell and the postsynaptic cell generally varied from $\pi/2$ to π depending on the initial rate of firing of the postsynaptic cell. Phase locking could also be demonstrated in cells which initially showed irregular firing due to uncorrelated synaptic input. The phase locking of cell firing observed in visual cortex in response to a visual stimulus may involve these facilitatory IPSP effects in combination with direct excitatory connections. Thalamic rhythms such as spindling involve inhibitory projections from the reticular nucleus that may utilize similar mechanisms.

203.1

CHROMAFFIN CELL GRAFTS TO RAT CEREBRAL CORTEX REVERSE LESION-INDUCED MEMORY DEFICITS: CHOLINERGIC DRUG CHALLENGE. S.A. Welner, Z.C. Koty* and P. Boksa. Douglas Hosp. Res. Ctr., Dept. Psychiatry, McGill Univ., Montreal, Quebec, Canada, H4H 1R3.

Lesions of the nucleus basalis magnocellularis (NBM) in the rat produce deficits in spatial memory, usually suggested to be due to a loss of cholinergic input to the cerebral cortex. In the present experiments, adrenal chromaffin cells, which are normally catecholaminergic but can express a cholinergic phenotype under certain conditions, were isolated from donor adult rats and transplanted to the cerebral cortex of bilaterally NBM-lesioned rats. After three months, the effects of grafts on memory impairments were tested in a T-maze alternation task; our previous finding that chromaffin cell grafts to lesioned animals completely reversed the spatial memory deficit seen in lesioned alone animals were confirmed (Brain Research, in press). In order to test whether maze performance in these rats could be modulated by cholinergic drugs, animals were challenged with the antagonist scopolamine (1.0 mg/kg), the acetylcholinesterase inhibitor physostigmine (0.05 mg/kg) and vehicle, injected i.p. to each rat 20 minutes prior to the test session on alternating days. Results showed that scopolamine worsened performance in all three groups, but this differnece was significant only for the control group ($p \leq 0.02$). Physostigmine significantly bettered performance on the memory task for the control group when compared to that under vehicle conditions ($p \leq 0.03$). For the lesioned alone and lesioned plus graft groups, a similar effect was suggested, but not significant. However, when compared to that under scopolamine, maze performance under physostigmine was significantly increased for control, lesioned and lesioned plus graft groups ($p \leq 0.001$, $p \leq 0.02$, $p \leq 0.008$, respectively). These results suggest that cholinergic modulation of spatial memory is present in lesioned alone and lesioned plus chromaffin cell grafted animals but to a lesser degree than in unlesioned-ungrafted controls.

203.2

TRANSPLANTS OF EMBRYONIC VENTRAL FOREBRAIN INTO CHOLINERGICALLY DEPLETED RATS RESTORE DIMINISHED METABOLIC UPTAKE IN BARREL CORTEX. S.E. Jacobs[1], A. Fine[2], D. Eslin[1*], and S.L. Juliano[1]. [1]Anatomy, USUHS, Bethesda, MD; [2]Physiol. and Biophys., Dalhousie U., Halifax, N.S.

Transplantation of acetylcholine (ACh)-rich fetal tissue into adult hosts can alter the behavior of cholinergically depleted or aged rats. The impact of such transplants on sensory systems, however, has not yet been identified. To address this issue, we studied the effects of embryonic ACh-rich or neocortical grafts on evoked metabolic activity in the barrel cortex of adult rats, which previously received basal forebrain lesions that depleted the cortex of ACh. Findings from earlier 2-deoxyglucose (2DG) studies indicate that cholinergic depletion leads to a decrease in barrel configuration. In the present studies, the animals received unilateral ibotenic acid lesions of the basal forebrain that depleted the cerebral cortex of ACh. One week later the lesions were followed by injecting cell suspension grafts of either embryonic ventral forebrain from 16 day old fetuses or neocortical tissue from young (13-14 day old) or older (19-20 day old) fetuses into the cholinergically depleted hemisphere. In rats that received basal forebrain grafts, the transplants innervated the previously ACh-depleted cortex with acetylcholinesterase-rich fibers, as demonstrated histochemically. 2DG studies in the same animals revealed that stimulation of multiple vibrissae led to metabolic uptake enhanced in dimension and intensity in barrels adjacent to ventral forebrain grafts compared with activated barrels in the same animals farther from the transplant. Surprisingly, in animals that received young cortical transplants, the evoked metabolic activity in the barrel field adjacent to the graft was enhanced compared to evoked activity in the normal hemisphere. Grafts of older neocortex did not appear to enhance evoked metabolic activity.

203.3

STRIATAL IMPLANTS PROVIDE LONG TERM PROTECTION AGAINST BEHAVIORAL, REGULATORY, AND MORPHOLOGICAL CHANGES INDUCED BY QUINOLINIC ACID. S.H. Pearlman[1], M. Levivier[2], and D.M. Gash[1]. Dept. of Neurobiology and Anatomy, University of Rochester, Rochester, NY 14642[1]; Dept. Neurosurgery, Erasme Hospital, Universite Libre de Bruxelles, Brussels, Belgium[2].

Our laboratory has previously demonstrated that transplants are able to protect the host from subsequent neurotoxic insult. We report here our continued studies to elucidate the mechanisms underlying this protective effect.

Adult female Long Evans rats (n=54) were divided into four groups: fetal striatum, gelfoam (control), gelfoam/aFGF, and non-grafted (control). Rats received two 2µl striatal implants followed 8-9 days later by a 240 nmol quinolinic acid challenge. Animals were tested for rotational asymmetries before grafting, 1 week post-implant, 14 days and 28 days post-lesion. Animals grafted with fetal striatum or gelfoam did not show changes in rotation after quinolinic acid lesions. The gelfoam/aFGF treated group did exhibit a small increase in turning behavior. Animals receiving fetal striatal grafts also demonstrated consistent protection in ambulatory, vertical and stereotypic locomotor activities. The adipsia and aphagia often associated with striatal lesions were also ameliorated. Morphometric analysis demonstrated that afferent, intrinsic and efferent systems are all better preserved in the grafted animals as compared to the non-grafted group. For example, both the endogenous cholinergic and enkephalinergic systems were better preserved in all three transplanted groups as compared to the non-grafted animals. These results support the hypothesis that there is a host generated response which is activated by the tissue implantation procedure alone (gelfoam group), but the most consistent and reproducible protection is found in those animals which received fetal striatal implants. Supported by NIH NS15109.

203.4

c-*fos* INDUCTION IN INTRASTRIATAL GRAFTS OF FETAL NIGRAL AND STRIATAL TISSUE: FUNCTIONAL ROLE OF D1 DOPAMINE RECEPTORS IN GRAFT-HOST INTERACTIONS. M.A. CENCI* R.J. MANDEL, P. KALÉN*, K. WICTORIN*, and A. BJÖRKLUND. University of Lund, Department of Medical Cell Research, Biskopsgatan 5, S-223 62 Lund, Sweden.

The proto-oncogene protein product, c-*fos*, has been reported to be induced by stimulation of striatal D1 receptors and c-*fos* induction in striatum is increased on the side mediating dopamine (DA) agonist-induced rotational behavior after DA denervation (Robertson et al., *Brain Res.* 503:346-349, 1989). In the present study, we examined c-*fos* induction in two different grafting models, heterotopic grafting of DA-rich fetal ventral mesencephalon (VM) into the DA denervated striatum and homotopic grafting of fetal striatum into the excitotoxically lesioned striatum. The induction of c-*fos* was studied immunohistochemically using a commercially available polyclonal antibody. Rats with unilateral 6-hydroxydopamine lesions treated with apomorphine (APO) displayed high levels of c-*fos* induction in the lesioned striatum while similarly lesioned rats treated with amphetamine (AMPH) displayed c-*fos* induction predominantly in the intact striatum. Behaviorally compensated unilateral DA depleted rats bearing VM grafts displayed a reinnervation dependent pattern of striatal c-*fos* induction that was normalized, i.e., high AMPH-induced c-*fos* but low APO-induced c-*fos* in the grafted striatum. Rats that were behaviorally overcompensated in response to AMPH (rotated contralateral to the graft) displayed higher striatal c-*fos* induction near the VM graft compared to their intact striatum. Rats with fetal striatal grafts placed in the ibotenic-acid lesioned striatum displayed AMPH-induced c-*fos* induction both in the intact striatum and within the grafts. Unlike the intact striatum, the c-*fos* positive nuclei within the striatal grafts tended to lie in clusters that coincided with patches of high AChE staining in adjacent sections. Thus, fetal DA-rich grafts may exert their functional effects at least partially through normalization of D1 receptor function. The data from the homotopic striatal grafts indicate that the D1 receptors are present in the grafts and can be affected by the host's DA projection.

203.5

SUBSTANCE-P-LIKE IMMUNOREACTIVE ELEMENTS IN FETAL STRIATAL GRAFTS IN THE RAT: A LIGHT AND ELECTRON MICROSCOPIC STUDY. G.A. Helm,* J.P. Bennett,* and J.A. Jane. Dept. of Neurological Surgery, University of Virginia, Charlottesville, VA 22908.

Fetal striatal neurons were transplanted into the ibotenic acid lesioned rat striatum. Three months after transplantation, the grafted tissue was processed for substance P-like immunoreactivity, and the tissue was examined at the light and electron microscopic levels. The study demonstrated that substance P-like immunoreactive (SP-LI) neurons measuring 10 to 25 microns were homogeneously present in the fetal striatal grafts. These immunoreactive neurons could be classified into two types based on their ultrastructural characteristics. Type I neurons contained an unindented nucleus which was surrounded by a moderate amount of cytoplasm, whereas Type II immunoreactive neurons contained an indented nucleus which was surrounded by copious cytoplasm. The dendrites of these neurons were contacted by multiple axon terminals which made both symmetric and asymmetric contacts. In addition, numerous SP-LI axon terminals forming symmetric junctions were present within the graft. Fifty-four percent of these SP-LI axon terminals made axodendritic contacts, 19% made axospinous contacts, and 27% made axosomatic contacts. The SP-LI axon terminals made axosomatic contacts with cells displaying both indented and unindented nuclei, demonstrating that both spiny and aspiny cells within the graft received substance P input. This study demonstrated that many of the neuroanatomical features of substance P immunoreactive elements in the normal rat striatum are repeated in mature fetal striatal grafts.

203.6

INCREASED TYROSINE HYDROXYLASE IMMUNOREACTIVITY (TH-IR) IN THE 6-OHDA LESIONED STRIATUM OF RATS IMPLANTED WITH AMNION CELLS. M.A. Palmatier, J.G. Sheng*, R.J. Plunkett* and I.J. Kopin. Clin. Neurosci., NINDS, NIH, Bethesda, MD. 20892.

Amnion cells (AmCs) produce an activity which, in vitro, increases process outgrowth in rat sympathetic neurons and appears to have similar effects in rat ventral mesencephalon. To determine if AmCs could induce sprouting of DAergic nerve fibers in vivo, the substantia nigra of rats were lesioned unilaterally with 6-OHDA. Live or killed AmCs were implanted into the denervated striata. Rats were assessed weekly for 1 month before and 4 months after implant for a turning response to amphetamine. The brains were processed for TH-IR.

Rats implanted with live AmCs (n=13) showed a significant decrease in the turning response to amphetamine post-implant and showed increased TH-IR in the lesioned striatum, particularly around the implants. These changes were not seen in rats implanted with killed AmCs (n=4).

These results suggest that implantation of AmCs in a parkinsonian animal model can lead to behavioral recovery and DAergic fiber growth in the denervated striatum. The NIH Guide for the Care and Use of Laboratory Animals was followed.

203.7

EFFERENTS FROM MOUSE FETAL STRIATAL GRAFTS RAPIDLY INNERVATE THE ADULT LESIONED RAT BRAIN, EVIDENCED BY A MOUSE-NEURON SPECIFIC MARKER.

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The developmental time-course and pattern of efferent fiber growth from cross-species cell suspension grafts of mouse fetal *striatum* (E13) implanted into adult ibotenic acid lesioned rat striatum were investigated using a cell surface monoclonal antibody (M6) specific for mouse CNS neurons (Lund et al, 1985).

As soon as *3-5 days* after implantation, fascicles of graft-derived fibers began to extend out of the implants, into the adjacent bundles of the host internal capsule. The graft-derived fibers notably extended along and inside the white matter fiber tracts on their way through the host brain, and by *8 days* post-grafting, efferent fibers reached the rostral globus pallidus, where small terminal-like networks began to form. The terminal-like areas were more dense at *2 weeks*, and present at all longer survival times (up to *15 weeks* after implantation), whereas the stainability of the efferent fibre fascicles was reduced over time, which, consistent with previous studies using this marker on retinal implants (Lund et al, 1985), could indicate a gradual myelination. Fetal *neocortical* control implants showed a greater growth capacity, whereas fetal *cerebellar* grafts did not extend efferents into the host brain.

203.8

EMBRYONIC MOTONEURONS GRAFTED INTO THE ADULT CNS CAN SURVIVE, DIFFERENTIATE AND MIGRATE

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Most experimental studies on grafting of neural tissue have involved the transplantation of a heterogeneous population of cells due to the difficulty of dissecting out a particular class of central neurons. Here we describe experiments in which an identified neuronal cell population has been grafted into the adult CNS. Embryonic mouse motoneurons were labelled by retrograde transport using a fluorescent tracer, dil and then partially purified on a Nycodenz density gradient or completely purified on a fluorescence activated cell sorter. Motoneurons were grafted into either the cervical, anterior horn region of the spinal cord or into the striatal region of the adult mouse brain. After 4 to 10 weeks of grafting, the tissues were analyzed for the presence of the fluorescent motoneurons. Neurons were found to survive, differentiate (i.e. ability to grow neurites) and migrate at least 2 mm in the spinal cord and 4 mm in the brain; they were also found in contralateral sites. These studies could be important for determining whether the grafted neurons are being guided by a neurotropic agent and whether this capacity to migrate could be exploited for developing more beneficial grafting procedures.

203.9

CO-TRANSPLANTATION OF EMBRYONIC NERVOUS TISSUE AND PERIPHERAL NERVE AUTOGRAFTS TO THE CERVICAL SPINAL CORD OF THE ADULT RAT.

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In order to study the possibilities of a reconstruction of the spinal cord and of its peripheral connections following severe injury, a cavity, made in the cervical enlargement, was filled with either solid heterotypic (cortex (CT), E14-E18) or homotypic (spinal cord (SC), E14) embryonic CNS tissue; or, alternatively, with peripheral embryonic tissue (dorsal root ganglia (DRG), E14). In addition, one end of an autologous peripheral nerve graft (PNG) was inserted in the center of the transplant, its other end being driven extraspinally and made blind.

From one to six months later, the animals were processed for GFAP and Myelin Basic Protein immunocytochemistry, HRP histochemistry following retrograde axonal transport from the PNG, and silver staining.

Integration of the three types of transplants with the host spinal cord was the rule. Surviving transplanted neurons differentiated processes, some of them becoming myelinated. Yet, growth of some of these processes into the PNG was extensive from DRG, moderate from SC and nonexistent from CT transplants, recalling the axonal regrowth of the corresponding fully differentiated neuronal populations of the adult animal, under similar conditions.

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203.10

ORGANOTYPIC NEURAL GRAFTS ARE BEST ACHIEVED IN NONHUMAN PRIMATES FROM EARLY GESTATION DONORS.

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Based on rodent experiments, it was assumed that the survival window for embryonic tissue was narrow, however our initial examination in nonhuman primates showed a much wider fetal window. The degree to which optimal survival is achieved, however, with different aged donors, and the extent to which grafts display morphological organotypy is not well understood. We examined a wide range of gestational ages, including 11 different crown rump lengths (CRL) from 1.3-21.0 cm in African Green monkeys. Grafts at 10-30 weeks of ventral mesencephalon, including the substantia nigra, the locus coeruleus and adjacent subcoeruleal region, ventral hypothalamus, including A-12 dopamine neurons and the cerebellar cortex were examined using tyrosine hydroxylase and routine stains. Optimal survival was seen from 1.3-11 cm CRL donors. Within this range, it was common to observe clusters of neurons arranged in patterns resembling their adult counterparts, e.g. grafts of mesencephalon contained clusters comparable to the zona compacta with dendrites similar to those that radiate into the zona reticulata. Similarly, pontine grafts contained juxtaposed clusters of densely packed, rounded neurons with few processes (resembling the locus coeruleus) and a more dispersed group of larger neurons with multipolar branching reminiscent of the subcoeruleus. Hypothalamic grafts contained small bipolar neurons similar to tuberoinfundibular dopamine neurons. Cerebellar grafts showed Purkinje neurons between molecular and granular cell layering. Such organotypy was not observed in late gestational grafts, although cell survival was seen; the numbers were greatly reduced in comparison to the best survival in younger donors. These data suggest that early gestational tissue from nonhuman primates produce the best survival and chances for functional interactions following transplantation.

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203.11

DONOR VESSELS IDENTIFIED USING MONOCLONAL ANTIBODIES IN CEREBROVENTRICULAR ALLOGRAFTS.

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Donor vessels in xenogeneic grafts to the CNS constitute only a proportion of graft vasculature; however, the fate of donor vessels in allogeneic grafts remains to be elucidated. To achieve this goal, MRC OX-27, a monoclonal antibody (Mab) specific for the MHC Class I of the PVG-RT^{c} rat strain, appeared an ideal candidate. Another Mab, MRC OX-26 which recognizes the rat transferrin receptor and appears to be only present on CNS blood-brain vessels, also was utilized to determine the overall vasculature of the grafts. One day neonatal PVG-RT1^{c} cortex was transplanted to the third ventricle of PVG-RT1^{u} hosts (differing only at their MHC) and vice-versa. Six months later the animals were sacrificed and their brains processed for immunohistochemistry. Host vessels always vascularized the grafts. Donor vessels were identified in 50% of the grafts, and a rich network of donor vessels was observed at the level of the median eminence (m.e.). A few OX-27 positive (donor) vessels were observed in the host m.e. These vessels were large and OX-26 negative, suggesting that the vessels lost their OX-26 phenotype and possibly became fenestrated. Apart from the m.e. region, no OX-27 positive (donor) vessels have been identified in the host brain. Supported by the I.S.R.T.

203.12

MORPHOLOGY OF BLOOD VESSELS SUPPLYING GRAFTED TISSUES/CELLS IS GRAFT DETERMINED.

R. Broadwell, B. Baker*, P. Ebert*, J. Villegas*, and A. Wolf. Univ. MD, Balto., MD 21201.

Data were presented previously suggesting that intracerebral CNS allografts possess blood-brain barrier (BBB) vessels contributed by donor and host; conversely, peripheral tissue grafts exhibit non-BBB vessels of only donor organ. We now focus on endothelia free, intracerebral cell suspensions and syngeneic, fetal CNS grafts placed under the kidney capsule of adult rats. Individual populations of purified neonatal astrocytes, fibroblasts, PC12, glioma, medulloblastoma, and neuroblastoma cell lines (2.5 x 10^5 cells) were delivered to the striatum of adult, homozygous athymic mice. Cell suspension grafts were vascularized by 3 days. With the exception of astrocytes, cell grafts contained vessels leaky to blood-borne HRP and exhibited open intercellular junctions; vessels supplying PC12 and neuroblastoma cell lines also were fenestrated. Host rejection of cell grafts was not evident. Vessels supplying CNS grafts to the kidney were leaky to HRP, but their endothelia were of the BBB type; the grafts were inhabited by host immune system cells identified immunohistochemically and by EM. Graft rejection most likely rendered graft vessels permeable to HRP. Intracerebral cell grafts in nonhomozygous nude rats also were rejected. The data confirm that the morphology of blood vessels supplying grafted tissue/cells is dictated by the graft and that the host immune response is a factor for acceptance of cell suspension grafts.

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204.1

WITHDRAWN

204.2

A POTENT OXYTOCIN ANTAGONIST REVERSES GONADAL STEROID FACILITATION OF SEXUAL BEHAVIOR. D.M. Witt, C.R. Harbaugh* and T.R. Insel. Section on Comparative Studies of Brain and Behavior, Laboratory of Clinical Science, NIMH, Poolesville, MD 20837.

Several studies suggest that the neuropeptide oxytocin (OT) is involved in the ovarian steroid regulation of sexual behavior in female rats. Recent results have shown that icv injections of OT facilitated sexual receptivity, as measured by lordosis quotient, in female rats that were maximally primed with ovarian steroids. However, it is not yet clear if OT has a physiological role in regulating sexual behavior. To determine if blocking endogenous oxytocin alters sexual behavior, we administered (icv) a potent oxytocin receptor antagonist, [d $(CH_2)_5$, Tyr $(Me)^2$, Thr^4, Tyr-NH_2 ^{9}]- OVT (herein called OTA), to female rats receiving differential gonadal steroid priming. Ovariectomized females were primed for two days with either 10 µg or 1 µg estradiol benzoate (EB) followed on the third day by progesterone stimulation (250 µg, P_4). In initial experiments with OTA (1 µg) administered 4 hours post P_4, just prior to behavioral testing, no significant effects were noted. Subsequently, OTA (1 µg) or artificial CSF was delivered by icv injection immediately prior to progesterone administration, 4 hours prior to behavioral testing. OTA reduced lordosis quotients by 40% in the 10 µg EB+P_4 and 63% in the 1 µg EB+P_4 groups. Significant reductions were also observed in proceptive behaviors following OTA administration in both high and low EB groups receiving P_4. In a subsequent experiment, lordosis quotients from ovariectomized females receiving EB (10 µg) without P_4 (or OTA) were nearly identical to the low EB+P_4 OTA-treated females. It appears that antagonism of endogenous OT reduced the effects of P_4 on reproductive behavior. This interaction may be complex however, as high doses of OTA given to EB-treated females without P_4 may significantly facilitate sexual receptivity.

204.3

EFFECTS OF OVARIAN HORMONES ON ELECTROPHYSIOLOGICAL ACTIONS OF OXYTOCIN ON HYPOTHALAMIC NEURONS *IN VITRO*. L.-M. Kow, A.E. Johnson[1], S. Ogawa, D.W. Pfaff The Rockefeller University, New York, NY 10021 and [1]SCSBB/LCS, NIMH, MD 20837

Since both estrogen (E) and, subsequently, progesterone (P) affect oxytocin (OT) receptors in the hypothalamus, and since infusion of OT into the hypothalamus facilitates lordosis, these ovarian hormones might alter OT action on hypothalamic neurons in a behaviorally-relevant way. This was investigated by recording single-unit activity of neurons in the ventrolateral portion of the ventromedial nucleus in hypothalamic slices, and subjecting these neurons to bath application of OT and related agents. Slices prepared from ovariectomized rats either treated with E (OVX+E) or not (OVX) were used to examine E effect. To help assess relevance to lordosis, the response of a neuron to OT was compared to its responses to two lordosis-relevant neurotransmitters, norepinephrine (NE) and acetylcholine (ACh). Perifused with OT at near-threshold concentration, $2X10^{-10}$M, only neurons from OVX+E preparations responded (11/50 units), whereas none of the 54 OVX units did ($p<0.001$). At 10^{-8}M, OT affected neurons from both groups, but the responsiveness was still higher ($p<0.05$) in OVX+E (24/44 units responded) than in OVX (14/48 units). Most (>80%) of the responses to OT were excitatory, and all the OT-responsive units tested also responded in the same way to a highly selective agonist, [Thr^4, Gly^7]-OT. Some weak modulation of responses to NE and ACh by OT has been observed, but was not affected by E. Neither affected was neuronal responsiveness to vasopressin, a peptide closely related chemically to OT. In OVX+E, but not OVX preparations, neurons responsive to OT were also more likely to respond to NE or ACh (both p's<0.05). Thus, estrogen not only enhanced neuronal responsiveness to OT but did so in the population of hypothalamic neurons responsive to lordosis-relevant transmitters NE and ACh. The effect of P is currently under study.

204.4

AN ESTROGEN-INDUCED HYPOTHALAMIC PROTEIN, HIP-70, IS AN ISOFORM OF THE PHOSPHOINOSITOL-SPECIFIC PHOSPHOLIPASE C-ALPHA ISOENZYME. C.V. Mobbs, U.E. Olazabal, and D.W. Pfaff, Rockefeller Univ., New York, NY 10021.

We recently described a protein, HIP-70, which is induced by estrogen in the ventromedial hypothalamus and by luteinizing hormone-releasing hormone (LHRH) in the pituitary (Science 247:1477). Recent computer searches have revealed that the N-terminal sequence of HIP-70 (DVLELTDENFESRVSDT) matches the N-terminal sequence of a phosphoinositol-specific phospholipase C isoenzyme, PLC-alpha. PLC generates the endogenous activator of protein kinase C (PKC), and we have shown that pharmacological activation of PKC facilitates the estrogen-dependent behavior, lordosis (Pharm. Biochem. Behav. 34:665). Furthermore, effects of LHRH and substance P, both of which facilitate lordosis, are mediated in part through the phosphoinositol pathway; PLC, like LHRH, can stimulate LH release. To corroborate the identity of HIP-70, we examined the relationship between HIP-70 and immunoreactive PLC-alpha isoforms with 2-D gel immunoblots. Proteins from the ventromedial hypothalamus of estrogen-treated rats were separated on 2-D gels and transferred onto polyvinylidifluoride. Filters were incubated with an antibody against PLC-alpha (gift of C.F. Bennett) diluted 1:1000 in 2% serum/Tween blocking buffer, then visualized by an anti-rabbit antibody conjugated with alkaline phosphatase. After development with NBT/BCIP, blots were stained with 0.1% India ink. Based on the protein pattern context provided by the ink stain, HIP-70 precisely co-migrated with the most basic of 4 major PLC-alpha immunoreactive isoforms detected by this procedure. We therefore propose that HIP-70 is an isoform of the PLC-alpha isoenzyme family, and that activation of this isoform by estrogen, LHRH, and substance P may mediate some effects of these substances on neuronal function and behavior.

204.5

EXPRESSION IN BRAIN AND OTHER TISSUES OF mRNA FOR AN ESTROGEN-INDUCED PROTEIN (HIP-70), AN ISOFORM OF PHOSPHOLIPASE C-ALPHA. M.G.Kaplitt, S.P.Kleopoulos*, D.W.Pfaff and C.V.Mobbs, Rockefeller University, New York,N.Y. 10021

We have recently described a protein induced by estrogen in ventromedial hypothalamus (VMH) and by LHRH in pituitary (Science 247:1477,1990). We have subsequently shown through sequence analysis and immunoreactivity studies that this protein, HIP-70, is an isoform of the phosphoinositide-specific phospholipase C-alpha (PLC-α) isoenzyme family (Mobbs, et.al., this volume). This study was undertaken in order to analyze brain and tissue distributions of mRNA for PLC-α and another isozyme, PLC-β, as well as to determine whether PLC-α induction by estrogen is controlled primarily at the mRNA level. Northern and slot blot analyses using probes from C.F.Bennett and C.A.Ross revealed that PLC-α mRNA levels were greatest in pituitary and uterus, high in VMH and preoptic area (POA), moderate in cerebral cortex and hippocampus, low in caudate and negligible in skeletal muscle. By contrast, PLC-β mRNA was highest in caudate, slightly lower in hippocampus and cortex, moderate in cerebellum, VMH and POA and barely detectable in other tissues such as pituitary, uterus, and muscle. Finally, although PLC-α mRNA levels were greatest in estrogen responsive tissues and brain regions, it does not appear likely that estrogen induction of PLC-α mRNA by itself can account for the observed induction of the HIP-70 protein isoform. Therefore, the induction of the HIP-70 protein isoform by estrogen may involve post-translational modifications of more acidic PLC-α isoforms, as previously proposed (Science, 1990).

204.6

PROGESTERONE-FACILITATED LORDOSIS BEHAVIOR DOES NOT APPEAR TO BE CORRELATED WITH MODIFICATION OF PROTEIN PATTERNS IN VENTROMEDIAL HYPOTHALAMUS OF THE FEMALE RAT. M.E. Montemayor, C.S. Giometti*, J. Taylor*, and E.J. Roy. Neurosci. Program, Univ. Illinois, Champaign, IL 61820 and Biol. & Med. Div., Argonne National Lab., Argonne, IL 60439.

Does progesterone modify protein patterns in the ventromedial hypothalamus (VMH)? OVX rats (N=14) were divided into two groups: estradiol only (4 ug/kg at 0 and 18 hrs) and an estradiol plus progesterone (2 mg/kg at 37 hrs). [35S]-cysteine & methionine (0.2 mCi/animal) were infused in the VMH of freely moving animals from 37 to 41 hrs. Following infusions, animals were tested for sexual behavior and sacrificed. The VMH was assayed for changes in the patterns of newly synthesized proteins using two-dimensional gel electrophoresis followed by fluorography. Pattern analysis was done using the Tycho II software system (Clin.Chem. 27(1981)1807).

No proteins were induced or lost as a result of being treated with progesterone following estradiol. Although several proteins exhibited changes that were statistically significant ($P<0.05$), an independent replication (N=12) of the experiment using 0.8 mCi of labeled amino acids/animal indicated that none of these changes was reproducible. Therefore, we concluded that progesterone does not cause detectable alterations in VMH protein expression among proteins in the range of 10-100 kDa and the apparent 4.8-6.7 pI range. (Supported by U.S. DOE, OHER, contract W-31-109-ENG-38)

204.7

ESTROGEN AND PROGESTERONE RECEPTOR IMMUNOREACTIVITY (ER-IR & PR-IR) IN THE BRAIN OF PRAIRIE VOLES EXPOSED TO DIFFERENT SOCIAL CONDITIONS. Q.C. Hnatczuk, C.A. Lisciotto, L.L.DonCarlos, C.S. Carter, and J.I. Morrell. Inst. Animal Behavior, Rutgers Univ, Newark, NJ 07102; Dept. Zoology, Univ. of Maryland, College Park, MD 20742.

The prairie vole's (Microtus ochrogaster) reproductive strategy is different from most mammals. It is reproductively quiescent until it contacts an unfamiliar vole of the opposite sex. The monogamous bonded pair undergoes a change in behavior and physiology, including mating and ovulation. To examine the effect of social stimuli on the brain, we used immunocytochemistry to visualize the ER-IR & PR-IR cells of paired (sexually receptive) and unpaired (naive) male and female voles. The largest number and most intensely labeled ER-IR cells were observed in the medial preoptic area (MPOA), bed nucleus of the stria terminalis (BNST),ventromedial nucleus (VMN) of the hypothalamus, the arcuate, and the medial amygdala. ER-IR cells were also seen in the central grey, lateral hypothalamus, lateral septum, the diagonal bands, the dorsomedial hypothalamus, zona incerta, superior colliculus, and the dorsal spinal cord. PR-IR was observed in the MPOA, BNST, the anterior, paraventricular and VMN of the hypothalamus and the medial amygdala. Social stimulation resulted in dramatic changes in the number of ER-IR neurons and in the intensity of ER labeling. Supported by Sigma Xi (OCH).

204.8

SEX DIFFERENCES IN THE NUMBER, DISTRIBUTION, AND PROJECTIONS OF TESTOSTERONE TARGET NEURONS IN THE MEDIAL PREOPTIC AREA AND BED NUCLEUS OF THE STRIA TERMINALIS OF RATS. C.A. Lisciotto and J.I. Morrell. Inst. Animal Behavior, Rutgers University, Newark, NJ 07102.

Retrograde tracing was combined with steroid autoradiography to investigate the projections of testosterone target neurons in preoptic, limbic and hypothalamic regions to the midbrain in male and female rats. Autoradiograms were prepared from the brains of GDX/ADX male and female rats that had received an injection of a fluorescent retrograde tracer into the midbrain, and an iv injection of [3H]-testosterone. Neurons that concentrate testosterone and project to the midbrain were observed in the bed nucleus of the stria terminalis (BST), the medial preoptic nucleus (MPN) and the ventro-medial nucleus of the hypothalamus. Male rats had more testosterone-concentrating neurons in the MPN and the BST than female rats. Moreover, the subset of testosterone-concentrating neurons in the MPN and BST that project to the midbrain was greater in male than female rats. Sex differences in the neuronal connectivity of androgen target neurons may underlie sex specific behavioral responsiveness to androgens. Supported by HD07243 to C.A.L. and HD22983 to J.I.M.

204.9

DEVELOPMENT OF SEXUALLY DIMORPHIC SPATIAL ABILITY: NEURAL SITES OF ESTRADIOL ACTION. Christina L. Williams, Rebecca A. Cohen, and Warren H. Meck. Departments of Psychology, Barnard College and Columbia University, New York, NY 10027.

Previous research has demonstrated that perinatal exposure to estradiol improves choice accuracy during acquisition of a 12-arm radial maze task in Sprague-Dawley rats (Williams et al., Behav. Neurosci., 1990). The current study was designed to examine the possible neural sites at which estradiol might act to cause organizational changes in visuospatial memory. Five groups of male Sprague-Dawley rats were treated as follows within 6 hrs of birth: HP-neonatally castrated and given bilateral implants of estradiol (E) aimed at the medial hippocampus; FC-neonatally castrated and given bilateral implants of E aimed at the frontal cortex; HY-neonatally castrated and given bilateral implants of E aimed at the ventromedial hypothalamus; CS-neonatally castrated; CN-castrated at puberty. At 60 days of age, subjects were trained on a 12-arm radial maze with 8-baited and 4-unbaited arms. After rats' choice performance reached steady-state reacquisition of the task was conducted in a novel training environment. Results revealed that CN, HP, and FC groups were significantly more accurate in choice performance than CS and HY groups during the initial acquisition phase; these differences disappeared at steady-state and re-emerged during the reacquisition phase. These data provide preliminary evidence to suggest that the hippocampus and frontal cortex are sites of estradiol action for the organization of sexually dimorphic visuospatial ability.

204.10

LESION OF THE HABENULAR COMPLEX DISRUPTS THE HORMONAL ONSET OF MATERNAL BEHAVIOR IN THE RAT. K.P.Corodimas, J.S. Rosenblatt,* A.D. Mayer* and J.I. Morrell. Inst. of Anim. Behav., Rutgers Univ., Newark, NJ 07102.

The habenular complex (Hbc) is reciprocally connected with the preoptic area, substantia nigra, and ventral tegmental area; regions key to the control of maternal behavior in the rat. This study explored whether the Hbc is part of a neural circuit necessary for the initiation of maternal behavior. On day 12 of pregnancy bilateral radiofrequency lesions were made in the Hbc, or as a control, just dorsal in the medial hippocampus. A third group served as unoperated controls. All animals were hysterectomized, ovariectomized, and given 20µg/kg estradiol benzoate on day 16 of pregnancy, then tested 48h later with pups for maternal behavior. Retrieval, nursing, and nest building were severely disrupted following Hbc lesions, whereas control groups displayed short-latency maternal behavior. Activity levels and body weight changes were similar across groups. These results suggest that the Hbc or axons traversing it are important for the onset of maternal behavior. Supported by Sigma Xi, Grants-in-Aid of Research (KPC).

204.11

INTRAUTERINE POSITION PHENOMENON AND PRECOPULATORY BEHAVIOR OF HOUSE MICE. B. M. Jubilan and J. G. Nyby. Dept. of Psychology, Lehigh University, Bethlehem, PA 18015.

Fetuses are exposed to different testosterone titers prenatally depending on intrauterine position. In general, fetuses located between two males (2Ms) are masculinized relative to those located between two females (0Ms). Fetuses next to single male (1Ms) are intermediate. Most studies examining the behavioral significance of intrauterine position have focused on copulatory behavior.

In the present study the effects of intrauterine position upon two precopulatory behaviors, sexual preference and ultrasonic courtship vocalizations were investigated. Three strains of mice were used. The anogenital distances were as predicted (2M>0M) but not statistically significant. Although the effects on sexual preferences were not profound, some subtle effects seemed evident: the 0M males among the males and the 2M females among the females appeared more discriminating in their preferences for male and female odors. The frequency of ultrasonic vocalizations was not significantly different for the different intrauterine categories of males. Genotype did not significantly contribute to the variability in either behavior.

In conclusion, with the genotype and sample sizes used intrauterine position did not appear to strongly affect the adult precopulatory behaviors or morphology assayed.

205.1

RETENTION-SPECIFIC DECREASES IN CA1 PKC FROM RABBITS 3 HOURS AFTER CLASSICAL CONDITIONING. J.L. Olds, B.G. Schreurs, M. Stultz* and D.L. Alkon. LMCN, NINDS, NIH. Bethesda MD. 20892.

It has been demonstrated that membrane-associated PKC (mPKC) undergoes massive changes in its distribution at 24 h and 72 h after 3 d of rabbit NM conditioning (Olds et al. Science 1989). The present study was designed to investigate shorter term changes in mPKC after NM conditioning. Rabbits received 3 d of training (P), or 3 d of unpaired trials (UP) or were left in their home cages (N). Three h after the last trial, animals from the P and UP groups were sacrificed and mPKC was quantified in all subjects using the [3H]-PDBU technique. Preliminary data shows that conditioned animals had signficantly lower mPKC in CA1 compared to control rabbits (P 20.71±.885, UP 24.0±1.1, N 24.1±1.6; $p<0.05$). We interpret these results to further confirm the involvement of PKC in memory storage processes (Alkon et al, 1989).

205.2

ACQUISITION-SPECIFIC CHANGES IN CA3 PKC FROM RABBITS UNDERGOING CLASSICAL CONDITIONING. A.M. Scharenberg, J.L. Olds, B.G. Schreurs, A.M. Craig* and D.L. Alkon. LMCN, NINDS, NIH. Bethesda MD. 20892.

It has been demonstrated that membrane-associated PKC (mPKC) undergoes an increase during retention after rabbit NM conditioning (Olds et al. Science 1989). The present study was designed to investigate PKC distributional changes during aquisition. Initially, a time point was selected for assessing PKC distributional changes. Rabbits received either 1 d or 2 d of NM training. These animals showed an increase in % CRs on day 1 of conditioning relative to the unpaired (UP) control group ($p<0.001$). Additionally, for the paired group, the % CRs on day 2 was increased relative to day 1 ($p<0.001$). A second experiment quantified mPKC in the brains of rabbits sacrificed immediately after receiving either 1 d of paired stimuli (n=10), 1 day of unpaired stimuli (n=6), or no behavioral manipulation (n=6). [3H]-PDBU binding was increased in the stratum oriens of CA3 for paired animals relative to either of the control groups (Paired 32.4±1.2, UP 27.2±1.4, Naive 27.6±0.9; $p=0.009$). We interpret this finding to further confirm the importance of PKC in associative learning.

205.3

A SIMPLE PDP MODEL SIMULATES SPATIAL CORRELATES OF HIPPOCAMPAL NEURONAL ACTIVITY. M.L. Shapiro, P.A. Hetherington*, H.B. Eichenbaum, and W.J. Fortin*. Department of Psychology, McGill University, Montreal, Quebec H3A 1B1.

Hippocampal place cells fire when behaving rats occupy selective regions of spatial environments, and seem to encode relationships among spatial stimuli. Here, a simple neural network model that computes locations from configurations of distal cues simulates many aspects of place fields and predicts quantitative relationships between place field and environmental properties.

A 3 layer neural network was trained to compute local representations of locations from visual angles of simulated cues using back propagation. Each input unit encoded the visual angle of one cue, and was connected to every association unit; each association unit was connected to every output unit. After training, the network accurately encoded locations, and the activation of units in the association layer resembled place field activity in the hippocampus. Like actual place cells, simulated units: (1) were active in selective locations; (2) were less accurate spatially than the network as a whole, thereby demonstrating coarse coding; (3) had directional, multiple subfields; and (4) persisted after single cue removal, showing graceful degradation. Also, like local groups of place cells, groups of simulated units had fields that covered most of the environment in an overlapped manner. Quantitative variants of the model predict that single place cells will have more subfields as the spatial complexity of an environment increases. Thus, a simple neural network model with 2 striking features of the hippocampal system, distributed connections and synaptic plasticity, can encode spatial locations using a representational scheme similar to that found in the hippocampus, and may predict and explain new properties of place fields.

205.4

THE SIGMA-PI MODEL NEURON: ROLES OF THE DENDRITIC TREE IN ASSOCIATIVE LEARNING. B. W. Mel, Computation and Neural Systems Program, Caltech, 216-76, Pasadena, CA, 91125.

A model for associative learning in cerebral neocortex has been previously proposed (Soc. Neurosci. Abst., 208.5, 1989). In this model, the extrinsically-projecting pyramidal cells of layers 2, 3, and 5 of association cortex are modeled as *sigma-pi* units, where a *sigma-pi* unit computes its activation level as a sum of contributions from a set of multiplicative (or locally-thresholded) clusters of synapses distributed throughout its dendritic tree. The model demonstrates how a broad class of biologically-relevant nonlinear associative learning problems can be solved in this system by modifying only a single layer of excitatory synapses under the control of a simple Hebb-type learning rule. The model also accounts for a variety of features of cortical anatomy, physiology, and biophysics whose relations to learning have remained poorly understood. These include, (1) three learning-related roles for the NMDA channel, one of them new, (2) the gross asymmetry in number and patterns of termination of excitatory vs. inhibitory synapses onto cortical pyramidal cells, as well as the apparent lack of plasticity at inhibitory synapses, (3) the replication of like-activated neurons beneath a single point in cerebral cortex, and in particular the clumping of apical dendrites of pyramidal cells on their rise to the cortical surface, (4) the complex 3-dimensional arborizations of axons and dendrites in layer 1, which give rise to a rich "combinatorial" association interface crucial to the current model, and (5) putative rules for activity-dependent axon growth and synaptogenesis during associative learning. Compartmental modeling studies are currently underway to test the crucial assumption of the *sigma-pi* learning scheme, namely the degree to which a complex dendritic tree can support a large number of independent AND-like operations among local clusters of synapses.

205.5

EMERGENCE OF BASIC-LEVEL RECOGNITION PHENOMENA FROM SIMULATION OF PRIMARY SENSORY CORTEX.
R.Granger, J.Ambros-Ingerson*, G.Lynch. Center for the Neurobiology of Learning and Memory, Univ. of Calif., Irvine, CA. 92717.

A simulation of the physiological activity of the two superficial layers of olfactory paleocortex (piriform cortex) has shown that the network yields coherent activity that can be interpreted as the storage, organization and retrieval of olfactory memories. Moreover, the repetitive sampling operation of the paleocortical network causes it to generate multiple responses to a single input. With learning via synaptic long-term potentiation (LTP), these responses become hierarchically ordered, such that the first-cycle response indicates coarse-grained cluster information whereas subsequent cycle responses are increasingly finer-grained (Ambros-Ingerson, Granger and Lynch, Science, 247:1344-48, 1990). These findings suggest that sensory cues might be "iteratively recognized" at a sequence of successively lower levels, with the first level recognized being a natural "entry level" for perceptual processing. Cognitive studies of visual and conceptual recognition in hierarchically organized domains suggest that such preferred levels do exist and exert a strong influence over early processing: people robustly prefer a "basic level" description (e.g., "bird" in the hierarchy "animal", "bird", "sparrow"), recognizing cues faster at this level and more frequently than either superordinate or subordinate names (Mervis and Rosch, Ann.Rev.Psych., 32:89–115, 1981). We tested the idea that the perceptual processing performed by primary sensory brain structures might yield analogs of basic level effects through simulations of piriform cortex using a stimulus structure from a study of basic level effects in human clasification (Corter, Gluck and Bower, Proc.Cog.Sci.Soc., 1988). We find that the first cycle piriform responses of the simulation do prefer the basic level, whereas subsequent responses prefer subordinate levels. This suggests that the unsupervised preprocessing performed by the pirform cortex is directly analogous to aspects of the cognitive phenomena of basic level superiority in early perceptual and conceptual processing. (Supported by ONR N00014-89-J-1255 and N00014-89-J-3179, and NSF IST-85-12419.)

205.6

VISUAL RESPONSE PROPERTY CHANGES OF THE MONKEY PREFRONTAL NEURONS DURING THE LEARNING OF A VISUAL GO/NO-GO TASK WITH EYE FIXATION. K. Kubota and A. Mikami. Dept. of Neurophysiol., Primate Res. Inst., Kyoto Univ., Inuyama, Aichi, 484, JAPAN.

During the learning of a visual discrimination task with GO/NO-GO performances, the number of task-related neurons increases progressively with increase of performance level (Neurosci. Res., 3:106, '85). To know how visual responsiveness develops during the learning, 3 Rhesus monkeys were trained to perform a visual GO/NO-GO task with eye fixation and neurons responding to visual cues were recorded from areas 46 and 8. While the monkey was fixating his eyes to a pair of slits with lever press, red or green cues (2.4 deg circle, 15 deg left to slits) was presented. On changing slit orientation, the monkey released his hand to red (GO trials), or withheld his hand to green (NO-GO trials). In undertrained stage 14 neurons obtained during 400 penetrations responded to either cue. And in welltrained stage 50 neurons obtained during 84 penetraions responded to either cue (N=38) or both cues (N=12). And about 2/3 of neurons had clear receptive field ipsi- and/or contralaterally. Thus, during the learning are emerging visual neurons, which receive facilitatory influences of wide receptive field and color-cue specific activations.

205.7

LEARNING-SPECIFIC DIFFERENCES IN PURKINJE CELLS OF LOBULE HVI: INTRACELLULAR RECORDING IN A RABBIT CEREBELLAR SLICE. B. G. Schreurs, J. V. Sanchez-Andres, and D. L. Alkon. Lab. of Molecular & Cellular Neurobiology, NIH, Bethesda, MD 20892.

We report the development of a rabbit cerebellar slice of lobule HVI and describe classical conditioning-specific differences in the threshold for dendritic spikes within Purkinje cells of ipsilateral lobule HVI.

Intracellular recordings made in rabbit Purkinje cell dendrites of lobule HVI revealed membrane potential, action potential, and input resistance values that were similar to values described for vermal Purkinje cells in guinea pig and rat. Activation of parallel fibers and climbing fibers produced synaptic potentials also similar to those previously described in other species: however, patterns of autorhythmicity were more marked than previously reported. Indeed, many of our recordings exhibited clear sequences of somatic, dendritic, and hyperpolarized phases for as long as the cell was held.

A comparison of Purkinje-cell dendritic activity in lobule HVI for animals subjected to classical conditioning, explicitly unpaired, or no treatment procedures revealed a significant ($p < .01$) decrease in the threshold for dendritic spikes in cells from conditioned animals (1.2 ± 0.18 nA; $n = 9$ cells) versus cells from unpaired (1.8 ± 0.22 nA; $n = 9$) and untreated animals (2.2 ± 0.25 nA, $n = 7$). There were no significant differences in membrane potential or input resistance among the treatment groups (p's > .10). The present results suggest a conditioning-specific increase in Purkinje-cell dendrite excitability in lobule HVI which may have consequences for classical conditioning of the rabbit nictitating membrane response.

205.8

ROLE OF CEREBELLUM IN CLASSICAL CONDITIONING J.C. Houk. Department of Physiology, Northwestern University Medical Center, Chicago, IL 60611.

The cerebellum is thought to be important in classical conditioning (cf. C. Yeo in Cerebellum and Neuronal Plasticity, Plenum Press, 1987; M.A. Gluck & R.F. Thompson in Neuroscience and Connectionist Theory, Lawrence Erlbaum Assoc., 1990). Several models assume that the US is transmitted to the cerebellum by climbing fibers and that both the CS-US association and the motor command that controls the CR are established within the cerebellum.

The adjustable pattern generator (APG) model of the cerebellum suggests a different role for the cerebellum in conditioning (J.C. Houk in Models of Brain Function, Cambridge Univ. Press, 1989; J.C. Houk, S.P. Singh, C. Fisher & A.G. Barto, in Neural Networks for Control, MIT Press, 1990). CSs and USs function as trigger signals that act on motor cortex, red nucleus and brainstem neurons. These inputs initiate activity in recurrent loops through the deep cerebellar nuclei; positive feedback in these loops is postulated to be the driving force for commanding movement (the CR). Climbing fibers train Purkinje cells to control this positive feedback and shape it into motor programs that control accurate movements.

Thus, the APG model proposes learning at two separate sites. CS-US associations are learned outside of the cerebellum by synaptic modification of sensory inputs to motor cortex, red nucleus and brainstem neurons, independent of climbing fiber activity. Learning accurate motor programs for CRs occurs in cerebellar parallel fiber synapses guided by climbing fiber activity.

205.9

RESPONSES OF PRIMATE NUCLEUS BASALIS NEURONS RELATED TO APPETITIVENESS, AVERSIVENESS, AND AROUSAL. R.T. Richardson and M.R. DeLong. Dept. Neurology, Johns Hopkins U., Baltimore, MD 21205

In monkeys performing various behavioral tasks, changes in the discharge rates of nucleus basalis neurons are primarily associated with stimuli related to a water reward. To determine whether these responses are related to the appetitive or arousing quality of the reward-related events, we studied the responses of basalis neurons to appetitive stimuli (water rewards), aversive stimuli (air puffs to the snout), and neutral somatosensory (passive displacements of the forearm) and auditory (pure tones) stimuli in two naive rhesus monkeys. With the neutral stimuli, 21% of 141 basalis neurons had significant changes in discharge rate following forearm displacement, and 3% of 74 neurons responded to tones. In contrast, 57% of 249 basalis neurons responded to water delivery, and 59% of 113 neurons responded to air puff. Of 85 neurons responding to either air puff or water, 61% had qualitatively similar responses to both stimuli (i.e. both increases or both decreases), 5% had opposite responses, 21% responded only to water, and 13% responded only to air puff. Moreover, 97 neurons were recorded during delivery of increasing volumes of water (0.1, 0.2, and 0.4 ml). Forty nine neurons were sensitive to the size of the water delivery in that they had progressively larger responses to the larger volumes. Of these neurons, 80% had qualitatively similar responses to the aversive air puff, thus suggesting that they were sensitive to the arousal component of the stimuli. In conclusion, a sub-population of basalis neurons appears to be differentially responsive to appetitive or aversive stimuli, but a larger sub-population appears to be responsive to both appetitive and aversive stimuli. The responses of neurons in the latter group appear to reflect the arousing properties of sensory stimuli.

205.10

SELECTIVE EFFECTS OF LIDOCAINE MICROINJECTIONS IN THE REGION OF THE SPINAL TRIGEMINAL NUCLEUS ON THE CONDITIONED AND UNCONDITIONED RESPONSES OF THE RABBIT NICTITATING MEMBRANE REFLEX. V. Bracha*, J.-Z. Wu*, M. Cartwright* and J. R. Bloedel. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ. 85013

Experiments were performed with intact rabbits to test the hypothesis that regions of the trigeminal nuclear complex are selectively involved in mediating conditioned responses (CR) following the classical conditioning of the nictitating membrane reflex (NMR). The NMR was conditioned in white adult New Zealand albino rabbits using a traditional delay paradigm over a seven day period. The conditioned stimulus, a 1 kHz, 85 db tone superimposed on 70 db white noise, was applied 350 msec prior to an unconditioned stimulus (UCS), a 100 ms duration airpuff. After each animal reached a criterion of 95% CS responding, a baseline series of three-trial sequences was performed. These sequences consisted of one paired CS-UCS trial, one airpuff UCS trial and one light UCS trial. The latter was used as a control to indicate whether changes in response amplitudes could be attributed to spread of lidocaine to output projections of the motor nuclei. The effects of microinjecting 0.3 to 1.0 ul of 4% buffered lidocaine were determined at sites located approximately 3 mm caudal to the facial nucleus. Injections at the lateral aspect of the spinal trigeminal nucleus reduced the amplitude and incidence of the CR and the amplitude of the UCR. In contrast, injections at the junction of the medial spinal trigeminal nucleus and the reticular formation resulted in a reduction in the incidence as well as the amplitude of the CR without a parallel effect on the UCR. The data suggest that the changes in neuronal interactions required for the execution of the conditioned NMR may occur at the initial stages of this reflex pathway. Supported by NIH Grant NS21958.

205.11

LEARNING-ASSOCIATED G-PROTEIN, CP20, INHIBITS ORGANELLE MOVEMENT WITHIN AXONS S. Moshiach, T. Nelson, J.V. Sanchez-Andres, & D.L. Alkon Lab. of Molecular & Cellular Neurobiology, NIH, Bethesda, MD 20892

The concentration of a 20,000 M.W. G-protein, cp20, was increased 24 hrs after classical conditioning of the mollusc *Hermissenda* but not after control paradigms (Nelson et al, Science, 1990). cp20 potently reduces the same two K^+ currents (in the type B cell) reduced by classical conditionings.

Here we asked whether cp20 might also regulate axonal transport and thereby perhaps initiate persistent structural changes measured on days after the conditioning. The movements of organelles (2-20μm) in length were visualized within sections of *Carcinus* axons which had been dissected free and exposed to Protease (2mg/ml, 5mins). Images were acquired with video-enhanced DIC, processed with an Image-1/AT analysis system (Universal Imaging), displayed on a SONY PVM-122 monitor and recorded at 30 frames/s. Organelle movements within a 4 min interval were quantified and counted using subtraction with shear as a filter to include only particles which could be unequivocally identified. cp20 (10μM) application was followed within 15 min by highly significant reduction ($p<.001$) of particles moving across a 10μm length of the axon, from 23.57 ± 1.34 to 12.58 ± 1.47 (N=7). Boiled cp20 application under identical conditions did not alter particle movement (20.5 ± 5.8 before vs. 19.25 ± 6.94 during application, N=4, N.S.). This cp20-induced regulation of organelle movement could, therefore, profoundly alter transport to cellular sites which undergo changes of excitability and structure during storage of associative memory.

206.1

SPINAL CORD REGENERATION IN AVULSION INJURIES OF THE BRACHIAL PLEXUS Dr. J. Terzis & Dr. H. Kai* EVMS, MRC, Norfolk, VA 23507

The functional deficit following avulsion injuries to the brachial plexus is devastating. To date, it has been impossible to use the axons of the avulsed motor neurons in reconstructive efforts.

We have established an avulsion model in the rat. The normal anatomy of the cervical cord will serve as the control group (A). Total avulsion of C5 to T1 will be carried out in group B, while transection of C5 to T1 will be carried out in group C and the involved spinal cord will be studied morphologically for the extent of injury in the ventral horn. In groups D and E, total avulsion and/or transection of the brachial plexus roots will be followed by implantation of C6 spinal nerve into the lateral column of the spinal cord at C7 level. HRP studies of the musculocutaneous nerve (MC) will lead to identification of the neuronal ventral horn pool in the normal and injured cervical spinal cord. Exact quantitation of the number of these neurons will be correlated with quantitative axonal morphometry of the MC nerve. This data will be correlated with muscle typing and quantitation of end plates in the biceps muscle. EMG and muscle function tests of the normal and reinnervated biceps will be done in all groups.

206.2

VENTRAL SPINAL CORD ISCHEMIA MODEL PRODUCED BY PHOTOCHEMICAL LASER OCCLUSION OF THE ANTERIOR SPINAL ARTERY. P.W. Madsen, R. Prado* B. Watson and A. Martinez. The Miami Project, Univ. of Miami Sch. of Medicine, Miami, FL 33136

Although spinal cord ischemia is a significant component of spinal injury, its precise role is poorly understood. Previous attempts to produce spinal ischemia by occlusion of the aorta in the rat produced inconsistent results. We report an effective model of rat spinal cord ischemia produced by occlusion of the cervical anterior spinal artery (ASA). Subtotal corpectomy of C5 or 6 was performed on anesthetized SD rats to expose the ASA which was occluded via the photochemical laser (Watson et al., Brain Res.). Following recovery, rats were subjected to sequential evaluation of motor (modified Tarlov scale, inclined plane and forepaw strength), and sensory function for one month post procedure. Maximal deficit was present within 72 hours post occlusion and the forelimbs were more severely affected than the hindlimbs. The motor deficits then improved until a plateau was reached at the second week. However, forelimb sensory function deteriorated after an early period of recovery during the first five days. This successful development of a rodent model of spinal cord ischemia will allow further characterization of the contribution of ischemia in spinal cord injury.

206.3

DORSAL SPINAL VENOUS OCCLUSION MODEL IN THE RAT: PRELIMINARY OBSERVATIONS. **A. Martinez-Arizala, J. Mora*, B. Green*, and N. Hayashi*. The Miami Project, University of Miami School of Med., Miami, Fl 33136.**

Although vascular disruption occurs with traumatic spinal injury, the effects and involvement of the spinal venous system are undefined. Improper venous drainage can certainly contribute to the development of tissue edema and can impair its resolution. In order to better understand spinal venous dysfunction, we developed a model of spinal venous occlusion in the rat. A T-7 to T-9 laminectomy was performed in anesthetized S-D rats and the dorsal spinal vein was focally coagulated at the caudal and distal ends of the laminectomy. Occasionally, the dorsal vein became visibly engorged and small hemorrhages were seen one to two hours after occlusion. During the first days following occlusion, all animals had hindlimb paralysis from which they slowly recovered. In addition, some animals also developed neurogenic bladders. Pathological examination of the tissues at 1 and 3 days post occlusion revealed marked hemorrhagic infarction and edema affecting primarily the dorsal columns and the dorsal gray matter. At one week these regions were necrotic and extensively infiltrated by macrophages. By one month pronounced tissue loss and some cystic cavitation was present in the lesioned areas. These results demonstrate that extensive behavioral and pathological changes can be associated with dorsal spinal cord venous dysfunction. Nonetheless, its precise in role in spinal injury still remains to be fully elucidated.

206.4

DISTRIBUTION AND CONCENTRATION OF ELEMENTS IN RAT SPINAL CORD AXONS AND GLIA. R.M. LoPachin, R.S. Lagasse, C.M. Castiglia*, M. Foster and A.J. Saubermann. Dept. Anesthesiology, SUNY, Stony Brook, NY11794.

Little information is available concerning the elemental composition of spinal cord axons. Acquiring such knowledge is important because elements participate in almost every aspect of axonal function, and because axonal degeneration is presumably mediated by disruption of subaxolemmal Ca, Na and K. To determine water content, and the concentrations (mmol/kg dry or wet weight) and distributions of elements in spinal cord axons, we used electron probe X-ray microanalysis (EPMA). Rat cervical spinal cords were rapidly excised, frozen and then cryosectioned (500 nm). Results show that the most prominent element in myelinated axons was K. Axoplasm of large and medium fibers contained similar concentrations of this element (e.g., 1947±61 dry wt, 175±6 wet wt), whereas small axons exhibited a lower K content (1391±100 dry wt, 149±11 wet wt). Regardless of axon size, low axoplasmic concentrations of Na, S, Ca and Mg were detected. Water content for all axons was approximately 90±1%. Dry wt elemental composition of mitochondria was similar to that of larger axons. However, with a water content of 69±3%, wet wt elemental concentrations were different from axons (e.g.,Kmito=523±35 wet wt). As expected myelin displayed high wet and dry wt concentrations of P with 33±3% water. Glial cell cytoplasm contained relatively high concentrations of both P and K with a water content of 68±4%. These results in spinal cord are remarkably similar to EPMA measurements of axons and glial cells in rat sciatic nerve (J. Neurochem. **51**: 764-775, 1988), and form the basis for studies of trauma- and chemical-induced injury in rat spinal cord. (supported by ES03830 and NS21455)

206.5

TIME - DEPENDENT LAMINECTOMY EFFECTS UPON ENERGY METABOLISM IN THE RAT SPINAL CORD. A.Mautes* and A.C. Nacimiento. Neurosurgical Research Laboratory, Saarland University Medical School, 6650 Homburg/Saar,FRG.

In a study of changes in energy metabolism in the spinal cord following compression trauma, a pretraumatic metabolic base line has to be defined, particularly to take into account the possible effects of laminectomy per se. Using a computer assisted bioluminescence image analysis technique (cf.Nacimiento et al.,Soc.f.Neurosci.Abstr. 1988,14,460.16)we measured simultaneously the regional content of ATP,glucose and lactate in tissue sections of the exposed lumbar,and of the unexposed thoracic cord as internal control. Immediate and delayed (3h) laminectomy effects were compared. Results: a) ATP content did not change significantly;b) glucose content increased after 3h exposure in both lumbar and thoracic sections to levels just short of significance. c) lactate increased significantly after 3h (i)in lumbar sections with and without exposure, and (ii) in thoracic sections after lumbar exposure. Thus the spinal cord energy state cannot be considered as normal under conditions usually prevailing in experimental spinal cord trauma investigations. Supported by DFG-Grant Na 115/5-1.

206.6

KETAMINE PREVENTS NEURONAL INJURY AND DEATH DURING MULTIPLE IMPACT RAPID ACCELERATION INJURY (RAI)
J. H. Lucas and A. Wolf. Dept. of Biological Sciences and Center for Network Neuroscience, Univ. of North Texas, Denton, TX 76203.

We have developed an in vitro model of RAI in which a ballistic pendulum is used to impact monolayer cultures of mammalian CNS cells. Impacts are delivered normal or tangential to the plane of growth at 3-5 sec intervals. Culture medium is removed during RAI (60 sec deprivation). Death of spinal cord (SC) neurons during tangential RAI occurred when the cumulative acceleration exceeded 350 g, and reached a maximum of 50% at 850-1700 g (5-10 impacts). Death was only 10% after multiple normal impacts or medium deprivation. Neuronal death occurred within 15 min with little additional loss at 24 h or 48 h. Common morphological changes observed in 393 selected neurons (11 cultures) after multiple tangential impacts were somal swelling, nuclear/ nucleolar shifting, increased nuclear prominence, and enucleation.

Measurements of somal dimensions of 162 neurons (4 cultures) subjected to 10 tangential impacts indicate that smaller neurons may be more sensitive to RAI. For neurons with avg. somal radii of 6-10 µm, 11-15 µm, 16-20 µm and 20-25 µm death was 53%, 45%, 39% and 17% respectively. However, because SC cultures contain a mixed neuronal population, it is possible that these data indicate a sensitivity of neuronal type rather than of size.

When the maximum nontoxic concentration of ketamine (100 µM) was added to 3 SC cultures, neuronal death after tangential RAI (10 impacts, 1700 g) was 15% compared to 44% death in the 3 impacted control (no ketamine) group. Death in 6 nonimpacted cultures (medium deprivation only) in the presence and absence of ketamine was 6% and 12% respectively. Ketamine also prevented morphological changes during RAI. These data suggest: 1) a particular neuronal type(s) may be more sensitive to multiple impact RAI, and 2) neuronal sensitivity to multiple impact RAI may depend on the density of the NMDA receptor/ion channel complexes in the dendrosomatic membranes. Supported by grants to G. Gross from NIH (PHS 23686) and the Hillcrest Foundation of Dallas, TX founded by Mrs. W. W. Caruth, Sr.

206.7

ANTIBODIES TO THE 68kD NEUROFILAMENT SUBUNIT READILY IDENTIFY TRAUMATICALLY INDUCED AXONAL SWELLINGS. A.Yaghmai* and J.T. Povlishock. Dept. of Anatomy, Medical College of Va., Virginia Commonwealth University, Richmond, VA 23298.

Reactive axonal change has long been recognized as a feature of traumatic brain injury and is a major determinate of morbidity. To follow the genesis of the reactive axonal change, various ultrastructural studies have relied on invasive techniques using anterograde axonal transport which, as such, exert some focal damage on the previously injured brain. To obviate this problem, we have utilized the neurofilamentous change associated with reactive axonal damage to provide a non-invasive way to follow the progression of axonal abnormality. To this end, anesthetized rats and cats were subjected to moderate fluid-percussion brain injury and allowed to survive for periods ranging from hours to days. At that time, the animals were reanesthetized, perfused with aldehydes and their brains processed for the immunocytochemical visualization of the neurofilament 68kD subunit as well as its phosphorylated and non-phosphorylated epitopes. Through this approach, all reactive axons revealed some immunoreactivity to all employed antibodies; however, the antibody to the 68kD subunit proved most sensitive. Not only did it allow for the consistent recognition of the reactive axons, but also, its use was not complicated by any background staining which was a significant problem in the case of the phosphorylated epitope. The results of this investigation suggest that antibodies to the 68kD subunit constitute excellent markers for the detection of reactive axonal change. Interestingly, the conspicuous presence of this subunit with traumatic brain injury may provide insight into the pathogenesis of the reactive axonal change. Specifically, it is posited that the striking immunoreactivity is associated with the unmasking of the 68kD subunit, perhaps, due to structural failure. Supported by NS-20193

206.8

SELECTIVE NEURONAL LOSS IN THE THALAMIC RETICULAR NUCLEUS FOLLOWING INERTIAL CLOSED HEAD INJURY IN PRIMATES. D. T. Ross, T. A. Gennarelli*, D.I. Graham*, and J.H. Adams* Head Injury Center, Division of Neurosurgery, University of Pennsylvania, Philadelphia, PA 19104, and Dept. of Pathology, Southern General Hospital, Glascow, Scotland.

The thalamic reticular nucleus (RT) is a pure population of GABAergic neurons which provide inhibitory control for thalamic relay nuclei. Due to its location, connections, and intrinsic properties, the RT has been implicated as playing an important role in attentional processes. The loss of RT neurons has been postulated as an organic basis for some types of post traumatic and post ischemic attentional deficits (Ross and Duhaime, *Brain Research, 501*:129-143, 1989). Attentional deficits are one of the most frequent persisting sequelae of even minor head injury but the biological basis of these deficits is poorly understood. Since primate inertial closed head injury duplicates the neuropathological and neurological aspects of human closed head injury, including post traumatic attentional deficits, we examined head injured primate brains for evidence of RT neuronal loss. In 9 of 10 cases examined selective degeneration of RT neurons was evident throughout the rostral region of the nucleus, the region associated with frontal and prefrontal cortices and related thalamic relay nuclei. The loss of these inhibitory thalamic reticular neurons and resultant thalamic and neocortical neuronal dysfunctions may underlie some forms of attentional deficits which persist following head injury. The selective vulnerability of RT neurons following head injury may, like RT loss due to ischemia and kainic acid toxicity, reflect their sensitivity to excitotoxic degeneration. (Supported by NIH Center Grant NS-08803-20)

206.9

A CONTROLLED CORTICAL CONTUSION MODEL OF TRAUMATIC BRAIN INJURY IN THE RAT: BIOMECHANICAL AND NEUROLOGICAL OBSERVATIONS. C.E. Dixon, G.B. Moore*, G.L. Clifton, D.C. Viano, J.W. Lighthall, S.A. Ridella, Worrel, P.J*.,and Hayes, R.L. Depts. Rehabilitation Medicine and Neurosurgery Medical College of Va., Virginia Commonwealth University, Richmond, VA 23298, and Biomedical Science Dept. General Motors Research Laboratories, Warren, MI 48090.

Although rat models have been useful in studying moderate traumatic brain injury, there is currently no model of severe head injury in the rat. The controlled cortical contusion model uses a constrained stroke pneumatic impactor to allow independent control of the contact velocity and compression parameters.

The first objective was to determine the relative contributions of contact velocity (V) and compression (C) to acute neurological deficits in response to controlled cortical impact. Sixty-two animals were injured at 1 of 18 different combinations of impact V and C parameters. Results showed that acute neurologic responses correlated better with velocity-compression product (VC) than with either V or C alone. At low contact V, functional impairment is best predicted by maximal C. However, as V increases, injury severity becomes a function of (VC), demonstrating the rate sensitivity of brain tissue.

The second objective was to characterize the chronic vestibulo-motor responses to 3 injury levels. Twenty-one rats were injured with a contact V of 6 meter/sec at one of three depths of C: 1, 2, or 3 mm. Performance on beam balance and walking tasks were measured daily for 5 days post-injury. Results show greater deficits with increasing depths of deformation.

The rat cortical contusion model produces a graded injury response that may more closely approximate severe human head injury than other rat brain injury models. Supported by CDC R49/CCR303547 and NIH 12587 and 21458.

206.10

RESIDUAL ATTENTIONAL IMPAIRMENTS AFTER PROLONGED COMA DUE TO TRAUMATIC BRAIN INJURY. R.F. Zec, J. Miller*, D. Zellers*, J. Belman*, J. Matthews*, D. Belman*, M. Payne*, S. Vicari*, R. Robb*, and S. Verhulst*. SIU Sch. of Med. Springfield, IL 62794.

The long-term consequences of severe closed head injury on attentional factors (encode, focus/execute, shift, sustain) was studied using tests specified in a recently proposed model of attention (Mirsky, 1989). The subjects were 32 patients with severe traumatic brain injury (inpatients + outpatients) and 42 control subjects. Age and education (t-tests), and gender ratio (Chi square) were not statistically different for the TBI and control groups. The mean length of coma for the TBI group was 65.4 (SD=70.0) days and time since injury was M=10.1 years; SD=6.5, range 3 to 28 years). Focusing/executing was severely impaired in the TBI group while encoding was only mildly impaired. Shifting and sustaining were intermediate and were moderately to severely impaired. Although the inpatient and outpatient TBI group did not differ significantly on a battery of memory test measures, the inpatients with TBI scored significantly poorer than the TBI outpatient group on the majority of the attentional test measures. The less severe attentional impairment in the TBI outpatient group is associated with their better outcome and may have prognostic value.

NERVE GROWTH FACTORS III

207.1

NGF RECEPTOR EXPRESSION IN BASAL FOREBRAIN AND PURKINJE CELLS AFTER PERINATAL HYPO- OR HYPERTHYROIDISM. B.C. Figueiredo, L. Garofalo, E.P. Pioro, P. Piccardo, A.C. Cuello. Dept. of Pharm. & Therap., McGill University, Montreal, P.Q., H3G 1Y6, Canada.

Early hypothyroidism produces deficits in protein synthesis in the developing brain. We compared NGF receptor (NGFr) expression in brains of such animals with that of control and those of thyroxine-treated. rats. Neonatal male rats were rendered hypothyroid (HO) by feeding the dams with a 0.4% propylthiouracil-enriched diet from embryonic day 19 until postnatal day (PD) 15 or PD 30. Other newborn rats were made hyper-thyroid (HR) by injecting them daily (s.c.) with 0.3 or 1.0 μg/gm b.wt. of thyroxine from PD0 to PD15 or PD30. Use of a mAb (192-IgG) recognizing rat NGFr (Chandler et al., *J.Biol.Chem.* 1984), revealed high levels of receptor immunoreactivity (IR) in Purkinje cells of HO rats on PD15 compared to control and HR animals. HO rats also demonstrated a sparse pattern of NGFr staining in the molecular layer. Further experiments will determine whether the increased NGFr-IR in the HO animals is a function of delayed development or is due to an absolute increase in NGFr levels. NGFr-containing basal forebrain neurons and their cortically-projecting fibers displayed relatively greater IR in HR animals when examined on PD15. Supported by MRC Can. & Centre of Excellence for Neural Repair and Functional Recovery.

207.2

GLIAL CELLS INCREASE THE PRODUCTION OF NERVE GROWTH FACTOR (NGF) FOLLOWING DESTRUCTION OF HIPPOCAMPAL NEURONS. C. Bakhit, M. Armanini, G.L. Bennett*, W-L.T. Wong*, S.E. Hansen*, and R. Taylor*. Genentech, Inc., South San Francisco, CA 94080.

The concept that septo-hippocampal cholinergic neurons depend on trophic support for their maintenance and survival was challenged by the recent finding that destruction of the target neurons did not result in the degeneration of the septal cholinergic neurons (Sofroniew et al., Science 247,338, 1990). This discrepancy could be resolved, however, if nonneuronal elements in the target field assume the role of providing trophic support following loss of neurons. To test this hypothesis, we used quinolinic acid to destroy hippocampal neurons. Stereotaxic injection of quinolinic acid in the dorsal hippocampus resulted in a significant increase in NGF-like immunoreactivity (LI) which peaked at 1 week but was still significantly elevated 3 months later. Simultaneous determination of choline acetyltransferase (ChAT) activity showed no significant changes from control. Immunocytochemical localization of NGF-LI showed staining of pyramidal and granule cell neurons in normal rats and loss of the neuronal staining after the lesion. However, intensely labeled astrocyte-like cells stained in the lesion area. Double labeling for NGF and the glial marker GFAP confirmed the glial source of NGF. Thus it appears that glial cells assume the role of providing trophic support following loss of target neurons.

207.3

NGF RELEASED FROM A POLYMER MATRIX PREVENTS LOSS OF ChAT EXPRESSION IN BASAL FOREBRAIN NEURONS FOLLOWING A FIMBRIA-FORNIX LESION. D. Hoffman, L. Wahlberg, and P. Aebischer. Section of Artificial Organs, Biomaterials and Cellular Technology, Brown University, Providence, RI 02912.

Following a unilateral fimbria-fornix lesion, the delivery of nerve growth factor (NGF) to the ipsilateral lateral ventricle of the rat can prevent the lesion-induced loss of choline acetyltransferase (ChAT) expression in the ipsilateral medial septum and vertical diagonal band region. In the present study, the ability of polymer rods to deliver NGF, and to prevent a decrease in basal forebrain ChAT expression following fimbria-fornix lesions was assessed. NGF was loaded into an ethylene vinyl acetate copolymer (EVAc) rod, fabricated by a melt-extrusion process. NGF release was established by the ability of the rods to induce neurite extension from PC12 cells and chick E12 dorsal root ganglia. Unilateral aspirative lesions of the fimbria-fornix were performed in adult rats, followed by implantation of a polymer rod into the ipsilateral lateral ventricle. Five animals received EVAc rods containing only the carrier molecule bovine serum albumin (BSA), and six received EVAc rods containing both BSA and NGF. After two weeks, ChAT-positive cells were counted in the medial septum and vertical diagonal band regions. Rats with NGF-releasing rods displayed ChAT(+) cell counts ipsilateral to the lesion equal to 88% of those on the contralateral side. In contrast, ChAT(+) cell numbers were 42% in animals with rods releasing BSA only ($p < 0.001$). No undue reaction to implanted rods was noted. Following a fimbria-fornix lesion, NGF released from polymer matrices effectively prevents a lesion-induced reduction in ChAT expression in basal forebrain neurons.

207.4

RESCUE OF TOXIC NEUROPATHY BY NERVE GROWTH FACTOR ADMINISTRATION. S. C. Apfel*, R. B. Lipton, J. C. Arezzo, J. A. Kessler. Depts. of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

Peripheral neuropathy is a dose limiting toxicity for a number of important therapeutic agents. For example, the new antitumor drug, taxol, causes a painful sensory neuropathy at therapeutic doses. In this study we have established a mouse model of taxol-induced neuropathy and have examined the effects of coadministration of nerve growth factor (NGF). Adult male mice were injected with taxol, taxol plus NGF, or vehicle, and were examined for behavioral, biochemical, and electrophysiologic manifestations of neuropathy. Taxol administration significantly ($p < 0.001$) elevated the mean tailflick threshold, a behavioral measure of nociception. Coadministration of NGF with taxol completely prevented any change in the tail-flick threshold. Taxol administration also reduced levels of the peptide neurotransmitter, substance P (SP) in the C6 dorsal root ganglion to 12% of control ($p < 0.001$). Coadministration of NGF completely prevented this decrease. Finally, electrical stimulation of the caudal nerve was measured in the tail. Treatment with taxol reduced the compound nerve amplitudes by 25% ($p < 0.05$), and coadministration of NGF completely prevented this decrease.

These results indicate that NGF is capable of preventing taxol neuropathy in mice. We are currently investigating other neurotoxins and neuronotrophic factors using these measures. The striking protective effect of NGF in preventing taxol neuropathy in mice dictates a trial in patients receiving taxol as a chemotherapeutic agent, and suggests that neuronotrophic factors may be useful for preventing the neurotoxicity of important therapeutic agents.

207.5

SUPPRESSION OF PC12 TUMORIGENESIS IN RAT BRAIN BY ADULT BRAIN EXTRACT: THE ROLE OF NGF. I.P. Finkelstein , H.S. U* and J.D. Hatton. Div. of Neurosurgery , UC San Diego, La Jolla, CA 92093.

Previous research has shown that co-injection of PC12 cells with an extract of adult rat brain into neonatal rat brains suppresses PC12 tumorigenicity in a dose-dependent manner. We examined the role of nerve growth factor (NGF), known to differentiate PC12 cells into a neuronal phenotype in vitro, as a tumor-suppressive agent in adult brain extract (ABE).

ABE was produced by homogenizing whole rat brains in PBS, then collecting and sterile filtering the supernatant after centrifugation. ABE was depleted of NGF by passage over a column of mouse anti-NGF antibodies coupled to CNBr-activated Sepharose 4B. Complete NGF depletion was verified by Western blot. Suspensions containing 10^4-10^5 PC12 cells in 4 ul DME medium supplemented with NGF-depleted ABE at 0%, 1%, 10%, 50% were injected with a hand-held Hamilton syringe into the right forebrains of 1-4 day old Sprague-Dawley rats. Animals were sacrificed 2, 4, and 6 days post-injection. Brains were fixed in 10% formalin, paraffin embedded, thin sectioned and examined after hematoxylin and eosin staining.

In this study (N= 42), more than 90% of the control animals receiving no ABE formed PC12 tumors, while 50 to 75% of experimental animals formed PC12 tumors. Unlike animals treated with unfractionated ABE, those co-injected with NGF-depleted ABE showed increased likelihood of tumor formation. The frequency of tumors in experimental animals suggests NGF plays a major role in the suppression of PC12 tumors . Since some tumor suppression was noted in the experimental groups, the involvement of factors other than NGF is indicated.

207.6

EFFECTS OF NERVE GROWTH FACTOR ON NEURONS FROM MEDIAL BASAL FOREBRAIN GROWN AS SOLITARY CELLS IN THREE-DIMENSIONAL CULTURE. P.W. Coates, E. Dunn* and M.S. Walker*. Dept. of Cell Biology & Anatomy, Texas Tech Univ. HSC, Lubbock, TX 79430.

Degeneration of neurons from medial basal forebrain (MBF) has been implicated in Alzheimer's disease. Contact with other cells - neurons and/or glia that provide nerve growth factor (NGF) - may ameliorate this loss. To determine whether the neurons can respond appropriately to NGF in the absence of cell contacts and which neuronal structure(s) may be affected at a cellular level, we cultured MBF neurons from late gestation rats in a model three-dimensional system for 72 hr without or with NGF (10 -100 ng/ml). In this system, neurons rapidly express characteristic features as unconnected solitary cells, including two distinct types of processes, an axon and dendrites. Indices of growth and differentiation of NGF-treated or untreated solitary neurons were quantitated using image analysis. Data were analyzed with non-parametric analysis of variance. There was a significant ($p<0.05$) increase in mean axon and total length of all processes, but not dendritic length with 100 ng NGF compared to control. The average number of primary processes did not change with any dose of NGF, but there was a significant increase in other indices of differentiation distal to primary processes (mean number of branch points, segments and terminals) with 10 and 100 ng. Differences were present only after 24 hr, indicating time is required for effects to be expressed at the cellular level. Results suggest that at least some neurons in this heterogeneous population from MBF are inherently NGF-sensitive and do not require synaptic or other cell contact-mediated mechanisms to respond. In this model it appears that NGF modifies specific neuronal structures and temporal patterns of development, i.e., axons and complexity of receptive fields. Since evidence suggests NGF acts on cholinergic neurons, work is in progress to determine whether solitary MBF neurons express cholinergic properties independent of cell contacts, and whether such neurons are associated with observed changes. (Support from AA/ITPA, NS20802 and HD22806.)

207.7

LONG TERM EFFECTS OF NERVE GROWTH FACTOR (NGF) AND ITS ANTISERUM ON SYMPATHETIC GANGLION CELL SURVIVAL AND MORPHOLOGY. K. G. Ruit and W.D. Snider, Department of Neurology, Washington Univ. Sch. Med., St. Louis, Mo. 63110

We have administered NGF and anti-NGF to neonatal mice to determine whether treatment during a critical period of development leads to permanent changes in sympathetic ganglion cell morphology and survival. Mice were treated daily with NGF or twice weekly with specific anti-serum for two to three weeks. Cell counts in NGF-treated animals showed markedly increased survival acutely. Superior cervical ganglia (SCG's) had a mean of 29,357 neurons, three times that of controls. In confirmation of our previous study, we also found major effects on dendritic morphology. SCG cells in treated animals had 49% more primary dendrites and 82% more dendritic branch points than controls. Administration of anti-NGF produced converse changes in morphology of surviving neurons. Two points not previously appreciated are that effects of NGF are more pronounced on dendritic branching than on dendritic length and that mouse SCG cells elaborate fewer primary dendrites than rat SCG cells even when exposed to similar concentrations of NGF.

Animals treated with NGF or anti-NGF were maintained for six months after treatment was terminated. At the end of six months, the mean number of neurons was 10,283, not significantly different from controls. Apparently all of the neurons which were saved by treatment with NGF during the first three postnatal weeks subsequently died when it was withdrawn. Cross sectional area of ganglion cell somata in NGF-treated animals was also not different from controls. Dendritic morphology in NGF and anti-NGF treated animals maintained for six months is presently under investigation.

We conclude that NGF exerts a physiologically relevant influence on ganglion cell dendritic growth. Interestingly, there appear to be intrinsic differences among species in the capacity of the cells to respond to NGF. NGF-induced changes regress almost completely over long time periods.

207.8

ANTI-NGF SHORTENS AP DURATION OF A SUBSET OF DORSAL ROOT GANGLION (DRG) CELLS MATURING IN VIVO. A. M. Ritter and L. M. Mendell, Dept. Neurobiol. and Behav., SUNY Stony Brook, NY 11790

Somal spike shape in DRG cells is heterogeneous, and related to peripheral receptor type: cells innervating high threshold mechanoreceptors (HTMRs) have broad, large amplitude spikes with long afterhyperpolarizations (AHPs) and an inflection on the falling phase, while those innervating low threshold mechanoreceptors (LTMRs) are smaller, narrower, and lack the inflection (Ritter and Mendell (1988), Soc. Neurosci. Abst. 14:695). As NGF has been shown to regulate electrical properties of DRG cells in vitro (Chalazonitis et al., (1987) PNAS 84:289), rats were treated starting at birth with anti-serum against mouse 2.5S NGF (5 μl/g every day for the first 7 days, then every other until the day of recording) to see if the different types of spike shape are regulated by NGF. Intracellular recordings were made from α-chloralose anaesthetized animals at 4-6 weeks of age and cells were identified as to peripheral receptor type. Compared to untreated controls or animals treated with pre-immune serum, it was found that the fall time of the spikes of Aβ and Aδ HTMRs decreased, without any accompanying changes in resting potential, input resistance, spike amplitude or AHP duration. Aβ LTMRs were unaffected by the treatment. It can be concluded that NGF may regulate electrical properties of some sensory neurons, and that its actions are not the same as on cells maintained in vitro whose spikes are broadened by anti-NGF treatment. Supported by MH 18018 and NS PO1 14899.

207.9

GRAFTED SEPTAL NEURONS ARE EXCITED BY NERVE GROWTH FACTOR
M. Eriksdotter-Nilsson1, M. R. Palmer2, A. Henschen1*, T. Ebendal3 and L. Olson1, 1Dept.of Histology and Neurobiology, Karolinska Institute, Stockholm, Sweden, 2Dept. of Pharmacology, Univ. Colorado Medical Center, Denver, CO, 3Dept. of Dev. Neurobiology, Uppsala Univ. Biomedical Ctr, Uppsala, Sweden

Nerve growth factor (NGF) is produced in hippocampus and cortex and is retrogradely transported to the cholinergic neurons in the basal forebrain. We have previously shown that during development septal grafts grow larger in the presence of NGF, partially due to an increase in the number of cholinergic neurons. NGF receptors are present on the membrane of cholinergic neurons in the medial septal nucleus and it is thought that the first step in NGF actions is to bind to these receptors. We have therefore studied the electrophysiological effects of NGF on rat septal and hippocampal neurons, which were grafted to the anterior chamber of the eye of adult Sprague-Dawley rats to permit superfusion of drugs. The hippocampal and septal grafts survived and grew well in oculo. They were studied after 2 months in the eye. The grafts were superfused with NGF (0.02-100 ug/ml) and neurons were recorded extracellularly. Doses ranging between 0.2-100 ug/ml of NGF caused excitations of neurons in septal grafts, whereas 0.02 ug/ml of NGF was without effect. A second application of NGF caused septal neurons to respond with a significantly larger excitation than was observed after the initial exposure. Superfusion of similar doses of NGF on hippocampal grafts did not elicit excitatory responses. Controls consisted of perfusion of cytochrome c and preabsorption of the NGF solution with antibody to the NGF receptor or antibody to NGF. These control perfusions did not cause excitations in the septal grafts. The grafted septal tissue contained clusters of acetylcholine (AChE)-positive neurons and a fiber network as seen using an antibody to AChE. A few AChE-positive cells were also seen in the hippocampal grafts, but the fiber network was not very prominent there. Biochemical measurements of AChE levels showed that septal grafts contained AChE in amounts comparable to those measured in situ. In conclusion, we have shown that NGF has the ability to excite septal neurons, which may be important for the understanding of the interactions of NGF with its receptor. (Supported by Swedish MRC 14X-03185 and NIH grant AG-04418).

207.10

STRESS-INDUCED CHANGES IN RAT BRAIN NERVE GROWTH FACTOR RECEPTOR P.J.Foreman, G.Taglialatela, D. McNeill, L. Angelucci, M.T. Ramacci & J.R. Perez-Polo. Univ. of TX Medical Branch, Galveston, Tx, 77550. In rodent brain the majority of nerve growth factor receptor (NGFR) synthesis is within cholinergic cells of the basal forebrain area (BFA). NGFR protein is highest in BFA, hippocampus (H) and cortex (Cx,) all related to limbic functions that regulate the response to stress. Previously, we found that after exposure to 1 hour of cold stress on 5 consecutive days, there was a 50% reduction in NGF binding to BFA and H but not Cx or cerebellum (Cb). Northern analysis revealed that after cold stress steady state levels of NGFR mRNA increase 2-fold in BFA. Using Mab 192, a monoclonal antibody to rat NGFR, we immunohistochemically labeled NGFR in BF and H. Image analysis showed a trend towards decreased NGFR antigen in BFA of stressed rats. We are attempting to confirm this by immunoprecipitation of labelled NGFR. We propose that after stress there is increased turnover of NGFR in BF and H and increased NGFR mRNA synthesis to replace reduced levels of NGFR activity. Supported by NINDS and the Sigma Tau Co.

207.11

NGF FACILITATES THE REGENERATION OF RAT SCIATIC NERVE ACROSS LONG NERVE GAPS. A. Derby**, G.E. Frierdich, G. Neises*, A. Benz* and D.G. Roufa. CNS Diseases Research, Searle R&D, St. Louis, MO 63198 and **Dept. of Biology UM-St. Louis, St. Louis, MO 63121.

Peripheral nerves possess limited intrinsic capability to regenerate. Rat sciatic nerve can regrow across 8mm nerve gap when the cut ends of the nerve are placed in silastic tube and fail to do so when the gap is increased to 13mm. We report here that the addition of NGF to the tube facilitated the regrowth of sciatic nerve across 13mm nerve gap.

A rat sciatic nerve was transected in the mid-thigh region, a 4-5mm section was removed and 15mm section of silastic tubing was implanted. The tube was prefilled with either 100ug NGF or Cyto C. The implants were removed 5 weeks later and the extent of neuronal regeneration within the tube was determined histologically. Only 1/15 implants treated with Cyto. C had a regenerate, and no myelinated fibers were present at the mid-point. In NGF treated implants 7/15 had regenerates, 5 regenerates had myelinated fibers. The mean number of myelinated fibers at the tube's mid-point was 4,266. These results provide strong support for the role of NGF in promoting regeneration of these neurons.

207.12

DIFFUSE ALTERATIONS IN NGF RECEPTORS ON SPINAL MOTONEURONS AFTER NERVE CRUSH INJURY. C.M. Bowe and N.H. Evans*, Section of Neurobiology and Department of Neurology, Brown University, Providence, R I 02912

Nerve growth factor (NGF), a neurotrophic protein with multiple effects on sensory, autonomic and cholinergic neurons, may also mediate motoneuron (MN) function. NGF receptor (NGF-r) mRNA is present in spinal MNs during early development and is increased in MNs with regenerating axons following nerve crush. In the present study, we examined the distribution of changes in MN NGF-r in the lumbar spinal cord after sciatic nerve injury.

Unilateral sciatic nerve crushes were performed on adult rats. Two weeks after nerve crush, lumbar $_{2\text{-}6}$ spinal cord segments were removed from experimental and control (unlesioned) rats. Cord sections were examined for the presence and distribution of NGF-r in the lateral and anterior horns. NGF-r were identified by a modification of methods described by Yan and Johnson (J. Neurosci.,8: 3461, 1988) employing a selective monoclonal antibody to NGF-r (MAB-192). Prominent labelling for NGF-r antibody was observed on MNs ipsilateral and contralateral to the crush injury throughout the lumbar spinal cord. Comparable labelling was not seen in sections from the control rat or in control sections not exposed to MAB-192. These observations indicate that alterations in MN NGF-r seen after axonal injury are not limited to neurons with regenerating axons but, rather, may represent a more diffuse response to axonal injury.

207.13

AXOTOMY OR TREATMENT WITH NERVE GROWTH FACTOR (NGF) INDUCES THE EXPRESSION OF NGF RECEPTOR IN ADULT SPINAL MOTOR NEURONS: IMPLICATIONS FOR THE PURSUIT OF A MOTOR NEURON TROPHIC FACTOR. D.L. Price, V.E. Koliatsos, T.O. Crawford, W.C. Mobley and W.L. Price*, Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

This investigation was designed to examine the potential influences of trophic factors on motor neurons in three experimental settings. First, adult rats were treated intraventricularly with NGF; lumbar motor neurons showed increased levels of NGF receptor immunoreactivity (NGF-IR). Second, sciatic nerves of rats were crushed and labeled with Fluoro-Gold (FG), and animals survived for 12 hours or for 1, 2, 7, 14, 21, 28, or 45 days; in FG-labeled motor neurons, NGF-IR appeared at day 2 postaxotomy, peaked between days 7 and 21, and persisted until day 45. Finally, to test the efficacy of trophic factors on motor neurons, we developed in adult rats an experimental model, i.e., proximal ventral rhizotomy, characterized by degeneration of motor neurons. Three weeks following this type of lesion, *ca.* 70-80% of motor neurons die, and remaining motor neurons show reduced basophilia and alterations in size and shape. This model can be used to assess the effects of NGF and other trophic factors on the survival of spinal motor neurons.

207.14

NERVE GROWTH FACTOR (NGF) PROMOTES THE RECOVERY OF NEOSTRIATAL CHOLINE ACETYLTRANSFERASE (ChAT) FOLLOWING A MECHANICAL LESION. M. Armanini, S. Feinglass*, C. A. Altar, C. Bakhit. Genentech Inc., South San Francisco, CA 94080.

Recent evidence has implicated NGF as a potential neurotrophic factor for cholinergic interneurons of the rat neostriatum (Mobley et al. 1989; Hagg et al. 1989; Williams et al. 1989). We investigated the effects of a mechanical lesion of the adult rat neostriatum on endogenous NGF levels, high affinity choline uptake (HACU), and ChAT activity and the effects of exogenous recombinant human (rh) NGF infusions in promoting the neurochemical recovery of cholinergic activity. A mechanical lesion of the Fisher 344 rat neostriatum was produced by moving a 25 gauge syringe tip 2 mm in the rostral-caudal and medial-lateral directions. NGF-like immunoreactivity (LI), measured by a specific two-site ELISA was increased as soon as 3 days following the lesion. ChAT activity was decreased at 3 days and returned to control values at 2 weeks. Continuous infusion into the lesion site of rhNGF (2.5 ug/rat/week) or a control protein (cytochrome c 2.5 ug/rat/week) resulted in a 25-30 % increase in ChAT activity by rhNGF at 2 and 4 weeks. In contrast HACU, which reflects cholinergic terminal density, was reduced by 28 % after the lesion, but did not recover as a result of the rhNGF infusion. These results demonstrate that rhNGF administration for 4 weeks is capable of inducing neostriatal ChAT activity but not inducing sprouting of cholinergic processes following a mechanical lesion.

207.15

CHRONIC INTRAVENTRICULAR INFUSIONS OF RECOMBINANT HUMAN ß-NGF INTO THE AGED RAT: A BEHAVIORAL AND MORPHOLOGICAL STUDY. K.S. Chen[1], J. Barnett*[2], H. Chan*[2], and F.H. Gage[1]. Dept. of Neurosciences (M-024), UCSD, La Jolla, CA[1] and Syntex Research, Palo Alto, CA[2].

Previous experiments have demonstrated that continuous intracerebral infusions of the 2.5S form of *mouse* ß-NGF can partially reverse impairments on a spatial memory task and the atrophy of cholinergic neurons in the basal forebrain region of aged rats. In this study recombinant *human* ß-NGF was chronically infused into the lateral ventricles of aged (24 month old) Sprague-Dawley rats. The NGF was administered for 28 days (0.71 µg/day) via a cannula connected to an osmotic minipump [Alzet]. These aged rats had been behaviorally characterized on the Morris water maze and had exhibited an impairment on the place navigation task compared to both young rats and a subgroup of aged unimpaired rats. Upon retesting on this task after implantation of the minipumps the NGF-treated aged rats showed improved retention of the platform location compared to the vehicle-treated aged rats.

An atrophy of cholinergic cells has also been demonstrated in aged behaviorally impaired rats. In this study cholinergic neurons were immunohistochemically stained with an anitbody to NGF-receptor (NGF-R). The mean size and number of labelled cells in the medial septal, diagonal band, and nucleus basalis regions were obtained for each animal using an image analysis system [Olympus]. There is a significant shrinkage in the size of the NGF-R-positive neurons in the aged impaired animals. Chronic infusions of NGF partially reversed the shrinkage of NGF-R-positive cells. The trophic effect was most evident on the side of the NGF infusion. These findings show that *human* recombinant ß-NGF, similiar to *mouse* ß-NGF, has a trophic effect upon atrophied neurons in the aged basal forebrain, and an ameliorative effect upon the retention deficit exhibited by these aged animals.

207.16

NGF PREVENTS THE RETROGRADE CELL LOSS OF RED NUCLEUS NEURONS AFTER SPINAL CORD INJURY AT BIRTH. Ellen Kunkel-Bagden and Barbara S. Bregman, Dept. of Anatomy and Cell Biology, Georgetown Univ., Washington, DC 22066.

Transplants of fetal spinal cord (target) tissue placed into the site of a spinal cord lesion in newborn animals prevent the retrograde cell death of the axotomized neurons and support the growth of axons across the site of injury. Non-target transplants are only able to support the temporary survival (7 days post-operative) of immature axotomized red nucleus (RN) neurons. Both target and non-target transplants potentially provide trophic support for the axotomized neurons and a favorable terrain for the damaged axons to traverse. In the present study we wished to determine if a trophic substance per se could prevent or delay the retrograde cell loss after neonatal spinal cord injury. A spinal cord "over-hemisection" was made at T6 in 20 rat pups at 2 days postnatal (HX) and matrigel was placed into the lesion site. Twenty normal pups served as controls (CON). NGF (1ug/2ul) or saline (SAL) was injected intraventricularly daily for five days. At 7 days, 30 days, and 12 weeks postnatal the survival of the neurons in the RN was assessed. After axotomy at birth, retrograde cell loss in the RN is apparent within 48 hrs after injury and is complete by 5 days post-operative. Saline treated animals (HX+SAL) show a 50% cell loss after injury, similar to lesion-only animals. At 7 days the number of neurons in the RN of the HX+NGF animals was similar to that in unlesioned control animals. Preliminary results indicate that this rescue of neurons in the RN by NGF is still evident at 30 days. Thus, NGF treatment rescues immature axotomized neurons from retrograde cell death. These results suggest that providing trophic support is sufficient to promote the survival of immature axotomized neurons. Supported by: NIH NS19259, NS01356, NS27054, and APA BA3-8802-1.

207.17

CHRONIC TREATMENT WITH NGF IMPROVES SPATIAL LEARNING AFTER NBM LESIONS. Ad J. A. M. Dekker, Donald J. Connor, Fred H. Gage, Leon J. Thal. Dept. of Neurology, VAMC, San Diego and Dept. of Neuroscience, UC San Diego.

Rats received bilateral lesions of the nucleus basalis magnocellularis by infusion of ibotenic acid (28 nmoles in two injections per side). Fourteen days later, osmotic minipumps, releasing NGF (0.3 ug/day) were implanted subcutaneously. Pumps were replaced every fourteen days. Acquisition of the Morris water maze task was started 1 month after the lesion, using 10 acquisition sessions, with two trials each, over two weeks. NBM-lesioned animals were significantly impaired in the acquisition of the task. Treatment with NGF reduced the average latency to find the platform by approximately 9 sec per trial. In a trial in which the platform was not present, lesioned rats swam closely to the edge (outer annulus), whereas rats treated with NGF swam significantly less in the outer annulus. The mechanism through which NGF infusion improves performance following ibotenic acid lesions is not known, since NGF did not increase ChAT activity in the cortex or the hippocampus. NGF may either affect ACh release, or ChAT levels outside the target areas. Alternatively, NGF might affect non-cholinergic neurons which are damaged by ibotenic acid.

207.18

EFFECTS OF LONG TERM LOSS OF HIPPOCAMPAL TARGET NEURONS ON SURVIVAL OF AFFERENT BASAL FOREBRAIN CHOLINERGIC NEURONS IN YOUNG ADULT AND AGED RATS. M.V. Sofroniew, J.D. Cooper*, C.N. Svendsen*, S. J. Stevens* and K.J. Baker*, Department of Anatomy, University of Cambridge, England.

Basal forebrain cholinergic neurons of the medial septum die following proximal axotomy of their projection to the hippocampus (Hp) in adult rats. This retrograde death can be prevented by intraventricular administration of exogenous NGF, suggesting that these neurons, which bear NGF receptors and transport labelled NGF, might normally be dependent for survival upon limiting amounts of NGF produced by target neurons in the Hp. We have found that unilateral excitotoxic ablation of nearly all Hp neurons does not affect the survival of septal cholinergic neurons for up to 120 days in young adult rats (Science 247:338,1990). To investigate this further, NMDA was injected unilaterally into the Hp in 9 sites, in young adult rats, 3-6 months (n=40), or aged rats, 24-27 months (n=12), of age. After survival times of up to 400 days for young rats, and up to 75 days for aged rats, Hp were stained for AChE, Nissl and GFAP, and the septal region for ChAT and NGF receptor. The amount of Hp tissue remaining, and the number, size and staining intensity of septal cholinergic neurons were quantitatively assessed by computerized image analysis. Some rats received Hp injections of the tracer, True blue, 7 days prior to NMDA, in order to compare any loss of ChAT-staining with loss of TB-labelled neurons. Due to the long survival times, complete sets of data are not yet available. Preliminary findings in 9 rats show a small loss (<20%) of ChAT-stained neurons in the septum of some, but not all, young adult rats 200-365 days after complete Hp lesions, and a moderate loss (<40%) of ChAT-stained neurons in the septum of some, but not all, aged rats with complete Hp lesions. These declines in ChAT-stained neurons are considerably less than that seen after axotomy (>75%). Comparison of loss of ChAT staining with loss of TB-labllled neurons, as a marker of cell death, is in progress.

207.19

EFFECTS OF NGF AND FETAL CELL TRANSPLANTS ON SPATIAL LEARNING AFTER HIPPOCAMPAL DAMAGE IN RATS. P. Tandon, S. Barone Jr., and H.A. Tilson. LMIN, NIEHS/NIH, Res. Tri. Pk, NC 27709.

NGF has been implicated in the growth and development of neurons. This study was performed to observe the effects of NGF and hippocampal transplants on functional deficits produced by hippocampal damage. Two groups of male Fischer-344 rats received bilateral injections of colchicine (COL, 2.5 ug/site) or artificial cerebrospinal fluid into the dentate gyrus. Two weeks post-lesion the animals received a suspension of fetal hippocampal cells (1 µl) or Earle's basic salt solution. Modified mini-osmotic pumps (0.25 µl) containing NGF (10 ng/µl) or cytochrome C (20 ng/µl, as control) were implanted at the same time. The animals were tested in the Morris water maze 6 or 12 weeks post-lesion. COL-lesioned rats were found to be impaired in the behavioral task. NGF treatment ameliorated this functional deficit. The presence of explants did not have any beneficial effect in the acquisition of this task. However, the group receiving NGF/explants performed better than the lesioned animals alone. Morphological examination done at the end of the study confirmed the presence of viable explants and COL-induced cell loss.

207.20

NERVE GROWTH FACTOR ACTION ON PC12 CELLS IS POTENTIATED BY ACETYL-L-CARNITINE TREATMENT. G.Taglialatela*, L.Angelucci*, M.T.Ramacci*, L.W.Thorpe, K.Werrbach-Perez* & J.R.Perez-Polo. Dept. of HBC&G, UTMB Galveston, Texas.

Some morphofunctional impairments of the central nervous system (CNS) during senescence may be due to reduced action of neuronotrophic agents, such as the nerve growth factor protein (NGF). We have previously shown that treatment with acetyl-l-carnitine (ALCAR) prevents various deficits of the CNS of aged rats. Here we have investigated if there is any direct effect of ALCAR on NGF and its receptor (NGFR). We used an in vitro model for cholinergic neurons - the rat pheochromocytoma (PC12) cell line. Treatment of PC12 cells with ALCAR for 6 days increased NGF binding in a dose-dependent fashion. NGF-induced neurite extension was increased by pretreatment with ALCAR at NGF concentrations that are normally not effective. Also, the ability of NGF to rescue serum-deprived PC12 was augmented 100 fold by ALCAR. Lastly, ALCAR enhanced choline acetyltranferase (ChAT) activity and protein synthesis in PC12 cells. These result suggest that one possible mechanism by which ALCAR prevents degenerative processes in the CNS during senescence may be the enhancement of neuronal responses to trophic agents.
Supported by NIH and the Sigma Tau Co., Italy.

208.1

CHARACTERIZATION OF THE HIGH AFFINITY NERVE GROWTH FACTOR RECEPTOR

S.O. Meakin* and E. M. Shooter. Department of Neurobiology, Stanford University School of Medicine, Stanford, CA, 94305

The NGF receptor subtypes on rat PC12 cells have been investigated by means of affinity labelling with ^{125}I-NGF and then subsequently analysed in vitro. Chemical crosslinking reveals that two distinct NGF-receptor complexes can be detected with apparent molecular weights of 100,000 and 158,000 for the low (LNGFR) and the high (HNGFR) affinity receptors respectively. Although partial proteolysis of each complex generates similar NGF-receptor peptides, suggesting a model in which the HNGFR represents a complex between the LNGFR and a second membrane associated molecule, two distinct antibodies against the LNGFR are found to immunoprecipitate the low affinity but not the high affinity receptor subtype. While the identity of the putative effector molecules(s) is as yet unknown, antibodies to src, ras, yes and raf-1 fail to immunoprecipitate the HNGFR complex suggesting that these protooncogene products are not the associated molecule. Interestingly, phosphotyrosine residues are found specifically on the HNGFR, but not the LNGFR, indicating that at least one protein in this complex contains p-Tyr residues and implicating tyrosine phosphorylation directly in the NGF induced signal transduction cascade.

208.2

POTENTIAL ENDOCYTOTIC-DEPENDENT MECHANISM FOR THE GENERATION OF TRUNCATED NERVE GROWTH FACTOR RECEPTORS.

A.A. Zupan and E.M. Johnson, Jr. Dept. of Pharmacology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

Our laboratory has described the existence of truncated forms of the human nerve growth factor receptor (hNGFR$_t$) in urine and amniotic fluid, as well as in media conditioned by human receptor-positive cell lines. The *in vivo* forms (45, 40, and 35 kDa) share in common their N-terminal amino acid sequences and do not appear to differ from each other significantly in glycosylation. We propose that these forms are portions of the receptor's extracellular domain generated by differential proteolytic cleavage proximal to the membrane spanning sequence. The elaboration of NGFR$_t$ in cell culture serves as a model system for studying generative mechanisms. A variety of protease inhibitors representing compounds from all inhibitor subclasses have been tested for their ability to prevent hNGFR$_t$ elaboration by A875 melanoma cells. While no specific inhibitor has been identified, lysosomal stabilizers (NH_4Cl, methylamine, chloroquine, etc.) reduce the amount of NGFR$_t$ detected by affinity-labeled crosslink immunoprecipitation of conditioned medium. This suggests the possible involvement of an endocytotic, membrane recycling event. Experiments to determine the C-terminal sequence of the *in vivo* forms are in progress. Supported by a grant from the Monsanto Corporation.

208.3

ISOLATION AND SEQUENCING OF A PUTATIVE PROMOTER REGION OF THE HUMAN NERVE GROWTH FACTOR GENE.

M. Fahnestock and D. Chen*. Molecular Biology Dept., SRI International, Menlo Park, CA 94025.

The gene coding for nerve growth factor (NGF), including its upstream promoter sequences, has previously been isolated from mouse and rat. The mouse NGF gene contains several small 5' exons more than 32 kb upstream of the coding region (Selby et al., Mol. Cell. Biol. 7:3057, 1987; Zheng & Heinrich, Mol. Brain Res. 3:133, 1988). However, the 5' exons and promoter of the human NGF gene, which also lie far upstream of the NGF coding region, have never been isolated. These sequences presumably carry cis-acting elements controlling NGF gene expression.

We have used PCR to amplify a putative NGF promoter region fragment from human genomic DNA. Our primers are based on mouse and rat NGF exon 1A and 1B sequences. We have amplified a 1.3 kb piece of DNA and have cloned and sequenced it. The DNA contains sequences typical of promoter regions such as TATA and CAAT boxes, but does not show a high degree of sequence homology to the mouse and rat NGF promoter regions. We discuss experiments designed to verify that the DNA fragment obtained by PCR is, indeed, part of the human NGF gene promoter.

208.4

NERVE GROWTH FACTOR (NGF) AND NGF RECEPTOR IMMUNOREACTIVITY IN THE ADULT RAT CNS.

J.M. Conner, T. Hagg, M. Manthorpe and S. Varon, Dept. Biol., M-001, UCSD, La Jolla, CA 92093

NGF, a neuronotrophic factor for the NGF receptor (NGFR)-bearing cholinergic neurons of the adult basal forebrain (BF), is presumably derived from their innervation territories - the hippocampal formation (HF), cerebral cortex and olfactory bulb. These CNS regions have high levels of extractable NGF and its mRNA. Using affinity purified antibodies against β-NGF and a sensitive immunohistochemical ABC-peroxidase method we localized NGF in the adult female Sprague-Dawley rat CNS. Contrary to previous reports we find NGF immunoreactivity restricted to a limited number of brain regions. 1. In the medial septum, diagonal band of Broca and nucleus basalis of Meynert NGF-reactivity was exclusively found within neuronal cell bodies and in one or more of their processes. These neurons had a morphology and distribution similar to that of the cholinergic ones found in adjacent sections stained with the 192-IgG monoclonal antibody against NGFR. 2. In the HF diffuse NGF-reactivity was primarily localized in the hylus of the dentate gyrus and extended within stratum radiatum of CA3 and CA2 where it was more intense. HF regions most densely innervated by NGFR-positive axons (i.e. the molecular layer of the dentate gyrus) were devoid of NGF staining. No NGF staining but many NGFR-positive fibers were found in the cerebral cortex. 3. In the olfactory bulb, NGF-positive profiles were detected in the granule and plexiform layers and surrounding the glomeruli. In contrast, intense NGFR-staining was only detected within the glomeruli. Thus, there appears to be an inverse relationship between the presence of detectable NGF and NGFR in the innervation territories of the cholinergic BF neurons. This suggests that brain regions more densely innervated by NGFR-positive cholinergic BF neurons may be subject to a higher rate of NGF removal by which these neurons take up NGF and transport it toward their cell bodies. Support: NINCDS NS16349, NS25011.

208.5

LOCALIZATION OF NERVE GROWTH FACTOR AND CHOLINERGIC m2 MUSCARINIC RECEPTOR mRNA IN ADULT RAT BASAL FOREBRAIN.

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Nerve growth factor (NGF) may play an important role in the development, function, and survival of cholinergic neurons located in the basal forebrain. These cholinergic neurons contain NGF receptors (NGFR) which bind, internalize, and transport retrogradely NGF from target tissue (i.e., hippocampus and cortex) to cell bodies in the basal forebrain. It has been reported that m2 muscarinic receptors (m2R) may be presynaptic autoreceptors located on cholinergic neurons. We used in situ hybridization histochemistry to identify cells in the adult rat basal forebrain that express m2R and NGFR mRNA. Probes complimentary to NGFR and m2R mRNA were transcribed using radiolabeled (35S) - and digoxigenin-UTP and hybridized to brain sections (16um thickness) for 3-4 hours. We found a similar distribution of NGFR and m2R mRNA in medial septum, vertical and horizontal limb of diagonal band, and nucleus basalis. In addition we identified cells in neostriatum which express m2R but not NGFR mRNA. Because of the similar distribution of m2R and NGFR mRNA, our results indicate the possible colocalization of these two receptors within individual cells in the basal forebrain. This connection between NGF and the muscarinic (i.e., m2) receptor system may further our understanding of the trophic mechanism(s) of NGF on these cholinergic neurons. Supported by NIH grant AG-08969.

208.6

NGF AND NICOTINIC ACHR EXPRESSION ON RAT NODOSE NEURONS IN CULTURE.

A. Mandelzys and E. Cooper. *Dept. of Physiol., McGill Univ., Montreal, Que. H3G 1Y6*

We have previously shown that NGF increases the proportion of neonatal rat nodose neurons expressing functional nicotinic receptors (nAChRs) when grown alone in culture without affecting neuronal survival. We have now quantified this receptor expression with whole-cell patch clamp techniques. Our experiments indicate that over 2 weeks in culture there is both an increase in the proportion of neurons expressing receptors (28% at day 4 to 64% on day 14), as well as a 5 fold increase in receptor density (3.7pA/pF to 20.7pA/pF) for sensitive neurons over the same time period. By comparison, neonatal rat sympathetic neurons which express nAChRs similar to those on nodose neurons have an initial nAChR density 11 fold greater than that for nodose and maintained this density for at least 2 weeks in culture. We have also addressed whether NGF needs to be continually present in the culture for this receptor expression. In some cultures NGF was withdrawn between day 8 and 14 after most neurons had expressed nAChRs: this resulted in a decrease in the proportion of neurons expressing nAChRs within 2 days. In other cultures, neurons were grown initially without NGF which prevented receptor expression, but began expressing nAChRs within days after addition of NGF. We conclude that NGF induces nAChR expression on nodose neurons and that NGF must be continually present to maintain this receptor expression. (Funded by FCAR,FRSQ and MRC)

208.7

PROTEIN KINASE C INVOLVEMENT IN THE NGF-INDUCTION OF THE METALLOPROTEINASE TRANSIN IN PC12 CELLS. G. CIMENT and C.M. MACHIDA*, Department of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, OR 97201.

In previous work, we found that nerve growth factor (NGF) induced the mRNA transcript encoding the metalloproteinase transin in PC12 cells. We report here that staurosporine -- a potent inhibitor of protein kinase C (PKC) -- blocks this NGF-induction of transin expression. In contrast, various PKC activators augmented the NGF-induction of transin expression, but did not cause induction in the absence of NGF. These effects of PKC inhibitors and activators on the NGF-induction of transin were also seen in transient transfection assays with a plasmid containing a 750 base pair region of the 5' flanking region of the transin gene fused to a reporter gene, indicating that this region of the transin promoter contains DNA sequences responsible for these effects of NGF and PKC.

These data indicate, therefore, that activation of PKC is necessary, but not sufficient, for the NGF induction of transin. These data suggest, moreover, that co-activation of multiple second messenger pathways, possibly involving various other types of protein kinases, is likely to be involved in the signalling pathway by which NGF induces transin mRNA expression in PC12 cells.

208.8

ANALYSIS OF THE INOSITOL PHOSPHATES PRODUCED BY TREATMENT OF PC12 CELLS WITH NERVE GROWTH FACTOR. M. L. Contreras. Dept. of Pharmacology and Toxicology, Michigan State University, East Lansing, MI 49924.

In PC12 cells, nerve growth factor (NGF) can stimulate the hydrolysis of the polyphosphoinositides (J. Neurochem. 48:1466, 1987). The NGF-induced hydrolysis of these lipids was further characterized to determine if NGF stimulates an increase in the levels of inositol 1,3,4,5-tetrakisphosphate ($Ins(1,3,4,5)P_4$), and in the levels of both of the isomers of inositol trisphosphate ($Ins(1,4,5)P_3$ and $Ins(1,3,4)P_3$). PC12 cells were incubated with ^{3}H-inositol for 2 days. Subsequently, the cells were treated with or without NGF for various periods of time, and the inositol phosphates were then separated by HPLC. NGF did stimulate an increase in the cellular levels of both isomers of ^{3}H-IP_3 and in the level of ^{3}H-$Ins(1,3,4,5)P_4$. The increase in the level of ^{3}H-$Ins(1,4,5)P_3$ was apparent as early as 5 sec after the addition of NGF. The appearance of a NGF-induced increase in the levels of ^{3}H-$Ins(1,3,4,5)P_4$ and ^{3}H-$Ins(1,3,4)P_3$ was slower and was sustained for several minutes. Supported by NSF grant BNS8909030.

208.9

PRODUCTION AND CHARACTERIZATION OF HUMAN RECOMBINANT NGF. S.L. Meyer, H. Sekhon*, R. Mudd*, R. Siman, C.A.H. Friedman*, K.V. Callison, A. Kish*, P. Vissavajjhala[2], A.H. Ross[2], and M.E. Lewis. Cephalon, Inc., West Chester, PA 19380 and [2]Worcester Foundation, Worcester, MA 01545.

Production of human recombinant NGF (hrNGF) has been previously hampered by the inability of prokaryotic and lower eukaryotic expression systems to produce biologically active NGF. We report here that expression of the portion of the human NGF gene encoding preproNGF using the polyhedrin promoter of baculovirus results in the production of a hrNGF protein that is correctly processed and fully biologically active. The protein can be purified from serum-free media following virus infection and was found to be correctly processed, having the same N-terminal sequence as predicted for mature hNGF. The biological activity of purified hrNGF was compared in a number of systems with that of purified mouse NGF and was consistently found to behave in a similar fashion. For example, hrNGF was shown to extend neurites on rat PC12 cells with a similar dose-response curve as mouse NGF and the neurite extension of both mouse and human NGF was blocked by a monoclonal anti-mouse NGF from Boehringer-Mannheim that partially cross-reacts with human NGF. The hrNGF also displaced radioactive mouse NGF from rat PC12 NGF receptors in a manner similar to mouse NGF. Availability of large quantities of consistently biologically-active hrNGF will greatly facilitate testing of NGF as a therapeutic in human neurodegenerative disorders, as well as allow detailed structure-function studies of this protein.

208.10

RAPID PURIFICATION OF MALE MOUSE SUBMAXILLARY GLAND NGF. N.A. Horn*, B. Matthews*, W. Engleman*, S. Rapp*, J.F. Zobel, N.M. Kimack and D.G. Roufa. Bioprocess Technology and Biological Sciences Monsanto Co. and CNS Diseases Research, Searle R&D, St. Louis, MO 63198.

Male mouse submaxillary glands are the richest known source of NGF. Commonly used protocols for the purification of 2.5S NGF employ gel filtration followed by ion-exchange chromatography. We report here a single, scalable ion-exchange method for the rapid isolation of active 2.5S murine NGF.

Submaxillary glands (200gm, wet weight) were homogenized, clarified and the nucleic acids precipitated. Following a second precipitation of impurities at pH 5.0, the extract was chromatographed on Pharmacia CM-Sepharose Fast Flow. The extract was loaded on a 7cm X 17cm column (500ml) equilibrated with 0.2M Acetic Acid pH 5.0 with NaOH. The column was washed with two salt steps and the NGF eluted with a salt gradient. At this scale the procedure yields 60-140mg of biologically active, greater than 85% pure 2.5S NGF as determined by SDS-PAGE, amino acid composition and N-terminal sequence analysis.

The procedure described represents an improvement over previously published protocols. A single, scalable column step with 1.5 days run time results in high yield of biologically active pure 2.5S NGF.

208.11

ACETYLCHOLINE RELEASE FOLLOWING CONTINUOUS INTRACEREBRAL ADMINISTRATION OF NERVE GROWTH FACTOR IN ADULT AND AGED FISHER 344 RATS. R.J.Rylett and L.R.Williams. Dept. of Physiology, Univ. Western Ontario, London, Canada, and CNS Diseases Research Unit, The Upjohn Co., Kalamazoo, MI.

Intraventricular infusion of NGF increases cholinergic neuronal markers ChAT and high-affinity choline uptake in frontal cortex, hippocampus and striatum of adult and aged Fisher 344 rats. We have now determined that this apparent enhancement of cholinergic phenotype results in increased ACh release. Potassium-evoked endogenous ACh release from slices of frontal cortex, hippocampus and striatum was quantitated by the chemiluminescent method of Israel and Lesbats (1982), and newly-synthesized ^{3}H-ACh release was measured from synaptosomes loaded with ^{3}H-choline. NGF was administered by Alzet minipump unilaterally into right lateral ventricle of 4 and 24 month Fisher 344 male rats for 2 weeks. Potassium-evoked endogenous ACh release from slices was increased 62 and 27% relative to controls in striatum of 4 and 24 month rats, respectively, following NGF treatment. Release of endogenous ACh from frontal cortex and hippocampus was not significantly different from controls. Qualitatively different results were obtained when release of newly-synthesized ^{3}H-ACh was measured from synaptosomes; ^{3}H-ACh release evoked by 35 mM K+ was increased in all three brain regions in both age groups of NGF-treated rats, with the rank order of increase being hippocampus > frontal cortex > striatum.

208.12

DEVELOPMENT OF POLYCLONAL ANTIBODIES AGAINST THE HUMAN NERVE GROWTH FACTOR RECEPTOR (NGFR). C.M.Lee, D. Frail, P. Distefano, M. Blake and J.F. McKelvy. Department of Biochemistry, The Chinese University of Hong Kong, Shatin, Hong Kong and Neuroscience Research Division, Abbott Laboratories, Abbott Park, Illinois 60064.

The currently available monoclonal antibodies for the human and rat NGFR are species specific and display little or no cross-reactivity with the NGFR from other species. The present study was undertaken to develop ploYclonal antibodies towards different domains of the human NGFR which may be useful in the immunocytochemical studies of NGFR in different species. Appropriate restriction fragments of the human NGFR cDNA (contained in a Bluescript plasmid kindly provided by Dr. Moses V. Chao, Cornell University Medical College) were inserted into the PATH1 TRP E "20" plasmid to produce 3 different constructs coding for TRPE-NGFR fusion proteins containing the cytoplasmic, full-length and extracellular domain of NGFR respectively. These engineered plasmids were used to transform E. Coli RRI and CAG456 and transformants carrying the appropriate constructs were cloned. The transformants carrying the construct for the cytoplasmic or the extracellular domain of NGFR produced large quantities of the respective TRPE-NGFR fusion proteins upon induction. These fusion proteins were used to immunize rabbits and antibodies to the human NGFR can be detected in the serum of these animals.

208.13

DIFFERENTIAL EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR IN DIFFERENT SUBCLASSES OF BASAL FOREBRAIN MAGNOCELLULAR NEURONS. G.K. Gouras, V.E. Koliatsos and D.L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

Cholinergic neurons of the basal forebrain magnocellular complex (BFMC), affected in Alzheimer's disease, respond to nerve growth factor (NGF). Much less is known about basal forebrain γ-aminobutyric acid (GABA)ergic neurons regarding involvement in disease or responsiveness to trophic factors. To examine the potential responses of these neurons to NGF, adjacent 6-μm sections of rat brain were labeled by immunocytochemistry and *in situ* hybridization for glutamic acid decarboxylase (GAD), NGF receptor (NGF-R), and choline acetyltransferase (ChAT); results indicated a nearly 100% colocalization of NGF-R with ChAT but not with GAD. NGF treatment of control animals did not appear to induce expression of NGF-R in noncholinergic neurons. Rats with fimbria-fornix lesions showed a 30-50% decrease in GAD-immunoreactive cells ipsilateral to the lesion; following fimbria-fornix lesions, NGF treatment did not rescue GABAergic neurons. Thus, GABAergic neurons of the BFMC do not appear to respond to NGF as do adjacent cholinergic neurons. We plan to evaluate the possible effects of other trophic factors on GABAergic neurons of the basal forebrain.

208.14

NERVE GROWTH FACTOR RECEPTOR IMMUNOREACTIVITY IN THE VISUAL CORTEX OF EMBRYONIC RHESUS MONKEYS. D.L. Meinecke and P. Rakic, Section of Neuroanatomy, Yale Sch. of Med., New Haven, CT 06510.

It is now known that CNS neurons contain nerve growth factor (NGF), but the functional implications are unclear. Some evidence suggests that NGF derived from sources such as the basal forebrain may play a role in fetal cortical development. For example, neurons of the basal forebrain are among the earliest generated cells of the telencephalon giving rise to some of the first projections to the developing cortex, and several studies with monoclonal antibodies show that while receptors for NGF are expressed through adulthood in basal forebrain, they are only transiently expressed in fetal telencephalon. Here we report the results of studies at the cellular and subcellular level using a monoclonal antibody to NGF receptor (ME20.4, Ross et al., PNAS #81, 1984) examining NGF receptor localization in fetal monkey visual cortex at stages of active cortical neurogenesis and migration. Light and electron microscopic observations show that many of the neurons located in the marginal and transient subplate zones are labeled. In addition, labeling occurs on many small processes in the intermediate zone which resemble growing axons. Immunolabeling does not appear to be correlated with synaptic sites, but is broadly localized on the plasma membranes of labeled neurons and processes. Migrating neurons and neurons located in the cortical plate are seldom labeled, but distal segments of radial glial processes in the cortical plate are positively labeled. The most intense labeling in the cortex is on the extracellular surfaces of endothelial cell membranes lining capillaries throughout the telencephalic wall. These findings support the possibility of multiple roles for NGF in cortical development considering the diverse sites of cortical NGF receptor localization. Furthermore, these results support the possibility of receptor mediated trophic interactions in the transient subplate zone with early NGF-containing projections from sources such as basal forebrain. Supported by grants form US Public Health Service.

208.15

SN-1,2-DIACYLGLYCEROL (DAG) IN SYNCHRONIZED PC12 CELLS DURING PROLIFERATION AND DIFFERENTIATION P. G. Holbrook and J. W. Daly Bldg 8A, Room 1A-15, NIH, Bethesda, MD 20892

DAG was measured in PC12 cells by a radioenzymatic assay [Preiss et al. (1986); JBC 261, 8597]. Levels in exponentially-growing cells maintained in serum-containing media were 1.3 ± 0.29 nmol DAG/100 nmol lipid phosphorous and decreased to 0.71 ± 0.14 nmol DAG/100 nmol lipid phosphorous when serum was removed. Exponentially-growing cells are in various stages of the cell-cycle. The effects of nerve growth factor (NGF) on differentiation may be cell-cycle specific [Rudkin et al. (1989); EMBO Journal 8, 3319]. PC12 cells were serum starved for 3 days prior to treatment with either serum-containing media, to elicit proliferation, or serum free media with NGF (50 ng/ml), to elicit differentiation. Cells appeared to be synchronized in the G_1 phase of the cell cycle; phospholipid content doubled 40 to 50 hours after readdition of serum, a time when mitosis is known to occur. DAG remained at basal levels up to 74 hours after the addition of serum but was elevated 5 to 6 fold 48 hours after NGF.

208.16

THE EFFECT OF THE ENDOGENOUS GANGLIOSIDE GM1 ON SENSITIVITY OF DORSAL ROOT GANGLIA NEURONS TO NERVE GROWTH FACTOR. D.F. Chen and F.J. Roisen. Department of Anatomical Sciences and Neurobiology, Univ. of Louisville, School of Medicine, Louisville, KY 40292.

Exogenous gangliosides potentiate the action of several neurotrophic factors including Nerve Growth Factor (NGF). Previously, we have shown that the ganglioside GM1 potentiated NGF-mediated neuritogenesis of chick embryonic sensory ganglia (DRG) during the periods when neuritogenesis was submaximal, embryonic day (ED) 7 & 11-13. During the peak of NGF-mediated development (ED 8-10), GM1 had no effect on neuritogenesis. The mechanism by which GM1 potentiates NGF-mediated development remains unclear. To determine whether the differences in the capacity of GM1 to enhance NGF-mediated development were related to endogenous levels of GM1 in the axolemma, a direct method of fluorescence using the cholera toxin B subunit labeled with FITC was employed, since the cholera toxin has been shown to have a high specificity for GM1. Trypsin dissociated DRG (ED 7-13) were cultured in medium containing NGF for up to 3 days. Specific fluorescence was detected on the perikaryal and neuritic surfaces. Nonneuronal cells remained unlabeled. Two different populations of neurons could be identified in the DRG based on the intensity of fluorescent labeling. These different neuronal types could not be distinguished by respective size. Overall, the fluorescence increased from ED 7-13. Our previous quantitative analysis with HPTLC binding with B-cholera toxin-horseradish peroxidase demonstrated that the concentration of endogenous GM1 in the mixed neuronal and nonneuronal population was specifically high at ED 7 and dramatically decreased to its lowest level on ED 8. Later on, the GM1 level increased markedly from ED 8-13. These results suggest that the mechanism by which GM1 potentiates neurotrophic factors is mediated through the neuronal membrane and that the concentration of endogenous GM1 regulates neuronal sensitivity to NGF. Supported by NIH NS24524.

208.17

NGF AUGMENTS THE CALCIUM BINDING PROTEIN, CALBINDIN-28K, IN RAT OLFACTORY BULB *in vivo*. A.M. Iacopino, S. Christakos, and C.A. Altar[1] Biochemistry Mol. Biololgy, UMDNJ, Newark, NJ and [1]Developmental Biology, Genentech, Inc., South San Francisco, CA 94080.

Calbindin-28K (CaBP) is a 28,000 MW calcium binding protein that protects against excitatory amino acid (EAA) induced neurodegeneration (K.G. Baimbridge and J. Kao, Soc. Neurosci. 14:1264). CaBP is widely distributed in brain and is found in areas that contain NGF and NGF receptor.

Via Alzet 2002 pumps, male rats received for 10 or 14 days a lateral ventricle infusion (n = 7/group) of 12 ul PBS/day containing either 1.0 or 1.5 ug cytochrome C (control) or an equal amount of recombinant human NGF (rhNGF). Six other animals received the rhNGF infusion into the left central neostriatum. CaBP was elevated by 70% and 86% ($p < 0.01$) in the olfactory bulb following rhNGF in each of two experiments and was not altered in the temporal cortex, hippocampus, olfactory tubercle or cerebellum and was not altered in the neostriatum following direct or icv injections.

These findings demonstrate that NGF can augment the content of CaBP in olfactory bulb neurons. In addition to its classic neurotrophic role, we propose that NGF, and possibly other neurotrophic factors such as FGF (M.P. Mattson et al, Soc. Neurosci. 15:1260) function as neuroprotective factors by augmenting the ability of CaBP-positive neurons to sequester cytoplasmic calcium and retard calcium-mediated neurodegeneration.

208.18

NGF AND FGF-LIKE ACTIVITY IN CSF OF HUMAN HEAD TRAUMA PATIENTS. S.L. Patterson[1], M.S. Grady[2], A.G. Bailliet[*1], and M. Bothwell[1], Dept. Physiology & Biophysics[1] and Dept. Neurosurgery[2], University of Washington, Seattle, WA 98195.

One of the factors determining the degree to which elements of central nervous system (CNS) can recover from injury may be the availability of neurotrophic substances. Neurotrophic activity has been reported in the fluid secreted into wounds in developing and adult rat brains (Nieto-Sampedro, M., et al., *Science*, 217:860-861, 1982), and in the CSF of human head trauma patients (Longo, F.M., et al., *Exp. Neurology*, 84:207-218, 1984). Since low levels of NGF are detectable in the CSF of young rats, we set out to determine if the neurotrophic activity associated with CSF following trauma might be attributable to the presence of NGF. We report here the presence of low levels of NGF in CSF samples collected from human head trauma patients soon after injury. The NGF was quantitated against a recombinant human NGF (Genentech) standard in an ELISA using antibodies against murine *B* NGF. When the CSF was assayed for the ability to promote neurite outgrowth from PC12 cells, neurite outgrowth was reduced, but not completely blocked, by antibodies to NGF, suggesting the possibility that there were other biologically active factors present. FGF is also reported to promote neurite outgrowth in PC12 cells. In an initial screening for the presence of FGF, we employed PC12 cells and NR119 cells, PC12 variants in which NGF, but not FGF, promotes neurite outgrowth. CSF from head trauma patients promoted greater neuritic growth from PC12 cells than from NR119 cells, suggesting that some of the biological activity associated with the trauma CSF may be due to basic FGF. Interestingly, there was no detectable NGF or FGF-like biological activity remaining in CSF collected from the same patients 2-3 days after the first sampling.

208.19

SELECTIVE INHIBITION OF NGF-MEDIATED MAP2 KINASE ACTIVATION BY K252a, K252b, AND STAUROSPORIN. L.K. TAYLOR AND G.E. LANDRETH. Alzheimer Research Laboratory. Departments of Neurology and Neurosciences, Case Western Reserve University, Cleveland, OH 44106.

The activation of MAP2 kinase is an early response following the treatment of PC12 cells with NGF. MAP2 kinase is activated through its phosphorylation by an as yet unidentified protein kinase. MAP2 kinase is also activated by EGF and bFGF treatment of PC12 cells. The means by which NGF activates MAP2 kinase is mechanistically distinct from those of EGF and bFGF. The antimicrobial alkaloid K252a, as previously reported, blocks the ability of NGF to activate MAP2 kinase as well as all other affects of NGF on these cells. Two structurally related alkaloids, K252 and staurosporin, are equally selective in their capacity to inhibit the NGF-mediated activation of MAP2 kinase.

K252b is not as potent of an inhibitor of MAP2 kinase activation as K252a. In vivo, K252b inhibits MAP2 kinase activation with an IC50 of 160 nM while in vitro inhibition of the kinase occurs at an IC50 of 360 nM. Staurosporin inhibits NGF-induced activation of MAP2 kinase in vivo with an IC50 of 10 nM, while producing little inhibition of the kinase in vivo at concentrations up to 400 nM. These data strongly suggest that NGF activation of MAP2 kinase is mediated through a protein kinase which is uniquely stimulated by NGF and not other growth factors. This alkaloid-sensitive kinase must be positioned at a very early point in the cascade of events following NGF receptor occupancy and may be the seminal event of NGF-action.

OTHER TROPHIC AGENTS I

209.1

TROPHIC MATERIAL WHICH ARE CAPABLE OF PROMOTING THE NEURITE OUTGROWTH OF RAT ADRENAL CHROMAFFIN CELLS. K. Shimoda and H.-Y.T. Yang, NIMH, Lab. of Biochem. Genetics, Neuroscience Center at St. Elizabeths, Washington, DC 20032

Adrenal chromaffin cells, which are derived from neural crest, can differentiate into sympathtic neuron like cells in responding to neuro-trophic factors. Previously, we have observed that 2 month old cultured chromaffin cells prepared from adult rats can extend neurites spontaneously if culture media are not changed for a week. Subsequent studies have suggested that trophic material, which is distinct from NGF but protein in nature, is produced or released and accumulated in the culture medium. In this study, this trophic material was further characterized and found to differ from bFGF, aFGF, EGF and insulin like growth factor because all these factors were inactive in inducing the neurites outgrowth from the primary culture of chromaffin cells prepared from adult rats. While the exact nature of this trophic material awaits further characterization, this neurite outgrowth promoting activity may be an interesting factor to be considered in adrenal gland transplantations in experimental Parkinsonian models.

209.2

CYTOKINE REGULATION OF NEUROPEPTIDE EXPRESSION IN CULTURED SYMPATHETIC NEURONS. M. Freidin* and J.A. Kessler. Depts. of Neurology and Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

We have been studying the effects of cytokines on neurotransmitter phenotypic expression cultures of the neonatal rat sympathetic superior cervical ganglion (SCG). In explants of the SCG, treatment with interleukin 1β (IL1-β) significantly increased levels of substance P (SP). Similarly, treatment of dissociated sympathetic neurons cultured in the presence of ganglion nonneuronal elevated levels of the peptide 2- to 10-fold. However, treatment of pure neuronal cultures with IL1-β failed to elevate levels of SP. Several other cytokines failed to influence sympathetic neuropeptide expression. However treatment with leukemia inhibitory factor (LIF) also elevated levels of SP in cultured neurons.

To examine the specificity of the effects of IL1-β, levels of neuropeptide Y (NPY) were also measured in these cultures. NPY is abundant in pure neuronal cultures and coculture with nonneuronal cells decreases levels by half. Treatment with IL1-β only slightly increased levels of NPY in contrast to the large increase to SP.

These observations suggest either that the effects of IL1-β reflect an indirect effect on nonneuronal cells or that an additional nonneuronal cell factor is necessary for the IL1-β effect on neurons. In conclusion, our observations suggest that IL1-β and LIF each influences neuropeptide expression by cultured sympathetic neurons. This supports the hypothesis that neuronal transmitter expression and function are regulated, in part, by interactions with specific immunoregulators.

209.3

WITHDRAWN

209.4

CILIARY NEUROTROPHIC FACTOR (CNTF) mRNA IS LOCALIZED TO NEURONS IN RAT HIPPOCAMPUS. G.M. Dobrea*, B.J. Wilcox and J.R. Unnerstall. Dept. of Neurology and The Alzheimer Center, Case Western Reserve University School of Medicine, Cleveland, OH 44106

CNTF is a 24 kd protein with trophic and differentiation effects on sympathetic and parasympathetic ganglia and astrocyte progenitor cells *in vitro*. The primary structure of CNTF has been confirmed using the DNA sequence obtained from mRNA extracted from rat sciatic nerve. We have utilized a 45mer oligonucleotide probe based on this DNA sequence to study the expression and cellular localization of CNTF mRNA in the adult rat CNS by *in situ* hybridization histochemistry. Following hybridization with [^{35}S]ATP labeled probe and washing under stringent conditions, sections through the rostral-caudal extent of the rat forebrain were dipped in molten emulsion. The autoradiograms were developed after 6-8 weeks. Specific clusters of grains were uniquely localized to neurons of the hippocampal formation. Occasional granule cells of the dentate gyrus and pyramidal cells of the hippocampus proper were labeled. Neurons of the CA2 transition zone and CA1 subfields were most noticeably labeled. This limited distribution was confirmed by Northern analysis. These data demonstrate that CNTF is expressed in specific neurons of the CNS. The limited distribution of CNTF mRNA in the adult hippocampus indicates that this neurotrophic factor has a selective role in maintaining neuronal viability and plasticity in this dynamic brain region.

209.5

IDENTIFICATION OF NEURONAL TARGETS FOR CNTF USING "EPITOPE TAGGING" TECHNOLOGY.

S.P. Squinto, T.A. Aldrich*, R.M. Lindsay, D.M. Morrissey, N. Panayotatos, S.M. Bianco*, M.E. Furth, and G.D.Yancopoulos. Regeneron Pharmaceuticals, Inc. 777 Old Saw Mill River Road, Tarrytown, NY 10591.

Ciliary neurotrophic factor (CNTF) promotes the survival of a variety of neuronal cell types and affects the differentiation of both embryonic sympathetic neurons and type-2 astrocytes. In the absence of antibodies to either CNTF or its receptor, the aim of these studies was to develop methods for the identification of other neuronal target cells with functional CNTF binding sites. We have engineered and expressed in *E. Coli* a "tagged" recombinant chimeric rat CNTF (rCNTF) which carries at its C-terminal a 10 amino acid epitope of the human c-*myc* gene. Purified rCNTF and rCNTF/myc were equally bioactive when assayed against E8 chick ciliary ganglia neurons. Using a rosetting assay we identified several human neuronal tumor cell lines as well as primary neurons derived from embryonic chick dorsal root ganglia that were positive targets for CNTF/myc binding. Functional binding of rCNTF/myc to these target neuronal cells was indicated by demonstrating a perfect correlation between rosette-positive cells and the rapid induction of either c-*fos* and or c-*jun* mRNA by CNTF in these target cells. This new methodology should prove useful in mapping the *in vivo* targets for CNTF.

209.6

EFFECTS OF CILIARY NEUROTROPHIC FACTOR (CNTF) ON VENTRAL SPINAL CORD NEURONS IN CULTURE.

V. Wong, R. Arriaga*, and R.M. Lindsay. Regeneron, Tarrytown, NY 10591.

CNTF was originally characterized as a target-derived neurotrophic factor supporting the survival of chick ciliary ganglion neurons in vitro. However, it showed a broad spectrum of additional activities including the survival of chick sensory and sympathetic neurons, the shifting of rat optic nerve O-2A progenitor cells towards type 2 astrocytes, and inducing cholinergic properties in sympathetic neurons. In this study, we examined the effects of CNTF on ventral spinal cord cultures which were enriched with somatic motorneurons. Treatment of CNTF on the day of plating resulted in a 5-fold increase in choline acetyltransferase (CAT) activity. The increase diminished, though significant, when treatment was postponed to day 1 or 2 after plating, but CAT activity remained at control levels when treatment was postponed to day 6. These observations suggest that CNTF enhances the survival of ventral cholinergic spinal cord neurons in culture. This increase in CAT activity was dose dependent and saturating effect of CNTF was reached at approximately 1ng/ml. Both neurofilament and protein levels were elevated in CNTF-treated cultures. NGF and bFGF had no effect on CAT activity, suggesting the specificity of CNTF on ventral cholinergic spinal cord neurons.

209.7

CHARACTERIZATION OF CILIARY NEURONOTROPHIC FACTOR IN RAT ASTROCYTES.

J.S. Rudge, R.F. Alderson, N.Ip and R.M. Lindsay. Regeneron Pharmaceuticals, Inc. 777 Old Saw Mill River Road, Tarrytown, NY 10591.

Ciliary Neuronotrophic Factor (CNTF) has been shown to have neuronal survival properties for a wide range of peripheral neurons and more recently central neurons (van de Stadt et al, 1990 Soc. Neurosci. Abs.) as well as a putative effect on type II astrocyte differentiation (Hughes et al, 1988 Nature, 335:70). The source of CNTF in the CNS is unknown but a potential candidate is the neonatal type I rat cortical astrocyte which has been shown *in vitro* to express biologically active CNTF (Rudge et al, 1983 Dev. Brain Res, 19:161). We have further characterized this astrocytic form of CNTF in rat hippocampal astrocyte cultures and find it to be present on the surface of type I astrocytes using specific antisera and immunocytochemistry. It was found to be identical to the 22.5Kd form of rat CNTF purified from *E. coli* lysates on Western blots. Messenger RNA (1.2Kb) for CNTF was also detected at high levels in these astrocytes. Further characterization of the regulation and expression of this molecule in astrocytes *in vitro* is underway.

209.8

CILIARY NEURONOTROPHIC FACTOR (CNTF) IMMUNOREACTIVITY IN THE RAT SCIATIC NERVE

M. Rende*, T. Hagg, E. Magal, S. Varon and M. Manthorpe Dept. Biol., M-001, Univ. Calif. San Diego, La Jolla, CA 92093

In vitro, CNTF has potent neuronotrophic activity for the cholinergic motor neurons of ciliary ganglion and also for sympathetic and dorsal root sensory ganglionic neurons. CNTF is very abundant in extracts of adult rat sciatic nerve and is produced by purified Schwann cell cultures. Two peptides were synthesized corresponding to internal residue Nos. 45-59 and carboxy terminal residue Nos. 181-200 of rat nerve CNTF (Stöckli et al, *Nature* 342: 920, 1989). These internal and carboxy-terminal sequences possess at least 80% and 50% sequence homology, respectively, among the cloned rat, rabbit and human CNTFs. Polyclonal rabbit anti-peptide antibodies were generated and affinity-purified with immobilized peptide. The antibodies reacted with rat sciatic nerve CNTF protein by ELISA and by immunosequestration of CNTF neurotrophic activity. Immunostaining was carried out on 5-20 µm paraformaldehyde-fixed cryostat sections using a sensitive ABC-peroxidase method. In normal adult rat sciatic nerve, CNTF immunoreactivity was restricted to nearly all Schwann cell bodies where it appeared to be particulate, intracellular and perinuclear. Staining was also observed in the Schwann cell cytoplasm surrounding the myelin sheaths. Electron microscopy is being undertaken to determine the intracellular location of Schwann cell CNTF. No staining was observed in axons, endothelial, epineural, perineurial or endoneurial cells nor in skeletal muscle innervated by sciatic nerve. This Schwann cell localization is in contrast to presence of CNTF staining of most CNS cells (Varon et al., *Soc. Neurosci. Abstr.*, 16, 1990). The exclusive and preponderant immunolocalization of CNTF to Schwann cells suggests that these cells produce CNTF in vivo where they may provide it to sensory, sympathetic and motor nerve axons. Supported by NINCDS NS16349, NS25011 and NSF BNS8808285, BNS8617034.

209.9

RAT HIPPOCAMPAL NEURONS IN CULTURE: EFFECTS OF CILIARY NEUROTROPHIC FACTOR (CNTF).

L van de Stadt, Y. Li*, R. M. Lindsay, R. F. Alderson, and N. Y. Ip. Regeneron Pharmaceuticals Inc., 777 Old Saw Mill River Rd., Tarrytown, NY 10591.

CNTF has been shown to act as a neurotrophic factor towards parasympathetic and sympathetic neurons in culture. The possibility that CNTF may have similar actions in the CNS has been examined. Hippocampal neurons derived from E18 rat embryos, when treated with CNTF for 8 days *in vitro*, showed enhanced neurite outgrowth. Utilizing an Elisa assay, we have demonstrated that CNTF treatment increased the levels of neurofilament protein (200kd) by 4-fold. In addition, the uptake of GABA via a neuron-specific high-affinity process, and GAD enzyme activity were increased in a dose-dependent fashion by CNTF. GABA uptake was maximally increased, 3-fold over non-treated controls, with 1ng/ml of CNTF. The ability of CNTF to induce GABA uptake is cell density dependent, with high plating densities being inhibitory. In addition, the response of GABAergic neurons to CNTF is not diminished in neuron-enriched cultures, suggesting that CNTF is not acting via glial cells.

209.10

REGULATION OF THE EXPRESSION OF SURFACE ADHESION MOLECULES ON CULTURED ASTROCYTES.

S. Choi-Kwon, R.E. Petroski, J.P. Grierson and H.M. Geller. Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School and The Graduate School, Rutgers University, 675 Hoes Lane, Piscataway, NJ 08854.

Basic Fibroblast Growth Factor (bFGF) has trophic actions on neurons and astrocytes. However, our data indicate that actions of bFGF on hypothalamic neurons might be mediated indirectly through its effects on astrocytes (see Petroski et al.). The present study focuses on the effects of bFGF and culture conditions on the expression of surface adhesion molecules by rat cerebral cortical astrocytes in culture. Confluent monolayers of astrocytes were grown for 7 days in FCS-containing medium (CM), chemically defined serum free medium (N2), or CM containing 5 ng/ml bFGF. The cells were stained in suspension with polyclonal antibodies directed against surface antigens (tenascin/cytotactin or NCAM) followed by a fluorescein conjugated secondary antibody. FACS analysis revealed that bFGF treatment significantly enhances the expression of tenascin/cytotactin and NCAM whereas N2 medium significantly decreased the expression of these adhesion molecules compared to those levels expressed in CM alone. The present data suggest that the trophic effects of serum-free medium or bFGF on neurons might be mediated by regulating the expression of adhesion molecules, thereby providing an altered substrate for neural attachment and neurite outgrowth. Supported by Grant # NS 25168 to HMG.

209.11

Biphasic Actions of Basic Fibroblast Growth Factor (bFGF) on the Development of Astrocytes R.E. Petroski, S. Choi-Kwon, J.P. Grierson and H.M. Geller. Dept. of Pharmacology, UMDNJ-Robert Wood Johnson Medical School and The Graduate School, Rutgers University, Piscataway, NJ 08854

bFGF is an angiogenic factor and has also been found to be neurotrophic in several brain regions. The specificity of its action has been questioned as it is found ubiquitously in the brain. We have examined the putative trophic effects of bFGF on developing neurons *in vitro* using a dissociated cell culture system derived from embryonic rat hypothalamus.

Treatment of these cultures with bFGF had no effect on neuronal survival in FCS or N2 media. However, a profound mitogenic effect on hypothalamic astrocytes in FCS was observed. Astrocyte proliferation was quantitated by loading fixed cells with propidium iodide and counting the nuclei. The optimum dose of bFGF (0.5 ng/ml) yielded an average count of greater than 10 times control. The dose-response was biphasic in that 5 ng/ml produced fewer counts. The mitogenic effect of bFGF was dependent on the presence of FCS and was completely abolished by treating the cultures with 1 μM Ara-C. We also tested the ability of bFGF to promote neuronal survival when the dissociate of embryonic hypothalamus was plated on a substrate of confluent cortical astrocytes. bFGF had no effect on neuronal survival and the highest dose (5 ng/ml) reduced neuronal counts. At this dose, bFGF induced morphological changes in that the flat astrocytes became "grainy" and more fibrillar.

We conclude that bFGF mediates a complex series of events in neural development. While bFGF acts as a potent mitogen for astrocytes, higher doses stimulate differentiation of the immature glial phenotype to one which is a poorer substrate for neuronal adhesion and survival. This may involve the altered expression of surface molecules (see Choi-Kwon et. al. this meeting). We have not been able to detect any direct trophic effect on hypothalamic neurons.

209.12

ONTOGENIC PROFILES OF RAT BRAIN INSULIN AND INSULIN-LIKE GROWTH FACTOR-I BINDING SITES. J.-G. Chabot, J. Tsang* and R. Quirion. Douglas Hospital Research Centre, Department of Psychiatry, McGill University, Montreal, Quebec, Canada.

In recent years, insulin and insulin-like growth factor-I (IGF-I) gene, protein expression and specific binding sites has been demonstrated in the mammalian central nervous system (CNS). Additionally, some results have suggested that insulin and IGF-I may act as neurotrophic and/or neuromodulatory substances in the brain. The autoradiographic distribution of both insulin and IGF-I binding sites was examined in the rat CNS during postnatal development and maturation. Under our incubation conditions, we observed a widespread and differential distribution of brain insulin and IGF-I binding sites during postnatal ontogeny. Both classes of sites appeared early postnatally and reached maximal adulthood levels over the third to fourth weeks. However, the respective ontogenic profiles of these sites differed between regions. For example, insulin binding appeared as early as days 1 to 4 in the cortex and olfactory bulb while it was absent up to day 7 in the thalamus. Moreover, the respective ontogenic appearance of insulin and IGF-I receptor sites was unique. For example, while IGF-I sites in the thalamus appeared on day 1, insulin binding sites were present in this area only at the end of the first week. This reveals that insulin and IGF-I binding sites represent two distinct populations of receptor sites. The presence of these binding sites in the adult CNS may also indicate possible neuromodulatory roles of insulin and IGF-I, in addition to their better known trophic actions in developing tissues.

209.13

INSULIN-LIKE GROWTH FACTOR II (IGF II) IN RAT BRAIN AFTER CEREBRAL ISCHEMIA. W.L.Russell, L.A.Phebus, K.S. Rash*, P.B. Summerlin*, A.P.Evan*, J.A.Clemens, D.P.Henry. Lilly Lab. for Clin. Res., Eli Lilly & Co., and Dept. of Anatomy, Indiana Univ. Sch. of Medicine, Indianapolis, IN 46202

IGF II is hypothesized to be a fetal growth factor. In adult rat brain, IGF II mRNA levels remain high after birth as the levels of both IGF II and IGF II mRNA decrease in other tissues. The function of IGF II in brain is unknown. Cortical ischemia was induced in 3 mo. old male rats by either a photochemical method or by middle cerebral artery occlusion. Rats were sacrificed at 0, 3, 6, or 9 days after ischemia. IGF II was extracted from cortex with a formic acid/acetone method as described previously (Abstr. Soc. Neurosci. 15(1), 231), and quantified by RIA. Three days after stroke, IGF II levels in the infarcted cortex (32.2 ± 3.7 ng/g tissue; mean±SEM; n=6) were higher than that in contralateral cortex (18.5 ± 3.2; $p<0.05$) and returned to normal by day 9. The distribution of IGF II was examined immunocytochemically using a monoclonal Ab to IGF II. One day after artery occlusion, enhanced immunoreactive staining was observed only in the infarcted hemisphere especially in the glia of the corpus callosum and anterior commissure. By the third day, pronounced IGF II immunoreactivity was observed in the infarcted cortex and probably resulted from IGF II produced in glial cells. Therefore, the production of IGF II is increased in response to cerebral ischemia, suggesting that IGF II may be involved in the tissue repair process after stroke.

209.14

EFFECT OF INSULIN ON MORPHOLOGICAL AND PHYSIOLOGICAL DIFFERENTIATION OF EMBRYONIC *DROSOPHILA* NEURONS. D. K. O'Dowd Department of Anatomy and Neurobiology, California College of Medicine, Irvine, CA 92717

Drosophila embryos can be dissociated and cultured *in vitro*. In the presence of 10-20% serum supplements morphologically undifferentiated gastrula cells give rise to both myotubes and neurons that express a variety of functional ion channels. In order to identify environmental factors that might be involved in the differentiation of these *Drosophila* cells I have examined cultures grown in a serum-free defined medium. Cells with neuronal morphology that express voltage-gated sodium channels are clearly differentiated 1-2 days after plating and can be maintained in culture for at least two weeks. However, there are few cells resembling myocytes or myotubes. Previous studies have shown that addition of exogenous insulin to serum-based media is important in the development of cultured myotubes but does not affect neuronal differentiation. Therefore, the effects of different concentrations of insulin on the differentiation of cells grown in defined media were examined. In contrast to earlier studies, the addition of insulin up to 10 uM failed to support morphological differentiation of myotubes and it did result in an increase in both the size of neuronal somata and processes. Supported by NS27501 and National Multiple Sclerosis Society RG 2160-A-1.

209.15

BASIC FIBROBLAST GROWTH FACTOR MODULATES ADHESIVE PROPERTIES OF CHICK NEURAL TUBE CELLS IN CULTURE. Y. Kinoshita, C. Kinoshita*, J. G. Heuer* and M. Bothwell. Dept. of Physiology & Biophysics, Univ. of Washington, Seattle, WA 98195.

Fibroblast growth factors (FGFs) have been shown to serve as mitogenic and trophic factors for neuronal as well as glial components of the CNS. Little is known, however, of their functional roles in early embryonic CNS development. Our *in situ* hybridization studies have revealed abundant FGF receptor mRNA expressed in the neural tube (NT), prompting us to examine the effect of basic FGF (bFGF) on NT cells' behavior in culture. Cells obtained from a thoracic region of E2.5 (26-30 somites) chick embryo NT were cultured on collagen substrate in F12 + 10% FBS and bFGF, when present, at 5 or 10 ng/mL. Addition of bFGF markedly improved cell spreading with half-maximal and maximal effects being attained at ~0.4 and 2 ng/mL; this effect was discernible 6-8 hrs after cell plating, abolished by cychloheximide treatment, and seen on collagen, laminin, or more poorly on fibronectin substrate but not on others tested (vitronectin, RGD-peptide, poly-lysine, etc), suggesting an involvement of a specific type(s) of the integrin family induced upon bFGF treatment. bFGF-treated cells showed enhanced cell attachment and spreading but similar cell aggregation ability as compared with control cells. NT (spinal cord) cells from older embryos (E3.5, E5, E8) were found to become less dependent on bFGF for spreading with age. In contrast, NT cells from younger embryos (8-10, 16-19 somites) required bFGF not only for cell spreading but also cell survival. Thus, the cell spreading ability of NT cells and its dependence on bFGF appeared to be highly developmentally regulated and the cell spreading response to bFGF is probably inherent to neuroepithelial cells. bFGF also promoted cell proliferation. These results suggest that, in an early stage of the NT development, bFGF is critically involved in the regulation of cell adhesive properties mainly in relation to extracellular matrices, thereby controlling proliferation, migration and differentiation of NT cells.

209.16

ACIDIC AND BASIC FGF EXERT TROPHIC EFFECTS ON INTRAOCULAR FETAL BRAIN TISSUE GRAFTS MB Giacobini*[1], B Hoffer[2], L Olson[1], [1]Dept. of Histology & Neurobiology, Karolinska Institutet, Stockholm Sweden, and [2]Dept. of Pharmacology, Univ. of Colorado, Denver, CO, USA

Basic fibroblast growth factor (bFGF) mRNA is present in considerable amounts in many areas of the central nervous system including cerebral cortex and bFGF has been suggested as a trophic factor for both developing and lesioned neurons. By using the in vivo model of intraocular transplantation with repeated injections of growth factors into the eye chamber, we have followed the survival and growth of small defined areas of the central nervous system under the influence of acidic FGF (aFGF) and bFGF. Acidic FGF significantly enhanced growth of transplanted parietal cortex (E19-20) and hippocampus (E19-21), but not spinal cord (E14). Acidic FGF brought about a volume increase of approx 100% of both parietal cortex and hippo-campus grafts. The trophic effects of bFGF stimulation were even more remarkable. Basic FGF enhanced growth of intraocularly transplanted cortex (E19) by up to 400% when compared to the vehicle alone, and bFGF was therefore clearly more potent than aFGF. Other CNS areas are now under investigation with bFGF stimulation. Effects on all areas tested so far were seen using 5-μl injections into the eye chamber of concentrations of both aFGF and bFGF down to at least 25 μg/ml. Histological and histochemical observations of the growth factor-stimulated grafts will also be presented. We conclude that both aFGF and bFGF are potent stimulators of volume growth of several but not all grafted fetal brain areas during development. These studies suggest that exogenous fibroblast growth factors might enhance survival and/or stimulate proliferation of cells in fetal brain tissue grafts.

209.17

Membrane-Associated Factor Inhibits Neuroblast Mitosis and Fosters Neuronal Differentiation. J-M. Lee, I.B. Black, E. DiCicco-Bloom and J.E. Adler Div. Dev. Neurology, Cornell Univ. Med. Coll., New York, NY 10021, Dept. Neurosci. and Cell Biol. UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ, and Dept. Neurology, Wayne State Univ. Sch. of Med., Detroit, MI.

Numerous studies have suggested that differentiation proceeds only after mitosis ceases in developing neurons. However, the mechanisms underlying these apparently obligatory sequential events are largely unexplored. We now present evidence that a single factor regulates both neuronal proliferation and neurotransmitter differentiation at distinct ontogenetic stages.

We have previously found that a membrane-associated protein factor induces the *de novo* appearance of choline acetyltransferase (CAT) activity in cultures of neonatal sympathetic neurons (Lee et al., Exp. Neurol. in press). To investigate the role of this factor during development, we defined a critical period during which the factor induced CAT activity. The factor elicited significant CAT induction in cultured neurons from embryonic day 20 (E20) to postnatal day (P7), in the absence of effects on neuronal survival. CAT activity was not altered at earlier or later stages. However, while E15.5 neurons failed to exhibit CAT induction by the factor, DNA synthesis was markedly reduced in this mitotically active population. Cell survival and morphology were unchanged. Thus, the factor appears to inhibit neuroblast mitosis at this embryonic age. These observations suggest that a single molecule may influence the cessation of neuroblast mitosis while reciprocally fostering neuronal differentiation. (Support: NIH grants NS10259 and HD23315)

209.18

ROLE OF MESENCEPHALIC GLIA IN THE NEURONOTROPHIC RESPONSE OF DOPAMINERGIC NEURONS TO GROWTH FACTORS. J. Engele and M.C. Bohn., Dept. of Neurobiology and Anatomy, University of Rochester, Med. Ctr., Rochester, N.Y. 14642.

Our previous studies demonstrated that the purified growth factors acidic FGF (aFGF), basic FGF (bFGF), and NGF, and conditioned media (CM) from the macroglia cell line B49, the microglia-like cell line R33, and the Schwannoma cell line JsC11 support the survival of dopaminergic neurons in serum-free low density cultures of the dissociated E14.5 rat mesencephalon. In the present study, we investigated whether these neuronotrophic effects are mediated via glial cells. The number of cells expressing the astroglial marker glial fibrillary acid protein (GFAP) increased 10-15-fold in mesencephalic cultures maintained for 8 days in the presence of all three CM and 4-5-fold after treatment with aFGF, bFGF, or NGF. The proliferative effect of aFGF, but not of bFGF, on glia was enhanced 100-fold by heparin. Moreover, medium conditioned by mesencephalic glia grown in aFGF and heparin stimulated the survival of dopaminergic neurons 2-3 fold without affecting glial number. In contrast, other growth factors, e.g. PDGF and Interleukin I, which had no survival-promoting effect on dopaminergic neurons, also did not enhance the number of GFAP-immunoreactive cells.

These results suggest that an array of factors which have neuronotrophic effects on dopamine neurons act indirectly by stimulating proliferation of glia and that mesencephalic glia may actually be a source of a dopaminergic trophic factor. Supported by NIH: NS25778, Amer. Parkinson's Disease Assoc., and DFG (En 187/1-1).

209.19

CHARACTERIZATION OF A SOMATOSTATIN STIMULATING ACTIVITY FOR CULTURED CILIARY GANGLION NEURONS. J.N.Coulombe, F.P.Eckenstein, and R.Nishi
Dept. of Cell Biology and Anatomy, Oregon Health Sciences University, Portland, Oregon. 97201

We previously found that induction and maintenance of somatostatin in cultured ciliary ganglion neurons (CGNs) depends upon the presence of soluble macromolecules released by cells of choroid layer. Here we present further characterization of this somatostatin stimulating activity (SSA). In vivo, only half of the CGNs innervate the choroid layer and express somatostatin, however, SSA can induce somatostatin expression in more than 90% of cultured CGNs. When ciliary neurons were labelled by retrograde transport after transection of the choroid nerves and cultured in the presence of SSA, many of the labeled ciliary neurons were found to contain somatostatin. Thus, SSA can induce neuropeptide expression in ciliary neurons which would not normally have expressed somatostatin. SSA can be obtained from choroid cells cultured in low serum (0.1% v/v) or serum-free medium and is ammonium sulfate precipitable. SSA is unusually heat stable and withstands 10' of exposure to 100^{o}C but loses activity after 30' at 100^{o}C. Using FPLC gel filtration chromatography we found that SSA has an apparent size of 30-40kD. These observations suggest that SSA is a protein serving an instructive role regulating neuropeptide expression in the avian ciliary ganglion.
Supported by EY06178 (JNC), NS25767 (RN) and the American Heart Association, Oregon Affiliate.

209.20

THE EFFECTS OF PRENATAL EXPOSURE TO DEXAMETHASONE (DEX), HALOPERIDOL (HAL) OR UNDERNUTRITION ON BRAIN GROWTH. R. Holson, F.M. Scalzo, S.F. Ali, R. Williams* and D.K. Hansen*. Division of Reproductive and Developmental Toxicology, National Center for Toxicological Research, Jefferson, AR 72079.

Exposure to many chemical substances during pregnancy acutely depresses maternal food intake and permanently stunts brain size in offspring. To better understand drug-induced brain growth impairment, it is necessary to examine the confounding effect of drug-induced maternal anorexia on pre- and post-natal brain growth. Reported here are the results of exposure to DEX (0.4 mg/kg over gestational days (GD) 9-14), HAL (5 mg/kg over GD 6-20), or pair-feeding (PF). DEX reduced GD 20 body weight (by 25%) while causing a 10% reduction in brain weight. PF controls showed a near-identical GD 20 decrease in brain weight. HAL reduced body weight at birth by 15% of control, and also caused a 10% reduction in brain weight. PF controls for HAL had a 5% reduction in brain weight at birth. Thereafter, brains of PF controls showed catch-up growth, attaining control levels by 4 weeks after birth. DEX and HAL offspring showed no such recovery. Prenatal DEX and HAL thus have a long-term depressive effect on brain weight that is not due to drug-induced maternal undernutrition.

209.21

LAMININ-LIKE NEURONAL IMMUNOREACTIVITY IN THE ADULT AND AGED RAT BRAIN USING ANTIBODIES AGAINST CHAIN-SPECIFIC SEQUENCES. M. Jucker, C.F. Hohmann, H.K. Kleinman*, and D.K. Ingram. Gerontol. Res. Ctr., NIA, NIH, Baltimore, MD 21224; Dept. Psychiat., Johns Hopkins Med. Sch., Baltimore, MD 21205; Lab. Dev. Biol. Anom., NIDR, NIH, Bethesda, MD 20892

Intraneuronal laminin-like immunoreactivity was examined by avidin-biotin peroxidase technique using polyclonal antibodies against EHS tumor-derived laminin (AB_{lam}) and against laminin-derived synthetic peptides (A-chain, AB_A) and fusion proteins (B1- and B2-chain; AB_{B1} and AB_{B2}). AB_A was raised against peptide PA22-2 which promotes neurite outgrowth in fetal septal cell cultures (Jucker *et al.*, Soc. Neurosci. Abstr. 15:575, 1989). Immunostaining with AB_{lam} revealed specific but widespread reactivity in most parts of the adult and aged rat brain confirming reports by others (Yamamoto *et al.*, J. Neurol. Sci. 84:1, 1988; Hagg *et al.*, Neuron 3:721, 1989). A similar immunoreactivity distribution was elicited by applying the peptide-specific AB. This immunoreactivity appeared even stronger, and light microscopy revealed distinctly labeled neuronal processes in several regions, including processes in the vertical diagonal band, and apical dendrites of cortical and hippocampal pyramidal cells. When AB_{lam} was preincubated with purified laminin, intraneuronal immunostaining was greatly diminished. Depending on fixation technique of the tissue, only intraneuronal or both intraneuronal and basement membrane laminin of vascular structures could be visualized. Results suggest that neuronal laminin-like molecules are similar to basement membrane laminin and share epitopes with all three laminin chains. Future studies will address further analysis of laminin-like neuronal immunoreactivity with aging and immunohistochemical mapping of injury-induced laminin-like molecules.

209.22

PARAMETERS AFFECTING MOTONEURON SURVIVAL IN VITRO. B.H.J. Juurlink, L.-C. Ang* and D.G. Munoz, Depts. of Anatomy and Pathology, University of Saskatchewan, Saskatoon, Canada S7N 0W0.

Although motoneurons can be isolated and grown in vitro, only a relatively small number survive for more than a few days. This poor survival may be related to problems in coping with the extensive damage the neurons have undergone during the isolation procedures. The objectives of this study were to examine the effects of gangliosides, pyruvate, α-ketoglutarate as well as alterations in the medium potassium levels on the survival of chick motoneurons in vitro. Motoneurons were isolated from E6 chick embryonic spinal cords and planted at low density [≈60 cells/mm^2] on polyornithine coated glass coverslips [Juurlink et al. 1990, J. Neurosci. Res., 26 (in press)]. The standard growth medium consisted of DMEM/F12 containing horse serum and muscle extract. Our observations demonstrated that the addition of GM1 ganglioside [10μM], pyruvate, α-ketoglutarate [2mM] or additional potassium [10-20mM] to the medium resulted in increased survival of motoneurons. The most crucial time for these additions appears to be the first 24 hours suggesting that they act in part to support repair of the damaged motoneurons. At 6 DIV motoneuron survival could be increased from the 20% under standard conditions to 40% when potassium was elevated and an additional energy source was present. Such cultures have been maintained for as long as 4 weeks. (Supported by the ALS Society of Canada).

209.23

EXPRESSION OF PROTEASE NEXIN-II IN THE NEURONS OF DORSAL ROOT GANGLIA IN MAN: A CORRELATIVE IMMUNOCYTOCHEMICAL AND IN-SITU HYBRIDIZATION STUDY. T.S. Kim*, B.H. Choi, W. Choe*, R.C. Kim, W. Van Nostrand*, S. Wagner* and D. Cunningham*, University of California, Irvine, CA 92717.

Protease nexin-II (PN-II) is a protease inhibitor that forms SDS-resistant inhibitory complexes with the epidermal growth factor-binding protein, the γ-subunit of nerve growth factor and trypsin. The deduced amino acid sequence of PN-II is identical to that of amyloid precursor protein (APP), and neuritic plaques of Alzheimer's disease is shown to be immunoreactive for PN-II. The presence of APP mRNA in the CNS has also been demonstrated. In order to examine expression of PN-II in the peripheral nervous system, freshly obtained human dorsal root ganglia were processed for light and EM immunocytochemical procedures using highly characterized monoclonal antiserum for PN-II, and in-situ hybridization using ^{35}S-RNA PN-II probes. Strong and highly specific immunoperoxidase and immunogold reactions for PN-II were demonstrated within the cytoplasm of neurons of dorsal root ganglia in cryostat sections, and vibratome sections fixed in 2% paraformaldehyde. In-situ hybridization demonstrated the presence of intense labeling of an antisense ^{35}S-RNA PN-II probe in the neurons. Labeling was not observed when the corresponding sense ^{35}S-RNA PN-II probe was used. These results indicate the presence of PN-II in the peripheral neurons of man, and provide a valuable information for elucidation of potential roles of PN-II in normal and abnormal functioning of the central and peripheral nervous system. (Supported by NIEHS grant ES 02928)

209.24

COMPLEX INTERACTIONS BETWEEN POLYAMINES AND CA-DEPENDENT PROTEOLYSIS. I. Najm, P. Vanderklish*, A. Etebari*, G. Lynch and M. Baudry, NIBS Program, University of Southern California, Los Angeles, CA 90089-2520 and Bonney Center, UC Irvine CA 92717.

Polyamines have been implicated in mechanisms of cell growth and differentiation but their mechanisms of action remain to be defined. Because they often mimic the effect of other cations and in particular calcium, we were interested in determining their actions on calcium-dependent proteolytic mechanisms.

Putrescine and spermidine did not modify the activity of purified calpain I, while spermine produced an inhibition at millimolar concentrations. None of the polyamines interfered with purified calpain II. On the other hand, spermine potentiated calpain activity from rat brain soluble fractions when using ^{14}C-casein as a substrate. Moreover, each of the three polyamines potentiated Ca-dependent proteolytic activity of rat brain soluble fractions as determined by the degradation of endogenous protein substrates, such as high-molecular weight microtubule-associated proteins and spectrin. The potentiating effect of polyamines was observed at physiological concentrations (100 μM). Preliminary experiments suggest that the complex effect of polyamines on cacium-dependent proteolysis might reflect their interaction with calpastatin, the endogenous calpain inhibitor. In any event, these results provide a potentially important new mode of action for polyamines in the regulation of protein degradation as well as protein synthesis.
Supported by grant NS 18427 to M.B.

209.25

IMMUNOCYTOCHEMICAL (ICC) LOCALIZATION OF INSULIN IN RABBIT SPINAL CORD NEURONS. C.K. Haun, G. Goubran*, W.K. Engel, V. Askanas and I.Tan*. Depts. of Anatomy & Cell Biology and of Neurology, USC School of Medicine, Los Angeles, CA 90033.

To trace the distribution of insulin administered intra- thecally (IT) in adult (2.5-4 kg) NZ white rabbits, ICC staining techniques were applied to lightly formalin-fixed cryostat sections of lumbosacral cord. ALZA 2ML2 pumps were filled with different doses of human insulin, recombinant, (Humulin BR, Lilly) to deliver 0.5-5.0 U/kg/d, IT, for 2 wks. (see Haun et al, Soc. Neurosci. Abstr. 15, 278, 1989). Alternatively, 25 or 50 U of insulin was given as a 0.25 or 0.5 ml bolus, and the animals were sacrificed after 3 or 1 h, respectively. Controls were cord specimens from untreated rabbits and from rabbits infused with artificial CSF; all were processed with the same techniques.

Mouse monoclonal anti-human-insulin-antibodies, detected by FITC and PAP methods, were present in large, medium and small neurons of ventral, intermediate and dorsal horns, and were strongly localized in the cytoplasm. Minor staining of glial elements in both gray and white matter was also seen. Rabbits given bolus IT insulin had 3X to 4X greater staining than controls. In rabbits infused chronically for 2 weeks or longer, staining varied from .75X to 2X that of controls.

TRANSPLANTATION: ANATOMICAL PROJECTIONS

210.1

FETAL RAT OLFACTORY BULB TRANSPLANTS SEND PROJECTIONS TO HOST OLFACTORY CORTEX. L. E. Westrum[1], J. N. Kott[1], H. Vickland[1], M. H. Hankin[2], and R. D. Lund[2]; [1]University of Washington, Seattle, WA 98195; [2]University of Pittsburgh, Pittsburgh, PA 15261.

We are using the rat olfactory system as a model to study developmental details of neurotransplantation. Time-mated dams received subcutaneous pulses of tritiated (3H) thymidine at embryonic (E) days 12-14. Olfactory bulbs (OB) from fetal rat donors of E14-15 were immediately grafted into neonatal rats in the site from which the host OB had been removed. Following survival times of 2-4 months, a small coagulation lesion was placed in the region of the transplanted OB and the subjects perfused after 2-3 days. The majority of the subjects showed variable sized "new" OBs. Alternate frozen saggital sections of egg yolk-embedded brains were processed for autoradiography (and Nissl stained) or silver stained for degeneration. Only OBs with extensive 3H label and precise lesions confined to the labeled areas were used. Degeneration could be traced from the lesion through the olfactory peduncle and into the superficial layers of the ipsilateral olfactory cortex. The results demonstrate "viable" donor OBs successfully grafted into hosts that send their axons to appropriate target areas of the host brain. (Sponsored by NIH Grants NS 09678 and NS 07144. LEW is a research affiliate of the CDMRC at the UW).

210.2

RETINAL TRANSPLANT-DERIVED SYNAPSES IN THE SUPERIOR COLLICULUS OF ANOPHTHALMIC MUTANT (OR^J) MICE. G.M. Horsburgh, M.H. Hankin and R.D. Lund, Department of Neurobiolgy, Anatomy & Cell Science, School of Medicine, Univ. of Pittsburgh, Pittsburgh, PA 15261.

The ocular retardation mutant mouse (or^J) undergoes early eye degeneration and, as a result, the retinal target nuclei are never innervated by optic axons. We have shown previously (Hankin and Lund, *Dev. Biol.* 138:136, 1990) that embyonic retinae from normal mice transplanted to the midbrain of newborn or^J mice are capable of developing robust projections to the superior colliculus (SC). In the present study, we have examined the ultrastructure of transplant-derived terminals using fiber tracing and conventional techniques.

Terminals of transplant origin are restriced to the superficial SC and display many characteristics of normal retinal terminals. They are typically larger than non-retinal terminals, contain large pale mitochondria and many synaptic vesicles, and make asymmetric synapses. Comparison of the proportions of asymmetric and symmetric synapses in the SC of normal (C57BL/6J) adult mice, of adult or^J mice in the absence of retinal transplants, and of adult or^J mice which received retinal transplants at birth has provided an indication of the degree of innervation by transplant projections. The superficial layers of the SC of or^J mice which did not receive transplants contained few asymmetric synapses (10-15% of synapses sampled), while in or^J mice with transplants the proportion of asymmetric synapses was up to four times higher, levels approaching those seen in normal mice.

These results show that retinae transplanted to genetically 'blind' animals not only project to appropriate target nuclei, but are also are capable of establishing synaptic connections in a manner which closely resembles the pattern seen in normal animals. Supported by NIH grants NS26777 (MHH) and EY05308 (RDL).

210.3

CHOLINERGIC FIBER GROWTH INTO FASCIA DENTATA TRANSPLANTS DIFFER BETWEEN NEWBORN AND ADULT RECIPIENT RATS. M. Schulz, T. Sørensen, N. Tønder* and J. Zimmer. PharmaBiotec, Inst. of Neurobiology, Univ. of Aarhus, Denmark.

Blocks of fascia dentata from newborn rats were grafted into the hippocampal area of newborn or adult rats, left to survive for 1-6 weeks before staining for AChE. In newborn recipients few or no AChE+ fibers were detected in the grafts (and the host fascia dentata) after 1 week. After 2 weeks AChE+ fibers were present throughout the grafts (with the highest density near the host-graft interface). A laminar distribution of the AChE+ fibers became visible in the host dentate molecular layer at this time. This was not yet seen in the similar aged transplants. After 3 weeks the AChE staining in the grafts had increased, and in grafts positioned to receive host perforant path fibers the AChE+ fibers displayed a laminar distribution as in the host fascia dentata. In adult recipients single AChE+ fibers were present in the grafts after 1 week. After 4-5 weeks the graft AChE staining was still patchy in some areas and not as dense as normal. Fimbria-close parts of the grafts were usually more heavily innervated than more distant parts. After 6 weeks the graft AChE staining was almost as dense as normal, but a laminar distribution was not achieved.

We conclude, that the growth of AChE+, host septohippocampal fibers into homotopic grafts of neonatal hippocampal tissue depends on the recipient age, which affects both the rate of ingrowth and the distribution of the ingrowing fibers.

210.4

INTRACEREBRAL TRANSPLANTS OF THE RAT FASCIA DENTATA - EFFECTS ON THE SYNAPTIC ORGANIZATION BY HOMO- AND HETEROTYPICAL AFFERENT CONNECTIONS. T. Sørensen and J. Zimmer. PharmaBiotec, Inst. of Neurobiology, Univ. of Aarhus, DK-8000 Aarhus C, Denmark.

We examined the synaptic morphology of fascia dentata transplants, which after grafting to newborn rats received major connections from the host brain. The transplants were placed either in host hippocampus, where they received homotypic entorhinal perforant path or commissural projections, or in the neocortex, where they received heterotypical callosal afferents in the outer parts of the dentate molecular layer. At an age of 3 months the ingrowth was shown in half of the recipient rats by anterograde, electron dense axonal degeneration, 3 days after transection of the host perforant path, hippocampal commissure or corpus callosum, respectively. For the other half of the recipient rats the ingrowth of host corresponding afferents was judged from the placement of the grafts and previous results. The ultrastructural results show that dentate transplants will accept both homotypic and heterotypic afferents. The synaptic contacts were of the morphological types seen in the normal fascia dentata. Preliminary quantitative results indicate that both homotypic and heterotypic afferents compensate for the reduction of synaptic density seen in isolated grafts.

Supported by the Danish MRC, the Lundbeck Foundation and the Aarhus University Research Foundation.

210.5

FETAL VENTRAL MESENCEPHALIC GRAFTS CONTAIN NON-DOPAMINERGIC NEURONS WHICH PROJECT TO THE HOST STRIATUM: COMBINED IMMUNOCYTOCHEMISTRY AND RETROGRADE TRACING WITH FLUOROGOLD. T.J. Mahalik, G.H. Clayton and T.E. Finger. Dept. of Cellular and Structural Biology, U. of Colorado Medical School. Denver, CO. 80262.

Grafts of fetal ventral mesencephalon have been used to correct the motor deficits in a rat model of Parkinson's disease. These grafts contain dopaminergic neurons which project to the host brain; however, it is clear that VM grafts are heterogeneous with respect to their neurotransmitter content. In this study we combined immunocytochemistry with retrograde tracing with fluorogold to chemically identify neurons in VM grafts that projected to the striatum of adult hosts.

Ventral mesencephalon from E14 fetuses was grafted to 6-hydroxydopamine-lesioned striatum of adult hosts; hosts were allowed to survive from 6 weeks to 1 year. Fluorogold was then injected into the striatum adjacent to VM grafts. After 4 days, rats were perfused and the brain was prepared for immunocytochemistry.

Immunocytochemistry showed that VM grafts contained cell bodies which were positive for either GAD, substance P, serotonin, enkephalin, or tyrosine hydroxylase. Double labeling with fluorogold revealed that, except for enkephalinergic cells, a subset of neurons of each type projected to the host striatum.

We have demonstrated that VM grafts contain a heterogeneous population of neurons. The major finding of our study is that a substantial number of non-dopaminergic neurons project to the host striatum. These results indicate that there is no "tight" control on the specificity of graft to host connections.

REGENERATION: SPECIFICITY AND FUNCTIONAL RECOVERY

211.1

NEURAL REGENERATION LEADS TO *DYSFUNCTION* IN LAMPREY SPINAL CORD. A.H.Cohen, M.T.Baker*, L.Guan*, T.Kiemel*. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

Because substantial regeneration of CNS neurons and their processes is so rare, it is seldom possible to ask if regeneration of central neurons leads to functional recovery. This lab and others have shown that after spinal transection, adult lampreys can regenerate CNS neuronal processes and these processes can contribute to the function of a behaviorally relevant central pattern generator. However, we here show that the regenerated neuronal processes also lead to ***dysfunction*** of that same circuit.

Isolated spinal cord-notochord preparations were made from adult lampreys which had had partial or complete spinal transections with 5, 8, or 10 months to recover. The quality of the preparations' most stable fictive swimming was assessed and compared to fully intact controls and controls with acute lesions comparable to the transections. At their best, the bursting of all groups of cords with regenerated fibers was significantly less stable than any group of control cords. The extent and location of the transection could worsen this effect, but no transected group was as stable as any control group. The phase delays across the transection site were also significantly more variable in the regenerated animals even in the presence of the intact fibers, i.e., prior to acutely cutting the formerly spared fibers. Again, this was true regardless of the control animals to which they were compared, including the animals with acute lesions comparable to the transections. Thus, the capacity of the intact fibers was functionally reduced by the neuronal regeneration which resulted from spinal transection. A mathematical model illustrates possible problems stemming from the regeneration of long fibers to explain the disturbance.

Support: NIH no. NS16803 and AFOSR contract F49620-88-C-0013 to AHC

211.2

ANALYSIS OF THE FUNCTIONAL ACTIVITY OF REGENERATING GIANT RETICULOSPINAL NEURONS DURING UNDULATORY BEHAVIOR IN LAMPREY. Joseph Ayers, Department of Biology and Marine Science Center, Northeastern University, East Point, Nahant, MA 01908.

Sea lamprey, Petromyzon marinus, can recover undulatory behavior following complete spinal cord transection. We have been developing procedures for the analysis of functional activity of regenerating giant reticulospinal neurons (RNs) to determine the relationships between functional recovery of neuron activity and overall behavioral recovery in ammocoete larvae. Specimens are prepared for recording from RN axons by implanting fine wire electrodes in the extradural space of the spinal cord. Correlated electrophysiological and kinematic recordings are obtained by recording the signals from the two electrodes on the audio tracks of a stereo video recorder while recording the undulatory movements on the video signal. We have adapted the program *NIH Image* to perform stop-frame analysis of undulatory movement. Correlated digital signal processing of the stereo electrophysiological signals allows us to extract the activity patterns of individual RNs and directly relate them to features of the ongoing undulations. In acute preparations where recordings are made anterior (25% of body length) to a caudal transection (75% of length), action potentials (APs) in RN's disappear over a time course of several hours and in 24 hours are no longer observed during swimming. Propagating APs in RNs are observed during swimming in intermediate stages of recovery although distal APs are considerably reduced in amplitude. In long term (>1 yr.) regenerated specimens a small proportion of reticulospinal axons exhibit large amplitude APs both proximal and distal to the lesion and occasional anterior propigating APs are observed. We conclude that it is feasable to study the functional activity of regenerating RNs and to relate these events to behavioral recovery. *Supported by NSF Grant DIR-8917532.*

211.3

REGENERATION OF NEUROMUSCULAR CONNECTIONS AFTER GANGLIA ALLOTRANSPLANTS IN THE CRAYFISH. K. Krause, D. Sheldon*, R. Kang* and S. J. Velez. Dept. of Biological Sciences, Dartmouth College, Hanover, N. H. 03755.

The superficial flexor nerve of the crayfish Procambarus clarkii is capable of regenerating a specific pattern of connections with its muscle target within 10 weeks after axotomy. We have postulated that the position-dependent pattern of connectivity in this system could be the result of a gradient over the muscle surface that provides positional cues to the growing axons. In the present work we use allotransplants to test this idea, forcing the nerve to grow into the muscle field in a direction that is opposite from normal. The third abdominal ganglia with the nerve attached to it was removed from one crayfish and transplanted to the lateral edge of a denervated muscle field of another crayfish. The host animal did not reject the transplant. At different intervals after the surgery, the muscles were exposed and the muscle fibers were impaled with microelectrodes to look for junction potentials (jp's) generated by the spontaneous activity of the transplanted neurons. At 4 weeks we detect small, long jp's (< 0.5 mV, >25 msec) near the insertion point of the nerve. By 6 weeks jp's are detected over many lateral fibers, some still small and long, others with normal characteristics (> 1 mV, <20 msec). Thus, these neurons can survive, grow and form new synaptic contacts when transplanted from one animal to another. We are now studying the connectivity patterns of these cells in order to test the gradient hypothesis.

211.4

SPECIFIC REGENERATION OF CUTANEOUS AFFERENTS IN THE FROG'S SPINAL CORD. S.C. Mears and E. Frank. Dept. of Neurobiology, Anatomy, and Cell Science, University of Pittsburgh School of Medicine, Pittsburgh, PA 15261.

Regeneration of nerve axons is not a common occurrence in the central nervous system of vertebrates. However, sensory axons, transected in the dorsal root, re-establish connections in the spinal cord of postmetamorphic bullfrogs. Although muscle sensory fibers are known to regenerate specifically, the specificity of regenerating cutaneous afferents has not been examined directly. We have used anatomical tracing techniques to determine where these afferents form their terminal arborizations.

The brachial dorsal root was repeatedly frozen with forceps cooled in liquid nitrogen and left to regenerate for several months. A cuff containing horseradish peroxidase and lysolecithin was then applied to the superior cutaneous branch of the radial nerve. Two weeks later the perfused spinal cord was serially sectioned in the transverse plane and reacted with tetramethyl-benzidine. Regenerated cutaneous afferents arborized specifically within the dorsal neuropil of the dorsal horn as in normal frogs. They did not penetrate more deeply into the spinal gray matter where muscle afferents terminate.

An interesting highlight involves the course taken by the regenerating fibers to reach the neuropil. In a previous study of regenerating muscle sensory fibers, the axons grew around the circumference of the spinal cord just under the pia and entered the spinal gray more ventrally to arborize in the ventral neuropil. Regenerating cutaneous afferents, however, took a normal course through the dorsal columns to the dorsal neuropil. These observations show that cutaneous afferent axons can regenerate and seek out their original target areas in the spinal cord. Supported by NS24373 to E.F.

211.5

FIN ORIENTATION AND REGENERATION OF ELECTRORECEPTORS IN GLASS CATFISH. M.M. Bever* and R.B. Borgens. Center for Paralysis Research, Purdue Univ., W. Lafayette, IN 47907.

Regeneration of electroreceptors (ERs) of adult glass catfish (Kryptopterus) was studied to understand the relative roles of epidermis and axons. In the anal fin, these receptors, innervated by 1 axon each, are found along the dorsal 1/3 of the caudal face of each fin ray.

We removed pieces (approx. 2 mm X 1 mm) of anal fin (thereby severing ER innervation) and replaced them with one of the following types of fin autografts: (A) normal orientation, with ERs, (B) reversed rostrocaudally (ERs along rostral fin ray face), (C) reversed dorsoventrally (ERs at distal end of transplant), (D) normal orientation, ERs absent.

ERs present at surgery disappeared by 1 week owing to nerve interruption. By 2-3 weeks, new receptors appeared in all graft types. In A grafts, most new ERs formed along caudal faces of fin rays. In B grafts, ERs formed along rostral faces. In C grafts, some ERs formed at the distal end of the graft. In D grafts, ERs formed mainly in the proximal portion of the graft. In all grafts, some ERs were also found in ectopic positions.

These results indicate that any epidermis (whether containing ERs or not) can form new ERs after induction by regenerating ER axons. Patterns of ER formation in type A, and B grafts are probably due to regenerating axons following old axon pathways. In addition, degenerated ERs may serve as targets for regenerating axons.

211.6

LOCAL REGENERATION IN THE RETINA OF THE GOLDFISH. P.F. Hitchcock, University of Michigan, Depts. of Ophthalmology and Anatomy and Cell Biology, Ann Arbor, MI.

To begin elucidating the mechanisms that control retinal regeneration in goldfish, small patches of retina were excised, and dopaminergic interplexiform cells were anatomically assayed in the regenerated patches.

Two groups were prepared. Group 1: A patch of retina was removed transclerally, and four months later the retinas were isolated, fixed, and processed as whole-mounts for anti-tyrosine hydroxylase (anti-TH) immunohistochemistry. Group 2: Patches of retina were excised three weeks after the eyes were injected with 15ug of 6-OHDA, to destroy the dopaminergic cells, and two months later these retinas were processed for anti-TH.

In group 1 (n=8), the regenerated patches contained qualitatively normal planimetric densities of TH-positive cells, although they were not as regularly arrayed as those outside the patch. In group 2 (n=3), the retinas were completely free of TH-positive cells, except at the proliferative margin and within the regenerated patches.

These results suggest that the same mechanisms that control de novo retinal growth at the margin also control retinal regeneration.

Supported by NIH grants EY07060 and EY07003 (CORE).

211.7

SPONTANEOUS AND ABERRANT REGENERATION OF OPTIC AXONS RESTORES VISUAL RESPONSE TO ADULT RAT SUPERIOR COLLICULUS. A.P.Foerster. Dept. of Biomed. Sci., McMaster University, Hamilton, Ontario L8N 3Z5

An implanted cutting device, that permanently identified the lesion site (APF, J. Comp. Neurol. 210:335, 1982), completely severed the brachium of the right superior colliculus (SC). HRP, traced from the left eye 9 months later, revealed that a newly routed pathway had developed from the severed tract. Beginning from a ventral location, it ascended to the laterodorsal thalamus and stria medullaris, then passed caudally around a huge lesion-related cavity to innervate the medial SC. Microelectrode mapping revealed a partial recovery of visual responses in this previously silenced SC. This remarkable example of delayed functional recovery (cf. APF, Exp. Brain Res. 79:564, 1990; Can. J. Physiol. Pharmacol. abstract in press) now appears to provide proof of spontaneous regeneration of severed axons in adult mammalian CNS. Moreover, they grew to their appropriate target. (MRC Canada)

211.8

SPONTANEOUS AXONAL REGENERATION IN THE CORPUS CALLOSUM (CC) OF THE ADULT RAT. L.G. Clements and A.P. Foerster. Dept. of Biomedical Sciences, McMaster University, Hamilton, Ontario CANADA L8N 3Z5

A double labeling approach was used, with one label taken up immediately by the cut axons, and the other at a later time by their distant terminals. Fluoro-Gold (FG) was applied via a razor-ended blade which cut the middle part of the CC. A series of 80 µm coronal sections revealed masses of labeled neurons in the contralateral cortex in register with the cut, and extending to <1 mm on either side of it. Rhodamine latex microspheres (RLM), injected into the ipsilateral cortex by half mm spaced injections, labeled 80-150 neurons/section in the contralateral cortex. When these injections were made 4 days after a blade cut, the count reduced to zero in a zone in approximate register with the central part of the lesion. This zero "window" was 270-750 µm after a 2 mm cut and about 1500 mm with a 3 mm cut. Some double labeling was seen in regions in register with the ends of the lesion, indicating FG uptake by intact axons. When the RLM were injected 4 weeks postlesion, the zero "window" was replaced by up to 100 or more RLM-containing neurons per section; up to 90% of these were double labeled. We suggest that a spontaneous and massive regeneration of severed CC axons had occurred. [Work supported by MRC Canada. LC is a MS Society (Canada) Student].

211.9

SEROTONIN-IMMUNOREACTIVE (5HT-IR) AXONS FORM SYNAPSES BEFORE WITHDRAWING FROM HIPPOCAMPUS AND CORTEX TRANSPLANTS IN SPINAL CORD OF YOUNG RATS. H. Bernstein-Goral, M.M. McAtee*, B.S. Bregman. Dept. Anatomy and Cell Biology, Georgetown University School of Medicine, Washington, D.C. 20007.

Bulbospinal 5HT-IR axons initially grow into both appropriate (spinal cord, SC) and inappropriate, (hippocampus, HC and cortex, CX) CNS transplants placed into the site of neonatal spinal cord lesions. 5HT-IR axons are withdrawn from inappropriate transplants between 21-26 days following transplantation. Only appropriate transplants (SC) can maintain 5HT ingrowth permanently. The present study has examined whether 5HT-IR fibers which transiently grow into hippocampus and cortex transplants form synaptic contacts prior to withdrawal. Fetal rat SC (E14), HC or CX (E18) was transplanted into mid-thoracic spinal cord "over-hemisections" in neonatal rat pups. Animals survived for 14 days post-operative (dpo, short term) or 8 weeks (long term). Transplants were stained for 5HT-IR and processed for ultrastructural analysis. At 14 dpo 5HT-IR fibers form synaptic contacts with dendrites and somata within the neuropil of both hippocampus and cortex transplants. At long survival times, 5HT-IR fibers are present only within spinal cord transplants, where they form axo-dendritic and axo-somatic synaptic contacts. These results demonstrate that although bulbospinal 5HT-IR fibers are not maintained in HC or CX, they initially innervate these inappropriate targets and form classical synaptic contacts prior to withdrawal. Supported by NIH NS 19259 and NS 01356.

211.10

THE NIGROSTRIATAL SYSTEM IN ORGANOTYPIC SLICE CULTURES: A TH-IMMUNOCYTOCHEMICAL STUDY. J. Zimmer, K. Østergaard and J.P. Schou*. PharmaBiotec, Inst. of Neurobiology, Univ. of Aarhus, DK-8000 Aarhus C, Denmark.

Organotypic slice cultures of rat ventral mesencephalon were used to study the development of dopaminergic (DA) neurons and fibers in the absence of their normal striatal target tissue and in co-cultures with striatal tissue. For exposure to minor or non-target areas co-cultures with hippocampus and cerebellum were used. Slices of rat VM, striatum, hippocampus and cerebellum were prepared from E21 to P7 rats and cultured by the roller tube technique for 3 to 60 days before immunocytochemical staining for tyrosine hydroxylase (TH), a marker of catecholaminergic neurons. The TH immunoreactive (TH-i), DA neurons of the substantia nigra (SN) retained their morphological in vivo characteristics in single slice cultures. The basic morphology of the neurons did not change when co-cultured with striatal slices, even in cases with extensive TH-i innervation of striatum. In co-cultures with hippocampus, a minor target for DA fibers, TH-i nerve fibers mainly grew into the opposing peripheral parts of the hippocampal slices. In co-cultures of VM and cerebellum (not a DA fiber target) only occasional ingrowth of single TH-i nerve fibers was observed. The observations, which suggest a target orientated growth of TH-i DA fibers, in some cases even with patch formation in the striatum, have clear implications for the use of VM slice cultures in studies of the plastic and regenerative properties of DA neurons.

211.11

A SIMPLE METHOD OF ASSESSING MOTOR AND SENSORY INNERVATION AFTER NERVE DAMAGE IN RAT SCIATIC NERVE MODEL. Y. Torigoe, C-Z. Hong, M. Gharibian*, R. Farboudmanesch*, J. Yu. Anat. & Neurobiol. and Dept. Phys. Med. & Rehab., Univ. Calif. Irvine, CA 92717

A cross extensor reflex (CER) method of evaluating functional recovery after nerve crush is compared with sciatic functional index (SFI) and traditional nerve conduction velocity (CV) method. In unanesthetized, gently restrained rats, one paw was electrically stimulated to elicit a CER in the contralateral leg. The evoked compound muscle action potentials (CMAP) were recorded from the contralateral leg muscles using surface recording electrodes that is unobtrusive to normal movements. After nerve crush, stimulation of the uninjured and recording from the injured leg tests for motor recovery; and stimulation of the injured and recording from the uninjured side tests for the sensory recovery. For each measurement, the average of 8 latencies of the first wave of CMAP was obtained for analysis. Eighteen days after nerve crush, SFI showed some functional return and in 25 days the SFI returned to nearly normal levels. The first measurable CMAP appeared approximately 30 days after nerve crush with the stimulation of the uninjured side (motor test) followed shortly, 33 days, by the stimulation of the injured side (sensory test). The amplitude of the CMAP increased and the latency shortened before finally returning nearly to its original values by 3 months. The major difference in the rate of recovery as seen by SFI and CER methods may be explained by longer length of time required for nerve regeneration to the most distal portions of the leg innervated by the sciatic nerve. Another difference may be attributable to SFI requiring only partial innervation for the walking behavior to appear normal, but for the normal reflex, the axons innervating the distal musculature and sensory innervation of the palmar surfaces must be fully regenerated to produce short latency muscle contractions. However, other tests including CV and CER methods, require full neural and muscular recovery before normal responses are achieved. We conclude that this animal model can be useful to assess the rate of both motor and sensory nerve recovery after injury with treatment of various physical or chemical agents, such as thermotherapy, electrotherapy, or application of topical medicines.

LONG-TERM POTENTIATION II

212.1

OPTICAL SYSTEM FOR REAL-TIME IMAGING OF ELECTRICAL ACTIVITY WITH A 128 x 128 PHOTOPIXEL ARRAY. G. Matsumoto and M. Ichikawa*. Electrotechnical Laboratory, Tsukuba, Ibaraki 305, JAPAN.

We have developed a compact optical recording sytstem for real-time imaging of electrical activity with a 128 x 128 photopixel array. Each pixel has a light-to-electricity conversion surface with 70 x 70 μm in size and a FET on the surface. The signals that the array receives simultaneously at the 128 x 128 pixels can be converted into electric ones and transfered to a computer system at the frame rate of 0.5 msec. The data are integrated during the period of time until they are transfered so that the signal-to-noise ratio becomes greatly improved. When the system was applied to guinea pig hippocampal slice preparation stained with RH-155, the optical change of 3×10^{-4} was detected without averaging. We found that the system was greatly useful to analyze functional connections of neuronal circuits of the hippocampal slice; that is, the spatio-tempral patterns of the activities thus obtained gave us a deep insight about (1) the main pathways of the information transmission in the slice preparation and (2) some feed-forward and feed-back inhibitory connections.

We acknowledge to Dr. Iijima for physiological experiments and Mr. Murayama (Fuji Photo Film Co.) for fabrication of the array.

212.2

MEASUREMENT OF CALCIUM TRANSIENTS IN PYRAMIDAL NEURONS OF HIPPOCAMPAL SLICES VISUALIZED BY CONFOCAL MICROSCOPY. R.J. Adams and T.J. Sejnowski. Computational Neurobiology Laboratory, The Salk Institute, La Jolla, Ca 92037.

Central to models of long-term potentiation is the role of postsynaptic elevations in calcium ion concentration in mediating synaptic modifications. The major path for this influx is thought to be through the NMDA receptor. Calcium influx through voltage-dependent calcium channels may also significantly contribute to the total cytoplasmic elevation. Initiation of various calcium-activated processes are then thought to give rise to an increase in synaptic efficacy.

We are investigating changes in intracellular calcium concentration in pyramidal neurons of hippocampal slices *in vitro*. Individual neurons are impaled using a glass microelectrode filled with the fluorescent calcium indicator fluo-3. A cell may then be filled with the dye by iontophoresis and followed physiologically during the course of the experiment. Fluorescent signals are monitored using a Bio-Rad MRC-600 laser scanning confocal microscope. Fluorescent measurements are made at high spatial resolution, with < 1 um depth of field. Individual scan lines may be taken at 2msec intervals to provide a fine dynamic record of the calcium changes within the cell. Electrical and calcium signals are recorded from cells activated either synaptically by axons stimulated with bipolar electrodes in the stratum radiatum or by depolarization by direct current injection through the intracellular electrode. Sustained elevations in calcium are seen in the basal and apical dendrites and the soma following high frequency stimulation under conditions that induce LTP.

212.3

CHARACTERIZATION OF CALCIUM ACCUMULATIONS IN HIPPOCAMPAL PYRAMIDAL CELLS W.G. Regehr & D.W. Tank
AT&T Bell Labs, Murray Hill NJ 07974.

Individual pyramidal cells in guinea-pig hippocampal slices were filled with fura-2 and calcium accumulations were measured in response to antidromic and synaptic stimulation using a cooled CCD and a photodiode array. The NMDA-receptor-antagonist AP5 blocked a component of accumulation localized to regions receiving synaptic input. This component was larger for higher stimulus frequencies. In the presence of AP5 similar distributions were observed for antidromic and orthodromic stimulation independent of the region receiving synaptic input. These accumulations peaked in proximal-apical and basal dendrites and were much smaller in the soma and distal dendrites. Antidromic stimulation produced calcium increases that were largely blocked by 500 μM Cd^{++}, partially blocked by either 2 μM nifedipine or 5 μM ω-conotoxin, and enhanced by 5 μM Bay-K 8644. These findings indicate that it is primarily influx through voltage-dependent calcium channels that produces AP5-insensitive accumulations. Intracellular injection of the sodium channel blocker QX-314 decreased AP5-insensitive accumulations without significantly altering the spatial distributions. The lack of appreciable calcium accumulations in the presence of AP5 in distal apical dendrites likely represents a much lower density of voltage-dependent calcium channels in this region. Experiments with FURAPTRA (K_d=50μM for Ca^{++}) confirm the basic findings obtained with fura-2 (K_d=200nM). We are currently characterizing calcium accumulations observed during associative LTP induction.

212.4

NMDA-Dependent LTP of Monosynaptic Entorhinal Input To CA3 and the Dentate Gyrus Alters Propagation of Activity Through the Trisynaptic Pathway. M.F. Yeckel and T.W. Berger, *Depts. of Behavioral Neuroscience and Psychiatry, University of Pittsburgh, Pittsburgh, PA, 15260.*

We have demonstrated recently that stimulation of entorhinal perforant path axons leads to monosynaptic single unit and population discharge in the three major subfields of the hippocampus (dentate, CA3 and CA1; Yeckel and Berger, *Proc. Natl. Acad. Sci.*, in press). Propagation of perforant path input through the trisynaptic pathway to CA3 and CA1 reachs suprathreshold levels only in response to frequencies of 10-20 Hz. Therefore, the modulatory influence of dentate granule cells and CA3 pyramidal cells on entorhinal input to CA1 cells is conditional on the pattern of input from entorhinal neurons. We examined the consequences of long-term potentiation (LTP) of monosynaptic entorhinal input on the propagation of activity through the trisynaptic pathway.

Trains of stimulation (10 Hz; 20-30 impulses) with intensities that evoked a 50% maximal response, were delivered to perforant path fibers of halothane anesthetized rabbits. The number of impulses necessary to evoke suprathreshold di- and trisynaptic responses, and the maximal amplitude of polysynaptic responses, were determined. Monosynaptic LTP was then induced with 400 Hz stimulation trains delivered to perforant path fibers. LTP could be blocked reversibly in the dentate gyrus and/or CA3 subfields with the NMDA-antagonist, d-APV (50-100 μM; n=8). Following LTP induction, 10 Hz stimulation trains were again delivered to perforant path fibers but with lower intensities so as to evoke the pre-LTP amplitude dentate population spike. Results showed that increasing the synaptic strength for monosynaptic input significantly decreased the number of impulses necesssary to evoke suprathreshold activation of di- and trisynaptic responses in the pyramidal cells. Additionally, the amplitude of the maximal polysynaptic response was increased (CA3: n=5; CA1: n=5). These data provide evidence that NMDA-mediated changes in synaptic efficacy alter frequency dependent propagation of activity through the trisynaptic pathway.
Supported by NIMH (MH00343) and NSF (BNS 8945137).

212.5

EXCITATORY SYNAPSES BETWEEN CA1 PYRAMIDAL NEURONES ARE MEDIATED BY BOTH NMDA AND NON-NMDA RECEPTORS. A.M. Thomson and S. Radpour. Dept. Physiology, Royal Free Hospital School of Medicine, London NW3 2PF UK.

Double recordings from pyramidal neurones in the CA1 region of hippocampal slices, with spike triggered averaging revealed single axon epsps that displayed voltage relations and sensitivity to antagonists typical of responses mediated in part by NMDA receptors. Co-activation of an epsp displaying these properties, at 1-2Hz, with a minimal Schaffer collateral epsp, led to lasting enhancement of the Schaffer epsp, but not of the local epsp. These results suggest that lasting enhancement of unitary Schaffer epsps can be achieved in the absence of generalized postsynaptic depolarization and without addition of GABA antagonists, provided an NMDA receptor mediated epsp is co-activated. Pairing of Schaffer epsps with local ipsps led to depression of Schaffer epsps. Preliminary experiments indicate that traditional protocols used for the induction of LTP in CA1 may be unsuccessful if local excitatory circuits are interrupted.

212.6

BLOCKADE OF FIMBRIAL RESPONSES DURING MOSSY FIBER TETANIZATION DOES NOT PREVENT HETEROSYNAPTIC FIMBRIAL LTP INDUCTION IN CA3 NEURONS. *G. Barrionuevo, X. Xie and J. E. Bradler.* Depts. of Behavioral Neuroscience & Psychiatry, University of Pittsburgh, Pgh, PA 15260.

We have previously shown that in the CA3 subfield, mossy fiber tetani induce heterosynaptic LTP in nontetanized fimbrial afferents[1]. Induction of heterosynaptic fimbrial LTP does not require NMDA receptor mediated transmission[2]; in contrast, homosynaptic fimbrial LTP evoked by tetanization of fimbrial afferents is blocked by APV[2]. This dissociation between homo- and heterosynaptic LTP induction argues against the involvement of CA3 collateral LTP in the heterosynaptic enhancement. However, mossy fiber-elicited quisqualate activities at the fimbrial input may be necessary for heterosynaptic LTP induction. In the present study, EPSPs from hippocampal CA3 neurons maintained in an *in vitro* slice preparation were evoked from two stimulation sites: i) the s. granulosum of the dentate gyrus, to activate mossy fibers; and ii) the ventral fimbria. Slices were superfused with medium containing D-APV (50 μM), and picrotoxin (10 μM). Prior to mossy fiber tetani, fimbrial responses were selectively blocked by focal applications of CNQX (40 μM) onto the s. oriens. Following washout of CNQX, fimbrial EPSPs expressed LTP (mean 38% of control, n=3). Focal application of CNQX alone did not have any long lasting effect on fimbrial synaptic efficacy (n=6). These results indicate that heterosynaptic fimbrial LTP proceeds in the absence of either NMDA or quisqualate receptor mediated activation.
Supported by grants RCDA NS01196, NS24288 and AFOSR.

[1]Bradler & Barrionuevo, Synapse 4:132-142 (1989).
[2]Bradler & Barrionuevo, Neuroscience, (1990), in press.

212.7

CHARACTERIZATION OF AN LTP-LIKE PHENOMENON INDUCED BY A TRANSIENT INCREASE IN EXTRACELLULAR POTASSIUM CONCENTRATION. M. W. Fleck, A. M. Palmer, & G. Barrionuevo. *Depts. of Behavioral Neuroscience & Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.*

Long-term potentiation (LTP) is a long-lasting, use-dependent increase in synaptic efficacy that is well characterized in the rat hippocampus and is typically induced by tetanic stimulation. LTP induction requires the concomitant depolarization of pre- and post-synaptic elements, and so should be inducible via potassium depolarization. Using the *in vitro* hippocampal slice preparation, we have induced an LTP-like phenomenon in the Schaffer collateral-CA1 synapses by transiently increasing potassium concentration (50 mM for 20 seconds) in the perfusion medium. Potassium-induced LTP, as monitored in stratum radiatum of the CA1 subregion, resembled that induced by tetanic stimulation in several ways: 1) the amplitude of the population EPSP increased by at least 20% (n=9), 2) this increase in population EPSP amplitude lasted for at least 20 minutes, 3) potassium-induced LTP prevented the subsequent induction of LTP by tetanic stimulation (n=2), and 4) induction was blocked by bath application of 20 μM D-APV (n=6). Thus, potassium-induced LTP and LTP induced by tetanic stimulation probably share a common induction mechanism. Preliminary results indicate that potassium-induced LTP provides a useful model for the *in vitro* analysis of transmitter release during LTP. Supported by grants NS01196 and NS24288.

212.8

ARACHIDONIC ACID IN HIPPOCAMPAL SLICES: ELECTROPHYSIOLOGICAL AND BIOCHEMICAL FINDINGS. L. Pellerin, M. Avoli and L.S. Wolfe. Neurobiology Unit, Montreal Neurological Institute, McGill Univ., Montreal, Canada H3A 2B4

Arachidonic acid (AA) metabolites have been proposed recently to be retrograde messenger in long term potentiation (LTP), a model of synaptic plasticity. Following NMDA receptor activation and AA release, AA or its metabolites act postsynaptically and/or diffuse and act on presynaptic terminals to increase transmitter release. We studied arachidonic acid metabolism in rat cerebral cortex and found glutamate and NMDA, but not kainate stimulated 12-(S)-HETE formation, one of the 12-lipoxygenase metabolites of AA. Using a different approach, we have examined the hippocampal slice, a well-characterized preparation for the study of LTP. AA (50 μM) was perfused on hippocampal slices maintained in vitro and extracellular responses of the CA1 region monitored. In 12 out of 18 experiments, a long-lasting potentiation of the stratum radiatum-induced response was observed. In five out of 18 experiments, arachidonic acid evoked a depression of the same response. When melittin, a bee venom peptide known to induce release of AA in immune cells, was applied to hippocampal slices a long-lasting potentiation was observed. Melittin induced AA release in hippocampal slices and the effect was dose-dependent. Glutamate and NMDA also released AA. Thus glutamate mobilizes AA from phospholipids which is then available for metabolism by lipoxygenases to products involved in neuromodulation at these synapses.

212.9

PHORBOL ESTERS ENHANCE SYNAPTIC TRANSMISSION IN WHOLE-CELL VOLTAGE CLAMP RECORDINGS: EVIDENCE FOR A PRE-SYNAPTIC ACTION. K.D. Parfitt and D.V. Madison, *Dept. of Molecular and Cellular Physiology, Stanford Univ. Sch. of Med., Stanford, CA 94305-5426.*

Bath application or presynaptic application of phorbol esters, activators of protein kinase C, potentiate synaptic transmission in hippocampal slices, mimicking tetanus-induced long term potentiation (LTP). We have used the whole-cell voltage clamp technique to study synaptic transmission in area CA1 of standard 400-500 um-thick hippocampal slices. LTP can only be induced in these recordings during approximately the first 30 min. after breaking into a cell, but can be maintained for hours once induced (Malinow and Tsien, in press). The loss of the ability to induce LTP after 30 min. of recording presumably results from the dialysis of postsynaptic intracellular contents with the contents of the pipette. In neurons dialyzed for >30 min., in which post-tetanic potentiation (PTP) but not LTP could be induced, bath application of phorbol diacetate (PDAc) was still capable of enhancing synaptic transmission. As has been observed with extracellular recording, this enhancement decays within 90 min. of PDAc washout. Previous studies suggest that a presynaptic protein kinase is involved in the expression of LTP, whereas postsynaptic kinases are involved in the induction of LTP (Malinow et al, 1989). Phorbol esters have many presynaptic actions, including the enhancement of the phosphorylation of synapsin, a mechanism that is thought to result in enhanced transmitter release. In addition, phorbol esters enhance depolarization-induced opening of N- and L-type calcium channels, which might also lead to enhanced transmitter release from presynaptic terminals. Since phorbol esters were capable of potentiating synaptic transmission even when the induction of LTP was blocked by postsynaptic dialysis, our data are consistent with the hypothesis that phorbol esters enhance synaptic transmission via a presynaptic mechanism.

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212.10

MECHANISM OF POST-TETANIC POTENTIATION OF QUANTAL SYNAPTIC TRANSMISSION IN THE DENTATE GYRUS OF THE RAT. A. Baskys, P. L. Carlen and J. M. Wojtowicz. Playfair Neuroscience Unit and Dept. of Physiology, Univ. Toronto, Toronto, Ontario M5T 2S8, Canada.

Synapses in the hippocampus exhibit a variety of plastic phenomena. In this study the rat hippocampal slice preparation in conjunction with quantal analysis was used to examine the mechanism of post-tetanic potentiation in granule neurons of the dentate. With the use of intracellular recordings we identified a population of spontaneous depolarizing potentials which were resistant to 10 μM bicuculline and therefore presumably originated from excitatory synapses. The potentials were true quantal events since they persisted in the presence of TTX and Mn^{2+} which blocked Na^{+} - and Ca^{2+} - dependent action potentials. The mean amplitude of these miniature EPSPs varied between 400-600 μV in 11 neurons studied thus far. Occasionally, "giant" quanta reaching up to 8 mV were observed. Tetanic stimulation at 400 Hz (8 trains lasting 20 ms each) of afferent axons contacting the granule neurons produced an increase in quantal content of evoked minimal EPSPs and the frequency of spontaneous quantal events. The mean amplitude of quanta remained unchanged. These results point to a presynaptic locus of the post-tetanic potentiation lasting for at least 15 min.

Supported by Ontario Mental Health Foundation, Medical Research Council of Canada and The Whitehall Foundation.

212.11

QUANTAL MODEL OF SYNAPTIC CURRENT FLUCTUATIONS IN HIPPOCAMPAL NEURONS. Z. Xiang*, E.W. Kairiss, C.L. Keenan, E.M. Landaw* and T.H. Brown. Dept. of Psychology, Yale Univ., New Haven, CT 06520 and Dept. of Biomathematics, Univ. of Calif., Los Angeles, CA 90024.

There has been considerable interest in the quantal mechanism responsible for certain forms of synaptic plasticity in the hippocampus--particularly long-term potentiation (LTP). Here we describe a general approach for fitting a quantal model of synaptic transmission to postsynaptic current fluctuations.

The full model incorporates (*i*) either a one-parameter Poisson or two-parameter binomial probability function to describe the number of quantal releases; (*ii*) a two-parameter Gaussian or a three-parameter gamma density function to describe the distribution of single-quantal amplitudes; and (*iii*) a two-parameter Gaussian density function to describe the noise. A maximum likelihood estimation procedure has been developed to fit jointly all model parameters to observed data. Individual parameters can be fixed to measured or hypothesized values. Likelihood ratio tests are used to test hypotheses about parameter subsets or to test changes in specific parameters in comparing two data sets. This methodology also provides standard errors and confidence intervals of estimated parameters. A modified Kolmogorov-Smirnov goodness-of-fit test is used to compare the empirical and theoretical cumulative probability distributions. In applying the model, we selected the mossy-fiber input to pyramidal neurons of the CA3 region because these synapses are electrotonically near the somatic recording site and because single quantal currents can be resolved in these cells.

Thus far, the model has been successful in providing adequate fits to all of the observed frequency distributions of currents. We are now comparing changes in estimated parameter values with changes expected to occur as a function of various experimental manipulations. This approach may be useful for evaluating the mechanism underlying the expression of LTP. (Supported by the AFOSR)

212.12

VOLTAGE-DEPENDENCE OF THE NMDA RECEPTOR-MEDIATED SYNAPTIC CONDUCTANCE IN HIPPOCAMPUS A.C. Nobre, Z. Xiang*, A.M. Zador and T.H. Brown. Dept. of Psychology, Yale Univ., New Haven, CT 06520.

The voltage-dependence of the N-methyl-D-aspartate (NMDA) receptor-mediated synaptic conductance is critical for explaining the induction of long-term potentiation (LTP) at certain Hebbian synapses of the hippocampus (reviewed in Brown et al., Ann. Rev. of Neurosci., 13:475, 1990). Here we characterize the voltage-dependence of this conductance in hippocampal pyramidal neurons of region CA1.

Synaptic currrents evoked by stimulation of Schaeffer collateral/commissural inputs were recorded from rat hippocampal slices. CNQX (10 - 12.5 μM) and picrotoxin (10 μM) were added to the bath and the extracellular Mg^{2+} concentration ($[Mg^{2+}]$) was varied from 1 to 3 mM. The synaptic current was measured at holding potentials ranging from -110 mV to +25 mV.

The three free parameters in the following equation were fit to the observed current-voltage relation:

$$I_{syn}(V_m) = \frac{E_{syn} - V_{syn}^*}{1 + \eta\,[Mg^{2+}]\exp(-\gamma V_{syn}^*)},$$

where I_{syn} is the recorded peak synaptic current, V_m is the holding potential, E_{syn} is the reversal potential, $V_{syn}^* = E_{rest} - \delta\cdot(E_{rest} - V_m)$ is the estimated voltage at the subsynaptic membrane, E_{rest} is the resting potential, and η, γ and δ are free parameters. The first two parameters arise from a model of channel function in which the rate constant for Mg^{2+} binding to the NMDA receptor-associated channel varies as a function of voltage. The third parameter compensates for the imperfect space clamp resulting from the electrotonic distance from the soma to the synapses.

This equation provided an adequate fit to the data and the resulting parameter estimates were used to help construct a computational model (see Zador et al., Soc. Neurosci. Abstr., 1990) of LTP induction. (Supported by AFOSR)

212.13

BIOPHYSICAL MODEL OF A HEBBIAN SYNAPSE. A.M. Zador, C.K. Koch and T.H. Brown. Dept. of Psychology, Yale Univ., New Haven, CT 06520 and Computation and Neural Systems Program, Calif. Inst. Tech., Pasadena, CA 91125.

The induction of long-term potentiation (LTP) in certain hippocampal synapses depends on activation of NMDA receptors and can be described as a "Hebbian" modification (reviewed in Brown et al., Ann. Rev. of Neurosci., 13:475, 1990).

We have constructed a biophysical model for the induction of this type of hippocampal LTP. The model is similar to that of Gamble and Koch (Science, 236:1311, 1987) in that it includes electrical and Ca^{2+} dynamics in the dendritic spine. The present model differs in that Ca^{2+} influx into the spine head is through NMDA receptor-gated channels rather than through voltage-dependent Ca channels. The parameters for the NMDA receptor-mediated conductance were within the range measured in hippocampal slices (see Nobre et al., Soc. Neurosci. Abstr., 1990).

Our model accounted for much of the phenomenology of LTP induction in pyramidal neurons of hippocampal region CA1 and granule cells of the dentate gyrus. The simulations suggested that spines may perform four important functions in this Ca^{2+}-dependent synaptic modification: *compartmentalizing* transient changes in $[Ca^{2+}]$, restricting the changes to spines associated with active synapses; *amplifying* the $[Ca^{2+}]$ changes relative to the case of a synapse on the dendritic shaft; *isolating* the spine head from changes in $[Ca^{2+}]$ at the dendritic shaft; and *steepening* the voltage dependence of the processes underlying LTP induction. The latter effect was important because the current-voltage relationship for the NMDA receptor-mediated conductance is not very steep (see Nobre et al., Soc. Neurosci. Abstr., 1990).

This proposed role of spines in the regulation of Ca^{2+} dynamics contrasts with traditional approaches to spine function that have stressed electrotonic properties. We have begun to use this model to explore the self-organizing properties of Hebbian synapses in realistic representations of hippocampal neurons (see Mainen et al., Soc. Neurosci. Abstr., 1990). (Supported by DARPA and ONR)

212.14

HEBBIAN SYNAPSES INDUCE FEATURE MOSAICS IN HIPPOCAMPAL DENDRITES. Z. F. Mainen*, A.M. Zador, B. J. Claiborne and T.H. Brown. Dept. of Psychology, Yale University, New Haven, CT 06520 and Div. of Life Sciences, University of Texas, San Antonio, TX 78285.

Networks of simple processing elements (PEs) can exhibit interesting self-organizing properties when the strengths of the interconnections are governed by a Hebbian modification algorithm (reviewed in Brown et al., Ann. Rev. of Neurosci., 13:475, 1990). In studies of "neural networks", the PEs and their interconnections are sometimes likened to abstract representations of neurons and their synapses, respectively. We have been exploring the computational consequences of more realistic representations of neurons containing Hebbian synapses.

Here we evaluated the proposal (Brown et al., In: Landfield & Deadweyler (Eds.) Long-Term Potentiation: from Biophysics to Behavior, 201, 1988) that Hebbian synapses can ". . . cause the formation of spatial domains (mosaics) of enhancement among electrotonically proximal and functionally related synapses." The effects of three kinds of parameters were explored: the structure and physiology of the neuron; the details of the rules for synaptic modification; and the types of spatiotemporal patterns of synaptic input to the neuron. Hippocampal granule cells were simulated using compartmental models that captured much of the known morphology and physiology. Synaptic enhancement was based on a biophysical model of LTP induction (see Zador et al., Soc. Neurosci. Abstr., 1990) plus various models of synaptic depression. The simulated "environment" consisted of numerous spatiotemporal patterns of synaptic input activity, denoting "features".

Over a wide range of conditions, patterned inputs did indeed induce mosaics of feature-specific clusters of strengthened synapses across the dendritic tree. The nature of the mosaic pattern was dependent on all three of the above categories of parameters. This form of self-organization may have interesting computational implications for hippocampal neurons. (Supported by ONR and DARPA)

212.15

INSIGHTS INTO LTP FROM COMPUTATIONAL MODELS. SYNAPTIC INPUT DISTRIBUTIONS AND SPINE HEAD [CA^{2+}]. W.R. Holmes and W.B Levy, Math. Research Branch, NIDDK, NIH, Bethesda, MD 20892, and Dept. of Neurosurgery, U. Virginia Med. School, Charlottesville, VA 22908.

Computational models of single dentate granule cells are helping us to understand the role of Ca^{2+} and NMDA receptors in the induction of long-term potentiation (J. Neurophysiol. 63(5)). In this published model all synaptic activation occurred on the same dendritic branch. We now generalize the model by activating inputs distributed throughout the cell.

When activated inputs were distributed evenly throughout the cell, the depolarization in the cell was close to uniform. Although the soma was more depolarized when inputs were evenly distributed rather than concentrated on one dendrite, the spine heads were less depolarized. Hence, for an equal number of active afferents, spine head [Ca^{2+}] changes were smaller than when synaptic inputs were localized to one dendrite. Yet, there was still a steep nonlinearity in spine head [Ca^{2+}] as a function of frequency and the number of co-activated afferents.

White et al. (PNAS 85:2368-2372) describe a requirement for spatial convergence of weak and strong inputs to produce associative potentiation of a weak input to the dentate gyrus. Our published model fails to reproduce this experimental result; the model predicts a cooperative interaction between strong and weak inputs that are spatially separated on the outer and middle thirds of the dendritic tree. We modified the model to include inhibition. Although this new model did not completely resolve the discrepancy, the results suggest that certain spatial patterns of dendritic inhibition might be part of the answer. (Supported in part by NS15488 and NIMH RSDA K02-MH00622 to WBL.)

DEVELOPMENT AND PLASTICITY—VISUAL SYSTEM: CORTICAL ANATOMY

213.1

NUMBERS OF BLOBS IN THE PRIMARY VISUAL CORTEX OF INFANT AND ADULT MONKEYS. A.-S. LaMantia and D. Purves, Department of Anatomy and Neurobiology, Washington University School of Medicine, St. Louis, Missouri 63110.

Several regions of the primate cerebral cortex are characterized by iterated circuits or modules. It is not known whether the entire complement of such circuits is present at birth, or, alternatively, whether modules are added gradually during postnatal development. Accordingly, we have examined a particularly accessible type of module in the monkey cortex, the cytochrome oxidase positive blobs of Area 17. Three neonatal and 3 adult rhesus monkeys were anesthetized and perfused with fixative. In the left hemisphere of each animal, the primary visual cortex was dissected, flattened, sectioned tangentially, and reacted for cytochrome oxidase to reveal the areal density of blobs. In the right hemisphere, serial coronal sections were cut through the occipito-parietal region to measure the extent of Area 17. Blob density did not differ significantly between infant and adult animals. In contrast, the area occupied by the primary visual cortex was found to increase by about 50% during postnatal maturation. Multiplying the blob density in the left hemisphere by the area of the primary visual cortex determined in the right shows that the total number of blobs in the rhesus monkey brain is about 8,400 at birth and 12,000 at maturity. Evidently, these complex iterated circuits are added postnatally.

213.2

AN IMMUNOHISTOCHEMICAL STUDY OF DEVELOPING HUMAN VISUAL CORTEX. L.S. Honig*, K. Herrmann and C.J. Shatz, Dept. of Neurobiology, Stanford University School of Medicine, Stanford, CA 94305.

The developing neocortex contains not only the immature neurons that will comprise the adult cortical layers, but also a transient zone, the subplate, destined to become the sub-cortical white matter. Studies in higher mammals have shown that the subplate contains, in addition to radial glia, migrating neurons and growing axons, a transient population of neurons that receive synapses. This special zone is also prominent in humans. To identify cellular elements and interactions in human cortical development, we examined visual cortex at conceptional ages 24 to 38 weeks (W), using neuron-specific (GAP-43 [D. Schreyer]; MAP-2 [R. Vallee]) and glial-directed (GFAP [L. Eng]; vimentin) monoclonal antibodies. At 24-38W, anti-vimentin densely immunostained radial glial cells in the ventricular and subventricular zones; subplate staining increased from 24W to 38W. Anti-GFAP stained astrocyte somata and processes strongly throughout cortex and subplate, intensifying from 24W to 38W. Immunoreactivity for GAP-43, an axonally transported growth associated protein, was most strongly present in a bilaminar pattern of fibers running parallel and just above the ventricular surface at 24W. The location of these fibers corresponds to the optic radiations and other long axonal projections. Subsequently, GAP-43 immunoreactivity increased in cortex and subplate, and by 38W became particularly intense in layers I (marginal zone) and IV. In contrast, reactivity to MAP-2 was strongest in the somata and processes of subplate neurons at 24W, and in neuronal cell bodies of the forming cortical plate, particularly the pyramidal cells of layer V. Staining of processes also was noted in the subventricular zone. Thus, the developmental pattern of immunostaining in humans for these antigens resembles that of other mammals, suggesting common cellular mechanisms in neocortical development. Supported by NIH EY02858 (CJS), Dana Fellowship (LSH), NATO (KH), and Fight for Sight (KH) grants.

213.3

CORRELATION OF GENICULOCORTICAL GROWTH INTO THE CORTICAL PLATE WITH THE MIGRATION OF THEIR LAYER 4 AND 6 TARGET CELLS. B.S. REINOSO and D.D.M. O'LEARY, Depts of Neurosurgery and of Anatomy & Neurobiology, Washington Univ Sch Med, St. Louis, MO 63110.

We have studied the timing of ingrowth of geniculocortical axons into the developing cortical plate in relation to the migration of their layer 4 and 6 target cells. Time-pregnant dams were injected intraperitoneally with the thymidine analogues tritiated thymidine ([3H]TdR) at E14 and 5-bromodeoxyuridine (BrdU) at E16 to label layer 6 and layer 4 neurons, respectively. Some embryos were removed by C-sections at ages 18-21; the remaining pups were delivered normally and analyzed on P30 to confirm the population of cortical neurons labeled by the thymidine analogues. The embryos and newborn pups (P0-P2) were perfused with 4% paraformaldehyde, their brains removed, transected rostral to the colliculi and DiI crystals placed in the region of the dorsal lateral geniculate nucleus (dLGN). The brains were stored in a 28-30o oven and vibratome-sectioned (100um thick) 1-2 months later. By E18, when geniculocortical (GC) projections are first seen in the occipital pole (Reinoso and O'Leary, Soc Neurosci. Abst., 1988), most layer 4 cells are still migratory although some have already reached superficial regions of the cortical plate (CP). By this time, layer 6 cells have already reached the deep cortical plate. At E19, GC axons are within the subplate area whereas many presumptive layer 4 cells have reached the cortical plate (some are still migrating). At these developmental stages, many GC axonal collaterals were noted within layer 6. Furthermore, some bipolar and subplate-like cells were labeled. GC axons invade layer 6 by E20 and start extending into layer 4 by birth (P0). By P2, GC projection to layer 4 is quite robust. Thus, GC axons do not "follow" layer 6 and 4 cells as they migrate through the intermediate zone and into the cortical plate. Rather, the nascent layers 6 and 4 are invaded by GC axons about 3 days after each of these neuronal populations reach their positions in the cortical plate.

213.4

DENDRITIC DEVELOPMENT OF LAYER III PYRAMIDAL CELLS IN KITTEN VISUAL CORTEX. N. Zec and S.B. Tieman, Dept of Pathology, Hershey Medical Ctr, Hershey PA 17033 and Neurobiology Research Ctr, SUNY, Albany NY 12222.

The cat's visual cortex is immature at birth and undergoes extensive postnatal development. For example, cells of layers II and III do not complete migration until about 3 wks postnatal (Shatz and Luskin, J. Neurosci. 1986, 6:3655). Despite the importance of dendritic growth for synaptic and functional development and the many studies on visual cortical development in the cat, there are few studies of dendritic development in this system. Accordingly, we have used the Golgi method to study the development of the dendrites of layer III pyramidal cells in the kitten's visual cortex. A series of kittens were deeply anesthetized and perfused with 10% formalin. Blocks of visual cortex were impregnated by the Golgi-Kopsch method and sectioned in the tangential plane. Layer III pyramidal cells were drawn with a camera lucida and analyzed by Sholl diagrams. In kittens less than 2 wks old, these cells are very immature, with only an apical dendrite and few or no basal dendrites. By 2 wks, all of the basal dendrites have appeared, but they have few branches and are tipped with growth cones. By 4 wks, they have finished branching, but continue to grow in length until, by 5-6 wks, they have reached their adult size. Thus, dendritic development occurs very rapidly over a time period that precedes and overlaps with the peak periods of synaptogenesis and of sensitivity to the effects of early visual experience. (Supported by NSF grants BNS 8217479 and 8811039 to SBT.)

213.5

MORPHOLOGY OF KITTEN VISUAL CORTICAL NEURONS DURING THE CRITICAL PERIOD, LABELLED BY INTRACELLULAR BIOCYTIN INJECTIONS. K. Grant, Y. Frégnac, F. Hester*, M. J. Friedlander, D. Smith*, and D. Debanne* Neurobiology R.Center, Univ. of Alabama at Birmingham, AL 35 294.

The activity of neurons of the primary visual cortex was recorded intracellularly in "in vitro" preparations taken from kittens at the peak of the critical period (5 weeks of age). Most cells, recorded in 450 µm thick slices, in standard bicuculline free ACSF, exhibited a "regular" pattern of excitability, as classically reported in adult neurons.

21 neurons were labelled by intracellular iontophoresis of biocytin (concentration 5%, dissolved in 0.05M TRIS buffer (pH= 7.4) containing 0.5M KCl; electrode resistance 60-120 MΩ, current pulses: -0.5/-1.0 nA, 200/300 ms duration, added on a constant -0.5 to -1.0 nA current). The tissue was fixed by immersion overnight in 4% paraformaldehyde and 0.1% glutaraldehyde in phosphate buffer, and resectioned at 80 µm after albumen/gelatin embedding. Slices were pretreated in alcohol to extinguish endogenous peroxidase activity. Labelling was revealed by incubation (40 mins on ice) in ABC complex (Vectastain) followed by reaction with DAB.

Biocytin labelling showed that neurons of many different morphological types had been recorded - spiny pyramidal cells, smooth cells, layer IV spiny stellate cells - although these showed rather uniform electrophysiological properties. Dendritic arborisations were comparable in their complexity to those found in the adult. Axonal arbors were labelled over several millimeters in these slices, revealing the presence of long horizontal connections (in both supra- and infragranular layers), as well as descending projections towards the optic radiations. In some cases horizontal axons showed a periodic branching pattern, with a spatial period similar to that of the intracortical orientation network. In supragranular spiny pyramidal cells, recurrent axonal collaterals projected back through the dendritic territory of the parent cell.

From these neurons identified at the peak the critical period for visual development, it is concluded that 1) their morphology and their axonal projections were essentially adult-like, and 2) most of them showed "regular" firing. It is therefore unlikely that neuronal susceptibility to activity-dependent changes during the critical period results from spontaneous bursting behavior, or from an immature morphology.

213.6

DEVELOPMENT OF SOMATIC INHIBITORY SYNAPSE COVERAGE ON THALAMIC RECIPIENT NEURONS OF MACAQUE MONKEY PRIMARY VISUAL CORTEX. J.S. Lund and T.R. Harper. Department of Psychiatry and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261

Two channels of visual input, differing in physiological properties, terminate on spiny stellate neurons in separate divisions (alpha and beta) of layer 4C in cortical area V1. We have examined the postnatal development of type II (inhibitory) synapses on the cell bodies of these neurons. A clear temporal and numerical correlation was found between the alpha and beta neurons in terms of the percent of their cell body surface area occupied by synapse apposition sites. This coverage rises swiftly after birth to a peak on both groups around 12 weeks postnatal, falling by 24 weeks to a level close to that found in the adult. This common developmental pattern of inhibitory coverage contrasts with the development of excitatory synapses and dendritic arbors which show quite different patterns of development between birth and thirty weeks of age on the alpha and beta neurons. This suggests that development of inhibitory coverage is coordinated between the two layers and does not directly relate to the ongoing differential development of excitatory contacts and dendritic arbors on the two cell groups. Supported by EY05282.

213.7

DEVELOPMENT OF SYNAPSES IN MACAQUE MONKEY STRIATE CORTEX SHOWS AN "INSIDE-OUT" PATTERN. B. Zielinski# and A.E. Hendrickson§, Dept. Biological Sciences #, Univ. Windsor, Windsor, Ontario N9B3P4 Canada and Dept. Biological Structure & Ophthalmology §, Univ. Washington, Seattle, WA 98195.

A quantitative study of synaptic density (contacts/100µm2) during development of primary visual cortex has found at gestational day (E) 75 that immature contacts occurred mainly in the marginal zone (adult layer 1) or in the subplate (adult white matter). Those few contacts in the cortical plate were confined to layer 6. A comparison of synaptic density and morphology in layer 2 vs layer 6 was done from E113 - adulthood. Before birth synapses in layer 2 contained fewer vesicles and had minimal synaptic densities, while those in layer 6 were much more mature. Synaptic density in upper layers is relatively low at E113 (2 vs. 8), and still lags slightly behind lower layers at E152 (25 vs 28) and postnatal (P) 2d (32 vs 37). By P 12 wks synaptic density is 30% higher in layer 2 (69 vs 49). By P 2 yr synaptic distribution has declined to the approximate level at birth (44 vs 33), is relatively even throughout all layers and is similar to adult density (37 vs 39). Between P 12 wk and P 6 yr asymmetric contacts in all layers acquire more pre- and postsynaptic membrane density that clearly differentiates between asymmetric and symmetric types.

These results confirm other work showing a transient overproduction of synapses in early postnatal monkey cortex. Our laminar analysis shows that visual cortex has a generalized "inside-out" prenatal synaptogenesis pattern which is more correlated with neuron age than input. After birth upper layers have the highest synaptic density, and may take slightly longer to reach final adult levels. (EY01208 and EY04536)

213.8

THE DEVELOPMENT OF CORTICOGENICULATE CONNECTIONS IN THE FERRET A. Claps[1] and V.A. Casagrande[1,2]. Dept. of Cell Biology[1] and Psychology[1,2], Vanderbilt Univ., Nashville, TN 37232.

In ferrets lateral geniculate nucleus (LGN) layers form between posnatal day 7 (P7) and P14 and do not form without retinal input (Guillery, *J.Neurosci.* 5:1370). However, the LGN also receives input from the visual cortex and it is unclear what role extraretinal input plays in LGN maturation. To address this issue, we studied the development of corticogeniculate axons in ferrets, by injecting WGA-HRP and ^{3}H proline into area 17, at weekly intervals from P0 to P28. In addition, we examined axonal morphology in adults following Biocytin injections and *in vitro* at P0 following DiI injections. In the adult, injections of WGA-HRP, produce columns of anterograde and retrograde label, that extend across all cell layers and interlaminar zones (ILZs). Injections of ^{3}H proline produce anterograde label only, that is densest within the ILZs. Corticogeniculate Biocytin labeled axons are structurally simple with *en passant* boutons and short collateral side branches (see also Condo et al. this meeting). At P1, the same tracers produce light diffuse label in the LGN. DiI at the same age labels many axons and cells; the axons that appear to be of cortical origin are fine and beaded. At P7, the corticogeniculate projections are more dense and organized into discrete but still uniform columns. Even at P14 when ILZs are evident corticogeniculate axons are still not concentrated in these zones. The corticogeniculate projection appears adult-like by P28. Thus, the temporal sequence of maturation of corticogeniculate axons does not seem to be correlated with ILZ formation. However, the results show that cortical axons are already in the LGN, at a time when they could influence the process of retinal afferent segregation. Supported by EY05038 and BNS 8708429.

213.9

MORPHOLOGY OF CORTICOGENICULATE AXONS IN ADULT FERRETS (*Mustela Putoris*). G.J. Condo,[1] A. Claps*,[1] and V.A. Casagrande[1,2]. Dept. of Cell Biology[1] and Psychology[2], Vanderbilt Univ., Nashville, TN 37232.

The lateral geniculate nucleus (LGN) receives a major input from visual cortex. While the function of this input is not fully understood, one approach to understanding the organization of this pathway is to determine the anatomical details of corticofugal axons. In this study we examined the structure of individual axons labelled in the LGN and adjacent nuclei following injections of Biocytin in area 17 of adult ferrets. The majority of corticogeniculate axons resemble the Type I axons described in the cat (Guillery, *J. Comp. Neurol.*, 128:21-50, 1966; Robson, *J. Comp. Neurol.*, 216: 89-103, 1983). These axons are of fine caliber and typically course perpendicular to the LGN laminae with terminals *en passant* and at the end of short collateral stocks in both the A- and C-laminae. Similar to the pattern seen following ^{3}H proline injections in developing ferrets (Claps and Casagrande, this meeting), a subset of axons have more extensive terminals in interlaminar and interleaflet zones and/or long lateral branches and terminals in the C-laminae. Single axons are often seen terminating in both the perigeniculate nucleus (PGN) and A-laminae of the LGN. In contrast, fine axons terminating in the medial interlaminar nucleus (MIN) appear to be a separate population, having more complex terminals which end in clusters of boutons aligned along a projection column. Large caliber axons terminating in the lateral posterior nucleus (LP) have larger boutons and more elaborate terminal fields than axons found in the LGN, PGN, or MIN. These results suggest that in the ferret the feedback pathway to the thalamus is complex and composed of several classes of axons with different terminal patterns. Supported by EY05038 and BNS 8708429.

213.10

POSTNATAL DEVELOPMENT OF VISUAL CALLOSAL PROJECTIONS IN THE STRIATE CORTEX OF THE RABBIT. A.M. Grigonis[1], Y. Wang*[2] and E.H. Murphy[2]. Departments of Anatomy, [1]Hahnemann University, Philadelphia, PA 19102-1192, and [2]Medical College of Pennsylvania, Philadelphia, PA 19129.

The cell distribution of the corpus callosum (CC) projection in the visual cortex of the adult rabbit is restricted to the 17/18 border. Previous studies have reported exuberant visual callosal projections, extending throughout area 17, on day 7, and mature, restricted callosal projections on day 15 (Chow, et al., *Exp. Brain Res.*, 42:126, 1981). We determined the postnatal development of callosal projections in normal Dutch-Belted rabbits at intermediate ages between 1 and 15 days. Multiple injections of HRP (Boehringer, 20% in H_2O) were made throughout one entire visual cortex (9 µl). Animals were perfused 24 hours later and the brains were cut and reacted with TMB. In three animals, following fixation, DiI was injected into one visual cortex, the brains were stored for six weeks, cut and observed under florescence microscopy. On the day of birth the tangential extent of the CC cell distribution was exuberant, 47% more than the adult tangential extent. At the time of eye opening, which occurs between days 10 and 11, the CC cell projection was also exuberant, 38% more than the tangential extent of the adult. Since we have previously demonstrated a maintenance of callosal exuberance in rabbit visual cortex following deprivation of visual experience, the present data support the hypothesis that the potential for visual experience to modify callosal development depends on the degree of callosal maturity at the time of eye opening. Supported by grant NS26989.

213.11

TRANSCALLOSALLY EVOKED RESPONSES IN THE VISUAL CORTEX OF NORMAL AND MONOCULARLY ENUCLEATED RABBITS. R.J. Clarke, B. Datskovsky*, A.M. Grigonis[1], and E.H. Murphy. Depts. of Anatomy, Medical College of Pennsylvania, Phila. PA, and [1]Hahnemann University, Phila., PA.

In the visual cortex of normal adult rabbits, callosal fibers originate and terminate in a narrow zone at the 17/18 border. In adult rabbits which are monocularly enucleated (ME) on the day of birth, the callosal zone maintains an immature pattern, extending into the medial regions of area 17 in the cortex contralateral to the enucleated eye. In order to determine the functional topographic organization of this expanded callosal zone, we investigated the region over which stimulation could evoke a transcallosal response. Visual field mapping was used to identify the 17/18 border electrophysiologically in the cortex of normal animals and in the cortex contralateral to the intact eye in ME rabbits. The electrode was maintained in this position to record responses to stimulation of the opposite cortex with bipolar electrodes placed in a homotopic location. The stimulating electrodes were then moved medially in 1mm steps, and the responses evoked at each location averaged. In the normal rabbit, stimulation at a position homolateral to the recording electrode evoked a consistent response with a positive deflection at a latency of 16-18ms. At a distance of 1mm or more from this optimal position, this short latency response was either absent or dramatically decreased in amplitude, reflecting the precise topographic projection pattern of the normal callosal projection. In ME rabbits, stimulation at a position homolateral to the recording electrode also evoked a consistent positive deflection response with a latency of 17-20ms, but responses of similar or only slightly decreased amplitude could be evoked when the stimulating electrodes were moved 2 or 3mm medial to this optimal position. Thus, the expanded callosal projection can be demonstrated electrophysiologically, suggesting that abnormal callosal projections in ME rabbits can mediate functional interactions between topographically disparate areas of the primary visual cortices. Supported by grant NS26989.

213.12

DEVELOPMENT OF OCCIPITAL CALLOSAL AXONS IN HAMSTER: ANTEROGRADE TRACING WITH DI-I. S.E. Fish, R.D. Mooney, and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699 and Dept. of Anatomy, Marshall University School of Medicine, Huntington, WV 25704.

Anterograde diffusion of Di-I was used to label callosal axons in hamsters from birth (P-0) through P-12. On P-0 and P-1, labelled fibers were visible crossing the midline in both the corpus callosum and anterior commissure, but only a very small number were seen in the cortex contralateral to the tracer deposits. As these ages, many fibers ended in elaborate growth cones. By P-2, many axons were visible in the white matter beneath presumptive area 17. These fibers gave off many branches extending short distances into the subplate. A smaller number of adult-like fibers with short side branches reached all the way to the pial surface. On P-2, long fibers with many side branches were also visible in the marginal layer paralleling the pial surface. By P-3, many more fibers extended from the white matter to the pia and they were similar to callosal fibers in adult hamsters. By P-4, numerous mature callosal axons could be seen and they appeared to be concentrated in the region of the presumptive area 17-18a border. At this age, axons in the white matter still gave off many short collaterals and a few fibers had long horizontal trajectories in layers V and VI. By P-6, most of the short branches of callosal axons had disappeared and the vast majority of labelled fibers were similar to those that have been observed in normal adult hamsters. Supported by EY 04170 and EY 08015.

213.13

NEONATAL HYPOXIA-ISCHEMIA INCREASES THE NUMBER OF VISUAL CALLOSAL PROJECTIONS IN THE CAT. B.L. Finlay, B. Miller, S. Nioka,* D. Nagy*, A. Zaman*, and B. Chance. Department of Psychology, Cornell University, Ithaca, NY, 14853, and Department of Biochemistry and Biophysics, University of Pennsylvania, Philadelphia, PA, 19104.

Perinatal hypoxic-ischemic insult has been shown to produce neuroanatomical and behavioral pathology. One of the main features of perinatal cortical development is the exuberance and retraction of transient callosal projections. We examined the effect of hypoxia-ischemia on developmental axon retraction.

Cats were subjected to hypoxia-ischemia by bilateral carotid artery ligation at 7 days of age. The cats were given neurological examinations, every week for 2 months and then bi-weekly up to 3 months of age, consisting of locomotor, superficial and deep reflex and sensory nerve function, including visual cliff and visual placing tasks. At 3 months of age, multiple HRP injections were made into the visual cortex of one hemisphere during aseptic surgery. The tangential extent of retrogradely labeled callosal cells was measured from the border between primary and secondary visual cortex, and the number of cells was counted in 200μm increments. A comparison of the distribution of callosally projecting cells was made between 4 hypoxic-ischemic animals and 3 controls.

Two hypoxic-ischemic animals failed the visual cliff task. These animals were also grossly hydrocephalic. Performance on other visual and behavioral tasks were normal. The hypoxic-ischemic animals had twice the number of callosally projecting cells. The overall tangential extent of callosal cells was not different than normals.

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213.14

DII LABELED INTERHEMISPHERIC FIBERS DURING CAT POSTNATAL DEVELOPMENT SHOW TRANSITORY CORPUS CALLOSUM AXON TERMINAL DISTRIBUTIONS THROUGHOUT STRIATE CORTEX WITHIN SUPRA- AND INFRAGRANULAR LAMINAE. A.J. Elberger. Dept. of Anatomy and Neurobiology, Univ. Tennessee, Memphis Memphis, TN 38163.

Previously it was shown that an intact corpus callosum (CC) during postnatal month 1 in cat is critical to normal visual functional development; this was hypothesized to be due to extensive, abundant CC/visual cortex interactions in early postnatal development (Elberger 1984; Elberger & Smith 1985). To examine this hypothesis CC axons/terminals were labeled by placing crystals of the lipophilic dye, DiI, in vitro in the mid-sagittal CC of normal cats 1-13 weeks old. The number and location of CC axons in striate cortex were compared at different developmental stages.

The results show that in the youngest cats, CC axons penetrate supra- and infragranular layers throughout the visual field representation; terminals are in layers 1-6. At increasing ages axons are reduced first in intermediate and then peripheral/central regions of the visual field representation. In older animals CC axons are located in striate cortex predominantly, but not exclusively, within the central visual field representation. This indicates that many transitory CC axons have the opportunity to interact with developing cortical cells representing all of the visual field. The abnormal visual development that follows neonatal CC section may be due to lack of CC and cortical interaction. Support by EY08466, (BRSG) RR05423.

213.15

Postnatal development of the connections of the feline cortical region extends along the anterior ectosylvian sulcus (AEs): WGA-HRP study. **Masao Norita, and Hideaki Shimizu*. Dept., Anat., & Embryol., Tokyo Metropolitan Institute for Neurosciences, Fuchu, Tokyo 183, Japan.**

Cortical and subcortical projections to and from the AEs in neonatal cats were studied with anterograde and retrograde WGA-HRP methods. The projections of AEs with cortical and subcortical structures are already present at birth, and the origins and terminations of the most of these projections are arranged topologically. Following deposit of the tracer in AEs, cortical label was found mainly in the presylvian sulcus, the cingulate gyrus, the cruciate sulcus, the medial prefrontal area, the splenial sulcus, the lateral suprasylvian sulcus, the posterior suprasylvian gyrus, the posterior rhinal sulcus; subcortically, thalamic label could be detected in the VM, LPm and LM-Sg, and axonal label was seen in the superior colliculus and the pontine nuclei. However, the cortical laminar distribution of the afferents arising from AEs changes with aging: in neonates, relatively dense label was found in layer I, the infragranular layers and white matter, while in adults labeling was seen mainly in the supragranular layers.

These data indicate that the major neuronal connection of AEs seems to be a prenatal event, while its corticocortical projection is still developing postnatally.

213.16

SEX DIFFERENCES IN CORTICAL DEVELOPMENT WITH VISUAL DEPRIVATION. D.E. Fleming, R.B. Burr* and R.W. Rhees Departments of Psychology and Zoology, Brigham Young University, Provo UT 84602

Control and visually deprived albino rats of both sexes were compared with respect to size (area) and thickness of Brodmann's areas 17, 18 and 18a of the cerebral cortex. Both groups were reared under conditions of a 12/12 light/dark schedule for 90 days, then were sacrificed. The light phase for the visually deprived group was dim red light. The cortical size and thickness measurements of the control males were greater than those of the control females. With visually deprived animals, both males and females had smaller cortical values than did the control animals. However, males and females of the deprivation group did not differ from each other in their cortical measurements. The control males had a modest R > L cortical asymmetry that was not present in female rats of either treatment group or in visually deprived males. These data suggest that varied levels of visual experience are important not only for maintaining brain size but also sex differences. (Funding provided by the College of Family, Home and Social Sciences, Brigham Young University.)

213.17

VISUAL CORTEX METABOLIC ACTIVITY INCREASES IN RESPONSE TO FLASHING DIFFUSE LIGHT IN NEONATAL RATS. A. C. Gafka and R. M. Cooper. Behavioral Neuroscience Research Group, Psychology Dept., University of Calgary, Calgary, Alberta, Canada, T2N 1N4.

Past 2-deoxyglucose (2-DG) work has shown that normal lab-reared adult rats monocularly exposed to flashing diffuse light (one eye covered with translucent mask) show increased metabolic activity in contralateral sub-cortical visual structures, with visual cortex remaining virtually unresponsive relative to the unstimulated hemisphere. In the present study, neonatal rats at the time of eye opening (14 d), to 3 weeks of age (28 d) were injected with 2-DG and monocularly exposed to 4 Hz flashing diffuse light (one eye covered by a translucent mask, the other by an opaque occluder). In all neonatal groups, flashing diffuse light significantly increased cortical metabolic activity in monocular area 17 and in area 18a of the hemisphere contralateral to the diffusely masked eye. These findings suggest that at the time of eye opening and shortly after, visual cortex neurons in the rat lack adult-like receptive field specificity; the development of response selectivity may be the result of experience, maturation, or both.

MEMBRANE COMPOSITION AND CELL SURFACE MACROMOLECULES I

214.1

A HIGH MOLECULAR WEIGHT CHONDROITIN SULFATE PROTEOGLYCAN FROM BRAIN IS RELATED TO THE LARGE AGGEGATING CARTILAGE PROTEOGLYCAN. H.J.L. Fryer, L. Molinaro*, and S. Hockfield. Sect. of Neuroanat., Yale Univ. Sch. of Med., New Haven, CT 06510.

Cat-301 is a monoclonal antibody which stains the surfaces of subsets of neurons in the CNS. The expression of the Cat-301 antigen coincides with the end of periods of synaptic plasticity during postnatal development and is dependant upon normal patterns of neuronal activity. We have recently shown (Neuron 2:1207) that the Cat-301 antigen is a high molecular weight chondroitin sulfate proteoglycan (CSPG).

Using Western blot analysis of tissue extracts from cat we have determined that a Cat-301 antigen, similar in molecular weight to the brain CSPG, is found in non-neural tissues including articular cartilage (AC). The AC antigen is similar to the brain CSPG in many respects. Both react with a second monoclonal antibody, Cat-304. Both are CSPGs, shown by a shift in apparent molecular weight of the antigen following digestion with chondroitinase ABC and by identification with antibodies to the stubs of chondroitin sulfate chains following chondroitinase digestion. The two CSPGs are distinct in that AC CSPG has a much higher buoyant density. Keratan sulfate substitution on the AC CSPG, which is not found on the brain CSPG, may account, in part, for this difference.

Cartilage contains high molecular weight CSPGs which bind to hyaluronic acid and form large aggregates. The AC CSPG which stains with Cat-301 belongs to this family of aggregating proteoglycans. In the presence of added hyaluronic acid the brain Cat-301 CSPG is found in higher density fractions of CsCl gradients, indicating that it too may be an aggregating proteoglycan. Our data suggest that the high molecular weight brain Cat-301 CSPG may belong to the family of large aggregating proteoglycans identified in other tissues. [Supported by EY06511]

214.2

DISTINCT NEURONAL SUBSETS EXPRESS ANTIGENICALLY DISTINCT SURFACE PROTEOGLYCANS IN MAMMALIAN CNS. Susan Hockfield, Sam Zaremba and Gail Kelly*. Sect. of Neuroanatomy, Yale Univ. School of Medicine, New Haven, CT, 06510.

Monoclonal antibody Cat-301 recognizes a suface-associated chondroitin sulfate proteoglycan. The Cat-301 proteoglycan is expressed on subsets of neurons in several areas of the mammalian CNS. We have demonstrated that the expression of the Cat-301 proteoglycan develops postnatally and is regulated by neuronal activity during a circumscribed period in development. We now report that Cat-301 is one member of a family of chemically related, neuronal cell surface-associated antigens. Antibodies to chondroitin sulfate moieties reveal related, but distinct, staining patterns to that observed with Cat-301. Three different antibodies all show the same surface-associated localization previously observed with Cat-301. This surface-associated staining develops postnatally, but with a time course different from that of Cat-301. Each antibody recognizes a subset of neurons distinct from the other and from that recognized by Cat-301. Consistent with this finding, we have identified several high molecular weight antigens on Western blots that react with antibodies to chondroitin sulfate but not with Cat-301.

These results are consistent with the possibility that distinct proteoglycans are present at the surfaces of functionally distinct neuronal subpopulations. Their spatial and temporal appearance suggests that they might play a role in specific recognition events during the acquisition of mature CNS structure. [Supported by EY06511]

214.3

KERATAN SULFATE PROTEOGLYCANS ASSOCIATED WITH NEURONAL SURFACES IN CNS. Sam Zaremba, Gail Kelly*, Robert Kalb and Susan Hockfield. Sect. of Neuroanatomy, Yale Univ. School of Medicine, New Haven, CT 06510.

In order to further our understanding of the molecular structure of the mature mammalian CNS we have been interested in the identification and characterization of CNS proteoglycans. Here we demonstrate that antibodies to keratan sulfate (KS) identify antigens on the surfaces of neuronal subpopulations in adult CNS of several mammalian species. KS immunoreactivity is discontinuous along the surface of neuronal cell bodies and extends a considerable distance along neuronal processes. We have shown that some of the KS reactive cells in hamster spinal cord are motorneurons by retrograde labeling with Fast Blue from the sciatic nerve. Staining is abolished by preadsorbtion of anti-KS antibodies with KS, demonstrating that the surface antigen is a KS-containing moiety.

Previous biochemical analyses demonstrated the presence of KS immunoreactivity in CNS but had not identified the macromolecules responsible for such reactivity. We have identified major anti-KS reactive molecules as 600-700 kd species on Western blots of unfractionated CNS homogenates. In addition, we have reported that one KS-positive molecule is part of the high molecular weight glycoconjugate immunoprecipitated by mAb VC1.1 (Zaremba et al, J. Neurosci, in press). Consistent with this identification, we also demonstrate that individual neurons in cat CNS can be double labeled with VC1.1 and anti-KS antibodies. The diversity of surface-localized proteoglycans (KS and other types) may be a means for generating specificity in recognition events in the developing CNS and stabilizing those connections in the adult CNS. [Supported by EY06511]

214.4

PG-1000: A GENERAL PROTEOGLYCAN COMPONENT OF BRAIN EXTRACELLULAR MATRIX ? M. Iwata, and S.S. Carlson. Dept. of Physiology and Biophysics, University of Washington, Seattle, WA.

PG-1000 (formerly TAP-1) is a large (10^6 daltons) chondroitin sulfate proteoglycan identified, purified, and characterized from electric organ. When the purified molecule is visualized in the electron microscope it has the "bottlebrush" structure of a large space-filling proteoglycan with a total length of about 350nm and about 20 side chains of 110 nm (Carlson and Wight, J Cell Biol. 105: 3075, 1987). Originally, it was proposed that this molecule might act as a nerve terminal anchorage protein, because of its apparent exclusive nerve terminal localization. However, using a new monoclonal antibody which identifies a protease sensitive epitope (T1) on PG-1000, we find that this proteoglycan is has a much wider cellular distribution. By immunocytochemistry it is present on both the synaptic and non-synaptic face of the electrocyte and the Schwann cell basement membrane in electric organ.

PG-1000 is present throughout the fish and mammalian brain. Immunocytochemical localization of the T1 antigenicity shows it to be present in both the gray and the white matter. Strong staining of T1 antigenicity is seen surrounding the large electromotor nucleus cells in the fish. In fish and mammalian brain extracts the T1 antigenicity behaves like a proteoglycan, eluting from DEAE Sephacel at about 0.7M NaCl with the pH 4.9. In addition, the fish brain antigen shows a shift in molecular weight upon digestion by chondroitin ABC lyase. Finally, PG-1000 can only be extracted from brain tissue under denaturing conditions. This is quite unlike most proteoglycans which have been studied in the brain; these are solubilized by salt extraction or non-denaturing detergents. In the brain, PG-1000 behaves like it does in peripheral nervous tissue, as a component of a detergent insoluble matrix.

Because PG-1000 appears to be present throughout the brain and acts like part of an insoluble matrix, we hypothesize that it is a general extracellular matrix component of the adult brain. Only a few general components of such a matrix are known

214.5

PROTEOGLYCAN-LIKE ANTIGEN MOLECULES IDENTIFIED BY MONOCLONAL ANTIBODIES GIVING PERINEURONAL STAINING IN THE RAT BRAIN. S.C.Fujita, J.Kudo, and S.Sano. Laboratory of Neurochemistry, Mitsubishi Kasei Institute of Life Sciences, 11 Minamiooya, Machida 194, Japan

Monoclonal antibodies(MAbs) 473 and 376 stain perimeters of limited numbers of central neurons that form partially overlapping subsets, and these immunoreactivities are obliterated by pretreatment of the sections with chondroitinase ABC. MAb 1B5 with established specificity to an epitope uncovered by chondroitinase ABC also stains a subset of central neurons in their perimeter. (Fujita et al., Neurosci. Res., 7, 117, (1989)) The antigens for these MAbs were sought by immunoblotting in the rat brain homogenate. Using acrylamide/agarose gels, diffuse bands of low mobility were found. The bands for MAbs 473 and 376 were not detected after, and that for MAb 1B5 was detected only after, treatment of the blots with chondroitinase ABC. The 473^+ and 376^+ bands were not found in tissue homogenates of rat liver, skeletal muscle, or kidney. Molecular weight of the antigens was estimated to be around 700kD, a size similar to that of Cat-301 antigen proteoglycan. (Zaremba et al., Neuron, 2, 1207 (1989))

214.6

WITHDRAWN

214.7

Ganglioside binding activities solubilized from rat brain membranes. P.J. Schwartz, I. Krop*, J.A. Mahoney*, P. Swank-Hill*, and R.L. Schnaar. Depts. of Pharmacol., Neurosci., and Toxicol., The Johns Hopkins Medical Institutions, Baltimore MD 21205

Gangliosides and complementary ganglioside binding proteins may be involved in neural membrane interactions. We synthesized and used conjugates of ganglioside G_{T1b} bound to radioiodinated bovine serum albumin to identify a high-affinity, ganglioside-specific binding protein on isolated rat brain membranes (Tiemeyer, M., et al. (1989) *J. Biol. Chem.* **264**, 1671).

Using differential detergent extraction, we defined two pools of ganglioside binding activity in crude rat brain P2 membranes. A "detergent sensitive" pool was extracted with 0.1% Triton X-100 in low ionic strength buffer. A "detergent resistant" pool required 0.5% Triton X-100 and 400 mM KCl for solubilization. Binding activities were assayed using a polyethylene glycol precipitation assay. The detergent resistant ganglioside binding activity was found predominantly on myelin membranes (Tiemeyer, M., et al. (1990) *J. Biol. Chem.* **265**, in press), while the detergent sensitive activity was enriched in synaptosomes. Both activities were inhibited by similar gangliosides and selected phospholipids. The two ganglioside binding pools may reflect different molecular receptors, or similar receptors associated with brain membranes in different ways. Supported by PHS Grants HD14010, GM07626 and ES07141.

214.8

DIRECT EVIDENCE FOR INVOLVEMENT OF MEMBRANE GLYCOSPHINGOLIPIDS IN SKELETAL MUSCLE DIFFERENTIATION. L.D. Cambron and K.C. Leskawa. Dept. Anatom. Sci. & Neurobiology, University of Louisville, Louisville, KY 40292

We have reported that differentiation of skeletal muscle cells in vitro was characterized by transient increases in glycosphingolipid synthesis at the time of contact and membrane fusion. This response was abberant in dystrophic avian myoblasts and absent in fusion defective varients. Also, normal myoblast contact was accompanied by a dramatic rise in lactosyltransferase activity (GalT-2), which was missing in fusion-defective cells (Leskawa et al., Soc. Neurosci., 1985, 1986, 1987, 1989).

To further explore roles of membrane GSLs in myoblast fusion, we examined the effect of an inhibitor of glucosylceramide synthase (Glc-T), D-threo-PDMP, on clonal (E63) myoblast fusion. Exposure of normal E63 rat myoblasts to PDMP resulted in an inhibition of fusion (20% of control). This inhibition was partially overcome by concurrent incubation of cells with PDMP and exogenous LacCer or ganglioside GM3 (60 to 70% of control). PDMP did not affect the synthesis of protein, DNA or phospholipids. Incorporation of serine into sphingolipids was unaffected.

These studies directly demonstrate a role for membrane glycosphingolipids in formation of multinucleated myotubes by skeletal myoblasts in vitro.

214.9

EXPRESSION OF CARBOHYDRATE ANTIGENS IN RAT OLFACTORY CELL CULTURES. G.A. Schwarting, M. Yamamoto, D. Gattey* and J.E. Crandall, E.K. Shriver Center, Waltham, MA 02254; and Program in Neuroscience, Harvard Medical Sch., Boston, MA.

We have previously described the expression of three unique carbohydrate antigens (CC1, CC2, and 1B2) in the rat olfactory system. CC1 antibody reacts with an N-acetylgalactosamine containing glycolipid which is expressed in the vomeronasal organ (VNO), vomeronasal nerve (VNN) and the accessory olfactory bulb (AOB). CC2 antibody, which reacts with α-galactose and α-fucose-containing glycoconjugates and 1B2 antibody, which react with a β-galactose terminal glycolipid, stain the VNO, VNN and AOB and also are on subsets of the main olfactory epithelium, olfactory nerve and olfactory bulb. Cells taken from embryonic day 15 rat olfactory epithelium are grown in serum free medium on a laminin substratum. Within a day of plating, the laminin surface is coated with a layer of non-neuronal cells. Olfactory epithelial cells grow on the surface of a non-neuronal monolayer either in large aggregates or as individual cells. Double label immunofluorescence studies have demonstrated that CC1 is co-expressed with $NCAM^+$ cell bodies at the periphery of $keratin^+$ aggregates. However, only the proximal portion of $NCAM^+$ processes are $CC1^+$ $CC2^+$ cells are $NCAM^+$ and $keratin^-$. At day 5 in culture they appear as columns of cells migrating away from the epithelial aggregates. 1B2 cells are $keratin^+$ and $NCAM^+$. These data suggest that 1B2 may be a marker for immature olfactory neurons.

214.10

CHARACTERIZATION OF PHOSPHOLIPIDS AND OTHER MEMBRANE COMPONENTS OF THE JELLYFISH CYANEA CAPILLATA. John A. Schetz and Peter A. V. Anderson. Department of Neuroscience, Univ. of Florida, Gainesville, FL 32610.

As a prerequisite for preparing artificial membranes for the study of a novel voltage-dependent Na^+ channel of the Scyphozoan jellyfish Cyanea capillata (P.A.V. Anderson (1987), J. exp. Biol. 133, 231-248), the composition of the neural membrane must be determined. Phospholipid, sterol and ganglioside contents of the membranes of nerve rich tissue were assessed. Membrane components were purified by a series of chemical extractions, separated by thin layer chromatography, and identified and quantified using various chemical tests. Preliminary data shows that whole jellyfish membranes contain at least phosphotidylcholines, phosphotidylserine, phosphotidylethanolamine, cholesterol, and various other sterols, but apparently no gangliosides. We conclude that these jellyfish either have unique or very low levels of gangliosides. Supported by NSF Grant BNS-8805885.

214.11

Electrical stimulation of rat striatal slices increases the level of lysophosphatidylcholine and decreases that of phosphocholine. S. A. Farber*, R. L. Buyukuysal*, B. E. Slack and R. J. Wurtman. Department of Brain and Cognitive Sciences, MIT. Cambridge, MA 02139, USA

We previously showed that electrically-stimulated rat brain slices continue to synthesize and release large amounts of acetylcholine (ACh) even when superfused without exogenous choline (Ch) (Ulus et al.,Brain Research, 484:217-227). Under these conditions membrane phospholipid levels decreased by 15%, but adding free Ch to the medium completely protected against this decrease. The present study examined the effects of electrical stimulation on levels of various choline-containing intermediates in phosphatidylcholine (PtdCh) synthesis and catabolism

Slices were superfused with Krebs-Ringer buffer containing 20 uM eserine, and electrically stimulated for 1 hour (1 Hz, 100 mAmp and 1 msec duration); perfusates were collected for later analysis of ACh and Ch levels. The slices were then incubated for 30 or 60 minutes with 20 uCi of [^{14}C]Ch (45 uM), and after labeling were washed, homogenized in methanol and water, and extracted with chloroform. The water soluble fraction was assayed for labeled and unlabeled phosphocholine (PCh), ACh and Ch; the organic phase was assayed for labeled and unlabeled PtdCh and other phospholipids.

We confirmed that electrical stimulation had no effect on tissue Ch levels but depleted membrane phospholipids by 16 - 37%. This treatment also increased the total lysoPtdCh levels from .062 ± .004 to .120 ± .016 nmols/ug DNA (94% ;P<.025) and reduced PCh from .20 ± .01 to .13 ± .02 nmols/ug DNA (33% ; P<.05). The accumulation of [^{14}C]PtdCh and [^{14}C]PCh were reduced by 40 and 32% respectively. Stimulation had no effect on the specific activity of [^{14}C]Ch in either the tissue or the incubation medium.

These findings suggest that both decreased PtdCh synthesis and increased degradation can mediate the depletion in this membrane phospholipid that occurs when the tissue is stimulated for prolonged periods in the absence of Ch.

214.12

DIFFERENT RESPONSE OF BRAIN AND HEART MITOCHONDRIAL CARDIOLIPIN FATTY ACID PROFILES TO ALTERATIONS IN DIETARY POLYUNSATURATED FATTY ACIDS. P.B. Lieberman, C.E. Greenwood and G.H. Anderson. Dept. Nutr. Sci., Fac. Med., Univ. of Toronto, Toronto, ONT, M5S 1A8.

We previously reported that alterations in the amount of dietary linoleic acid (18:2n-6) induced large and rapid changes in brain mitochondrial (MI) cardiolipin (CL) 18:2n-6 content in rapidly growing rats (Dyer and Greenwood, 1989). MI membrane characteristics vary tremendously amongst different tissues. For example, brain MI have relatively less cristae and hence less inner MI membrane where CL is localized, less CL, and less CL 18:2n-6 compared to heart. Hence, MI membrane response to the same dietary challenge may vary amongst tissues. To compare the relative effect of dietary polyunsaturated fatty acids (PUFA) on MI with differing membrane characteristics, the fatty acid profile of MI CL from rat heart and brain were measured 4 weeks after feeding 20% (w/w) fat diets with 3-13% PUFA. Heart had significantly higher amounts of MI CL 18:2n-6 (45% vs. 10%, heart and brain MI, respectively) and PUFA (55% vs. 35%) and lower levels of saturated (20% vs. 24%) and monounsaturated fatty acids (18% vs. 38%) and 20:4n-6 (4% vs. 16%) than brain. Absolute changes in MI 18:2n-6 levels associated with dietary PUFA were higher in heart than brain (increased from 35% to 55% and 8% to 16%, respectively), while the relative changes were similar. While both dietary 18:2n-6 and 18:3n-3 were positively correlated ($p < 0.0001$) with MI membrane 18:2n-6, dietary 18:2n-6 was a slightly better predictor of MI 18:2n-6 than was dietary 18:3n-3. Dietary 18:2 n-6 and 18:3n-3 accounted for 81% and 74% of the variance in brain MI 18:2 n-6 and 38% and 33% in heart MI 18:2n-6, respectively. These results show that brain MI membrane 18:2n-6 is sensitive to dietary fat manipulation but, heart MI 18:2n-6 might be exquisitely sensitive expressed as larger absolute changes in membrane 18:2n-6. The effects of dietary fat manipulations on CL fatty acid profiles are of specific interest as they may alter the function of CL-dependent enzymes and impact on MI respiratory efficiency. (ILSI-NF and NSERC funded)

214.13

ANALYSIS OF MEMBRANE PHOSPHOLIPIDS IN ALZHEIMER'S DISEASE BRAIN BY ^{31}P NMR. Pettegrew JW, Panchalingam K, Strychor S and Branthoover G. Laboratory of Neurophysics, Dept of Psychiatry, U. of Pittsburgh, Pittsburgh, Pa. 15261

Recent *in vivo* and *in vitro* nuclear magnetic resonance (NMR) studies demonstrate elevation of phosphomonoesters and phosphodiesters in Alzheimer's disease (AD) brain. This would indicate an abnormality in brain phospholipid synthesis. In this study, membrane phospholipids of Alzheimer's Disease autopsy brain tissues were analyzed by ^{31}P NMR technique. Quantitative assessment of phosphatidylcholine (PtdC), phosphatidylethanolamine (PtdE), phosphatidylserine (PtdS), phosphatidylinositol (PtdI), phosphatidic acid (PtdA), cardiolipin, sphingomyelin, and some of their lyso- and plasmalogen derivatives were made. The results demonstrate a reduction in PtdC, PtdE (p=0.02) and PtdA in AD brain (21 samples, 10 brains) compared to non-AD diseased controls (16 samples, 8 brains) of the same age group. The phospholipid PtdI is not altered. PtdS, sphingomyelin (p=0.03), PtdE plasmalogen and cardiolipin are elevated. Correlation studies indicate PtdC is negatively correlated (p=0.002, r=0.8) with the number of senile plaques (SP) per x 200 magnification between cortical layers II and IV in the same brain region. This would indicate a reduced synthesis of this phospholipid as the disease progresses. Cardiolipin which is present only in the inner mitochondrial membrane is positively correlated with the number of SP (p=0.01, r=0.8) indicating that this lipid synthesis is elevated with the progression of the disease. The possible implications of these findings will be discussed.

214.14

CALSEQUESTRIN AND CEREBELLAR CA^{2+} STORES. P. Volpe, E. Damiani* and A. Margreth*. UTMB, Galveston, TX, and CNR Unit for Muscle Biology and Physiopathology, Università di Padova,Italy.

Calsequestrin (CS) is the Ca^{2+}-binding protein (CBP) of sarcoplasmic reticulum of striated muscle. The existence in non-muscle cells of a protein, sharing with CS structural and functional properties, is still debated. We have re-investigated this problem, in chicken cerebellum microsomes. By ^{45}Ca- overlay, we identified a 51 kDa CBP which shared with chicken muscle CS Stains All-staining properties and pH-dependent electrophoretic behavior. By Western blot, this protein was found to be immunologically related to chicken muscle CS. The 51 kDa CBP exhibited Ca^{2+}-dependent hydrophobic changes, as demonstrated by Ca^{2+}-dependent elution from phenyl-Sepharose column. Finally, the amino terminal sequences and Cleveland's peptide maps of chicken muscle CS and of the cerebellum 51kDa CBP were found to be identical. Thus, chicken cerebellum contains a protein truly belonging to the CS class. A cerebellum microsomal fraction obtained by differential centrifugation, was enriched in CS, Ca^{2+}-pump and inositol 1,4,5-trisphosphate receptor. (NIH GM40068-02).

214.15

A OUABAIN-SENSITIVE NA,K-ATPASE IN TENTACLES OF THE SEA ANAMONE STOICHACTIS HELIANTHUS. Susan C. Specht, Roberto López Rosado, Rosa Figueroa Nieves and Sonia Soto. Department of Pharmacology and Institute of Neurobiology, University of Puerto Rico, Medical Sciences Campus, San Juan, Puerto Rico 00936

Innervated portions of one species of jellyfish (Cyanea capillata) and two sea anemones (Aiptasia tagetes and Stoichactis helianthus) were examined for evidence of a ouabain-inhibited, Na,K-stimulated ATPase. Robust activity was found only in the innervated tentacles of Stoichactis helianthus, a large sea anemone whose sting produces a powerful and persistent burning sensation. Na,K-ATPase activity was completely inhibited by ouabain or strophanthidin at 10^{-3} M. The K_i for strophanthidin (prepared in dimethylformamide) was approximately 10^{-6} M, assayed for K-stimulated p-nitrophenyl phosphatase activity; in rat, the K_i determined by this assay is about one order of magnitude higher than if assayed for Na,K-ATPase activity. SDS-PAGE of the crude preparation revealed a protein band in the region of 90 kD. (Supported by NIH grants NS-07464 to R. Orkand and SO6 RR 08224 to E. Santiago Delpin).

214.16

PERIPHERAL BENZODIAZEPINE RECEPTOR LIGANDS INDUCE MORPHOLOGICAL CHANGES IN MITOCHONDRIA OF CULTURED GLIOMA CELLS. T. Shiraishi, K.L. Black and K. Ikezaki . Div.of Neurosurgery, Univ. of Calif., Los Angeles, Sch.of Med., Los Angeles, CA 90024

To further elucidate the cellular biological function of the peripheral benzodiazepine receptor which localize to the mitochondrial outer membrane, morphological changes of mitochondria after treatment with peripheral benzodiazepine ligands were examined. C6 and T98G glioma cell lines were incubated with the medium containing low (10nM) or high (10μM) concentration of PK11195(PK), Ro5-4864(Ro) (peripheral type ligand) and clonazepam(CL) (central type ligand) for 24 or 48 h. After exposure to R123(10μg/ml) for 30 min at 37°C, cells were examined by fluorescent microscopy. Prior to treatment, mitochondria were diffuse throughout the cell in filamentous pattern. After 24 h of exposure to low concentration of PK and Ro, the mitochondria became more compact in the perinuclear region. After 48 h exposure, mitochondria recovered to their filamentous diffuse pattern. CL treatment had no influence on morphology. Electron micrographs showed that the cells after low PK treatment for 24 h had increased numbers of swollen mitochondria. These morphological changes might relate mitochondrial activity.

214.17

DIFFERENTIAL DISTRIBUTION OF (Na,K)-ATPase α ISOFORMS IN THE CENTRAL NERVOUS SYSTEM G. Siegel, V.Hieber, D.J. Fink, and M.Mata. VAMC and U.Michigan, Ann Arbor,MI 48104

In order to study the expression of the different α isoforms of (Na,K)-ATPase in the central nervous system of the rat, we used riboprobes prepared from the unique 3′ untranslated region of α1 cDNA, and the translated region of α3 cDNA in *in situ* and dot blot hybridization assays of the brain.

α1 was found predominantly in the cerebral cortex where it labeled pyramidal neurons in the superficial layers. α1 was also found in neurons of the dentate gyrus of hippocampus, and in the locus ceruleus and inferior olives of the brain stem. In contrast, α3 was found in pyramidal neurons in the deep layers of cerebral cortex, in both pyramidal and dentate gyrus neurons of the hippocampus, and in neurons of most subcortical structures of the thalamus, basal ganglia and brainstem nuclei. There was no substantial labeling of glial cells, nor of vascular endothelial cells with either probe, but the ependyma of choroid plexus contained only α1.

In the cerebellum, Purkinje cells showed predominantly α3, as did stellate and basket cells. The granule cells contained both α1 and α3.

These experiments show that both α1 and α3 isoforms of (Na,K)-ATPase are found in neurons of the CNS, though the distribution of the two isoforms differs.

214.18

DIFFERENTIAL DISTRIBUTION OF (Na,K)-ATPase α ISOFORMS IN THE PERIPHERAL NERVOUS SYSTEM. V.Hieber, G. Siegel, D.J. Fink and M.Mata. VAMC and U.Michigan., Ann Arbor,MI 48104

mRNA transcripts for 3 isoforms of the α subunit of (Na,K)-ATPase have been identified in the nervous system (designated α1, α2, and α3). The α1 isoform in rodent is more resistant to ouabain inhibition and binding than are α2 or α3.

In order to study the localization and expression of the different α isoforms, we prepared probes from the unique 3′ untranslated region of α1 cDNA, and from the translated region of α3 cDNA. These probes were used in dot blot and *in situ* hybridization assays of rat spinal cord, dorsal root ganglia (DRG), and peripheral nerve.

Within the ventral horn of spinal cord, a discrete subset of laterally placed motor neurons contained α1, while α3 was found in all motor neurons including those containing α1. In the DRG, most neurons, but no satellite cells, contained both α1 and α3. However, some DRG cells had α3 but not α1. Schwann cells were labeled with α1 probe in a perinuclear distribution, but contained no detectable α3. Data from dot blot analysis agreed with the *in situ* hybridization pattern.

These experiments imply that: (1) The riboprobes are able to distinguish α1 from α3 isoform mRNAs, (2) Both α1 and α3 isoforms of (Na,K)-ATPase are found in neurons, and (3) Schwann cells contain the α1 isoform.

214.19

BIOCHEMICAL STUDIES ON RAT BRAIN TRANSGLUTAMINASE. Y. Takeuchi, P.J. Birckbichler and M.K. Patterson, Jr. Biomedical Division, The Samuel Roberts Noble Foundation, Inc., Box 2180, Ardmore, OK 73402.

Transglutaminases (TGase) are a class of calcium-dependent enzymes that catalyze an acyl transfer reaction between peptide-bound glutaminyl moieties and primary amines including the ε-amino group of peptide-bound lysine. Since little is known about the involvement of TGase in CNS function, we initiated studies on rat brain TGase. The tissue was homogenized and TGase activity was found in both soluble and particulate fractions following ultracentrifugation. Two immunoreactive protein bands (79 kDa and 75 kDa) were detected in both fractions following SDS-PAGE and Western blotting using an antibody specific for "tissue" TGase. While the catalytic activity was approximately equally distributed between the two fractions, considerably higher amounts of immunoreactive material were found in the soluble fraction. The soluble form of the enzyme was inhibited by GTP and attempts at purification were made using a GTP-agarose column. Solubilization of the particulate form was accomplished with detergents with an apparent increase in activity suggesting masking of catalytic sites in particulate structures. Studies utilizing purified rat brain TGase should be useful in investigating the possible interaction between the enzyme and neurotransmitter(s) or neuropeptide(s).

214.20

LOCALIZATION AND STRUCTURE OF RUBROPHILIN. B.W. Moore, H.A. Rosenthal and K.E. Isenberg. Dept. Psychiatry, Washington University Sch. of Med., St. Louis, MO 63110.

Rubrophilin is a 53Kd membrane-associated nervous-system-specific protein. Immunohistochemistry using polyclonal antisera with fetal rat brain cultures demonstrated exclusive staining of neurons. On polyacrylamide SDS gels rubrophilin stains red by an identified component of Coomassie Brilliant Blue R-250 and it has a distinctive amino acid composition. Rubrophilin was purified to homogeneity and shown to have a blocked N-terminus. The purified protein was digested to completion with the protease AspN (Boehringer-Mannheim) and peptides purified for sequencing by reversed phase high pressure liquid chromatography. Sequences of six peptides confirm the peculiar composition of rubrophilin. Search of the Genbank database by standard techniques did not reveal any significant homologies. Its distinctive biochemical properties, its specificity to neuronal membranes, and its presence in several vertebrates suggests an important function for rubrophilin in the nervous system.

SYNAPTIC STRUCTURE AND FUNCTION I

215.1

FREQUENCY DEPENDENT LOSS OF SYNAPTIC VESICLE SUBCLASSES. G.Q.Fox. AbG. 161, Max-Planck-Inst. für biophysikalische Chemie, 3400 Göttingen, FRG.

The presynaptic terminals of Torpedo electric organ have a synaptic vesicle population composed of different size classes. The 68 and 90 nm subclasses are known or suspected of containing ACh and their numbers are influenced by electrical and chemical stimulation. Electrical stimulation at 10, 1 and 0.1 Hz to less than 20% of the evoked response of the organ(fatigue) has been found to produce specific declines in vesicle numbers of these 2 subclasses with 10 Hz effecting the 68 nm vesicles, 0.1 Hz the 90 nm vesicles and 1 Hz producing an intermediate condition. No change was found in the amount of terminal membrane nor could it be demonstrated that vesicles were able to incorporate a dextran marker during 10 and 1 Hz stimulation.

Overshoots of control numbers of 68 nm vesicles were obtained after 3.5 h of recovery, confirming previous observations of the presence of a 68 nm endocytotic mechansim. This mechanism is not to be confused with recycling processes postulated to be operative during stimulation.

215.2

HIGH LEVELS OF SYNAPSIN II IN OLFACTORY NERVE TERMINALS IN THE OLFACTORY BULB. T.E. Finger and M.D. Browning, *Dept. Cellular & Structural Biology and Dept. Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver CO 80262*

Synapsin I and synapsin II are synaptic vesicle-associated phosphoproteins that are thought to play a role in regulating some aspects of synaptic transmission. The ratio of synapsin I to synapsin II is a constant 2:1 in virtually all areas of the brain. One salient exception to this rule is the olfactory bulb where levels of synapsin II match or exceed those of synapsin I. We have recently examined the olfactory bulb with antibodies specific for synapsin I and for synapsin II, and the results of these studies may provide an explanation for the unusually high levels of synapsin II in the olfactory bulb. Immunocytochemistry of the olfactory bulb reveals that the glomeruli have higher levels of synapsin II-like immunoreactivity than do the outer plexiform layer or other layers of the bulb. Conversely, synapsin I-immunoreactivity is lower in the glomeruli than in the outer plexiform layer. Thus the outer plexiform layer and deeper layers of the bulb react like other areas of the CNS such as cerebral cortex; the glomeruli are unusual in reacting more heavily for synapsin II than synapsin I. Microdissection studies corroborate the immunocytochemical data. Western blots of tissue dissected from the central two-thirds of the bulb (including the granule cells, mitral cells and inner half of the outer plexiform layer) show the usual 2:1 ratio of synapsin I: synapsin II. In contrast, blots of tissue taken from the outer third of the bulb (glomeruli and outer half of the outer plexiform layer) exhibit a 1.3:1 ratio. These results suggest that olfactory receptor nerve terminals within the glomeruli contain high levels of synapsin II in comparison to synapsin I. This unusual ratio is more similar to the ratio of synapsins in ganglia and effector organs than the ratio in the CNS.

Supported by PHS grants DC00244 to TEF and DK40483 & NS26377 to MDB.

215.3

MICROSCOPIC STUDIES OF LOW pH COMPARTMENTS IN NEURONS AND PHOTORECEPTORS. E.Augenbraun* and E.Holtzman. Dept. Biol. Sci., Columbia Univ. NY 10027.

Previously Sulzer and Holtzman identified low pH compartments in the presynaptic terminals of frog retinal photoreceptors by staining with weak base vital dyes and by vacuologenic effects of ammonia. The present work extends these findings: 1) We find that chloroquine has vacuologenic effects on presumptive acidified compartments in photoreceptors; these effects are evident at much lower concentrations than are needed with ammonia so chloroquine should prove more widely useful than ammonia. 2) We demonstrate accumulation of the weak base DAMP in photoreceptors and in cultured hippocampal neurons. DAMP is aldehyde fixable and immunocytochemically demonstrable, and is potentially usable for electron microscopy (cf Anderson). Our best results are obtained when HRP is included in the medium used to administer DAMP; evidently HRP provides sites to which DAMP can be fixed within acidified endocytic structures. DAMP staining is seen in structures within cell bodies, in photoreceptor terminals, and in "varicosities" along hippocampal processes that appear to include regions rich in synaptic vesicles.

Supported by EY 03168 (NEI). We thank Drs. S. Rayport and D. Sulzer for advice and assistance.

215.4

IMMUNOEXTRACTION OF BRAIN NA/CA EXCHANGER WITH ANTIBODIES TO A 36-KDA PROTEIN. M.L. Michaelis, C.K. Jayawickreme*, S. Schueler*, M.S. Hurlbert*, and C. Guilly*. Dept. Pharmacology and Ctr. Biomed. Res., Univ. of Kansas, Lawrence, KS 66047.

The Na/Ca antiporter is a plasma membrane system which plays a role in both Ca^{2+} influx and efflux. Attempts to isolate the antiporter protein have been limited due to the lack of selective high affinity ligands. Through the use of several chromatographic techniques with solubilized bovine synaptic plasma membranes, we have consistently found an enrichment in 2 protein bands (36 and 50 KDa) when the antiporter activity was enriched (J. Cell Biol. 1989, 107, 127a). We have now electroeluted those bands from SDS-PAGE gels and immunized rabbits. The IgG's produced against each of the proteins exhibited strong immunoreactivity in both ELISA assays and Western blots with solubilized membranes. And, more importantly, the anti-36K antibodies immunoextracted greater than 95% of solubilized membrane antiporter activity while the preimmune IgG's extracted none. Since the protein is not inactivated by low pH, we recovered almost all of the immunoextracted activity with a sodium acetate, pH 3.5 wash of the column. Our observations strongly suggest that these 2 proteins are involved in antiporter activity in brain plasma membranes. (NIAAA grant #AA04732 and ARO grant #DAAL03-88-K-0017).

215.5

CYTOPLASMIC ORGANIZATION IN THE DENDRITIC SPINES OF RAT HIPPOCAMPAL CORTEX IN ORGANOTYPIC CULTURE. Dennis M. D. Landis, Lucas D. Pozzo Miller* and L.A. Weinstein*. Depts. Neurology and Neurosciences, School of Medicine, Case Western Reserve University, Cleveland, OH 44106

We have previously used rapid freezing, freeze fracture and shallow etching to show that the cytoplasm of Purkinje cell dendritic spines in mouse cerebellar cortex contains a core of actin-like microfilaments, and more peripherally distributed filamentous proteins of unknown nature (*J. Cell Biol.* 97 : 1169, 1983). The structure of the post-synaptic density has at least two components, and the relative amounts of the two components vary as different spines are compared (*Synapse* 1: 552, 1987).

The dendritic spines of CA1 hippocampal cells are located too far from the brain's surface to be accessible with our usual freezing methods. We therefore prepared organotypic cultures of hippocampal slices obtained from 6-7 day old rats and maintained for 14-21 days in vitro. These cultures were rapidly frozen, and prepared by freeze fracture or freeze substitution techniques.

The cytoplasm of hippocampal dendritic spines in these cultures also contains a core of actin-like filaments, and filamentous proteins just below the membrane of the spine. The postsynaptic density appears to have a lattice-like substructure with adherent globular proteins, and the relative amounts of these structures vary spine to spine. These features of cytoplasmic organization thus appear to be characteristic of dendritic spines in the central nervous system.

215.6

IMMUNOHISTOCHEMICAL IDENTIFICATION OF A POSTSYNAPTIC COMPONENT OF THE NEUROMUSCULAR JUNCTION. S. H. Astrow and W. J. Thompson. Dept. of Zoology, Univ. of Texas, Austin, TX 78712.

We prepared monoclonal antibodies against a membrane-enriched preparation derived from neonatal rat muscles and obtained one (3G2) which recognizes a molecule at the neuromuscular junction in adult animals. The epitope appears to be intracellular, since staining of intact muscles with 3G2 is only obtained after permeabilization. The antigen may be unique to neuromuscular synapses as staining is not detectable in sections of other tissues including smooth muscle, spinal cord and autonomic ganglia. To determine when the antigen appears in developing muscles we double-labeled hindlimb sections with antibody 3G2 and rhodamine-conjugated α-bungarotoxin (R-BTX; an acetylcholine receptor ligand). At embryonic day 17 (E17), R-BTX diffusely labels the surface of many fibers, but there is no corresponding 3G2 staining. Rather, the antibody stains the cytoplasm of each fiber, near myotendonous junctions. Although acetylcholine receptor clusters are prominent at E20, 3G2 staining is not apparent at these sites until about postnatal day 4 (P4). At earlier times, 3G2 antigen is also present extrajunctionally in some small (presumably secondary) muscle fibers scattered throughout the muscle. By P7, staining of extrajunctional regions is not above background and 3G2 staining closely follows the distribution of R-BTX. In contrast with many other postsynaptic molecules localized to the neuromuscular junction, 3G2 antigen rapidly disappears upon denervation. Thus, this molecule appears to be a novel junctional component that may play a role in the stabilization of neuromuscular synapses.

215.7

THE 17K Mr PROTEIN, A MAJOR SPECIFIC SUBSTRATE FOR PROTEIN KINASE C FOUND IN RAT POSTSYNAPTIC DENSITY FRACTION. T. Suzuki[1]*, S. Dohmae[1]*, P. Siekevitz, and R. Tanaka[1]*. [1]Dept. of Biochem., Nagoya City Univ. Med. Sch., Nagoya 467, Japan. [2]Rockefeller Univ., New York, NY 10021

Postsynaptic density (PSD) fraction isolated from rat cerebral cortex contained the activity of kinase C, which phosphorylated 17K Mr protein endogenous to the fraction. The electrophoretic mobility of the protein was slightly smaller than that of myelin basic protein and the protein was not contained in the purified rat myelin. The isoelectric point of the 17K Mr protein was in neutral range, while that of myelin basic protein was calculated to be 12.24. The 17K Mr protein did not cross-react with anti-myelin basic protein antibody. Thus, the 17K Mr protein was clearly distinct from myelin basic protein. Phosphorylation of the protein by the endogenous kinase C was greater in the PSD isolated from hippocampus and from cerebellum than in the fraction from cerebral cortex.

215.8

EVIDENCE THAT THE MAJOR POSTSYNAPTIC DENSITY PROTEIN (mPSDp) MAY BE DISTINCT FROM THE ALPHA SUBUNIT OF CALMODULIN KINASE II (α-CK II). K. Wu, Y. Huang*, J. E. Adler and J. B. Black Div. Dev. Neurology, Cornell Univ. Med. Coll., New York, N.Y., Dept. Neurosci. and Cell Biol. ,UMDNJ/ Robert Wood Johnson Med. Sch., Piscataway, N.J., Div. Neurosci., New York State Psych. Inst., New York, N.Y. and Dept. Neurology, Wayne State University School of Medicine, Detroit, MI.

Increasing evidence suggests that the mPSDp plays a critical role in synaptic communication and plasticity. The mPSDp and α-CK II have long been considered to be identical. However, the two proteins do differ in solubility and antigenicity, raising the possibility of nonidentity. To further define the relationship between the two proteins, we purified the mPSDp to homogeneity from adult rat cerebral cortex, and compared the proteins (α-CK II and its polyclonal antiserum were kindly supplied by Drs. Cernik and Greengard, Rockefeller Univ.). The PI of the mPSDp was 6.2, whereas that of α-CK II was 7.2, suggesting physicochemical differences. Purified mPSDp bound calmodulin (CaM) in the presence of Ca^{2+}, and autophosphorylated in a Ca^{2+}/CaM-dependent manner. These activities were inhibited by a polyclonal antiserum against the mPSDp (Ab-mPSDp). Ab-mPSDp recognized the mPSDp in the purified state or in total synaptic membrane. However, Ab-mPSDp did not react with α-CK II, and Ab-α-CK II reacted only weakly with mPSDp, suggesting similarity but not identity of the proteins. Finally, V-8 digestion of mPSDp revealed at least an 8-amino acid sequence not present in α-CK II. These results suggest that mPSDp and α-CK II may be similar, but not identical.

215.9

NEUROCHEMICAL AND MORPHOLOGICAL COMPARISONS OF POSTSYNAPTIC DENSITIES (PSDs) ISOLATED FROM RAT AND HUMAN BRAIN. Y. Huang*, M. Stanley and K. Wu Div. Neurosci., N.Y.S. Psych. Inst., Dept. Psych. & Pharmacol., Columbia Univ., New York, N.Y. and Div. Dev. Neurology, Cornell Univ. Med. College, New York, N.Y.

Growing evidence suggests that the PSD, a subcellular organelle attached to postsynaptic membrane at a chemical synapse, plays a critical role in synaptic communication and plasticity. During our characterization of PSDs from human and rat brains, we found, among others, interesting differences in electron microscopic morphology of the two entities. PSDs were isolated from cerebral cortex of frozen brain according to the procedures of Carlin et al. (J. Cell Biol. 86:831-843, 1980). The SDS-gel profiles of human and rat PSDs revealed similar proteins but with different quantities. Electron microscopic examination indicated that most rat PSDs appeared as concave-shaped, single stranded bars while human PSDs showed mixture of straight, single stranded as well as double stranded bars. The double stranded bars seen in human PSDs probably resulted from association of two PSDs from different synapses. Alternatively, they may be the presynaptic dense projection and postsynaptic density present in the same synapses. Overall, the double stranded bars could be derived from the symmetric synapses while the single stranded bars were from the asymmetric synapses. The underlying cause of the relative abundance of double stranded bars in human preparation remains to be determined. Cyclic AMP- and Ca^{2+}/calmodulin (CaM)-dependent protein kinase activities, as well as CaM-binding proteins, in the PSD preparations from the two species were also determined and compared.

PRESYNAPTIC MECHANISMS II

216.1

HISTAMINE H_3 RECEPTORS IN THE CNS: RECEPTOR BINDING, BIOCHEMICAL RESPONSE AND MOLECULAR MODELING. S. Ghodsi-Hovsepian, D. Ngur*, P. Weiland*, J. Woodruff*, G.J. Durant* and W. Hoss. Center for Drug Design and Development and Department of Medicinal and Biological Chemistry, The University of Toledo, Toledo, OH 43606.

Histamine H_3 receptors inhibit the release of histamine presynaptically. Unlike other histamine receptors (H_1 and H_2), H_3 receptors have not been well characterized. Previously we have shown that H_3 agonists stimulate low-K_m GTPase activity in the brain (S. Ghodsi-Hovsepian et al., Soc. Neurosci. Abstr., 1989), indicating that H_3 receptors are coupled to their effectors through GTP-binding proteins. A binding assay for histamine H_3 receptors utilizing [^{3}H]-N$^{\alpha}$-methylhistamine as the radioligand and unlabeled α-methylhistamine to account for nonspecific binding was established. Specific binding to rat cortical membrane receptors was of high affinity (overall K_d = 0.5 nM) and saturable. Nonspecific binding was 20-30% of the total binding at the K_d value. A Scatchard plot of the specific binding was curved, suggesting the presence of more than one affinity state for the receptor, more than one receptor or negative cooperativity. The specific binding of [^{3}H]-N$^{\alpha}$-methylhistamine to rat cortical membranes was completely inhibited by burimamide, which is an antagonist at H_3 receptors (as well as H_2 receptors), in the concentration-dependent manner. To determine if H_3 receptors were linked to phosphoinositide (PI) turnover in the brain, the effects of histamine and α-methylhistamine were determined in rat cortical slices. Although histamine produced a concentration-dependent increase in this preparation, α-methylhistamine was inactive over the concentration range of 10 - 500 μM, suggesting that H_3 receptors do not attenuate histamine release in the brain by a mechanism involving the stimulation of PI turnover. Low-energy conformations of histamine and various methyl- and dimethylhistamine derviatives were calculated using C. Stills program MacroModel and compared with their agonist activities at H_3 receptors. The data predict that of the five low-energy (within 20 kJ of the global minimum) conformations of histamine, a conformation with the amino group and imidazole ring in a *trans* conformation with respect to the ethane bond in the side chain may be associated with activity at H_3 receptors.

216.2

THE ROLE AND POTENTIAL INTERACTION OF ADENYLATE CYCLASE, PROTEIN KINASE C, AND CALMODULIN ON ANGIOTENSIN RELEASE FROM DISSOCIATED BRAIN CELL CULTURES. A.P. Gadbut, S.M. Cash*, T.R. Radice*, J.A. Noble* and J.A. Weyhenmeyer. Neuroscience Program, Univ. of Illinois, Urbana, IL 61801.

Several studies have suggested that angiotensin (ANG) can be released from CNS tissue in a neurotransmitter-like fashion. We have shown previously that ANG release is dependent upon the influx of Ca^{2+} through primarily N-type voltage gated calcium channels and the subsequent activation of calmodulin (Gadbut et al., FASEB J., 4:A1199, 1990). In this study, we examined the effects of forskolin and the phorbol ester, TPA, on ANG release from dissociated cell cultures of fetal rat hypothalamus/brainstem. Increasing concentrations of forskolin (2 μM-200 μM) stimulated ANG release in a dose dependent manner. The addition of cholera toxin also resulted in ANG release, providing further evidence for an adenylate cyclase/G_s coupled mechanism. In contrast, ANG release was not effected following the addition of TPA under either ionically neutral or stimulating conditions. Finally ANG release was attenuated in the presence of 20 μM forskolin/25 μM W-7 as compared to 20 μM forskolin alone. These results suggest that both the adenylate cyclase and intracellular Ca^{2+} (calmodulin) are important in the regulation of ANG release from dissociated cultures of fetal rat brain cells.

This study was supported by NSF grant BNS 17117.

216.3

FURTHER CHARACTERIZATION OF DEPHOSPHORYLATION OF B-50 IN SYNAPTIC PLASMA MEMBRANES FROM RAT BRAIN. Y. F. Han and L. A. Dokas#. Departments of Biochemistry and Neurology#, Medical College of Ohio, Toledo, OH 43699.

B-50 is a neuronal-specific, presynaptic membrane-associated protein. Although protein kinase C-mediated B-50 phosphorylation has been well characterized, the dephosphorylation of B-50 has not been as extensively studied. Previous work from this laboratory has suggested in synaptic plasma membranes (SPM) the existence of membrane-bound phosphatase(s) that dephosphorylate B-50. The present study further characterizes B-50 phosphatase activity in SPM by using okadaic acid (OA), a new specific inhibitor of type 1 and type 2A protein phosphatases. At low concentrations of [γ^{32}P]ATP, phosphorylation of B-50 reaches a maximal value at 30 seconds, followed by dephosphorylation. OA, added 30 sec. after the initiation of phosphorylation, partially prevented the dephosphorylation of B-50 at 2 nM, a dose which inhibits type 2A phosphatase. At the higher concentrations of 1 μM, a dose of OA which inhibits type I phosphatase, a more pronounced effect on B-50 dephosphorylation is seen. Spermine at 2 mM could antagonize the inhibitory effects induced by 2 nM OA. Heat-stable protein phosphatase inhibitor 2 (I-2) also inhibited dephosphorylation of B-50. The effects of OA and I-2 on B-50 phosphatase activity were additive. The B-50 phosphatase activity that remained in the presence of OA and I-2 was found to be sensitive to chelation of divalent cations. These results indicate that type 1 phosphatase is the major B-50 phosphatase in SPM and that type 2A and a cation-sensitive form might account for the remainder. (Supported by grants from NIH (NS23598) and the Ohio Department of Aging.)

216.4

NEURONAL EXPRESSION OF PARVALBUMIN AND CALCIUM/CALMODULIN DEPENDENT PROTEIN KINASE II FROM HSV-1 VECTORS. **M.J. During, A.I. Geller, A. Freese, and R. Neve. Dept. Neurosurg., Yale Sch. Med., New Haven CT 06510; Dana Farber Can. Inst., Boston MA 02115; HST, MIT, Cambridge MA 02139; and Dept. Psychobiol., U CA, Irvine CA 92717.**

We are using defective Herpes Simplex Virus (HSV-1) vectors to study neuronal physiology by altering signal transduction pathways including calcium calmodulin dependent protein kinase II, parvalbumin, and protein kinase C. The calcium/calmodulin binding domain and the catalytic domain of calcium/calmodulin dependent protein kinase II are on separate regions of the protein; following limited proteolysis the catalytic domain of calcium calmodulin protein kinase II is active in the absence of calcium. We have expressed the catalytic domain of calcium/calmodulin dependent protein kinase II from a HSV-1 vector (pHSVCaCK) in PC 12 cells as demonstrated by in situ hybridization. pHSVCaCK increases protein phosphorylation and neurotransmitter release from PC 12 cells and from sympathetic neurons.

Parvalbumin, a calcium binding protein, is found in most rapid firing GABAergic neurons; expression of parvalbumin in neurons which do not normally contain this protein may alter the physiological properties of those neurons. Expression of Parvalbumin from a HSV-1 vector (pHSVparv) has been demonstrated by immunofluorescence in PC12 cells; its effects on PC 12 cells and rat sympathetic neurons are now under study. Additional HSV-1 vectors containing the catalytic domain of protein kinase C have been constructed and are now being studied. We conclude that HSV-1 vectors expressing proteins involved in signal transduction can modify neuronal physiology and may reveal the roles of various second messenger pathways in producing stable, long term modification of neuronal function.

216.5

STABLE MODIFICATION OF RAT SYMPATHETIC NEURON PHYSIOLOGY AND NEUROTRANSMITTER RELEASE BY EXPRESSION OF ADENYLATE CYCLASE FROM A HSV-1 VECTOR. A.I. Geller, A. Freese, K. Neve, M.J. During, and R. Neve. Dana Farber Can. Inst., Boston MA 02115; HST, MIT, Cambridge MA 02139; VA Med. Cntr, Portland OR; Dept. Neurosurg., Yale Sch. Med., New Haven CT 06510; and Dept. Psychobiol., U CA, Irvine CA 92717.

To study neuronal signal transduction mechanisms and synaptic plasticity, we are using defective Herpes Simplex Virus (HSV-1) vectors to express in neurons the catalytic domains of second messenger enzymes such as adenylate cyclase. These catalytic domains are no longer regulated but are continuously active. The resulting increase in activity of a second messenger enzyme allows us to study its role in such processes as neurotransmitter release. We have established that HSV-1 vectors can stably express E. coli ß-galactosidase in cultured PNS and CNS neurons (Science 241, 1667, 1988; Proc. Natl. Acad. Sci. 87, 1149, 1990); and following stereotactic injection into the adult rat brain, in neurons around and projecting to the injection site. Using a HSV-1 vector, we now express the catalytic domain of yeast adenylate cyclase in PC 12 cells and rat sympathetic neurons. Expression of the catalytic domain of adenylate cyclase in PC 12 cells resulted in a 20 fold increase in cAMP concentration, an increase in protein phosphorylation similar to that obtained using dibutyryl cAMP, and a 2 fold increase in monoamine neurotransmitter release. Adenylate cyclase was also expressed in sympathetic neurons; the yeast adenylate cyclase protein and the resulting increase in cAMP concentration were localized to the cell body as determined by immunofluorescence and an increase in protein phosphorylation was also observed. Of interest, a 3 fold increase in monoamine neurotransmitter release was obtained; the increase in neurotransmitter release was calcium dependent, was inhibited by tetrodotoxin, and was stable for at least one week. In conclusion, elevation of cAMP concentration is a possible mechanism to achieve stable, long term modification of neuronal function. Furthermore, HSV-1 vectors expressing catalytic fragments of other second messenger enzymes can be used to further study neuronal physiology.

216.6

ASSOCIATION OF RAB3 WITH THE SYNAPTIC VESICLE MEMBRANE. P.A. Johnston*, G.A. Mignery*, R. Jahn+, K. Robinson* and T. C. Südhof. Howard Hughes Medical Institute, and Department of Molecular Genetics, UT Southwestern Medical Center, Dallas, TX, 75235; +MPI für Psychiatrie, Planegg-Martinsried, 8033, FRG.

The mechanism of membrane attachment of rab3, a synaptic vesicle-specific GTP-binding protein, was investigated. Rab3 is covalently modified by a posttranslational process causing it to behave like an integral membrane protein in the synaptic vesicle membrane (Fischer v. Mollard et al., *Proc. Natl. Acad. Sci* 87:1988, 1990). Antibodies were generated against amino- and carboxy-terminal peptides of rab3 and used to test the association of rab3 fragments with the membrane after partial proteolysis. A mutant form of rab3 that lacks the carboxy-terminal cysteine residues was transfected into cells to test the role of those cysteine residues in membrane attachment. And finally, the release of rab3 from synaptic vesicles after different chemical treatments was studied. Results from these experiments together indicate that rab3 is attached to the synaptic vesicle membrane via its carboxy-terminal cysteines by an unknown covalent hydrophobic modification that differs from farnesylation and palmitoylation.

216.7

MODULATION OF ELECTRICALLY STIMULATED ^{3}H-DA RELEASE FROM RAT STRIATAL SLICES BY DOPAMINERGIC (DA) AGENTS. LJ Wichlinski, RH Song*, JH Gordon & JZ Fields. Res Svce 151, VA Hosp, Hines, IL 60141 and Dept of Pharmacology, Loyola Univ Med Sch, Maywood, IL 60153.

Pre-synaptic DA autoreceptors (auto-r) on nigrostriatal terminals mediate inhibition of DA release. Using inhibition by apomorphine (APO), of electrically evoked ^{3}H-DA release, these auto-r have been studied using superfused striatal slices from rabbit and rat with controversial results in rat. We incubated rat slices in ^{3}H-DA and superfused with Krebs buffer. Slices were stimulated (unipolar pulses, 10 Hz, 23 mA, 12-16 V, 4 ms for 5 min) 90 min later (S_1) & again at 150 min (S_2). APO was added to the superfusate 20 min before S_2. The dependent measure was the ratio S_2/S_1 of the values for % of total tissue [^{3}H] released. APO (10^{-7} and 10^{-6} M) reduced the S_2/S_1 ratio relative to controls ($p < .05$), while the change at 10^{-5} M APO was also lower but not significant. The median S_2/S_1 ratio was 0.65 for vehicle slices (n=22), 0.35 for 10^{-7} M APO (n=7), 0.30 for 10^{-6} M APO (n=11), and 0.43 for 10^{-5} M (n=8). Our results confirm DA auto-R inhibition of electrically evoked ^{3}H-DA release by APO in rat striatum. Further studies are currently in progress to investigate the effects on DA auto-receptors of the peptide cyclo(leu-gly), a neuromodulator which we showed down-regulates DA receptors. (Supported by grants from the VA, Scottish Rite Schizophrenia Res Pgm-NMJ, & NS26449).

216.8

DIFFERENTIAL REGULATION OF MAZINDOL (MAZ) AND AMPHETAMINE (AMPH) BINDING SITES ON DOPAMINE (DA)-TRANSPORTER COMPLEX (DTC) IN RAT STRIATUM. I. Shimizu* and C. Prasad. Dept. Medicine, LSUMC, New Orleans, LA 70112.

DTC modulates inactivation and recycling of DA released into synaptic area. Available data suggest association between DA-reuptake and entities such as Na^+/K^+ ATPase and binding sites for DA uptake inhibitors (e.g. cocaine, MAZ, AMPH). Studies into pharmacologic potencies of phenylethylamine derivatives and their ability to inhibit ^{3}H-AMPH or ^{3}H-MAZ binding suggest that both of these ligands label the same site. To further examine relationship between these two binding sites on DTC, following experiments were conducted. Rats were treated intraperitoneally with vehicle, cyclo(His-Pro)(CHP)(2mg/Kg/day X 7 days), MAZ (0.5 mg/Kg/day X 7 days), or AMPH (12, 24 and 30mg/Kg/day for 3, 3 and 4 days respectively), and killed 1-2 days after last treatment. Striata were dissected and Bmax of ^{3}H-MAZ- and ^{3}H- AMPH- binding sites determined. Both CHP and MAZ elevated MAZ- and decreased AMPH- binding sites, whereas AMPH decreased both binding sites. The results of these studies suggest that in striatum ^{3}H-MAZ and ^{3}H-AMPH may not label same population of sites.

216.9

DIFFERENTIAL IONIC REGULATION OF THE DOPAMINE TRANSPORTER BINDING IN THE RAT AND HUMAN FRONTAL CORTEX. A. Hitri, D. Venable* and R.J. Wyatt. Neuropsychiatry Branch, NIMH Neuroscience Center at St. Elizabeths, Washington, D.C. 20032.

In the striatum, GBR 12935 dopamine transporter binding is sodium-facilitated, while in the frontal cortex (FC) it is sodium-inhibited. We have studied 3H-GBR 12935 binding in the rat and human FC as a function of sodium concentration with and without potassium. For rat and human, in the absence of potassium increasing sodium concentrations decreased the Bmax, without changing the Kd. In the human FC, inclusion of 5 mM KCl reversed the sodium-induced inhibition. In the absence of Kd, using 30 mM NaCl the Bmax was 203, while with KCl it was 92 fmoles/mg. Using 120 mM NaCl the Bmax was 112 without, and 261 fmoles/mg with KCl. In the rat FC the sodium inhibition was not affected by potassium ions. Benztropine was more potent with 30 than with 120 mM NaCl, in the absence of KCl in human FC. The addition of 5 mM KCl reversed the sodium effect; 30 mM NaCl shifted the curve to the right compared with 120 mM NaCl. In the rat, benztropine inhibition was more potent in 30 than in 120 mM NaCl, and was not reversed with potassium ions. The data indicate a differential regulatory effect of sodium and potassium ions on the dopamine transport or in rat and human FC.

217.1

Ca-DEPENDENT POTASSIUM CURRENT IN PRESYNAPTIC MOTOR TERMINALS IN *Drosophila* LARVAE. M. Gho* and B. Ganetzky, Lab. of Genetics. University of Madison-Wisconsin, Madison WI 53706.

Transmitter release at the presynaptic terminal is triggered by the entry of Ca^{2+} in response to depolarization. Repolarization of the terminal regulates transmitter release. At the *Drosophila* neuromuscular junction, several different potassium currents have been proposed to play a role in repolarization. Mutations that eliminate specific ionic currents can be used to dissect the functional roles of these different currents. For exemple, the mutation *slowpoke* (*slo*) has been shown to eliminate a fast Ca^{2+}-dependent potassium current (I_{CF}) from larval and adult muscles. Here we investigate the effect of this mutant on repolarization of the nerve terminal.

Synaptic currents evoked by nerve stimulation were measured in voltage-clamped body wall muscles of mutant and normal larvae. In 5.4 mM [Ca^{2+}] Ringer's, elimination of either I_A alone or I_{CF} alone had no marked effect on synaptic transmission. However, simultaneous elimination of both currents resulted in greatly prolonged synaptic current. *slo* exerted its effects directly on the terminal reather than by broadening axonal action potentials because the same results were observed when the *slo* terminals were depolarized electrotonically. Furthermore, under these conditions, better voltage control of the presynaptic terminal could be obtained in the *Sh; slo* double mutant than in *Sh* mutant or normal larvae, suggesting the absence of an additional repolarizing mechanisms in *slo*. These results indicate that *slo* mutants affect I_{CF} in presynaptic terminals as well as in muscles and that I_{CF} along with I_A, plays an important role in repolarization of the presynaptic terminal.

217.2

COMPARATIVE EFFECT OF VESAMICOL AND TROXYPYRROLIUM ON MINIATURE END-PLATE CURRENTS IN THE RAT. I.G. Marshall*, T. Searl*, C. Prior* and K. Pemberton* (SPON: Brain Research Association). Dept. of Physiology and Pharmacology, University of Strathclyde, Glasgow, U.K.

(-)-Vesamicol (VES) selectively inhibits the transport of acetylcholine (ACh) into rapidly recycling synaptic vesicles (Marshall, I.G., *et al.*, *J. Physiol.*, in press, 1990), as evidenced by the production of two populations of miniature end-plate currents (MEPCs). However, it is not clear whether preformed ACh stores are affected by VES. In an attempt to determine this we compared the effects of VES on transmitter release to those of troxypyrrolium (TXP) an inhibitor of the nerve terminal high affinity choline uptake system. MEPCs were recorded for a 4 minute period following drug application from cut rat hemi-diaphragm muscle fibers voltage-clamped at -55 mV. The motor nerve was then stimulated at 10 Hz for 5 minutes, and MEPCs were recorded again for a further 4 min period directly on cessation of stimulation. Stimulation in controls had no effect on the mean MEPC amplitude (pre-stim., 2·34 ± 0·17 nA; post-stim., 2·08 ± 0·15 nA, n=7). As reported previously, stimulation in VES (50 nM) produced a decrease in mean MEPC amplitude with a bimodal amplitude distribution. Stimulation in TXP (40 μM) caused reductions in MEPC amplitude from 2·63 ± 0·23 nA to 1·82 ± 0·14 nA (n=4), but had no effect on the distribution of amplitudes (CV, pre-stim., 0·24 ± 0·02; post-stim. 0·27 ± 0·05, n=4). Since, in the presence of VES preformed quantal size is unaffected, ACh exchange between the cytoplasm and preformed vesicles must be inhibited by VES, if at all, in both directions equally. However, the uniform reduction in MEPC amplitudes following stimulation in the presence of TXP suggests that both preformed and recycling vesicles continually exchange ACh with the cytoplasmic pool.

Supported by grants from the Wellcome Trust and MRC.

217.3

ANALYSIS OF MINIATURE END-PLATE CURRENT (MEPC) DECAY KINETICS REVEALS TWO POPULATIONS OF QUANTA RELEASED FOLLOWING ACUTE AND CHRONIC EXPOSURE TO THE PARALYTIC AGENT 2,4-DITHIOBIURET (DTB). J.M. Spitsbergen and W.D. Atchison, Dept. Pharmacol./Toxicol., Neurosci. Program, Mich. State Univ., E. Lansing, MI 48824.

Rats treated with small daily doses of DTB develop neuromuscular weakness following 4 to 6 days of treatment. Previous intracellular recording studies demonstrated an increase in the frequency of abnormally large slow miniature end-plate potentials (MEPPs) in muscles from rats exhibiting muscle weakness following chronic treatment with DTB and in muscles from rats exhibiting no weakness following a single dose of DTB. In addition, a generalized slowing of the rise and decay times for all MEPPs was observed. Since prolongation of rise and decay times for synaptic potentials are some of the earliest alterations observed following DTB exposure and may represent subtle effects on transmission which could lead to the deficits observed in chronically-treated rats, we wished to study these changes in more detail. MEPCs were recorded, using two microelectrode voltage clamp, from hemidiaphragms of rats following acute and chronic exposure to DTB. One hr following exposure to DTB the frequency of MEPCs with amplitudes more than 2 times the mode was increased from 5.1% of all MEPCs in controls to 28.4%. By 24 hr after treatment abnormal MEPCs comprised 32.4% of all MEPCs, while in muscles from chronically treated rats the frequency was 12%. Initially the decay time constant (τ) for MEPCs appeared larger in muscles from chronically-treated rats, however, if the abnormal MEPCs are not included in the calculation of τ then τ_{MEPC} is decreased in muscles from chronically-treated rats. τ_{MEPC} is also decreased in muscles taken 24 hr following a single dose of DTB. Thus, DTB alters kinetics of current flow at the neuromuscular junction, albeit in a manner different from that predicted from effects of DTB on MEPP and EPP rise and decay times. (Supported by NIH grant NS20683 and a Muscular Dystrophy Association grant.)

217.4

ABNORMAL MINIATURE POTENTIALS OCCUR PRIOR TO NEUROMUSCULAR WEAKNESS INDUCED IN RATS BY DITHIOBIURET. W.D. Atchison, Dept. Pharm./Tox., & Neurosci. Prgm. Mich. State Univ. E. Lansing, MI. 48824.

The purpose of the present study was to investigate whether the large amplitude, slow rise and decay miniature end-plate potentials (MEPPs) that accompany neuromuscular depression caused by dithiobiuret (DTB) occur prior to block of neuromuscular transmission. Experiments were conducted using transected hemidiaphragm muscles of rats treated for 7-8 days with 1 mg/kg/day, ip of DTB or with 0.9% NaCl (1 ml/kg/day) as control and conventional intracellular recording techniques. At the time of observable hindlimb muscle weakness in DTB-treated rats, quantal content of EPPs from diaphragm end-plates was not different from control. In DTB-treated hemidiaphragms exposed to solutions containing elevated Mg^{2+} (6 mM) and lowered Ca^{2+} concentrations (1 mM), stimulation of the motor nerve from DTB-poisoned rats was associated with decreased quantal content compared to similarly-treated control preparations. Prolongation of rise and decay times of MEPPs occurred from end-plates of DTB-treated rats irrespective of whether or not low Ca^{2+}/high Mg^{2+} solutions were used. However, these effects were more pronounced when low Ca^{2+}/high Mg^{2+} solutions were used. Diaphragm-derived end-plates of the DTB-treated group were also characterized frequently by the presence of very large amplitude MEPPs with prolonged decay times. The overall percentage of the total population of MEPPs which these abnormal MEPPs made up in the DTB-treated rats was increased dramatically by exposure to low [Ca^{2+}]/high [Mg^{2+}] solutions. In DTB-treated preparations treated with normal Ca/Mg solutions, these MEPPs of amplitude > 1 mV made up 19% of all MEPPs, compared to <1% in controls. Conversely, in DTB preparations treated with low Ca/high Mg solutions, 44% of all MEPPs had amplitudes > 1 mV while only 4% of control MEPPS had similar amplitudes. Thus, aberrant MEPPS occur at motor terminals treated with DTB prior to failure of evoked release, and may represent an early subtle effect of DTB on the release process. Supported by NIH grant NS20683 and a grant from the Muscular Dystrophy Association.

217.5

A KNOWN CHAOTIC SYSTEM MIMICS THE GENERATION OF MEPPs AND SUB-MEPPs. J.Vautrin, J.Holsapple and M.E. Kriebel. Dept. of Physiology. SUNY Health Science Center at Syracuse, Syracuse, NY 13210.

Unitary end plate potentials and spontaneous end plate potentials (MEPPs) recorded at the neuromuscular junction were attributed to the secretion of preformed quantal packets of ACh (Fatt & Katz, J.Physiol., 117: 109, 1952). However, studies at higher resolving power reveal ACh subpackets (ACh-SPs) that generate 3.2 nano-siemens postsynaptic conductances (Erxleben & Kriebel, J. Physiol., 400: 645, 1988) which are not explained by the quantal hypothesis. All MEPPs (bell-, sub-, skew-, slow, giant) can be described by combinations of ACh-SPs in different numbers and intervals. Previously we have shown that most treatments increasing ACh release rapidly change ACh-SP composition of MEPPs. Our goal is to identify the process that generates and combines ACh-SPs. Droplet formation (see "The Dripping Faucet as a Model Chaotic System" R. Shaw, Aerial Press, 1984) mimics the changes in the ACh-SP composition of MEPPs. A stream of water produces quantal and subquantal drops which "combine" in a manner similar to that seen for MEPPs. MEPP and drop amplitude and interval dependencies suggests that both are generated dynamically by a flow. A.F.M. and NIH NS25683.

217.6

FREQUENCY FACILITATION IS NOT CAUSED BY RESIDUAL IONIZED CALCIUM AT THE FROG NEUROMUSCULAR JUNCTION. R. Robitaille and M.P. Charlton. Dept. of Physiology, Univ. of Toronto, Toronto, Canada, M5S 1A8.

Frequency facilitation of transmitter release may be caused by the summation of Ca ions entering the synapse with the residual free Ca ions left from prior activity. This hypothesis was tested in conditions of increased intracellular Ca buffering capacity by loading the frog NMJs with the Ca chelator dimethyl-BAPTA (DMBAPTA). Mathematical models predict that mobile buffers should alter the distribution of free Ca and increase its rate of decay from the release sites (Duffy et al. Soc. Neurosc. Abst. p475, 1989). Therefore, the amount of facilitation should be reduced and its rate of decay increased in the presence of the Ca chelator. Experiments were performed in low Ca-High Mg Ringer and transmitter release was monitored using conventional recording techniques.Loading the NMJ with the Ca chelator reduced the size of the EPPs by 50%. These effects are presynaptic in origin since MEPP amplitude was not affected by the buffer. The amount of facilitation as well as the rate of decay were greatly reduced in presence of the Ca chelator. The reduction in the decay rate of facilitation does not agree with the predictions from the residual free Ca hypothesis and, therefore, other mechanisms must be considered. Supported by the Medical Research Council of Canada.

217.7

CALCIUM CURRENTS IN CULTURED EMBRYONIC MOTOR NERVE TERMINALS. S.C. Hulsizer, S.D. Meriney and A.D. Grinnell. Jerry Lewis Neuromuscular Res. Ctr., UCLA School of Medicine, Los Angeles, CA 90024.

We have chosen to study the calcium currents in presynaptic structures of embryonic Xenopus laevis nerve-muscle cultures, since the mature motor nerve terminal is not amenable to patch clamp techniques. After plating, spinal cord neurons send out neurites which grow to nearby muscle cells and make functional synapses within minutes after contact (Kidokoro and Yeh, 1982). One to two days later, electron microscopic observations reveal presynaptic specializations, reminiscent of those seen at mature synapses (Weldon and Cohen, 1979). We have made "whole-cell" patch clamp recordings from varicose enlargements (5-7 μm in diameter) of neurites which are found either isolated, or on muscle cells. Using barium as the permeate ion, and 140 ms depolarizing pulses from a holding potential of -60 mV, we have seen both inactivating and non-inactivating current types. We will present comparisons of the biophysical and pharmacological properties of the different calcium currents recorded from isolated varicosities and those associated with muscle cells. Supported by grants from the MDA, NIH and NSF.

217.8

A MATHEMATICAL PROCEDURE FOR CONSTRUCTION OF DELAY HISTOGRAMS OF NEUROTRANSMITTER RELEASE. Y.Aumann* and H.Parnas. Department of Neurobiology, The Hebrew University of Jerusalem, Jerusalem 91904, ISRAEL.

A procedure for construction of delay histograms of neurotransmitter release, from the epc and mepc has been developed. The relationship of the delay histogram to the epc and the mepc is described in a set of linear equations. With the epc and the mepc obtained experimentally (or otherwise), solution of these equations offers a simple, quick and highly automated procedure for determining the delay histogram. No approximation of the mepc shape is required.

Previously, direct experimental techniques have been described for the histogram construction. Aside from being highly tedious and time consuming, these techniques are limited to very low quantal contents. The proposed procedure does not share this restriction.

Methods to overcome noise effects were developed and tested.

The results obtained by the suggested procedure were compared to those obtained experimentally. Reasonable agreement was achieved.

217.9

MECHANISM OF REPETITIVE NERVE FIRING INDUCED BY ANTICHOLINESTERASE AND POTASSIUM CHANNEL BLOCKERS AT THE FROG NERVE TERMINALS. R.A. Maselli and B.J. Distad*. Dept. of Neurology, University of Chicago, Chicago, IL 60637.

The mechanism underlying the repetitive nerve firing induced by anticholinesterases is not well understood. It has been speculated that repetitive nerve action potentials may result from an impaired process of nerve terminal repolarization secondary to K^+ channel blockage. In order to test this hypothesis we performed focal recordings of presynaptic currents in the cutaneous pectoris muscle of the frog in the presence of 10 uM d-tubocurarine. Under these conditions 4-aminopyridine and guanidine produced repetitive nerve firing and effectively blocked fast K^+ currents in the nerve terminals. On the other hand, neither the irreversible cholinesterase inhibitor diisopropylfluorophosphate nor neostigmine induced repetitive nerve firing or resulted in significant K^+ current blockage. These results highlight the difference between the mechanisms of repetitive nerve firing induced by anticholinesterases and K^+ channel blockers and suggest that endplate potentials of prolonged duration in unblocked preparations are required for the generation of anticholinesterase induced repetitive nerve firing.

217.10

DIVALENT IONS USED AS CA CHANNEL BLOCKERS ALLOW Ca^{2+} INFLUX IN PRESYNAPTIC BOUTONS OF CRAYFISH AS MEASURED WITH FURA-2. R.M. Mulkey and R.S. Zucker. Dept. of Mol. & Cell Biology, Univ. of Calif., Berkeley, CA 94720.

Mn^{2+} and Mg^{2+} have been used at the crayfish neuromuscular junction as Ca channel blockers to study 1) the Ca dependence of long term facilitation (Wojtowicz and Atwood, J. Neurosci., 8(12):4667,1988) and 2) the voltage dependence of transmitter release (Hochner et al., Nature, 342:433,1989). Fura-2 was used to measure the internal Ca^{2+} concentration at crayfish presynaptic terminals during high frequency stimulation (100 Hz) in the presence of Mn^{2+} or Mg^{2+} and transmitter release was measured postsynaptically. High frequency stimulation in 6 mM Mn^{2+}, with no added Ca^{2+}, or 2mM Mn^{2+}, with .2mM external Ca^{2+}, produced a 250 nM to 1.2 μM increase in intracellular Ca^{2+} respectively, accompanied by evoked release. Likewise, high frequency stimulation in 12.5 mM Mg^{2+} with no added Ca^{2+} gave a 400 - 800 nM rise in internal Ca^{2+} and produced evoked release. When high frequency stimulation was given in zero Ca^{2+},plus either 2mM EGTA or 13.5 mM Co^{2+}, a 30 - 70 nM rise in intracellular Ca^{2+} was seen with no evidence of evoked release. These results indicate that Mn^{2+} and Mg^{2+} do not block Ca^{2+} influx and evoked release at high frequency stimulation, whereas zero Ca^{2+} with EGTA or Co^{2+} keep Ca^{2+} influx to a minimum with no evoked release.

217.11

A SLOW COMPONENT OF FACILITATION IS LINEARLY RELATED TO PRESYNAPTIC CALCIUM AT CRAYFISH NEUROMUSCULAR JUNCTION. [1,2]K.R. Delaney, [2]R.Llinas and [1]D.W. Tank [1]AT&T Bell Labs Murray Hill NJ, 07974 and [2]New York Univ. NY, 10016

Simultaneous fura-2 measurements of presynaptic intracellular calcium concentrations ($[Ca^{2+}]_i$) and postsynaptic excitatory junction potentials (ejps) were made at neuromuscular synapses of crayfish walking leg. We have quantitatively related residual $[Ca^{2+}]_i$ to facilitation of evoked transmitter release under normal and altered calcium loading and handling conditions. We observe that a component of facilitation, (τ = 1-5 seconds), which develops during, and decays after, 1Hz to 16 Hz trains of action potentials, is linearly related to residual $[Ca^{2+}]_i$ measured in presynaptic terminals. The linear relationship persists and is more accurately measured when the time constants of development and decay of facilitation are increased 5-8 fold by increasing intraterminal calcium buffering capacity with injection of EDTA or EGTA. Consistent with this relationship between $[Ca^{2+}]_i$ and facilitation, broadening the action potential with TEA increases both the accumulation of calcium and the amount of steady-state facilitation seen during tetanic stimulation by the same factor. A linear relationship between facilitation and $[Ca^{2+}]_i$ is also seen if residual $[Ca^{2+}]_i$ is increased through inhibition of Na^+/Ca^{2+} exchange by ouabain-mediated Na^+ accumulation. Our data indicate that models relating $[Ca^{2+}]_i$ to transmitter release should treat this slow component of facilitation as an independent multiplicative factor linearly related to residual $[Ca^{2+}]_i$ over the range examined (150 to 800 nM). The data suggest that a binding site or enzyme with a relatively high affinity for calcium is responsible for one component of facilitation while calcium binding to a low affinity site(s) is required for release. K.D supported by an MRC Canada Postdoctoral Fellowship

217.12

PHOSPHATASE INHIBITOR OKADAIC ACID (OA) INCREASES SYNAPTIC TRANSMISSION AT THE CRAYFISH NEUROMUSCULAR JUNCTION (NMJ). J.E. Swain and M.P. Charlton. Department of Physiology, University of Toronto, Toronto, Ontario, M5S-1A8.

Protein phosphorylation is controlled by kinases and phosphatases. We assayed the role of phosphatases in synaptic transmission at the crayfish NMJ using the permeable phosphatase inhibitor OA. Excitatory postsynaptic potentials (EPSP) evoked by electrical nerve stimulation were recorded as a measure of neurotransmitter release. OA caused a dose-dependent, reversible increase in EPSP amplitude (174% @ 1uM, 401% @ 10uM). At low concentrations, there was an increase in spontaneous neurotransmitter release (>100%), and no change in muscle input resistance. However, at higher concentrations, the muscle input resistance also increased (26% @ 10uM). Thus, at lower concentrations, OA has presynaptic effects, but at higher concentrations, there are both pre- and postsynaptic effects. OA also caused a decrease in facilitation (3 pulses @ 100Hz; 33% @ 1uM, 34% @ 10uM). Studies of the presynaptic action potential and calcium levels in the presence of OA are in progress. Supported by the MRC of Canada, and the Savoy Foundation-Epilepsy.

217.13

BLOCK OF PRESYNAPTIC Ca^{++} CURRENTS BY HYPERTONICITY D.C. Brosius*, J.T. Hackett and J.B. Tuttle. Depts of Physiol. and Neurosci., Univ. of Virginia, Health Science Center, Charlottesville, Va 22908

Hypertonic (HOSM) solutions block neuromuscular transmission presynaptically but enhance spontaneous quantal release. The nerve terminal Ca^{++}-channel is a likely site for this presynaptic modulation of release. Synaptic Ca^{++}-currents were recorded from dissociated chick ciliary ganglion neurons. Synaptic transmission was measured as postsynaptic currents using whole cell patch recording from chick pectoral myotubes co-cultured with the neurons.

Ca^{++} currents were characterized by their I-V relationships and kinetics of activation ($\tau = 1.91 \pm 0.03$ msec, n=9), inactivation ($\tau = 57.2 \pm 3.2$ msec, n=8) and deactivation ($\tau = 0.63 \pm 0.08$ msec, n=6). Only a single type of Ca^{++} current was observed. Extracellular Cd^{++} blocked depolarization evoked Ca^{++} currents (IC_{50} = 6 uM), but did not block repolarization evoked tail currents. Evoked transmission was blocked by 20 uM Cd^{++} but spontaneous release was not affected. HOSM bathing solution (tonicity adjusted to 660 mOsm with sucrose) blocked depolarization-evoked release while consistently enhancing spontaneous release. HOSM solutions reduced depolarization-evoked Ca^{++} currents by 27% but did not affect tail current amplitude. Neither Cd^{++} nor hypertonicty affected Ca^{++} current kinetics. Measurements of free intracellular Ca^{++} using indo-1 showed that HOSM solutions increase $[Ca^{++}]_i$ even in the absence of extracellular Ca^{++}.

In conclusion: HOSM solutions may inhibit evoked release by blocking the Ca^{++} channels coupled to the release machinery, and may enhance spontaneous release by increasing the resting free $[Ca^{++}]_i$. Supported by NSF BNS 87-08162 and NIH NS25669.

217.14

CYCLIC AMP AND THE MOBILIZATION OF TRANSMITTER STORES. W.F. Dryden. Dept. Pharmacology, University of Alberta, Edmonton, Alberta, Canada T6G 2H7.

Quantal release of transmitter at the neuromuscular synapse is increased by measures that raise the level of cytosolic cyclic AMP (cAMP) (Dryden, W.F. *et al. Can. J. Physiol. Pharmacol.*, 66:207, 1987) and mobilization of the secondary transmitter store is apparently dependent on cAMP dependent protein kinase (Dryden, W.F. & Marshall, I.G. *Soc. Neurosci. Abst.*, 15: 190.7, 1989) The present experiments were conducted to examine depletion of the immediately available store of neurotransmitter, and mobilization of the secondary store under conditions of raised cytosolic cAMP. End plate potentials (EPP's) were recorded from cut murine hemidiaphragms superfused with Bretag's SIF solution containing 2.4 mM Ca^{++}, 0.3 μM alcuronium and 200 μM troxypyrrolium. The quantal content of EPP's during 1 s trains of stimuli at various frequencies and in paired pulses of varying interpulse intervals were evaluated before and following treatment with either stimulants of cAMP production (forskolin, fluoroaluminate) or phosphodiesterase inhibitors (IBMX, SQ20,009). As expected, the quantal content of the first EPP of a train was increased, but the loss of quantal content in subsequent EPP's in trains and paired pulses (rundown) was much greater that control. Transmission was, however, maintained at a depressed level. These results suggest that cAMP facilitates transfer of quanta into the immediately available store from the secondary store which becomes relatively depleted. Mobilization from the secondary store may still occur to maintain transmission, but to a reduced extent.

217.15

DEPOLARIZATION IN POTASSIUM PROPIONATE WITH ELEVATED CALCIUM DEPLETES MOTOR NERVE TERMINALS AT TWITCH ENDPLATES BUT NOT AT TONIC ENDPLATES. L.M. Coniglio and R.L. Parsons, University of Vermont College of Medicine, Burlington, VT 05452

We showed previously that in isotonic potassium propionate (KP) with normal concentrations of Ca2+ and Mg2+, the rate of quantal release (estimated from MEPC frequency) increased at snake twitch and tonic muscle fiber endplates. Ultrastructurally, terminals did not deplete, consistent with a sustained elevation of MEPC frequency over several hours (Coniglio et.al. Neuroscience Absracts 15:257, 1989). In the present study, snake nerve-muscle preparations were maintained in KP with elevated Ca2+ (3.6mM) and no Mg2+. Under these conditions, MEPC frequency at twitch endplates increased greatly, then declined, so that at most endplates, no MEPCs were recorded after 2 hours. The mean MEPC amplitude also declined over time in this solution. Ultrastructurally, terminals showed depletion of synaptic vesicles after 6 hours. MEPC frequency at tonic endplates also increased under these conditions, but remained elevated for at least 18 hours. No change in mean MEPC amplitude was observed. Ultrastructurally, nerve terminals at tonic endplates contained numerous vesicles after 6 hours. We conclude that depolarization of nerve terminals in KP containing 3.6mM Ca2+ and no Mg2+ depletes terminals at twitch endplates but not those at tonic endplates.
This research was supported by an MDA grant.

217.16

TETRAHYDROAMINOACRIDINE (THA) INCREASES NEUROSECRETION AT A CHOLINERGIC SYNAPSE BY INCREASING PROBABILITY OF TRANSMITTER RELEASE. S.D. Provan* and M.D. Miyamoto. Dept. of Pharmacol., E. Tenn. State Univ., Johnson City, TN 37614.

THA is a long-acting, membrane permeable anticholinesterase which has been tried with varying success to offset the cholinergic deficit in Alzheimer's Disease. We have examined the effects of THA at the motor nerve ending to elucidate the mechanism of its reported clinical action. Miniature endplate potentials (mepps) were recorded from isolated frog cutaneous pectoris, and unbiased estimates of m (no. of quanta released), p (probability of release), and n (no. of activated release sites) made using a new method (Physiologist 31: A84, 1988). $[K^+]$ was raised to 10 mM to increase mepp frequency and ensure usable (binomial) results. Data were recorded at 5 min intervals, after 10 min equilibration. THA (3 μM) caused a monotonic increase in m (25% after 20 min), which was due to an increase in p, as n was unaffected. By contrast, physostigmine (3 μM) produced a 20% decrease in m, which was due primarily to a decrease in p. Neostigmine, which does not enter the nerve ending, had no effect on p. Spatial variance in p was unchanged in all experiments. Although THA and physostigmine are both membrane permeating anticholinesterases, their opposing effects on p suggest fundamental differences in their presynaptic actions. This may involve influx of Ca^{2+}_o and/or release of Ca^{2+} from intracellular stores (Supported by NIH NS22457).

217.17

PRESYNAPTIC REGULATION OF NEUROMUSCULAR TRANSMISSION BY NICOTINIC AND MUSCARINIC RECEPTORS. D.F. Wilson and R.H. Thomsen* Dept. of Zoology, Miami Univ., Oxford, OH 45056

The presynaptic effects of the muscarinic antagonist, atropine and the nicotinic antagonists, hexamethonium and tubocurarine were tested on evoked transmitter release at the neuromuscular junction using intracellular recording techniques to identify the role of presynaptic receptors in regulating evoked transmitter release. End-plate potentials (EPPs) and miniature end-plate potentials (MEPPs) were recorded from isolated cut-muscle rat diaphragm-phrenic nerve preparations to measure quantal release. In the presence of 400 uM hexamethonium a significant increase in quantum content (42%) was observed with a single stimulus relative to control but not during repetitive stimulation (50 Hz) and enhanced the magnitude of tetanic fade from 0.61 to 0.42. This concentration significantly depressed the amplitude of the MEPPs but not the first EPP. In the presence of 75 nM tubocurarine, the quantum content of the first EPP was significantly increased by 36% and the plateau EPPs by 18%. Tetanic fade was significantly enhanced from 0.61 to 0.53. This concentration of tubocurarine significantly depressed the amplitude of the MEPPs but not the first EPP. Atropine (2.5 uM and 7.5 uM) was also tested on the neuromuscular junction but failed to significantly alter any of these parameters. The failure of atropine to alter transmitter release suggests that the nerve terminal lacks muscarinic receptors. These results support the hypothesis that acetylcholine released from the nerve terminal normally has a negative feedback effect by depressing transmitter release via nicotinic receptors. (Supported by NIH Grant NS-27260)

217.18

MUSCARINIC AUTORECEPTORS ARE PRESENT ON CENTRAL AND PERIPHERAL CHOLINERGIC NERVES INNERVATING THE GUINEA PIG INFERIOR MESENTERIC GANGLION (IMG). H. P. Parkman and J. H. Szurszewski. Department of Physiology and Biophysics, Mayo Medical School, Rochester, MN 55905.

The aim of this study was to determine if cholinergic nerves innervating the IMG contain presynaptic autoreceptors which regulate release of acetylcholine (ACh). Isolated ganglia with attached lumbar colonic nerves (LCNs) and splanchnic nerves (SpNs) were loaded with ^{3}H-choline (750 nM, 80 Ci/mmole) followed by washing with 50 μM eserine and 10 μM hemicholinium-3. The amount of ^{3}H-ACh and ^{3}H-choline released during 5 minute periods was determined by radio-thin layer chromatography. Addition of atropine (1 μM) augmented the release of ^{3}H-ACh during SpN stimulation by 55% ($p<0.01$) and during LCN stimulation by 78% ($p<0.01$). When eserine was not present in the superfusate, the release of ^{3}H-choline plus ^{3}H-ACh was still augmented by the presence of atropine (1 μM) during stimulation of the central SpNs (35%, $p<0.01$) and during stimulation of the peripheral LCNs (24%, $.05<p<.10$). These results suggest that both the peripheral LCNs and central SpNs contain presynaptic muscarinic receptors which inhibit release of acetylcholine from cholinergic nerves. Furthermore, the results suggest that these autoreceptors may function in vivo when acetylcholinesterases are present to metabolize the released acetylcholine. (Supported by NIH DK 17632 and DK 07198.)

217.19

INHIBITION OF TACHYKININ RELEASE FROM ENTERIC NERVE ENDINGS BY ADENOSINE A^1 AND A_2 RECEPTOR AGONISTS. R.M. Broad, T.J. McDonald* and M.A. Cook. Depts. of Medicine and Pharmacology, U. of Western Ont, London, Canada, N6A 5C1.

Previous work has shown Ca^{2+}-sensitive evoked release of the tachykinins (TK) Substance P (SP) and Neurokinin-A (NKA) from perifused guinea pig (g.p.) enteric nerve varicosities (enteric synaptosomes). Recent experiments using the A_2-selective agonist [2-p-carboxyethyl]-phenylamino-5'-N-carboxamidoadenosine (CGS 21680, 10μM) demonstrated inhibition of release of both SP (86.3 ± 13.7%) and NKA (84.1 ± 15.9%) from synaptosomes as well as inhibition of field stimulated contractions of the intact g.p. ileum (EC_{50}=9.6x10^{-6}M). In synaptosomes, determinations in the presence of incrementing concentrations of the adenosine analogs CPA and NECA revealed the graded nature of the responses with EC_{50}'s for inhibition of SP release of 3.44x10^{-8}M and 7.6x10^{-8}M respectively. Pre-incubation with pertussis toxin (500 ng/ml) had no effect on the ability of CPA (50 nM) to inhibit the evoked release of these TK's. Pre-treatment of the synaptosomes with dibutyryl-cAMP (1 mM) similarly had no effect on the ability of CPA to inhibit evoked TK release. These data suggest the presence of both subtypes of adenosine receptors functionally linked to the release of TK's from enteric nerves. These data do not support a role for intracellular cAMP in mediating the response and do not support involvement of a susceptible G-protein. Supported by MRC, Canada.

POTASSIUM CHANNELS III

218.1

VOLTAGE-CLAMP ANALYSIS OF POTASSIUM CURRENTS IN BASOLATERAL AMYGDALA NEURONS. H.C. Moises and M.D. Womble. Dept. of Physiology, Univ. of Michigan, Ann Arbor, MI 48109.

Neurons of the basolateral amygdala (BLA) receive an extensive cholinergic input from the basal forebrain. We previously reported (Washburn & Moises, Neurosci. Abstr. 15:193, 1989) that application of the cholinomimetic carbachol produces a slow depolarization and reduces both accommodation and the slow afterhyperpolarization (AHP) in these cells. In this study, the single electrode voltage-clamp technique was used to survey membrane currents in BLA neurons in rat brain slices as a prelude to identifing targets of cholinergic action. M-current (Im) was seen as a voltage-sensitive inward current relaxation during hyperpolarizing steps from a holding potential (Vh) of -40 mV. Im was not affected by extracellular Cs (2 mM) or TEA (5 mM). Two Ca-activated K currents were identified underlying the AHP: the rapidly decaying Ic and the slowly decaying Iahp. Both were blocked by Cd (100 uM) but only Ic was blocked by TEA. Neither were affected by extracellular Cs. Negative steps from a Vh of -80 mV produced a slowly activating inward current, Iq, which was blocked by 2 mM extracellular Cs, while large depolarizations from this Vh induced Ia, a rapidly decaying outward current. Iq and Ia were insensitive to Cd and TEA. Thus, BLA neurons have many of the same K currents seen in other cortical neurons, several of which have been identified as targets of cholinergic modulation (ie., Iahp and Im). (Supported by NIDA grant #03365.)

218.2

SPIKE FREQUENCY ADAPTATION OF RAT DORSAL ROOT AXONS FOLLOWING NEUROMA FORMATION IN SCIATIC NERVE D.A. Utzschneider, R.B. Bhisitkul, J.D. Kocsis, **Dept. of Neurology, Yale Med. Sch. and VA Med. Ctr., West Haven, CT. 06516.**

Peripheral axons display a number of hyperexcitability phenomena following nerve ligation and neuroma formation. Much of this activity is attributed to altered electrophysiological properties of the axon region near the site of the transection. To study proximal sensory axon regions following nerve injury, dorsal roots of adult rats were studied in vitro in a sucrose gap chamber twenty days after sciatic nerve ligation. Current clamp of normal dorsal roots reveals a prominent inward rectification upon hyperpolarizing constant current passage. In general, the axons give rise to a single action potential during passage of modest levels of depolarization, but repetitive action potential discharge does occur during passage of large currents. The burst is marked by high frequency discharge which accomodates after 5-10 spikes. This is in contrast to dorsal roots obtained from rats where neuroma was present. Although inward rectification was still present in these roots, even large depolarizing current steps could not elicit repetitive firing. Dorsal roots obtained from rats whose sciatic nerves were crushed and allowed to regenerate showed reduced burst activity to depolarization as compared to control animals, but exhibited greater burst activity than the neuroma group. The increased spike frequency adaptation of sensory axons proximal to the site of neuroma indicates that the axonal hyperexcitability associated with neuroma formation is not generated over this region of the injured axon. Brismar et al. (BRAIN RES 378:347-356, 1986) have demonstrated an increase in axonal K and leak conductance proximal to the site of neuroma, possibly by loosening of paranodal myelin. An increased K and leak conductance could account for the reduction in the ability of these axons to generate bursts of action potentials.

218.3

A VOLTAGE- AND TIME-DEPENDENT RECTIFICATION IN RAT DORSAL ROOTS. B.D. Birch, J.D. Kocsis, F. DiGregorio*, R.B. Bhisitkul and S.G. Waxman, **Dept. of Neurology, Yale Med. Sch. and VA Med. Ctr., West Haven, CT. 06516.**

An increase in membrane conductance in response to hyperpolarization, which has been referred to as inward (anomalous) rectification, is observed in many types of biological membranes. In this study we investigated the ionic, pharmacological, and kinetic properties of inward rectification in rat dorsal root axons using the sucrose gap technique.

Spinal roots were excised from rats and placed in a sucrose gap chamber. Constant hyperpolarizing current pulses elicited a time-dependent hyperpolarization characterized by a relaxation of membrane voltage toward resting potential followed by a slower decay away from resting potential during the current pulse. The relaxation in the hyperpolarization was reduced, but not eliminated, by the removal of either potassium or sodium from the perfusate. Removal of both potassium and sodium virtually abolished the relaxation and decay, indicating that the conductance is dependent on sodium and potassium. Pharmacologic blockade of voltage-dependent sodium channels with tetrodotoxin and potassium channels with tetraethylammonium or 4-aminopyridine did not reduce the relaxation or decay, suggesting that inward rectification is not mediated by these channels. Inward rectification was partially blocked by barium and completely abolished by cesium as seen in rat optic nerve (Eng et al. J Physiol 421:185-202, 1990). Simultaneous pulse experiments reveal that inward rectification becomes progressively refractory to activation during the decay phase; current pulses given at the onset of the decay elict a voltage relaxation whereas pulses given later during the decay do not. This phenomenon suggests that the voltage decay during the current pulse represents inactivation of a conductance which mediates inward rectification.

218.4

WHY IS THE INPUT CONDUCTANCE OF HIPPOCAMPAL NEURONES IMPALED WITH MICROELECTRODES SO MUCH HIGHER THAN WHEN GIGA-SEAL PATCH PIPETTES ARE USED?

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In preparations where both microelectrode (µ-el.) and whole-cell patch (giga-seal) recordings have been done, the latter technique usually yields about tenfold higher input resistance (Rinp) than the former (e.g.in hippocampal neurons; Edwards et al., Pfluegers Arch 414:600). If the giga-seal value is representative, it has many implications for our understanding of the functions of the cells, and several ideas based on µ-electrode data may have to be revised, e.g. regarding the resting potential (RMP) mechanism, electrotonic structure, and the impact of synapses, ion channels and pumps.

Until the reason for the Rinp difference is understood, however, one cannot be sure which technique is most reliable. The difference is often attributed to a leak through the hole made by the µ-electrode. However, such a hole (probably nonselective and accounting for ~90% of the input conductance) should cause a severe depolarization (e.g. from about - 70 to - 5 mV, if Rinp drops from about 500 to 50 MΩ, as seems to occur in hippocampal neurons), but this is not observed. Possible explanations for this discrepancy include: the impalement could activate either (1) a K conductance (e.g. by depolarization, or influx of Ca or Na ions), or (2) a conductance for a passively distributed ion (e.g. Cl^-), or (3) a pump which maintains the RMP in spite of a non selective leak. However, none of these ideas seem to be supported by the available data, at least for hippocampal neurons: (1) the resting K conductance shows little or no voltage-dependence, and the low Rinp and RMP with µ-el.s seems to persist in Ca-free medium and after injection of Ca-buffers, which abolish the Ca-activated K currents I_C and I_{AHP}; (2) injection of Cl^- ions have little or no effect; (3) inhibiting the Na/K pump with cooling or drugs does not immediately depolarize the cell.

Thus, the discrepancy beteween µ-el. and giga-seal does not seem to be satisfactorily explained. We have therefore started to re-examine this problem.

Supported by the Norwegian medical research council (RMF/NAVF).

218.5

A TRANSIENT VOLTAGE-DEPENDENT OUTWARD CURRENT RECORDED FROM RAT CEREBELLAR PURKINJE CELLS (PCs) UNDER VOLTAGE CLAMP. S.-J. Li*, Y. Wang*, H.K. Strahlendorf and J.C. Strahlendorf, Depts. of Physiol. and Neurol., Texas Tech Univ. Hlth. Sci. Ctr., Lubbock, TX 79430.

Current-clamp recordings of turtle PCs demonstrate a transient hyperpolarization (TH) that outlasts the electrotonic response of the membrane to hyperpolarizing current injection (Hounsgaard, 1988). This TH displays voltage, time and ionic dependencies suggestive of the transient outward K current (I_A) recorded in many neurons. Recordings of rat PCs in our laboratory also revealed this TH and it is augmented by serotonin. PCs were voltage-clamped with a single electrode system and superfused with modified Kreb's solution containing 2.5 mM Ni^{2+}, 10 mM TEA, 0 Ca^{2+} and 3 µM TTX. From a holding potential (HP) of -70 mV, 10 mV depolarizing steps elicited a transient outward current (TOC) at steps more positive to -50 mV that declined to a steady state over a period of about 200 msec. At holding potentials near -45 mV TOC was substantially inactivated. Hyperpolarizing commands to near -100 mV from a HP of -50 mV also revealed the TOC upon return to the HP for steps more negative than -60 mV. Bath application of 500 µM 4-AP markedly reduced the TOC. Our results suggest the TH seen in PCs may be mediated by this TOC which is similar to I_A seen in other cells. Supported by NS 19296 and the Tx. Adv. Res. Prog., Grant 010674-020.

218.6

AMINOPYRIDINE BLOCK OF K CHANNELS IN NEUROBLASTOMA CELLS. J. K. Hirsh * and F. N. Quandt Multiple Sclerosis Center and Dept. of Physiology, Rush University, Chicago, Il. 60612

The action of 4-aminopyridine (4-AP) and derivatives on voltage-dependent K channels was examined using whole cell and excised membrane patch clamp techniques applied to N1E-115 neuroblastoma cells grown in tissue culture. Half maximal block of the steady state current measured with whole cell voltage clamp was obtained at a concentration of 0.35 mM when 4-AP was added to the external solution. Approximately 10 percent of the current was resistant to 4-AP. The time course of K current in the presence of submaximal doses of 4-AP was altered. First, a slow phase of outward K current developed in response to a step depolarization after exposure to 4-AP. Second, the decay of current during a maintained depolarization was eliminated. Block by 4-AP did not decrease when depolarizations were applied repetitively at intervals between 1000 to 60 mS. The alterations in whole cell current are consistent with the presence of a channel type with slow activation and inactivation kinetics, which is resistant to 4-AP. However, single channel analysis indicated that block by 4-AP is modulated under certain conditions. 4-AP effects on single delayed rectifier channels were studied using inside-out membrane patches, since K channel current was more sensitive to application of 4-AP to the inside surface of the membrane. With current through the K channel carried by Rb, the latency of opening of K channels in inside-out excised membranes increased in the presence of 4-AP. 4-AP produced a component in the closed time histogram with a duration of 200 mSec, presumably representing the blocked state. 4-AP had little effect on the open time or amplitude of single channel current. Supported by the National Multiple Sclerosis Society.

218.7

WHOLE-CELL CLAMP OF RAT THALAMIC NEURONS REVEALS TWO DISTINCT TRANSIENT K CURRENTS J.R. Huguenard, D.A. Coulter and D.A. Prince. Dept. Neurology, Stanford Univ. Medical Center, Stanford, CA 94305.

We have recently described a prominent transient Ca current (I_T) in relay neurons (RNs) that is both necessary and sufficient for the generation of Ca-dependent, low-threshold spikes (LTS; Coulter, *et al., J. Physiol.* 414:587,1989). However, these cells are also characterized by K conductances that are activated concurrently with I_T, and thus tend to slow or retard the development of Ca-dependent spikes. In order to further understand the regulation of LTS burst firing in RNs, we examined the various K currents activated in the voltage range near threshold for LTS activation.

RNs were obtained from rat VB complex by acute isolation, and current recordings were made via whole-cell clamp. The pipette solution consisted of KCl, EGTA-Ca, $MgCl_2$ and HEPES, Na currents were blocked with TTX, and Ca currents were minimized by using low (0.5 - 1 mM) extracellular Ca. Most experiments were performed 22-24°C. Depolarizations to between -50 and 0 mV from a holding potential of -90 mV produced an outward current that was largely inactivating during a 300 ms depolarization. A rapidly activating (peak < 4ms) current, I_A, was activated at a threshold near -60 mV, and inactivated with a time constant (τ) of 20-30ms. I_A was half-inactivated at a holding potential of -83 mV, was blocked by 4AP (5mM), but not by TEA. In addition to I_A, a second, smaller inactivating current (I_{A-2}) was also present. In comparison to I_A, I_{A-2} was steady-state inactivating, ($V_{½}$ = -80 mV). However I_{A-2} peaked relatively slowly (200 ms), inactivated slowly (τ > 3s), activated with a slightly higher threshold (-40 mV) and was blocked by TEA but not by 4AP. Both currents were K dependent, with reversal potentials governed by the Nernst relationship.

Since I_T, I_A and I_{A-2} are partially inactivated at rest (≈ -70 mV), hyperpolarizing influences will promote subsequent activation of all three currents. Implications of the interactions of these conductances for LTS generation will be discussed. Supported by NIH grants NS06477 and NS12151.

218.8

EXPRESSION OF POTASSIUM A CURRENT CHANNELS IN *XENOPUS* MYOCYTES DURING DIFFERENTIATION IN CULTURE. U. Ernsberger & N.C. Spitzer. Department of Biology and Center for Molecular Genetics, UCSD, La Jolla CA 92093.

The developmental regulation of potassium currents plays a major role in the differentiation of electrical activity in excitable cells. Outward currents carried by potassium can significantly influence the extent of calcium influx through voltage dependent channels, and thus may affect a variety of processes regulated by calcium. To understand the maturation of potassium currents, we have analyzed single potassium channels in inside-out patches from Xenopus myocytes differentiating *in vitro*.

Under conditions that suppress the activity of calcium and calcium-dependent channels, ~90% of the channels from 1 day old myocytes are selective to potassium ions and are referred to as potassium channels. Although ~70% of the patches from one day old myocytes showed no ion channel activity between -80 and +40mV, ~80% of the patches with activity showed superimposed channel openings. The pattern of activity in patches with channel openings to only a single current level suggest that the superimposed openings do not reflect substates of a single active channel but clustering of independent channels.

The abundance of channels with properties characteristic of potassium A current increases by roughly one order of magnitude between 9 and 30 hrs in culture. The A current channels, with conductances of ~20pS, are inactivated at depolarized potentials. When depolarized from holding potentials of -80 or -120mV, they activate within milliseconds, at activation rates that are voltage dependent. Several other channel classes were distinguished on the basis of conductance, dwell time distributions and inactivation behavior. Ensemble average currents indicate that these channels do not contribute to the voltage-dependent A and delayed rectifier currents. There is little or no increase in their density during the period in which the A current density increases. Thus the A current channels are likely to play an increasing functional role during this developmental period.

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218.9

UNITARY 'A' CURRENT CHANNELS IN CENTRAL NEURONS. I.D. Forsythe, P. Linsdell & P.R. Stanfield. Dept. of Physiology, University of Leicester, P.O. Box 138, Leicester LE1 9HN. UK.

Ion channels underlying the transient outward potassium current (Connor & Stevens, J. Physiol. 213: 21, '71) were studied in locus coeruleus and hippocampal neurons grown in dissociated cell culture, using the patch-clamp technique in the 'on-cell' and 'inside-out' configuration. Patch electrodes contained 140 or 3 mM KCl. 'Inside-out' patches were perfused with 140 mM KCl using three flow pipes, through which different media were perfused at a constant temperature of 20 ± 0.1 °C. The current-voltage relation of the channels were studied in response to both step and ramp voltage protocols.

Peak open probabilities were in the range of 0.6-0.9, while activation and inactivation of unitary channels were similar to whole cell 'A' currents. In the 'on cell' configuration single channels had peak conductances of 41.3 ± 1.1 pS (n=4, ±SEM) and 15 ± 0.6 pS (n=7), $[K^+]_o$ was 140 and 3 mM, respectively. The unitary conductance rectified in a voltage dependent manner at positive potentials. With 'inside-out' patches, channels had a linear I/V and conductance of 17.8 ± 1.9 pS when perfused on the inside with medium containing 140 mM K^+ and no added Mg^{2+}, Ca^{2+} or Na^+ ($[K^+]_o$ = 3 mM). Addition of 2 mM Mg^{2+} or 10 mM Na^+ to the internal perfusate reduced the unitary amplitude and increased open state noise in a voltage dependent manner, with K_ds at 0 mV, of 15.5 and 76 mM, respectively. This rectification was similar to that observed in 'on-cell' patches. This phenomenon could serve to limit the contribution of the 'A' current to repolarization during the overshoot of an action potential. Kinetic analysis shows a single open state and at least three closed states, plus an inactivated state. A subconductance state with approximately 60% of the fully open conductance is also observed.

218.10

DEVELOPMENTAL EXPRESSION OF A SLOWLY-INACTIVATING, VOLTAGE-DEPENDENT K CURRENT IN ACUTELY-DISSOCIATED NEOSTRIATAL NEURONS. D. J. Surmeier, R. C. Foehring, A. Stefani, S. T. Kitai. Department of Anatomy and Neurobiology, College of Medicine, The University of Tennessee, Memphis, Memphis, TN 38163

Cultured embryonic rat neostriatal neurons have two voltage-dependent K currents: A current (IA) and delayed rectifier (Surmeier et al. Neurosci. Lett. 103: 331, 1989). We have found evidence for a third current in neurons dissociated from postnatal rats older than 2-3 weeks. This current is similar to the D current described in CA1 neurons by Storm (Nature 336: 379, 1988) but in neostriatal neurons this current is more TEA-sensitive and recovers more rapidly from inactivation.

Conventional whole cell recording techniques were used on acutely dissociated rat striatal neurons at room temperature. Neurons were dissociated from rats between P1 and P120 with a protocol modified from Mody et al. (Neurosci. Lett. 96: 70, 1989). Extracellular solutions were designed to minimize the contribution of Cl and Ca currents.

Whole-cell recordings of neurons from rats older than P14 revealed two K currents which inactivated at potentials positive to -40mV. Half-inactivation voltages of the two currents were around -50 and -90 mV. These currents differed in inactivation recovery kinetics (at -100 mV, time constants were 20-50 ms and 80-1500 ms), deactivation kinetics and sensitivity to 4AP and TEA. (Supported by NINDS grant NS 26473 and NS 20702).

218.11

HYPERPOLARISATION ACTIVATED INWARD CURRENT IN HISTAMINE NEURONS OF RAT HYPOTHALAMUS. Anita Kamondi and Peter B. Reiner, Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C. V6T 1W5

The hyperpolarisation activated inward current (I_h) was studied in histaminergic tuberomammillary (TM) neurons using single electrode voltage clamp technique in an *in vitro* slice preparation of the rat hypothalamus. Hyperpolarizing voltage steps from a holding potential of -60 mV evoked an inward current in a time and voltage dependent manner, causing inward rectification of the steady state current-voltage relationship measured between -60 and -130 mV. The time constant of the fully activated current was ~300 ms and the current did not inactivate. The activation curve for I_h fitted to the Boltzman equation showed a $V_{1/2}$=-100 mV, and k=1.12. The reversal potential of I_h was close to -35 mV measured in control extracellular solution containing 2.5 mM K^+ and 156.2 mM Na^+. When the external potassium concentration was raised to 10 mM, I_h increased and the reversal potential shifted in a depolarized direction. Partial replacement of the external sodium by choline decreased both the instantaneous and steady state current and displaced the reversal potential in a hyperpolarized direction. Removal of extracellular Ca^+ and Cl^- did not affect I_h. Changes in the external ionic concentration did not affect the voltage dependence of I_h. Extracellularly applied Cs^+ (1 mM) reversibly inhibited the current. External Ba^+ (2 mM) caused an outward current at holding potential -60 mV, decreased the instantaneous conductance, but did not block I_h.

Supported by the MRC.

218.12

PATCH CLAMP STUDIES ON CULTURED SMOOTH MUSCLE CELLS FROM RAT CEREBRAL ARTERIES. Y. Wang and D.A. Mathers, Physiology Department, University of British Columbia, Vancouver, B.C. V6T 1W5 Canada.

Electrical properties of smooth muscle cells (SMCs) cultured from conducting vessels of the rat cerebral circulation were studied using standard patch clamp methods. Cells were dispersed to small clumps by treatment of basilar, middle and posterior cerebral arteries with 0.3% collagenase at 37°C. Whole-cell current clamp recording showed that the average resting potential (RMP) of the cells declined slowly with passage of time in vitro. After 3-4 days in culture, the mean RMP was -44 ± 3.2 mV (mean ± S.E.M.) with mean input resistance 2.85 ± 0.675 GΩ. Cell-attached patch recording showed biphasic currents indicative of spontaneous action potential generation in only a few percent of cells tested. Application of 10 μM serotonin induced this type of activity in 5/10 cells tested. Serotonin also activated a high conductance membrane channel with kinetic and conductance properties resembling the calcium dependent potassium channel of rat myotubes and aorta SMCs.

218.13

NONSTATIONARY FLUCTUATION ANALYSIS OF M-CURRENTS IN BULLFROG SYMPATHETIC AND RAT PC-12 CELLS. N.V. Marrion*, A. Villarroel*, W. Gruner* and P.R. Adams. Howard Hughes Medical Institute, SUNY at Stony Brook, NY 11794.

We have used nonstationary, ensemble variance measurements (Sigworth, *J.Physiol.*, 307:97, 1980) to estimate single channel conductance for the M-channel in both bullfrog sympathetic neurons and rat PC-12 cells. M-current (I_M) was activated by whole-cell voltage-clamping both cell types at positive holding potentials (between -40 and -30 mV) and deactivated by 20 mV hyperpolarizing voltage steps.

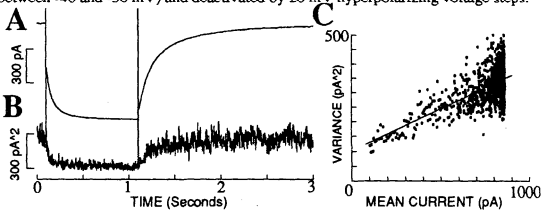

Figure A shows the average membrane current trace of 84 records obtained by stepping the voltage of a bullfrog sympathetic neuron from -35 mV to -55 mV for 1 second. The variance, computed from 21 groups of four records, fell in a time-dependent manner as M-channels closed during the voltage step and increased as M-channels reopened at the cessation of the pulse (figure B). Linear regression of variance versus mean current during the first 400 ms of repolarization (figure C) gave a single channel conductance estimate of approximately 5 pS (reversal potential -98 mV). Similar results were obtained from PC-12 cells. These results accord with single channel recordings from bullfrog sympathetic neurons (Gruner et al, *Soc. Neurosci. Abstr.*, 15:990, 1989) and suggest that the M-channel in PC-12 cells may also have similar properties.

218.14

POTASSIUM CURRENTS AND SENSORY CODING IN THE TOADFISH SACCULAR HAIR CELLS. A. Steinacker and A. Romero*, Dept. Otolaryngology, Washington University School of Medicine, St. Louis, MO 63110.

Sensory coding of characteristic frequency (CF) in auditory hair cells has been modelled around the interaction of an outward calcium activated potassium current (**IKCa**) and an inward calcium current to produce resonance at the CF. Using whole cell patch clamp analysis of hair cells of the putative auditory end-organ, the saccule, of the toadfish (Opsanus tau), we have found two distinct classes of hair cells, on the basis of their outward currents. There are only two major outward currents of the hair cell of the saccule of the toadfish **IKCa** and a voltage gated potassium current (**IK**). The temporal and voltage sensitivity of kinetics of activation and deactivation of these two currents are markedly different. One class of saccular cells contains only **IKCa** and no **IK**. The second class contains **IK** in addition to **IKCa**. (They may both additionally contain a small A current). These two classes of cells must code auditory stimuli in different ways. We have measured the kinetics of activation and deactivation of these two currents as they appear in individual cells to ascertain whether their rates are linked in individual cells and find that they are. This suggests that the two currents are functionally linked in sensory processing. The role of **IKCa** alone and these two currents in tandem in resonance phenomena was also investigated since the current model for resonance is based on the kinetics of only **IKCa**. We conclude that at least two forms of sensory processing are taking place in the hair cell. Supported by DRF.

218.15

TWO CALCIUM-ACTIVATED POTASSIUM CONDUCTANCES GENERATE THE POST-STIMULUS HYPERPOLARIZATION IN RAT LOCUS COERULEUS NEURONS. S.S. Osmanovic and S.A. Shefner. Dept. Patho-Physiology, Medical Faculty Belgrade, Yugoslavia and Dept. Physiology and Biophysics, University of Illinois College of Medicine, Chicago, IL 60612.

The properties and ionic mechanisms underlying the post-stimulus hyperpolarization (PSH) which follows trains of action potentials in rat locus coeruleus (LC) neurons were investigated. Intracellular recordings were made from LC neurons in a totally submerged brain slice preparation. The PSH following trains of action potentials evoked by small depolarizing currents was monophasic. When larger depolarizing stimuli were used, an additional, earlier, phase of PSH occurred immediately following the train. Thus, PSH elicited with stronger stimuli consisted of two distinct components: a faster, early (PSH_E) and a slower, late one (PSH_L). The time course of PSH decay could be fit by a biexponential function. Artificial CSF containing low Ca^{2+} (0.25 mM) and high Mg^{2+} (20 mM), or Cd^{2+} (0.5-1 mM) blocked both components (n=5). Apamin (2-500 μM) selectively blocked PSH_E (n=22). TEA (2-6 mM) decreased the amplitude of PSH_L, but not PSH_E and prolonged both components. Addition of apamin after TEA caused complete block of PSH and the appearance of a large, long-lasting depolarization (PSD). This depolarization was associated with firing and decreased input resistance. External Ba^{2+} (1-2 mM) depressed both PSH_E and PSH_L (n=5), but for short trains the reduction was greater for PSH_E. With longer trains in Ba^{2+}, both phases of PSH were abolished, being replaced by PSD. These data suggest that PSH in LC neurons is mediated by two different Ca^{2+}-activated K conductances. PSH_E is mediated by an apamin-sensitive conductance, which is blocked by Ba^{2+} but not TEA. PSH_L is mediated by an apamin-insensitive conductance which is blocked by Ba^{2+} and reduced by TEA. Grant support: US PHS AA 5846.

218.16

LARGE CONDUCTANCE POTASSIUM CHANNELS IN DISSOCIATED RAT SUBSTANTIA NIGRA NEURONS. X. Gu, K. Breedlove*, S.G. Speciale, A.L. Blatz and D.C. German, Depts. of Psychiatry and Physiology, UT Southwestern Medical Center, Dallas, TX 75235.

Cell-attached patch-clamp recordings of dissociated rat substantia nigra neurons revealed the presence of large-conductance K channels. Single neurons from the zona compacta were isolated using either a trypsin or protease dissociation method. At least one-half of the neurons exhibited large inward currents which were active as soon as seals were formed. These channels were active at the normal resting membrane potential, and exhibited single channel currents of 5-6 pA under the following conditions: bath solution (mM) - 150 NaCl, 4 KCl, 2 $CaCl_2$, 5 TES, pH 7.0; pipette solution - 140 KCl, 1 EGTA, 5 TES, pH 7.0. Assuming a resting membrane potential of -60 mV, the single channel slope conductance was 100-150 pS. These channels were observed whether or not the D2 dopamine agonist quinpirole (1-10 uM) was present in the pipette solution. Recordings from excised, inside-out patches demonstrated that these channels were sensitive to uM concentrations of intracellular Ca, and were voltage dependent. The observation that these channels are active at normal resting membrane potentials suggests that they are either more sensitive to intracellular Ca than BK channels in tissue-cultured skeletal muscle, or that under typical dissociation conditions the neurons become loaded with Ca. Research supported by MH-30546, DA-05314 and GM-39731.

218.17

CA^{2+}-ACTIVATED K^+ CURRENTS IN ACUTELY ISOLATED EMBRYONIC CHICK CILIARY GANGLION NEURONS. S. E. Dryer, M. E. Wisgirda* and M. Dourado*. Department of Biological Science, Florida State University, Tallahassee, FL 32306.

Patch clamp recordings were made from neurons obtained from E11-E14 embryos. Depolarizing voltage steps from holding potentials of <-50 mV evoked K^+ currents that were abolished by perfusion with Ca^{2+}-free salines ($I_{K(Ca)}$). Application of 10 MM TEA completely blocked $I_{K(Ca)}$. 1 MM TEA caused 60 - 90% block of $I_{K(Ca)}$. The effects of TEA were completely reversible. Saturating concentrations of apamin (200 NM) blocked 40-70% of the TEA-sensitive $I_{K(Ca)}$. Apamin effects were not reversible in 20 minutes. At least three different $I_{K(Ca)}$ conductance states were detected in inside-out patches. These had conductances of 190 pS, 110 pS, and 45 pS with $[K]_o$ = 150 MM and $[K]_i$ = 75 MM. The 45 pS state showed the greatest sensitivity to Ca^{2+}. Three different $I_{K(Ca)}$ conductance states were observed in cell-attached and outside-out patches. $I_{K(Ca)}$ channels in outside-out patches were completely blocked by 10 MM TEA, and partially blocked by 1 MM TEA. Current-clamp recordings showed that both $I_{K(V)}$ and $I_{K(Ca)}$ contribute to spike repolarization and the a.h.p. Surprisingly, 200 NM apamin produced only a small and inconsistent reduction in the a.h.p. Supported by NS-27013.

218.18

COMPARISON OF VOLTAGE-DEPENDENT POTASSIUM CURRENTS IN RAT LACTOTROPHS AND CLONAL PITUITARY CELLS (GH3). S.M. Simasko. Dept. of Physiology, School of Medicine, S.U.N.Y. at Buffalo, Buffalo, NY 14214.

The activity of voltage-dependent potassium channels are an important element in the control of membrane potential and hence Ca^{2+} influx through voltage-dependent calcium channels. In this study whole-cell patch-clamp techniques were used to study voltage-dependent potassium currents in rat lactotrophs identified by reverse-hemolytic plaque assay and in the rat pituitary cell line GH3. Ca^{2+} currents and Ca^{2+}- activated K^+ currents were eliminated by recording in Ca^{2+}-free bath buffer (replaced with Mg^{2+}). In both cell types, when the cells were held at -76 mV and then pulsed to positive potentials, an outward current activated that then relaxed to a sustained level. The inactivating portions of the current traces could be fit to a double exponential (time constants of ~75 msec and ~4 sec in both cell types). The relative proportion of fast inactivating current (I_{Kf}) and slow inactivating current (I_{Ks}) varied from cell to cell, suggesting that these currents are due to different channels. In both cell types I_{Kf} was completely inactivated when the holding potential was reduced to -40 mV. Half-maximal conductance of I_{Kf} in both cell types occurred at ~0 mV. However, whereas recovery from inactivation of I_{Kf} in GH3 cells was slow and best fit by two exponentials (time constants of 350 msec and 7 sec), the recovery from inactivation in lactotrophs was rapid and best fit by a single exponential (time constant of 90 msec). The more rapid recovery from inactivation of I_{Kf} in lactotrophs would suggest that this current is much more influential in the control of membrane potential in these cells. The basis for the differences in recovery rate is at present unknown.

218.19

VOLTAGE-GATED IONIC CURRENTS IN EPITHELIAL SENSORY CELLS FROM SEA ANEMONE TENTACLES. D. A. Hessinger * and P. Brehm. Dept. of Physiology, Loma Linda University School of Medicine, Loma Linda, CA 92350 and Dept. of Neurobiology and Behavior, SUNY Stony Brook, Stony Brook, NY 11794.

Nematocysts, the eversible prey-capturing organelles of cnidocytes, are triggered to discharge by calcium-dependent exocytosis following mechanical and chemical stimulation. The sensory receptors for these stimuli reside on adjacent supporting cells (SCs) in sea anemone fishing tentacles. Using whole cell, voltage-clamp techniques on SCs isolated from excised anemone (Haliplanella luciae) tentacles we have identified and partially characterized three voltage-dependent currents: (i) transient outward potassium ; (ii) late outward, carried in part by potassium; and (iii) inward calcium. Both transient and late outward currents are eliminated by substituting either $CsCl_2$ or N-methylglucamine (NMG)-Cl for internal KCl. The transient outward current is blocked by external 4 mM 4-aminopyridine (4-AP) while causing an unexpected reciprocal increase in the late outward current. The late outward current is dependent on divalent cations; substitution of barium by calcium results in a 2 to 3 fold increase in late outward current with no corresponding change in transient current. The voltage dependent inward current is carried by calcium or barium and is blocked by external 2 mM $CdCl_2$. Given the central role of calcium to nematocyst discharge and the role of SC sensory receptors in controlling discharge, it is likely that modulation of calcium action potentials in these cells via sensory stimulation may regulate exocytosis in adjacent cnidocytes. [Supported by NSF grants 8609859 (DAH) & 8503159 (PB)].

218.20

VOLTAGE-ACTIVATED IONIC CURRENTS FROM MYOEPITHELIAL CELLS ISOLATED FROM THE SEA ANEMONE *CALLIACTIS TRICOLOR*. M.A. Holman and Peter A.V. Anderson. Whitney Laboratory and Depts. of Physiology and Neuroscience, Univ. of Florida, St. Augustine, FL 32086.

The Cnidaria are the most primitive phylum to possess a recognizable nervous system and are thus useful models for studying the evolution of the nervous system and electrical excitability. Little is known about the Anthozoa, the most primitive of the cnidarians. We have examined the electrical properties of myoepithelial cells from the sea anemone *Calliactis tricolor.* These cells have resting potentials in the range of -60 to -90 mV and produce fast action potentials when depolarized. The ionic currents underlying this excitability were examined using the whole-cell patch clamp configuration.

These cells possess two inward currents, both carried by Ca^{++}. These were distinguishable by their tail currents, and had the unusual ionic permeability sequence $Ca^{++} > Ba^{++} > Sr^{++}$. We identified five outward K^+ currents: 1) a fast, transient 4-AP sensitive current, 2) a Ca^{++}-dependent K^+ current, 3) a fast transient K^+ with slow inactivation, 4) a steady-state K^+ component, and 5) a small, very fast transient outward current that was insensitive to internal Cs/TEA, but was shown not to be Cl^--dependent. With a few exceptions, these currents are similar to those seen in other organisms.

Supported by NSF grant BNS-8805885

CALCIUM CHANNELS: PHARMACOLOGY

219.1

NEOMYCIN INHIBITS VOLTAGE-SENSITIVE CALCIUM CHANNELS (VSCC) WHICH ARE INSENSITIVE TO ω-CONOTOXIN GVIA (ω-CT) AND DIHYDROPYRIDINES (DHP). R.A. Keith, T.J. Mangano, W.C. Moore, C. Thompson, J. Patel and A.I. Salama. Department of Pharmacology, ICI Americas Inc., Wilmington, DE 19897.

The effects of neomycin, ω-CT and DHP on VSCC-dependent neuronal responses were compared. ω-CT and DHP partially inhibited the K^+-evoked (K^+-E) release of ^{3}H-norepinephrine (NE) from rat cortical slices and the K^+-E increase in intracellular calcium ($[Ca^{++}]i$) in neocortical neurons in primary culture, but had no effect on the K^+-E release of ^{3}H-D-aspartate (asp) from hippocampal slices. This suggests that K^+-E release of ^{3}H-NE and the K^+-E increase in $[Ca^{++}]i$ are only partially dependent on L- and N-type VSCC, whereas the K^+-E release of ^{3}H-D-asp has no dependence on N- or L-type VSCC. In contrast, neomycin caused a concentration-dependent and virtually complete inhibition of K^+-E ^{3}H-NE and ^{3}H-asp release and K^+-E increase in $[Ca^{++}]i$ (IC_{50} values 100-300 μM). At 1 mM neomycin there was only a slight inhibition (8%) of carbachol-induced phosphoinositide turnover, suggesting direct inhibition of phospholipase C is not the mechanism underlying the inhibition of K^+-E responses. Thus neomycin inhibited depolarization-induced neurotransmitter release and calcium influx that was not inhibited by ω-CT and DHP. This suggests that neomycin inhibits a neuronal voltage-sensitive calcium influx pathway that is critical for neurotransmitter release yet distinct from N-or L-type VSCC.

219.2

ACTIVATION OF NIMODIPINE-SENSITIVE L-TYPE CALCIUM CHANNELS ON BRAIN DOPAMINERGIC TERMINALS BY POTASSIUM PLUS PICOMOLE OF MPP^+. C. C. Chiueh and S.-J. Huang*, Lab. of Cerebral Metabolism, NIMH, Bethesda, Maryland 20892.

MPP^+ (1-methyl-4-phenylpyridinium ion) is the major cerebral metabolite of MPTP and is retained in monkey dopaminergic nerve terminals.

Millimolar MPP^+ increases Ca^{2+} influx and dopamine (DA) overflow in the lateral striatum which might lead to a selective lesioning of nigrostriatal neurons (Miyake et al., 1990). In this study, MPP^+ (5 to 500 pmol) caused only a monophasic DA efflux from striatum of anesthetized rats into a microdialysis probe. K^+ depolarization of nerve terminals (15 to 90 mM for 5 min) enhanced DA response to MPP^+. Therefore, similar to BAY K 8644, picomoles of MPP^+ can activate nimodipine-sensitive L-type calcium channels only when membrane potential is being depolarized by K^+.

This K^+-induced potentiation of MPP^+-evoked DA release depended on extracellular Ca^{2+} and was reduced one half by nimodipine (10 μM). This enhanced DA efflux was augmented further by 4-aminopyridine (1 mM), a K^+ channel blocker. These results indicate that MPP^+ could alter the gating mode of voltage-regulated L-type calcium channels on brain dopaminergic terminals. Furthermore, MPP^+ might also function like an organic cation and interact with K^+ channel.

219.3

HIGH THRESHOLD CALCIUM CURRENTS IN RABBIT SENSORY NEURONS:DIFFERENCES IN SENSITIVITY TO ω-CONOTOXIN (ω-CTX) AND NIMODIPINE (NIM). P.E. TanPiengco* and R.T. McCarthy. Inst. for Preclinical Pharmacol., Miles Inc., West Haven, CT. 06516

The patch voltage clamp in the whole cell and cell attached modes has been used to measure Ca^{++} channel currents (I_{Ba}) in freshly dispersed dorsal root ganglia (DRG) cells. A variable amount of high threshold peak inward current was inhibited by ω-CTX (5μm;n=10). In some cells this inhibition was complete even for current elicited from depolarized potentials (VH=-30 mV). In such cells, BAY K 8644 (BK;100 nM) had no effect. When half-maximal I_{Ba} inactivation was very positive (V1/2 = -30mV), ω-CTX block was minimal. This ω-CTX resistant current (I_R) remained unaffected by higher concentrations of ω-CTX (10-20μM). I_R contained dihydropyridine (DHP) sensitive I_{Ba}; inhibited by NIM and enhanced by BK. Single channels (VH=-50mV; 28pS; 110 Ba), enhanced by pretreatment with BK, could be potently inhibited by NIM. However, in both whole cell and cell attached records a small component of I_R was also resistant to NIM(200nM;≅70% block). These results suggest that pretreatment with ω-CTX can be used to more easily study DHP sensitive currents and demonstrate high affinity of NIM for L-type Ca^{++} channels.

219.4

[3H]-NIMODIPINE IDENTIFIES A LOW AFFINITY DIHYDROPYRIDINE BINDING SITE IN DEVELOPING CORTICAL ASTROCYTES. M.C. Howell*[1], M.J. Litzinger[2] and H.S. White[1]. [1]Dept. of Pharmacol. & Toxicol. and [2]Depts. of Biol., Physiol.and Pediatrics & Neurology, Univ. of Utah, S. L. C., UT 84108

Nimodipine has been shown previously to block K^+-stimulated $^{45}Ca^{2+}$ influx in mouse cortical astrocytes in nanomolar concentrations thereby suggesting that astrocytes express a high affinity dihydropyridine receptor (Hertz et al., J. Neurosci. Res. 22(2):209,1989). Previous binding studies with [3H]-nimodipine in mouse spinal cord astrocytes have only identified a low affinity binding site. The present study examined the developmental profile of [3H]-nimodipine binding in cultured mouse cortical astrocytes. Results obtained from displacement studies identified the presence of a low affinity binding site (micromolar) throughout development (2 to 9 weeks in culture). Binding was maximal between 3 and 4 weeks in culture. This low affinity binding was also confirmed in 3 to 4 week old cultures using the dihydropyridine ligand [3H]-PN-200. These results are consistent with those of Bender and Hertz (Eur. J. P'Col. 110:287,1985).

Younger cultures displayed a greater sensitivity to displacement by unlabeled nimodipine than did older cultures. This observation would suggest that astrocytes are better able to regulate extracellular calcium concentrations during certain periods of development. Whether or not astrocytic regulation of extracellular calcium plays an important role in neuro-developmental processes such as neurite extension which are dependent in part on extracellular calcium has yet to be elucidated but is worthy of further investigation. (Supported by NIH grant 2-RO1-NS-22200).

219.5

EFFECTS OF NIMODIPINE ON RABBIT CA1 HIPPOCAMPAL NEURONS. J.R. Moyer, Jr. J. Black' & J.F. Disterhoft. Depts. of Cell, Molecular & Structural Biology and of 'Pharmacology, Northwestern Univ. Med. Sch., Chicago, IL 60611.

Nimodipine, a dihydropyridine calcium channel antagonist, facilitates acquisition of trace eye-blink conditioning in aging rabbits (Deyo *et al.*, 1989). This task depends upon an intact hippocampus for acquisition (Moyer *et al.*, 1990), and results in learning-specific reductions of the afterhyperpolarization (AHP) following a burst of 4 action potentials in CA1 (deJonge *et al.*, 1990). Intravenous administration of nimodipine alters spontaneous activity of hippocampal neurons in an aging- and dose-dependant fashion *in vivo* (Thompson *et al.*, 1989). Since nimodipine also has vasodilatory effects, the present experiments investigate whether nimodipine may alter cellular activity by acting directly on hippocampal neurons *in vitro*.

	ELECTROPHYSIOLOGICAL CHARACTERISTICS				
	Max AHP size @ RMP	Area of AHP (800 ms)	# of Spikes to 800 ms pulse	Max AHP size (depolarized)	area of AHP (depolarized)
Mean Change after 10μM Nimodipine	-31.5 ± 10%	-47.3 ± 13%	+76.4 ± 1.3%	-26 ± 2.8%	-33.2 ± 4%

Current clamp recordings were taken from 25 CA1 neurons in submerged 400 μm slices from young adult rabbits at 31°C. Only cells with an input resistance of greater than 30 MΩ, a resting potential of at least -55 mV, and an action potential amplitude greater than 70 mV were studied. Nimodipine: 1) decreased the peak voltage of the afterhyperpolarization following a train of 4 action potentials (both at rest and depolarized 10 mV from rest); 2) decreased the overall integrated area of the AHP and 3) decreased spike-accommodation to an 800ms depolarizing pulse.

Additional studies will be conducted on CA1 neurons from aging rabbits. These data are consistent with the hypothesis that nimodipine exerts its behavioral effects via a direct action on calcium-mediated events in hippocampal neurons.

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219.6

DIHYDROPYRIDINE EFFECTS ON NON-INACTIVATING CALCIUM CHANNELS IN CA1 NEURONS J. Black[1], J.F. Disterhoft[2], & J.Z. Yeh*[1], Departments of [1]Pharmacology and [2]Cell, Molecular, and Structural Biology, Northwestern University Medical School, Chicago, IL 60611, U.S.A.

The dihydropyridine calcium channel antagonist nimodipine has been shown to enhance learning in aging rabbits tested in a hippocampally-dependent conditioning task (Deyo et al., 1989). Further, i.v. nimodipine administration alters spontaneous firing of CA1 neurons recorded *in vivo* (Thompson et al., 1989). We have used whole-cell voltage-clamp techniques to test for a direct effect on Ca^{2+} channels of CA1 neurons. Acutely dissociated CA1 hippocampal neurons from young guinea pigs were prepared per Kay and Wong (1986). Extracellular recording solution contained (in mM): TEA·Cl, 140; $BaCl_2$, 3; $MgCl_2$, 1; HEPES, 10, glucose, 6. Pipette solution contained (in mM): N-methyl-glucamine·Cl, 110; EGTA, 5; MgATP, 5; HEPES, 10; phosphocreatine 10. Under these conditions CA1 cells exhibited a non-inactivating ('L-type') calcium channel current, which was activated at potentials more positive than -50mV, reaching peak at -20mV. This current was reversibly blocked to 80% of control by 10μM nimodipine (control vs drug paired t =-2.679, DF=6, p<0.05). Nimodipine did not alter the apparent voltage-dependence of activation or inactivation of the Ba^{2+} current, but did reduce calculated peak Ba^{2+} permeability. In contrast, 10μM Bay K 8644 reversibly shifted the voltage-dependence of activation approximately 8mV in the hyperpolarizing direction (paired t =6.165, DF=5, p<0.01) and markedly prolonged the tail current. As a result of this shift, current during a pulse to -20mV was increased to 175% of control (paired t =6.562, DF=5, p<0.01). These results suggest that the dihydropyridines have direct effects upon calcium channels in CA1 hippocampal neurons.

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219.7

PHENCYCLIDINE BLOCK OF CALCIUM CURRENT IN ACUTELY DISSOCIATED HIPPOCAMPAL CA1 NEURONS: SPECIFIC EFFECTS ON CHANNEL GATING. J.M.H. ffrench-Mullen[1] and M.A. Rogawski[2]. [1]Laboratory of Neurophysiology and [2]Neuronal Excitability Section, Medical Neurology Branch, NINDS, NIH, Bethesda, MD 20892.

CA1 neurons were enzymatically dissociated from the hippocampi of adult guinea-pigs and whole-cell patch-clamped in solutions containing 3 mM external Ba^{2+} and internal Cs^{2+}, BAPTA and Mg^{2+}-ATP. I_{Ba} was examined with 5 ms to 1 s depolarizing command steps from a holding potential of -80 mV. The dissociative anesthetic phencyclidine (PCP) reversibly depressed I_{Ba} with an IC_{50} of 7 μM. The inhibition of I_{Ba} was voltage-dependent so that the fractional block decreased at positive potentials. Analysis according to the method of Woodhull indicated that the PCP blocking site senses 56% of the transmembrane electrostatic field. Time-dependent activation, inactivation and deactivation of I_{Ba} were unaffected by PCP. Steady-state inactivation of the current showed an inverted U-shaped voltage dependence, similar to that observed in sympathetic neurons by Jones and Marks (*J. Gen. Physiol.* **94**: 169-182, 1989), which is compatible with a 3-state cyclic model of channel gating (with rate constants depending only upon voltage). Kinetic modeling suggested that PCP acts specifically to slow the transition of the channel from the inactivated to the open state. Our data indicate that PCP produces a potent and specific block of voltage-dependent Ca^{2+} channels in mammalian CNS neurons, possibly by affecting a single step in the gating process.

219.8

FUNNEL-WEB TOXIN BUT NOT ω-CONOTOXIN BLOCKS HIGH THRESHOLD Ca^{++} CURRENTS IN INTERMEDIATE PITUITARY (IP). P.J. Williams, Q.J.Pittman, B.A MacVicar and *R.R. Llinas. Neuroscience Research Group, Univ Calgary, Calgary Canada, & *New York Univ Med Centre, New York, U.S.A.

We have previously identified three Ca^{++} conductances in cells of the IP based on their different activation and inactivation thresholds. We used single electrode voltage clamp and bath application of pharmacological agents to further characterize these currents in cells of the isolated intact intermediate pituitary. BAY K8644 (1μM), a dihydropyridine agonist which increases current through L channels, produced an increase in the high threshold non-inactivating current of 46 ±25% (mean ± S.D,n=4). Funnel-web spider toxin (FTX, 1:1000 dilution, Llinas et al, Ann N.Y. Acad Sci (1989) 560, 103) decreased the peak high threshold current by 49 ±26% (mean ±S.D,n=5). Effects were seen within 1 min after application of the toxin and were maximal after 6 min. Currents remaining after FTX were predominantly non-inactivating, indicating that FTX inhibited the high threshold inactivating current. In contrast to FTX, ω-conotoxin (5μM) had no effect on any of the three Ca^{++} currents, even after 20 minutes exposure. This suggests that the high threshold inactivating current may not be identical to the N channel described in SCG neurons but may be more similar to the P channel found in cerebellar Purkinje cells. Our results suggest the presence of three separate Ca^{++} currents in IP cells having characteristics in common with T, L, and P channels.

219.9

HYDROXYPEREZONE ACTION ON GUINEA PIG PAPILLARY MUSCLE. X. García, E. Gijón and Alcántara*. Dept. Of Physiol. and Dept. of Pharmacol. Sch. of Med. Universidad Nacional Autónoma de México, Ap. P. 70-250, México, D.F. 04510. MEXICO.

Perezone causes positive inotropic effect on rat auricular muscle and increases its contraction duration. It increases auricular action potential duration and amplitude (Gijón, E., García, X. and Alcántara, G. Res. C. Nacl. Farmacol. 13:8, 1989). The purpose of this study was to know if the perezone isomer, hydroxyperezone shares some of the perezone properties, on 300 g Hartley male guinea pig papillary muscles prepared for isometric and intracellular recordings, they were electrically driven. Hydroxyperezone like perezone produces a positive inotropic effect and increases papillary action potential duration, it may be observed an increase in threshold for electrical stimulation, with partial blockade 1:2. Action potential duration recovers and reduces up to 34% of control values, which hardly doubles with adrenaline. These results show that hydroxyperezone behave like perezone, it probably affect the papillary muscle by interfering with the calcium movement from the internal deposits and on the muscle membrane.

219.10

DIFLUNISAL ACTION ON PAPILLARY MUSCLE FROM THE GUINEA PIG. E. Gijón, E. Castillejos* and García, X. Dept. of Physiol. Sch. of Med. Universidad Nacional Autónoma de México, Ap. P. 70-250, México, D.F. 04510. MEXICO.

Diflunisal causes a contraction followed by a relaxation in intestinal muscle (Gijón, E., Castillejos, E. and García, X. Abs. Soc. Neurosc. 14(2):1072(432.13), 1988). Diflunisal decreases the transport rate and calcium accumulation by the sarcoplasmic reticulum (Holguín, J.A. Biochem. Pharmac. 37(21):4035-4040, 1988). Diflunisal acting as a calcium ionophore would induce positive inotropic effects in cardiac muscle. Papillary muscles from 350 g Hartley male guinea pig were prepared for isometric and intracellular recordings, they were electrically driven. It is shown that diflunisal 0.39 mM, from Merck Sharp and Dohme de México, causes a positive inotropic effect, a decrease in basal muscle tension and an increase in action potential duration, followed by a negative inotropic effect, an increase in basal muscle tension and a decrease in action potential duration and amplitude. These results suggest a calcium ionophore action of diflunisal at the cardiac muscle membranes.

219.11

Voltage-dependence of μ-opioid inhibition of calcium channel current in SH-SY5Y cells. C. Hammond*, G. Henderson*, E.P. Seward,* and C. Kennedy* Department of Pharmacology, University of Cambridge, Cambridge CB2 1QJ, U.K.

In the human neuroblastoma cell line SH-SY5Y μ-opioid receptor activation results in a G protein-mediated inhibition of the whole cell, peak inward N calcium channel current (Henderson and Seward, J. Physiol. 422: 19P,1990). The present experiments were designed to examine the voltage-dependence of the opioid inhibition of the calcium channel current.

Inward calcium channel currents (carried by barium) or outward calcium channel currents (carried by caesium) were evoked by depolarising voltage steps from -90 to +10 or +100mV respectively. Both the inward and outward currents were markedly reduced by ω Conus toxin (1μM) and abolished by lanthanum (100μM). The μ-opioid agonist [D-Ala^2,N-MePhe^4,glyol]enkephalin (DAMGO, 200nM) produced a marked inhibition (48 ± 7%, mean ± standard error, n=7) of the inward current but was ineffective in inhibiting the outward current. In addition to reducing the amplitude of the peak inward current, DAMGO also markedly reduced its rate of rise. There was no discernable effect of DAMGO on the rate of rise of the outward current. Taken together these results indicate that the opioid inhibition of the calcium channel current in SH-SY5Y cells is reversed by membrane depolarisation.

(Supported by grants from the MRC)

219.12

PHARMACOLOGICAL ANALYSIS OF CALCIUM CURRENTS IN IDENTIFIED EMBRYONIC MOUSE MOTONEURONS. M. Mynlieff and K.G. Beam. Dept. of Physiology, Colorado State Univ., Fort Collins, CO 80523.

The application of calcium channel antagonists at the neuromuscular junction has been used by other investigators to attempt to identify specific calcium channels involved in neurotransmitter release. There is relatively little known, however, about the specificity of these calcium channel antagonists in motoneurons. The purpose of this study was to determine the specificity of various calcium channel antagonists and agonists on E14 mouse motoneuron calcium currents. Motoneurons were identified by retrograde labelling with the carbocyanine dye dil prior to dissociation. Voltage clamp "whole-cell" patch recording was used to measure calcium currents 24 hours after dissociation. Previous work in our laboratory has shown that the motoneurons possess 3 different calcium currents which can be distinguished on the basis of kinetics and voltage dependence. There was a low-voltage-activated transient current, with characteristics similar to the T current described in sensory neurons, as well as a high-voltage-activated (HVA) transient current and an HVA current which was sustained for at least 200 ms. Amiloride (1-2 mM) caused a ~50 % block of the T current without affecting the HVA currents. Sandoz +202-791 (100 nM - 2 uM), a dihydropyridine agonist, specifically increased the sustained component of the HVA current and shifted its activation to more negative potentials (10 mM barium as charge carrier). Thus, L-type channels contribute to the sustained current. Omega-conotoxin GVIA (10 uM) blocked the T current 90.1 ± 4.9% (n=7), the HVA transient current 75.8 ± 3.7% (n=8), and the HVA sustained current 68.4 ± 5.0% (n=8).

Supported by NIH grant NS-26416 to KGB and NIH fellowship NS-08769 to MM.

219.13

PHARMACOLOGICAL EVIDENCE FOR A THIRD COMPONENT OF CALCIUM CURRENT IN RAT RETINAL GANGLION CELLS. H-S. Vincent Chen and Stuart A. Lipton. Dept. of Biol. Chemistry and Molec. Pharmacology, Harvard Medical School & Dept. of Neurology, Children's Hospital, Boston, MA 02115.

At least two types of unitary calcium channels have been identified in postnatal rat retinal ganglion cells (RGCs) (Karschin & Lipton, *J. Physiol.* 1989;418:379): a T-type and an L-type [dihydropyridine (DHP)-sensitive]. However, the existence of an N-type channel, a major component of calcium current in other neurons, is still unclear. In isolated rat RGCs, we used the whole cell patch-clamp method with the DHP agonist (+)-(S)-202-791 (1 μM) and synthetic ω-conotoxin (ω-CgTx; 10 μM, Peninsula) to separate the various components of calcium current (Plummer et al., *Neuron* 1989;2:1453). In 16 of 22 RGCs (73%), ω-CgTx irreversibly blocked the peak Ba^{2+} current (I_{Ba}) by 10-30% but did not affect tail currents prolonged by the DHP agonist. The irreversible block of a component of I_{Ba} suggests the existence of an N-type component. Approximately 20% (3/16) of the ω-CgTx-sensitive cells had an additional I_{Ba} component that was blocked reversibly; in 27% (6/22) of the RGCs, ω-CgTx had no effect. In some cells, the DHP agonist dramatically prolonged the tail current but only modestly increased the peak current even after ω-CgTx block. Furthermore, in these same cells the current remaining after block by ω-CgTx was mostly inactivated at a holding potential of -40 mV. Since N-type current is largely inactivated by this voltage protocol, the current remaining after ω-CgTx block in these cells was probably mostly N-type. We conclude that at least some rat RGCs have a third component (probably N-type) of Ca current, a portion of which is insensitive to ω-CgTx.

219.14

HALOTHANE BLOCKS LOW-VOLTAGE-ACTIVATED CALCIUM CURRENTS IN RAT SENSORY NEURONS. M. Takenoshita* and J.H. Steinbach. Anesthesiology Research Unit, Washington U. Sch. Med., St. Louis, MO 63110.

Volatile anesthetics may act by blocking at least some neuronal voltage gated calcium currents (I-Ca; Krnjevic and Puil, Can J Phys Pharm, 66, 1570). We studied I-Ca in dissociated neonatal rat dorsal root ganglion cells (Schroeder et al, J Neurosci, 10, 947). Cells were whole-cell voltage clamped 12-24 hours after dissociation. Halothane (HAL) was applied from a closed perfusion system; bath concentrations were assayed by gas chromatography. Low-voltage-activated I-Ca (LVA I-Ca; I-Ca elicited at -30mV from a potential of -80mV) was reversibly blocked by low concentations of HAL (EC50 of 50-100 μM). The block developed within 10 sec of application of HAL, but often required several minutes for reversal. The block by HAL apparently did not result from a shift in the voltage dependence of inactivation nor of activation for LVA I-Ca. Isoflurane was slightly less effective on a molar basis than HAL, but produced qualitatively identical effects. High-voltage-activated I-Ca (I-Ca at +20mV from -80mV) was less strongly blocked by HAL or isoflurane in these experiments. Estimated clinically effective concentrations of HAL are 200-800 μM.

219.15

DOES H_2O_2 DECREASE I_{Ca} BY REGULATING $[Ca]_i$ IN LOBSTER NEURONS? R.S. Hernandez, R.J. Lowy, D.A. Miller, D.R. Livengood. Physiol. Dept., AFRRI, Bethesda, MD 20814.

Free radical damage is implicated in such pathological insults as toxins, reperfusion injury, and radiation exposure. H_2O_2 is an active oxygen compound that can generate free radicals. We previously reported that H_2O_2 blocks I_{Ca} by 50-100% in the lobster cardiac ganglion. We proposed that an increase in $[Ca]_i$ was responsible (Biop. J. 53: 555a, 1988). Neurons #1 and #2 of the ganglion were ligated and bathed in lobster saline with TEA, 4-AP, and TTX at 18°C for 30 min., followed by superfusion with 3.5 mM H_2O_2 added to the saline for 30 min., and then washed with control saline. Using a 2-electrode voltage clamp to measure I_{Ca}, we observed that the block developed within 10-15 min. and progressed with time. In parallel experiments $[Ca]_i$ levels were determined by pressure injecting cells with the Ca sensitive dye Fura-2 (free acid pentapotassium salt). Fluorescence changes were quantified using a low-light-level video microscope and image processing system. Perfusion with H_2O_2 saline for 30 min. failed to alter $[Ca]_i$ from control values. Cells and dye responded to 100 mM KCl saline exposure, resulting in the expected large reversible increase in $[Ca]_i$. In vitro exposure of Fura-2 acid to hydroxyl free radical generating conditions failed to alter the amount or Kd of the dye. These results provide evidence that $[Ca]_i$ does not increase during the same free radical treatment conditions that result in I_{Ca} block.

219.16

H_2O_2 BLOCKS I_{Ca} IN LIGATED LOBSTER NEURONS AT SITES REMOTE FROM THE SOMA. D.A. Miller, R.J. Lowy, R.S. Hernandez, and D.R. Livengood. Physiol. Dept., AFRRI, Bethesda MD 20814

We have previously reported that H_2O_2, a model for free radical damage, produces a time and concentration dependent block of the early inward calcium current (I_{Ca}) in ligated neurons of the lobster cardiac ganglion (Biophys. J. 53: 555a, 1988). In the present study, the anterior two giant neurons were ligated approximately 0.3 mm from the soma. TTX, TEA and 4-AP were used to block Na and K currents. I_{Ca} evoked in response to voltage steps to -20 mV or greater from a holding potential of -50 mV was without initial delay. However, with voltage steps to -45 or -40 mV from holding potentials of both -50 and -80 mV, I_{Ca} had a delayed time of onset and frequently had two or more components, suggestive of one or more remote, non-space clamped loci of origin of I_{Ca}. 3.5 mM H_2O_2 produced a progressive time dependent block of I_{Ca} without appreciable alteration of the shape or time of onset. Subsequent to electrical measurements, some neurons were pressure injected with Lucifer Yellow (1% in 200 mM KCl) and examined using a low-light-level video microscope and image processing system. Simultaneous viewing of bright field and fluorescent images showed that dye was untraceable past the axon ligature but was present in a number of fine "dendritic" processes. The dendrites are suggested as a possible remote, non-spaced clamped locus for the H_2O_2 sensitive I_{Ca}.

219.17

GADOLINIUM NON-SELECTIVELY BLOCKS BOTH N-TYPE AND L-TYPE CALCIUM CHANNELS. T.A. Brown, L.M. Boland, and R. Dingledine. Dept. Pharmacology, Univ. North Carolina-Chapel Hill and Dept. Neurobiology, Harvard Medical School, Boston, MA, 02115.

The effect of gadolinium (Gd) and other lanthanides on voltage-activated barium (Ba) currents in a rat dorsal root ganglion (DRG) cell line (F11-B9), rat and frog DRG neurons, and rat atrial and ventricular myocytes was studied using the whole-cell patch clamp technique. In contrast to the Gd-resistant current reported for NG108-15 cells (Docherty, J. Physiol., 398: 33-47, 1988), all current components in these cells could be completely inhibited in a dose-dependent manner by Gd. F11-B9 cells express two current components (sustained and transient) which are both blocked by Gd. The dihydropyridine-sensitive sustained current component (30 mM Ba) was maximally blocked by 30 μM Gd with an IC50 of 0.36 μM, and the dihydropyridine-insensitive transient component was maximally blocked by 30 μM Gd with an IC50 of 0.95 μM. Complete block of rat and frog DRG currents (5 mM Ba), which consist of both Ω-conotoxin- and dihydropyridine-sensitive components, was achieved with 1 μM Gd. L-type current from atrial and ventricular myocytes (5-10 mM Ba) could also be abolished with 1 μM Gd. Other lanthanide cations also blocked all Ba currents in F11-B9 cells. Lutetium blocked all of the sustained and transient current at 100 μM with IC50s of 0.31 μM and 1.26 μM, respectively. Lanthanum provided complete block of the sustained current at 10 μM with an IC50 of 0.14 μM and of the transient current at 100 μM with an IC50 of 1.73 μM. These findings suggest that Gd blocks both N-type and L-type currents. (Supported by NS23804)

219.18

CALCIUM CHANNEL BLOCKING ACTIONS OF CHARGED AND UNCHARGED MERCURIALS IN RAT FOREBRAIN SYNAPTOSOMES. S.J. Hewett, M.E. Welke and W.D. Atchison. Dept. Pharm./Tox., Mich. State Univ., E. Lansing, MI 48824.

The relative blocking efficacy of methylmercury (MeHg), ethylmercury (EtHg), divalent mercury (Hg^{2+}), dimethylmercury (2MeHg), p-chloromercuribenzoate (PCMB) and p-chloromercuriphenylsulfonate (PCMBS) on Ca^{2+} influx into rat forebrain synaptosomes was assessed to provide information as to the mechanism(s) by which mercurials interact with membrane Ca channels. $^{45}Ca^{2+}$ uptake into synaptosomes was measured after 1 ("fast, inactivating phase") and 10 ("total") sec of depolarization by 77.5 mM KCl and during 10 sec of depolarization into synaptosomes that were previously depolarized for 10 sec in the presence of 77.5 mM K^+ but no external Ca ("slow, noninactivating phase"). In the range tested (10-500 μM), total uptake, as well as the "fast" and "slow" components of $^{45}Ca^{2+}$ influx into synaptosomes, were blocked in a concentration-dependent manner by MeHg, EtHg and Hg^{2+} (>10 μM). In contrast, 2MeHg, PCMB, and PCMBS (10-500 μM) caused no appreciable reduction in any phase of uptake. To characterize the interaction of charged mercurials with nerve terminal Ca^{2+} channels, experiments were designed to determine whether charged mercurials blocked Ca^{2+} channels in a voltage-dependent manner. The magnitude of depolarization caused by each K^+ concentration and thus the voltage-dependence of influx was estimated by incubating synaptosomes for 2 sec in Na-free K^+ solutions (5mM-60mM) containing 1 μCi $^{45}Ca^{2+}$ ± mercurials (100 μM). Of the three mercurials tested (MeHg, EtHg, Hg^{2+}), only MeHg blocked uptake of $^{45}Ca^{2+}$ in a voltage-dependent manner. Uptake in synaptosomes exposed to 100 μm MeHg was reduced significantly from controls at all K^+ concentrations tested with a clear voltage-dependence of block being exhibited between 5 and 50mM KCl. In addition, uptake in 37.5 and 50 mM K^+ was significantly reduced from uptake in 5mM K^+. Thus mercurials of dissimilar charge and lipophilicity, differentially affect $^{45}Ca^{2+}$ influx into the synaptosome and likely have different mechanisms of action at synaptosomal membranes. (Supported by NIEHS grant ES03299.)

219.19

METHYLMERCURY BLOCKS CURRENTS MEDIATED BY VOLTAGE-DEPENDENT Ca CHANNELS IN NERVE GROWTH FACTOR-DIFFERENTIATED PHEOCHROMOCYTOMA CELLS. T.J. Shafer and W.D. Atchison Dept. of Pharm./Tox. and Ctr. for Environ. Tox., Michigan State Univ., E. Lansing, MI 48824

The effect of the neurotoxic organic heavy metal methylmercury (MeHg) on Ca channels was examined by measuring effects of MeHg on currents carried by 20 mM Ba in pheochromocytoma (PC12) cells using whole cell patch voltage-clamp. Cells cultured in the presence of nerve growth factor for 3-5 days exhibited voltage-dependent currents containing inactivating and non-inactivating components in response to depolarizing voltage steps from a membrane holding potential of -70 mV; the non-inactivating component was not observed during depolarizing steps from a holding potential of -40 mV. The non-inactivating component of current accounted for approximately 10% of the total current observed. Peak current amplitudes were observed following voltage steps to +10 mV from a holding potential of -70mV. Addition of 40-100 μM MeHg to the recording medium resulted in a rapid block of inactivating and non-inactivating Ba currents which was apparent after 10-15 sec and complete within 1 min. Within this concentration range and duration of exposure, MeHg did not cause alterations in the membrane capacitance nor alter nonspecific leak currents in PC12 cells. These results indicate that PC12 cells cultured in nerve growth factor for 3-5 days may contain two types of Ca channels, and that MeHg reduces inactivating and non-inactivating Ba currents by interacting with voltage-dependent Ca channels in PC12 cells, not by disruption of membrane electrical properties. (Supported by NIH grant ES03299; TJS is the recipient of a Student Fellowship sponsored by Hoffman-LaRoche, Inc.)

219.20

LEAD BLOCKS VOLTAGE GATED CALCIUM CHANNELS OF CULTURED DORSAL ROOT GANGLION NEURONS. M. L. Evans, D. Büsselberg, C. Trautman and D. O. Carpenter. Wadsworth Labs and School of Public Health, Albany, NY 12201 and Johannes Gutenberg University Mainz, D-6500 Mainz, FRG.

We have previously demonstrated Pb^{2+} to be a potent and reversible blocker of voltage dependent calcium currents in Aplysia neurons, and now report similar effects in mammalian neurons. Dorsal root ganglion cells were cultured from neonatal rats and used for whole cell patch clamp recordings after 1 to 10 days in culture. Calcium channel currents were recorded in the following external solution (mM): TEA Cl 130, HEPES 10, glucose 10, $BaCl_2$ 10, $MgCl_2$ 1, TTX 0.02. A cesium based internal solution containing 2 mM ATP was used. Depolarizing steps of 80 mV applied for 100 ms from a holding potential of -80 mV activated a calcium channel current with both activating and non-inactivating components. Pb^{2+} produced a dose-dependent reduction of the current amplitude with threshold less than 0.2 μM and greater than 95% reduction at 20 μM. The IC_{50} was 0.64 μM. The block reached a steady state within 2 min and was partly reversible after 5 min wash. Over the 100 ms duration of the current the degree of time dependent inactivation was greater in Pb^{2+}. The current voltage relationship for the peak current was not altered by Pb^{2+} at 0.5 μM. Voltage dependent sodium but not potassium currents were reduced by about 5% at 2 μM Pb^{2+}.

219.21

A VOLTAGE-INDEPENDENT, DIVALENT-SELECTIVE ION CHANNEL IS BLOCKED BY INTRACELLULAR MAGNESIUM. J. A. Strong, Dept. of Biol. Sci., Purdue Univ., W. Lafayette IN 47907.

Previous reports[1-2] have described a voltage-independent, 24 pS divalent-selective ion channel in neurons of the mollusc *Lymnaea*. Though present in high density the channel rarely opens during cell-attached recording (using 45 mM Ba as the charge carrier) but is unmasked by formation of a cell-free patch. I now report that application of 1 mM Mg to the cytoplasmic face of cell-free patches causes a complete, rapid, reversible block of this channel. A partial block is seen in 0.1 mM Mg, which enhances long channel closings without affecting the size or duration of openings. Hence, Mg may be the physiological substance which normally inhibits the channel (based on values of intracellular Mg measured in other cells). Selectivity experiments show that the size of the single channel current saturates at relatively low concentrations of extracellular Ba. In the range of 5-15 mM Ba, the size of the single channel current increases with increasing Ba, and is independent of the monovalent cation present (Na or TRIS). In some experiments a second, smaller Mg-sensitive channel is also seen, which may be analogous to a Mg-sensitive, non-specific cation channel recently described in lobster neurons[3]. REFS: [1]Yazejian & Byerly, J.Membr.Biol 107:63 [2]Scott & Strong, Soc Neurosci Abstr.15:824 [3] McClintock & Ache, J. Membr. Biol 113:115.

219.22

TRIVALENT LANTHANIDES BLOCK MULTIPLE CALCIUM CHANNELS IN SECRETORY CELLS. B.A. Biagi, and J.J. Enyeart*. Departments of Physiology and Pharmacology, The Ohio State University, Columbus, OH 43210.

Several types of voltage gated calcium channels have been identified in endocrine and neuroendocrine secretory cells. These channels differ in their sensitivity to inorganic Ca^{2+} antagonists. We have studied the effects of the trivalent lanthanides, gadolinium (Gd^{3+}) and lanthanum (La^{3+}), on whole cell calcium currents in rat pituitary GH_4C_1 and rMTC6-23(clone 6) thyroid C-cell lines. In both cell types, 5 μM Gd^{3+} completely blocked T-type Ca^{2+} currents in a reversible manner. In comparison, 5 μM Ni^{2+} inhibited T-type current by 12% while 50 μM produced a 60% block. Block of L-type Ca^{2+} current was promoted by channel activation. With long depolarizing test pulses, 200 nM Gd^{3+} inhibited L current completely. Other trivalent cations, including lanthanum and yttrium, blocked both currents with similar characteristics and potency. In C cells, a third current component with characteristics of N-type Ca^{2+} channels was also blocked by the trivalents. These results indicate that Gd^{3+} and other related trivalent cations are nonselective inhibitors of low and high threshold Ca^{2+} channels in endocrine and neuroendocrine cells. In this respect, they are among the most potent inorganic Ca^{2+} antagonists yet discovered.

PEPTIDES—RECEPTORS: OTHER

220.1

SEX DIFFERENCES IN THE REGULATION OF OXYTOCIN RECEPTOR BINDING BY OVARIAN STEROIDS IN THE VENTROMEDIAL HYPOTHALAMIC NUCLEUS OF THE RAT. H. Coirini, A.E. Johnson, M. Schumacher and B.S. McEwen. Lab of Neuroendocrinology, The Rockefeller University, New York, NY 10021 and SCSBB/LCS, NIMH, Poolesville, MD 20837.

The facilitation of sexual receptivity by oxytocin is related to the regulation of oxytocin receptors (OTR) in the ventromedial nucleus (VMN) by ovarian steroids. In female rats, estradiol benzoate (EB) dramatically increases OTR binding in this brain region while progesterone (P) not only modulates binding by also increases the area occupied by OTR. In a previous study, we showed that while males are relatively insensitive to the facilitative effects of EB and P on sexual receptivity, they are equally sensitive to EB with respect to the induction of [^{3}H]-Oxytocin binding in the VMN (Neuroendocrinology **50**:193, 1989) In the present study, we compared the effects of P on OTR binding in male and female rats primed with EB to determine if sex differences in the regulation of OTR by P are related to these behavioral differences.

Adult male and female rats were gonadectomized and adrenalectomized and were treated one week later for two successive days with either 10 µg EB or oil. Forty four hours later, half of the EB treated animals were injected with P (0.5 mg) and the others with vehicle. All the animals were killed 4 hrs later and brain prepared for labelling with ^{125}I-OVTA (a ligand of high affinity and selectivity for OTR) using autoradiographic methods. In agreement with a previous study, P increased the region of OTR binding in and around the VMN in EB primed females (Proc Natl Acad Sci, **86**:6798, 1989). However, P did not alter the distribution of OTR in males. These results suggest that the relative insensitivity of males to ovarian hormones with respect to the induction of sexual receptivity may be related to sex differences in the modulatory effects of P on OTR binding.

220.2

ESTROGEN INCREASES OXYTOCIN RECEPTOR AFFINITY IN THE MEDIAL PREOPTIC AREA. Pedersen, C. A., Walker, C. H., Mason, G. A. and Caldwell, J. D.; Dept. of Psychiatry and BDRC, School of Medicine, Univ. of North Carolina, Chapel Hill, NC. 27599-7250

In this study we used a radiolabelled OXT analogue (OTA), ornithine-vasotocin, to study OXT receptor dynamics in the medial preoptic area-anterior hypothalamus (MPOA-AH), medial basal hypothalamus (MBH) and hippocampus of ovariectomized rats injected im once daily for three days with an oil vehicle or with 0.5 ug or 5 ug estradiol benzoate (EB). We found that, at a concentration of 0.1 nM ^{125}I-OTA, membranes from animals treated with 5 ug EB showed significantly more binding than did membranes from oil treated controls. Scatchard analyses showed that the 5 ug EB dose signi-ficantly increased OXT receptor affinity over oil vehicle controls in membrane fractions taken from the MPOA-AH (K_D = 0.21 $\pm$ .01 nM for controls versus K_D = 0.12 $\pm$ .01 for 5 ug EB); similar changes in binding affinity in the MBH were not significant. There were no changes in binding affinity in hippocampal tissue across estrogen treatments. There was a significant reduction in receptor densities in the MPOA-AH (B_{max}'s were 17.1 fmol/mg protein $\pm$ 1.6, 12.5 $\pm$ .9 and 11.9 $\pm$ 1.0 for oil, 0.5 ug and 5 ug EB, respectively); with a similar but non-significant decrease in the MBH. OXT and a uterotonic antagonist analogue PM-OXT both competed with ^{125}I-OTA; however, the OXT metabolite OXT_{4-9} had no effect. MPOA-AH OXT receptors may mediate the receptivity facilitating effects of central OXT.

220.3

MEAL-DEPENDENT CHANGES IN CENTRAL BOMBESIN RECEPTORS

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The objective of this experiment was to explore the possibility that changes in bombesin (BN)-receptor binding may be an important variable in the satiety response. Male Sprague-Dawley rats (300-350 g) were used for this experiment. Pairs of animals were food deprived for 12 hr and only one from the pair allowed to eat ad libitum for a period of 35 min. One pair was processed per day until n = 8 in both the pre- and post-prandial groups. Animals were sacrificed, brains rapidly removed and frozen. Fresh-frozen brains were sliced from interaural 14.2 mm to 2.96 mm, (16 um thickness) and processed for in vitro autoradiography, using a buffer containing ^{125}I-Tyr^4-BN (9000 cpm/50 ul buffer). Brain slices were applied, in pre- and post-prandial subject pairs to Amersham (RPN.10; Arlington, Illinois) Hyperfilm-βmax film. Quantitative analysis of the autoradiography films was performed using ^{125}I standards cross-calibrated with ^{14}C standards (Baskin et al, 1989), and an image analysis system (MCID; Imaging Research Inc., St. Catherines, Ontario, Canada). T-Test for correlated samples was used to analyze this data. Our results indicate a generalized increase in receptor density post-prandially, although statistically significant ($p < .05$) increases were discovered in hypothalamic nuclei (medial preoptic area; periventricular nuclei), cingulate and rhinal cortical areas, fundus striatum, central amygdaloid nucleus, vertical limb of the diagonal band, septal hippocampal nuclei and, most notably, within the hippocampus and nucleus accumbens ($p < .005$). Thus, BN receptor binding characteristics can change rather rapidly in relation to the feeding state. If increased binding does reflect increased sensitivity then these data indicate that rats become more sensitive to the peptide effects post-prandially; a suggestion which would support BN's suggested role as a satiety peptide.

(This research was supported by grants from NSERC and MRC)

220.4

DISTINCT DISTRIBUTION OF BOMBESIN AND NEUROMEDIN B BINDING SITES IN THE RAT BRAIN. E.E. Ladenheim, R.T. Jensen*, S.A. Mantey*, P.R. McHugh and T.H. Moran. Dept. Psychiatry, Johns Hopkins Univ. Sch. Med., Baltimore, MD 21205 & NIDDKD, NIH, Bethesda, MD 20892.

Bombesin/gastrin releasing peptide (BBS/GRP) and neuromedin B (NMB) are structurally related compounds which are biologically active in the rat central nervous system. Despite their similarity, differences in their regional distribution and bioactivity suggest that they may possess separate receptor populations. In order to investigate this possibility, we examined the regional distribution of binding sites for ^{125}I-[Tyr^4]BBS and ^{125}I N-D-Tyr-NMB in the rat brain using receptor autoradiography. Our results indicate that the distribution of BBS binding sites is distinct from that of NMB. In the forebrain, NMB binding sites were observed in the olfactory bulbs, dentate gyrus, pyriform cortex, septum and thalamic nuclei. By comparison, BBS binding sites were found in the nucleus accumbens, hippocampus, median preoptic nucleus, arcuate nucleus, median emminence and paraventricular nucleus. Binding differences were also observed in brainstem structures. NMB binding sites were located in the interpeduncular nucleus, dorsal raphe nucleus and the nucleus of the solitary tract. BBS binding sites were seen in the central gray and the caudal aspect of the spinal trigeminal nucleus. The differences in the distribution of binding sites for BBS and NMB suggest that they may function as distinct neuropeptides in the rat central nervous system.

220.5

EFFECTS OF ALKYLATING AND REDUCING AGENTS ON NEUROTENSIN BINDING TO PORCINE BRAIN MEMBRANE RECEPTORS. S.P. Mitra, R.E. Carraway and C.F. Ferris. Dept. of Physiology, Univ. of Massachusetts Med. Ctr., Worcester, MA. 01655.

The importance of sulfhydryl (-SH) & disulfide (S-S) groups in the binding of ^{125}I-labeled neurotensin (NT) to porcine brain membranes was investigated at 4 C. Pretreatments (1 hr, 4 C) with reducing agents e.g., dithiothreitol (DTT), enhanced binding by ~2-fold (ED_{50}, 10μM), whereas alkylating agents e.g., N-ethyl-maleimide (NEM), reduced binding (ED_{50}, ~100μM), even when washed away. This appeared to result from alterred receptor function since the stability of ^{125}I-labeled NT was not changed. Scatchard analysis indicated that 1 mM DTT increased by ~60% and 2 mM NEM decreased by ~40% the number of high affinity (Kd,~0.4 nM) and low affinity (Kd,~10 nM) binding sites with little effect on the Kd's. The additional receptors gained by exposure to DTT were not sensitive to NEM unless pretreated with DTT, suggesting that reduction of S-S in latent receptors gave active receptors with -SH. Preincubation of receptors with 10 nM NT prior to treatment with NEM blocked the inhibitory effect on NEM on binding, suggesting that the critical -SH group(s) were located at the NT-binding site. These findings are consistent with the idea that -SH group(s) within the binding pocket of the NT-receptor have critical role(s) in the binding reaction. NIH DK28565.

220.6

FURTHER CHARACTERIZATION OF NEUROTENSIN RECEPTORS IN MICE BRED FOR DIFFERENCES IN SENSITIVITY TO ETHANOL. A.D. Campbell, B.C. Jones, and V.G. Erwin. Alcohol Research Center, School of Pharmacy, University of Colorado, Boulder, CO 80309.

The tridecapeptide neurotensin (NT) is widely distributed in brain and may serve as a neurotransmitter or neuromodulator in systems affected by ethanol. Studies in this laboratory have shown that NT produces a dose-dependent increase in ethanol sensitivity in SS but not LS mice, which were selectively bred for their differences in sensitivity to ethanol. Our studies have shown B_{max} values for ^{3}H-NT binding to be greater in SS mice in every brain region assayed. Scatchard analysis revealed two distinct binding sites for NT (K_D's 0.3 and 5 nM), and the histamine (H_1) antagonist levocabastine appeared to inhibit NT binding to the low affinity (NT_L) form of the receptor. Scatchard analysis in the presence of 50 μM levocabastine revealed one site , with K_D and B_{max} values similar to the high affinity form (NT_H) of the receptor. Using levocabastine, we characterized ^{3}H-NT receptor binding in nine brain regions. Significant regional differences in both NT_H and NT_L receptors were found between LS and SS mice. Furthermore, we observed line differences in the ratio of NT_H and NT_L receptors in several brain regions, including entorhinal cortex and caudate putamen. The differences in the NT receptors between brains of LS and SS mice further support the role of NT in ethanol actions. (Supported by NIAAA Grants Number 003527, 00079 and 07330).

220.7

CGRP BINDING SITES IN THE NUCLEUS ACCUMBENS: ONTOGENIC APPEARANCE, PHARMACOLOGICAL PROFILE AND PHYLOGENIC DIFFERENCES. T.Dennis[1], A.Fournier[2], S.Guard[1], S.St Pierre[2], R.Quirion[1]. [1]Douglas Hospital Res. Ctr. & Dept. of Psychiatry, McGill University, Montreal, Canada, H3A 1A1. [2]INRS-Santé, Pointe Claire, P.Q., Canada.

Autoradiographic studies have previously demonstrated high densities of [^{125}I]CGRP binding sites in rat nucleus accumbens (Sexton *et al.*, *Neurochem Int.*, 12:323, 1988) while the neurobehavioral effects of this peptide are suggestive of a possible modulatory role on dopaminergic neurons (Jolicoeur *et al.*, *Reg. Peptides*, submitted, 1990).

We now report that the use of quantitative autoradiography showed that [^{125}I]hCGRPα binding sites in rat n. accumbens rapidly increased in density between postnatal days 4 and 14. Furthermore, regional competition studies in adult rat brain showed that salmon calcitonin was almost as effective as hCGRPα in competing for [^{125}I]hCGRPα binding sites in n. accumbens but was without affinity in other regions such as the mesolimbic cortex confirming the existence of atypical CGRP sites in the n. accumbens.

Marked species differences in [^{125}I]hCGRPα binding were observed in n. accumbens. In the rat, a high density of labelling was seen in the medial posterior region of n. accumbens, while low levels were found in striatum and fronto-parietal cortex. Similar distributions, of varying densities, were observed in mice and guinea pig. Very high densities of CGRP sites were observed in rabbit n. accumbens, caudate nucleus and putamen, while low levels were present in cortex. Very high densities of [^{125}I]hCGRPα binding sites were localized throughout the basal ganglia and cortical ribbon of pig brain. In both monkey and human brain, only very low densities of specific binding sites were seen in n. accumbens and surrounding structures. Thus, marked species differences are apparent. This may have physiological and behavioral implications.

Supported by the Medical Research Council of Canada and the Canadian Heart Foundation.

220.8

BRADYKININ RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES. A.E. McEachern, E. Shelton*, K. Jarnagin* and R.W. Aldrich. Dept. of Molecular and Cellular Physiology, Stanford University, Stanford, CA 94305 and Syntex Research, Palo Alto, CA 94304.

Activation of bradykinin receptors (BKR) elicits phosphoinositide turnover and increases in intracellular Ca^{++}. We have expressed BKRs by injecting poly A^+ mRNA derived from a number of tissue sources into *Xenopus* oocytes. BK-induced inward Ca^{++}-activated Cl^- currents were measured by two electrode voltage clamp. Only rat uterus mRNA produced large, consistent responses 2-4 days after injection. Responses had lag times after BK application on the order of seconds and generally included a fast, transient component followed by a slower current upon which fast oscillations were often superimposed. Guinea pig ileum mRNA produced a smaller response. mRNA from many tissues and cell lines gave very small and inconsistent responses (<10 nA): rat neural tissue (olfactory bulb, midbrain, cerebellum, first cervical dorsal root ganglion), guinea pig brain, and HSD-1, PC12-BK, NG108, and MDCK cell lines. mRNA from rat thalamus and cortex produced no detectable response.

Pharmacological studies indicate that BK interacts with a B2-type receptor and not with Angiotensin Converting Enzyme. The EC_{50} for BK is about 100 nM. These BKRs may be translated from exogenous mRNA, however, the possibility exists that injected mRNA activates transcription from an endogenous *Xenopus* BKR gene or translation, processing or expression of endogenous BKRs. Supported by the American Cancer Society.

220.9

HIGHLY POTENT GROWTH HORMONE-RELEASING FACTOR (GRF) ANALOGS AND ENZYMATICALLY CLEAVED FRAGMENTS: CORRELATION BETWEEN RECEPTOR BINDING AND ACTIVITY. T.F. Mowles*, Y. Lee*, J. Rivier*, A.B. Davidson and R.M. Campbell*. Dept. of Animal Science Research, Hoffmann-La Roche Inc, Nutley, NJ 07110.

Scatchard analysis of [His^1, ^{125}I-Tyr^{10}, Nle^{27}]-hGRF(1-32)-NH_2 binding to rat anterior pituitary homogenates indicates a single high affinity class of receptors (54.40 ± 12.68 fmol/pituitary; K_d = 202 ± 27 pM). Binding of [His^1, ^{125}I-Tyr^{10}, Nle^{27}]-hGRF(1-32)-NH_2 was highly specific, as homologous (secretin, glucagon, VIP and PHI [1-27]) and non-homologous (SRIF[1-14], TRH, bGH) peptides (≤ 1 μM) did not displace the bound radioligand. With all hGRF analogs examined, *in vitro* GH-releasing activity was directly correlated with GRF receptor binding affinity. Deamination of Tyr^1 did not significantly affect hGRF(1-29)-NH_2 binding, while D-Ala^2 (replacement of L-Ala^2) increased affinity ≈2-fold. hGRF(1-29)-NH_2 analogs with Ala^{15}-substitution (for Gly^{15}) displayed 4-5 times higher affinity for the GRF receptor relative to hGRF(1-44)-NH_2. Replacement of Gly^{15} with Sar^{15}, known to disrupt helix formation, resulted in a dramatic loss of GH-releasing activity and receptor binding. The present data supports the proposal that Ala^{15}-substitution increases receptor affinity, and hence potency, due to increased amphiphilic α-helical interactions. Furthermore, hGRF(1-44)-NH_2 and hGRF(1-29)-NH_2 fragments, representative of plasma DPP IV and trypsin-like cleavage products, are virtually inactive *in vitro* as a consequence of greatly diminished binding to the GRF receptor.

220.10

INTRACELLULAR EVENTS INDUCED BY AN ANTIOPIATE PEPTIDE (Tyr-MIF-1) IN A HUMAN NEUROBLASTOMA SH-SY5Y CELL LINE [1,3]S.L. Chang, [2,3,4]J.E. Zadina, and [4]J. Doucet*, [1]Depts. of Anatomy, [2]Medicine and [3]Neuroscience Training Program, Tulane Univ. Sch. of Med., New Orleans, LA 70112; [4]VA Med. Ctr., New Orleans, LA 70146; [5]Dept. Biochem. Molec. Biol., Louisiana State Univ. Med. Ctr., New Orleans, LA 70119.

Tyr-MIF-1 (Tyr-Pro-Leu-Gly-NH_2) is a naturally- occuring antiopiate peptide and has been suggested to play a biological role in opiate addiction. Zadina et al. (Excerpta Medica, 1990, in press) have recently shown Tyr-MIF-1 binding sites on SH-SY5Y cells, a neuroblastoma cell line which has predominently mu-type opiate receptors. In this study, we explored intracellular events induced by Tyr-MIF-1 in SH-SY5Y cells, presumably through activation of Tyr-MIF-1 binding sites. The SH-SY5Y cells were grown at 37°C in EMEM:F12 (1:1) medium supplemented with 10% FCS in a CO_2 incubator until 80% confluency. The cells were incubated with Tyr-MIF-1 in concentrations of 10^{-5}, 10^{-7} and 10^{-10}M for 1 hour before harvest. Hybridization of the total cellular RNA prepared from cells with a [^{32}P]-labeled c-fos cRNA probe (SP65asfos) revealed 25%, 60% and 43% increases after treatment with 10^{-5}M, 10^{-7}M, and 10^{-10}M Tyr-MIF-1. Tyr-MIF-1 at 10^{-6}M also elevated the cytosolic calcium concentrations, as measured with an indo-1/AM calcium probe, in undifferentiated SHSY-5Y cells. In cells differentiated with retinoic acid, Tyr-MIF-1 decreased intracellular Ca^{+2} concentrations.

220.11

MONOCLONAL ANTI-IDIOTYPIC ANTIBODIES AGAINST DES-ENKEPHALIN-γ-ENDORPHIN (DEγE) INTERACT WITH THE PUTATIVE RECEPTOR FOR γ-TYPE ENDORPHINS

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Des-enkephalin-γ-endorphin (DEγE) is an opioid-inactive fragment of ß-endorphin, which elicits certain behavioral effects upon central or peripheral administration in rats. These effects are independent of opioid mechanisms, and are shared with other non-opioid γ-type endorphins. High affinity binding sites for γ-type endorphins are present in rat forebrain regions in extremely low concentrations. For further study of these sites monoclonal anti-idiotypic antibodies were raised against a monoclonal anti-DEγE antibody specifically recognizing the C-terminal part of DEγE. The anti-ID MoAb CR14.1 was selected, characterized by RIA- and ELISA-techniques, and found to act as a functional antagonist for DEγE in vivo in rats. CR14.1 itself has no affinity for γ-type endorphins. Binding sites for intrinsically labeled [^{35}S]CR14.1 in rat brain cryosections had an anatomical distribution similar to those for [^{35}S]Met-DEγE. Furthermore, CR14.1 completely inhibited the binding of [^{35}S]Met-DEγE to rat brain binding sites, whereas a control serum did not. CR14.1 specifically recognized two protein bands on western blots from rat brain membranes, with apparent molecular weights of 59 kD and 83 kD. Finally, CR14.1 interfered with affinity labeling of [^{35}S]Met-DEγE to a 60 kD protein band. These results suggest that CR14.1 recognizes the putative receptor for non-opioid γ-type endorphins.

220.12

SUBSTANCE P RECEPTOR DESENSITIZATION: INVOLVEMENT OF A GTP BINDING PROTEIN.

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The repeated administration of intrathecal substance P (SP) has been shown to produce behavioral "desensitization" characterized by an increase in the tail flick latency and attenuated SP-induced biting and scratching. It has been suggested that the mechanism of "desensitization" involves activation of an endogenous opioid system. We examined the effects of repeated intrathecal SP administration on SP receptor binding in the rat dorsal horn.

Preliminary studies of SP receptor binding using ^{3}H-SP as a probe indicated two populations of binding sites (nonlinear regression analysis). We focused on the high affinity site by using ^{125}I-SP. Repeated intrathecal injection of SP (0.7 μM) reduced the number of high affinity ^{125}I-SP binding sites as compared to controls (intrathecal saline). Intrathecal SP also increased the K_D for this high affinity binding component. The addition of 5'-guanylylimidodiphosphate (10^{-4} M), a nonhydrolyzable GTP analog, produced a similar decrease in ^{125}I-SP binding affinity.

These data indicate that repeated intrathecal administration of SP alters SP receptor activity. These changes may reflect the development of a desensitized state. The altered agonist affinity seen under desensitizing conditions raises the intriguing possibility that desensitization in this system involves an alteration in receptor-G protein coupling.

220.13

EFFECTS OF CATIONS AND NUCLEOTIDES ON BINDING OF AN AMINOTERMINAL FRAGMENT OF SUBSTANCE P IN MOUSE BRAIN MEMBRANES.

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We have recently shown that the SP aminoterminal heptapeptide, [^{3}H]-SP1-7, labeled a homogeneous population of binding sites (B_{max} = 29.2 fmol/mg protein) with high affinity (K_d = 2.5 nM) in the mouse brain membranes. To determine whether SP1-7 binding reflects an interaction with G protein-linked receptors, we studied the effects of cations and nucleotides on [^{3}H]SP1-7 binding. Using EDTA-washed membranes that are nominally free of divalent cations, [Ca^{2+}] over a range of 1 to100 μM increased specific [^{3}H]SP1-7 binding, while 0.5 to 10 mM Ca^{2+} was inhibitory. Mg^{2+} (1μM - 100mM) and K^{+} (1μM - 10mM) decreased specific binding in a concentration-dependent manner. Na^{+} had no effect on [^{3}H]SP1-7 specific binding at concentrations up to 100 mM, but 250mM decreased binding by 33% compared to controls incubated under conditions of no additonal cations. Addition of GTP or ATP in the incubation medium decreased both K_d and B_{max} of the ligand, implicating modulatory roles for both nucleotides in specific binding of [^{3}H]SP1-7. Maximally effective concentrations of either Ca^{2+} or Mg^{2+} alone (0.1 μM) had no effect on [^{3}H]SP1-7 binding parameters, but Na^{+} alone decreased the B_{max}. ***In the presence of 250 mM Na^{+} or100 μM Mg^{2+}, the inhibitory effects of GTP were abolished.*** There appears to be a negative cooperativity in guanine nucleotide and cations modulation of SP aminoterminal specific binding to the SP N-terminal receptor complex . NIDA04090, DA04190 and DA00124.

220.14

CROSSLINKING OF THE SUBSTANCE P RECEPTOR TO A 43 kDa POLYPEPTIDE: A POTENTIAL G PROTEIN ALPHA SUBUNIT.

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We have previously shown, through a reconstitution assay, that association of the substance P (SP) receptor with a G protein is required for high affinity binding of SP to its receptor. In the present study, we have utilized chemical crosslinking strategies to characterize the G protein that couples to the SP receptor. [^{125}I]-p-benzoyl-phe^{8}-SP, a photoreactive derivative of SP, is covalently incorporated into the SP receptor. The photolabelling is GTP-sensitive, indicating that the G protein is associated with the receptor under the conditions employed in photolabelling. Subsequent incubation of the photolabelled receptor with a homobifunctional crosslinking reagent, followed by SDS/PAGE analysis, results in the appearance of a band on autoradiograms which has an Mr that is approximately 43 kDa greater than that of the receptor. This molecular weight is typical of G protein alpha subunits. Chemical crosslinking of the photolabelled receptor is blocked by the incubation of the photolabelled receptor with a nonhydrolyzable analog of GTP prior to the addition of the chemical crosslinker, whereas incubation of the photo labelled receptor with a nonhydrolyzable analog of ATP has no effect. These data suggest that the photolabelled, crosslinked receptor represents a ternary complex of SP, the SP receptor and the alpha subunit of a G protein.

220.15

NEUROKININ (NK) MODULATION OF Na, Cl, and HCO_3 TRANSPORT IN PROXIMAL JEJUNUM MUCOSA (PJM) OF PIGS.

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Substance P (SP) and related NKs exist in neurons terminating in jejunal mucosa. We characterized NK effects on ion transport across pig PJM, a tissue whose function is similar to humans. Muscle-free PJM was mounted in Ussing chambers. NKs increased short-circuit current (I_{sc}), a measure of active ion transport, maximally by ≃ 60 - 70 μA/cm² with an order of potency: SP > NKA > NKB and respective ED_{50}s of 13, 73 and 1281 nM. SP activity was reduced by 30% after atropine-induced blockade of muscarinic cholinoceptors, but remained unaltered by H_1-histamine antagonists. Tetrodotoxin (TTX; 0.1 μM) respectively reduced NKA and SP efficacy by 50 and 75% and nearly abolished NKB activity. Rank NK potency in the presence of TTX was NKA ≥ SP >> NKB. The NaCl influx blocker bumetanide (10 μM) or external Cl removal decreased SP action on I_{sc}. Radiotracer flux and pH-stat titration analyses revealed that SP increases net Na and HCO_3 secretion, effects inhibited by TTX or by 10 μM acetazolamide in HCO_3-free media. Thus, NKs in pig PJM produce Cl secretion through interactions with a non-neuronal NK-1 receptor. In contrast, NKs act through a different receptor type situated on enteric neurons to stimulate Na-HCO_3 secretion and regulate luminal pH in small bowel.

220.16

RECEPTOR-SELECTIVE ANALOGS DEMONSTRATE NPY/PYY RECEPTOR HETEROGENEITY IN HUMAN NEUROBLASTOMA CELL LINES AND RAT BRAIN.

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Neuropeptide Y (NPY) receptors are heterogeneous consisting of two subclasses, Y1 and Y2 (Wahlestedt, et al., *Reg. Pept.* 13:317, 1986). We sought evidence for differential expression of NPY receptor subtypes in human neuroblastoma (NB) cell lines and rat brain. Cells from SK-N-MC or SK-N-BE(2) NB lines were incubated with ^{125}I-peptide YY (PYY) which labels NPY and PYY binding sites (Lynch, et al., *J. Neurosci.* 9:2607, 1989). pNPY, pPYY, the Y1-selective agonist p[Ile^{31},Pro^{34}]NPY, and the Y2-selective agonist pNPY 13-36 were used as displacing ligands. In SK-N-MC cells the K_i for pNPY was 2.1±0.18 nM, for pPYY 0.86±0.12 nM. p[Ile^{31},Pro^{34}]NPY was approximately equipotent (1.7±0.21 nM) while pNPY 13-36, was much less potent (880±260 nM) suggesting preferential expression of the Y1-receptor. In contrast, SK-N-BE(2) cells displayed binding characteristics indicating preferential expression of Y2-receptors (K_i values for pNPY, pPYY, p[Ile^{31},Pro^{34}]NPY and pNPY 13-36 were 0.87±0.15, 0.49±0.11, 430±170 and 5.4±0.4, respectively). Similar results were obtained using ^{125}I-NPY. Comparable competitive binding studies in membranes or sections of rat brain revealed selective expression of Y2-receptors in hippocampus. The autoradiography analysis also indicated further regional receptor heterogeneity. We conclude that Y1- and Y2-receptors may be independently expressed in NB lines and brain.

220.17

CHARACTERIZATION OF TWO NEUROPEPTIDE Y BINDING PROTEINS IN BOVINE TISSUES BY AFFINITY-LABELING. W. Li', R.G. MacDonald" and T.D. Hexum. Depts. of Pharmacol. and Biochem.', Univ. Neb. Med. Ctr., Omaha, NE 68198-6260.

Neuropeptide Y (NPY) is a 36 amino acid messenger that has a variety of effects including stimulation of feeding behavior and inhibition of norepinephrine release; its binding sites have been identified in several tissues. To elucidate NPY receptor structure, we affinity cross-linked ^{125}I-NPY to receptors present in hippocampal or adrenal medulla membranes using disuccinimidyl suberate (DSS, 1 mM). SDS-polyacrylamide gel electrophoresis followed by autoradiography revealed the presence of a different NPY binding protein in each tissue. A 50 kD ^{125}I-NPY-labeled species was present in bovine hippocampal membranes. Competitive inhibition by unlabeled NPY resulted in an IC_{50} of 8.0 x 10^{-10} M. ^{125}I-peptide YY (PYY) also bound and could be cross-linked to this receptor. Either unlabeled NPY or PYY could displace the binding of both ^{125}I-NPY and ^{125}I-PYY and NPY > NPY^{13-36} > NPY^{18-36} ≥ NPY^{20-36} ≥ NPY^{26-36}in displacing ^{125}I-NPY. Cross-linking of ^{125}I-NPY to bovine adrenal medulla membranes identified a 90 kD species which had an IC_{50} of 1.1 x 10^{-7} M for unlabeled NPY. ^{125}I-PYY was not cross-linked to this protein. NPY ≥ NPY^{13-36} > NPY^{18-36} > NPY^{20-36} ≥ NPY^{26-36}. Pancreatic polypeptide (bovine, human or avian) did not displace the binding of ^{125}I-NPY on either of these receptors. (Work was supported by American Heart Assoc., Inc.)

PEPTIDES: PHYSIOLOGICAL EFFECTS II

221.1

PLASMA VASOPRESSIN INCREASES AFTER INTRAVENTRICULAR DES-LEU-ANGIOTENSIN I. D.G. Changaris, J.R. Claybaugh*#, and R.S. Levy. Depts. Neurology & Biochemistry & Lab. Biol. Psychiatry, University of Louisville School of Medicine, Louisville, KY 40292; #Dept. Clinical Investigation, Tripler Army Medical Center, Honolulu, Hawaii 96859.

Intracerebroventricular (ICV) injection of angiotensin II (AII) induces vasopressin secretion. An alternate pathway in the brain for the synthesis of AII requires the intermediate nonapeptide, des-leu angiotensin I (des-leu AI). To confirm that a second carboxyl cleavage causes vasopressin secretion, we compared the plasma vasopressin levels after ICV injection of des-leu AI and the D-substituted analog, des-leu D-His AI.

Twenty-four male Sprague Dawley rats with ventriculostomies received random ICV injections of either 3.3 nmole des-leu AI or des-leu D-His AI. Rats were sacrificed at 2, 6, and 12 min. by decapitation. Core blood was collected and, after centrifugation, the plasma was frozen for subsequent radioimmunoassay. Those rats receiving the L analog nonapeptide had significantly higher plasma vasopressin levels at 6 & 12 min ($p<0.05$) than those receiving the D analog. Since the octapeptide AII causes vasopressin secretion, these data suggest that the nonapeptide requires a single carboxyl hydrolysis to generate AII and the D for L substitution blocks this hydrolysis. (Supported by NIH-CIDA 531621; VA-DOD 002; and the Glenmore Foundation.)

221.2

PHYSIOLOGICAL ACTIONS OF FIBROBLAST GROWTH FACTOR (FGF) IN CENTRAL NERVOUS SYSTEM. Y. Oomura, K. Sasaki*, T. Muto*, K. Suzuki*, K. Hanai[1]*, I. Tooyama[2]*, H. Kimura[2]* and N. Yanaihara[3]*. Toyama Med. & Pharmaceu. Univ., Toyama 930-01, [1]Fac. Sci., Kyushu Univ., Fukuoka 812, [2]Shiga Univ. Med. Sci., Shiga 520-01, [3]Univ. Shizuoka Sch. Pharmaceu. Sci., Shizuoka 422, Japan.

Glucose effects on the release of acidic FGF (aFGF) into cerebrospinal fluid (CSF) from the ependimal cells which produce aFGF, and the effects of aFGF, basic FGF (bFGF) and related peptides on food intake and neuronal damage by ischemia were examined. The aFGF in CSF of rats was increased 1000 times in a 2 hour period after glucose infusions into the 3rd ventricle (3V). Food intake was dose-dependently decreased by infusions of aFGF or bFGF into the 3V of rats, and increased by bilateral infusions of their antibody into the lateral hypothalamus. The potency of aFGF on food intake inhibition was twice as compared with that of bFGF. When peptides, which consist of 1 to 15 (N-aFGF) or 114 to 140 (C-aFGF) amino acids of aFGF, were infused into the 3V to investigate the active portion of aFGF, N-aFGF decreased food intake dose-dependently, but C-aFGF did not. Continuous infusions of aFGF into the lateral ventricle of the gerbil prevented the delayed neuronal death of hippocampal CA1 pyramidal cells induced by 5 min ischemia. The results suggest that FGF has various physiological actions in the central nervous system.

221.3

BOMBESIN ACTIVATES TUBEROINFUNDIBULAR (TI) AND TUBEROHYPOPHYSIAL (TH) DOPAMINE (DA) NEURONS AND DECREASES BASAL AND STRESS-INDUCED SECRETION OF PROLACTIN AND α-MELANOCYTE STIMULATING HORMONE (αMSH) IN THE MALE RAT. T.W. Toney, J.M. Manzanares, K.J. Lookingland and K.E. Moore, Dept. of Pharmacology, Mich. State Univ., East Lansing, MI 48824

The purpose of the present study was to examine the effects of bombesin on the activity of TIDA and THDA neurons, and the secretion of prolactin and αMSH in non-stressed and stressed (30 min supine restraint) male rats. For comparison, the effects of bombesin on mesotelencephalic DA neurons were also examined. The activity of DA neurons was estimated by measuring the concentrations of 3,4-dihydroxyphenylacetic acid and the accumulation of 3,4-dihydroxyphenylalanine following administration of a decarboxylase inhibitor in brain regions containing terminals of these neurons. In non-stressed rats bombesin (0.3-3.0 µg, icv) decreased plasma levels of prolactin and αMSH and increased the activity of TIDA neurons terminating in the median eminence and THDA neurons in the intermediate lobe (IL), but had no effect on mesotelencephalic DA neurons or THDA neurons projecting to the neural lobe. In stressed animals bombesin (2.5 µg; icv) increased the activity of TIDA and IL THDA neurons, and reduced plasma levels of prolactin and αMSH. These results suggest that bombesin-induced suppression of basal and stress-induced prolactin and αMSH secretion is due, in part, to activation of TIDA and IL THDA neurons. (Supported by ADAMHA Grant MH 42802.)

221.4

CATECHOLAMINERGIC AND ENKEPHALINERGIC MEDIATION OF THE HYPOTHALAMIC ACTIONS OF ATRIAL NATRIURETIC PEPTIDE. F.-L.S. Huang* and W.K. Samson, Anatomy and Neurobiology, Univ. of MO, School of Medicine, Columbia, MO 65212.

Atrial natriuretic peptide (ANP) acts within the hypothalamus to inhibit luteinizing hormone (LH) and prolactin (PRL) secretion (Endo. 122:1573; Neuroendo. 47:268). Both dopaminergic and opioidergic mechanisms have been suggested. Third cerebroventricular (3V) injection of 2nmole ANP reduced LH ($p<0.0001$) and PRL ($p<0.05$) levels in conscious, ovariectomized female rats. Dopamine turnover was stimulated significantly in the arcuate (ARC) and paraventricular (PVN) nuclei. Bilateral injection of 0.1nmole ANP into either the PVN or ARC significantly reduced plasma LH levels, indicating potential sites of action since preoptic nucleus infusions were ineffective. The LH inhibitory effect of ANP was mimicked by 3V injection of 1nmole the enkephalin analog, DP^2DP^5ENK. The effects on LH secretion of both ANP and DP^2DP^5ENK were blocked by pretreatment with 50 ng naltrindole, a specific delta opioid antagonist. The PRL lowering effect of ANP was not altered by naltrindole pretreatment; however, the PRL stimulatory effect of enkephalin was blocked. These studies support our hypothesis that the effect of ANP on PRL is mediated via increased DA turnover and establishes an enkephalinergic mechanism for the inhibitory hypothalamic effect of the peptide on LH secretion. Supported by NIH Grant HD25373.

221.5

THE DEPRESSOR ACTION OF INTRATHECAL NEUROPEPTIDE Y (NPY) IS MEDIATED BY SPINAL Y_2 RECEPTORS. X. Chen, M.M. Knuepfer and T.C. Westfall Dept. Pharmacol. St. Louis Univ. Sch. Med. St. Louis, MO 63104

Previously we have shown that the intrathecal (int) injection of NPY produces a decrease in AP. In the present study we examined the nature of the receptor subtype mediating the depressor effect of int NPY as well as the effect of the peptide on the spinal micro-vasculature. For int injections, one PE 10 catheter was inserted down the spinal subarachnoid space (about T10). Drugs were dissolved in saline and slowly injected at a volume of 10-15 μl. A laminectomy was performed to expose the dorsal surface of the spinal cord between T_{11} and L_1. Spinal vascular blood flow was measured by a laser Doppler flowmeter. The left carotid artery was cannulated for AP measurement. Int NPY C-terminal fragments (NPY^{11-36}, NPY^{14-36} and NPY^{18-36}) produced concentration dependent depressor effects suggesting that the depressor effect of int NPY may be mediated by a Y_2 receptor. Co-administration of the same amount (0.1 nmol) of NPY and NPY^{14-36} or NPY^{18-36} did not affect the depressor effect of NPY. The int injection of NPY (0.1 nmol) significantly decreased AP and concomitantly, gradually decreased spinal vascular resistance suggesting that the depressor effect of int NPY was not due to local ischemia or neuronal damage by this vasoconstrictor (Supported by HL 26319 and HL 35202).

221.6

FACILITATION OF BARORECEPTOR REFLEX RESPONSE BY ENDOGENOUS SOMATOSTATIN IN THE RAT. Julie Y.H. Chan, S.S.Lin* and Samuel H.H. Chan. Taipei Veterans General Hospital and National Yang-Ming Medical College, Taipei, Taiwan, R.O.C.

We evaluated the participation of endogenous brain somatostatin (SOM) in the modulation of baroreceptor reflex (BRR) response, using Sprague-Dawley rats anesthetized with pentobarbital sodium. Intracerebroventricular (i.c.v.) application of SOM (2 or 4 nmol) promoted a significant elevation in BRR response. Blocking the endogenous SOM activity with its specific antagonist, cyclo-(7-aminoheptanoyl-phenylalanyl-D-tryptophyl-lysyl-threonyl [benzyl]) (2 nmol, i.c.v.), or antibody against SOM (1:20, i.c.v.), on the other hand, appreciably attenuated the same response. These modulatory effects on the BRR were essentially duplicated upon bilateral microinjections of SOM (320 pmol), SOM antagonist (320 pmol) or anti-SOM (1:20) into the caudal portion of the nucleus tractus solitarius (NTS), the terminal site for baroreceptor afferents. Furthermore, microinjection of pertussis toxin (25 ng) or n-ethylmaleimide (2 nmol) into the bilateral NTS also reversed the facilitatory action of SOM on the BRR response. These results suggest that neurons in the central nervous system that contain SOM may participate in cardiovascular control by tonically facilitating the BRR, possibly by exerting an influence on the NTS, in a signal transduction process that may involve a pertussis toxin sensitive GTP-binding regulatory protein(s), possibly Gi.

221.7

MICROINFUSIONS OF AII AND AIII INTO THE PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS (PVN) INDUCE DIPSOGENIC AND PRESSOR RESPONSES IN ALERT RATS. Laurie L. Jensen, Joseph W. Harding, and John W. Wright. Washington State University, Pullman, WA., 99163.

Microiontophoretic applications of angiotensin II (AII) and angiotensin III (AIII) have been shown to excite cells in the PVN (Harding & Felix, 1987). Brosnihan et al. (1987) reported increases in blood pressure following PVN microinjections of AII(800 fmoles). Further, our laboratory has observed dose-dependent increases to microinfusions of AII and AIII into the PVN of anesthetized rats (Jensen, et al., 1989). In the present investigation, alert normotensive rats received PVN microinfusions of AII and AIII at doses of 0, 10, 50, 100, and 250 pmoles in a total volume of 50 nl aCSF. Preliminary data indicate that PVN microinfusions of AII and AIII increase blood pressure and induce dipsogenic responses in a dose-response fashion. These results indicate an important role for the PVN in central angiotensin control of blood pressure and body water balance.

221.8

RELATIONSHIP BETWEEN THE CENTRAL KININ SYSTEM AND BLOOD PRESSURE IN SPONTANEOUSLY HYPERTENSIVE RAT. K.Kariya, M.Sasaki*, A.Yamauchi* and N.Gemba* Dept. of Pharmacology, Fac. of Pharm. Sci., Kobe-Gakuin Univ., Kobe 673 JAPAN

We have reported that bradykinin(BK, Neuropharmacol., 20, 1221, 1981) and kininogenase(KL, Japan.J.Pharmacol., 43, 129, 1987)injected intraventricularlly produced a dual pressor response in Sprague-Dawley rat. In this study, we observed about the sensitivity on blood pressure related to the central kinin system using spontaneously hypertensive rat(SHR) and WKY as the control.

Intraventricular injection of BK(1-5 nmole) caused a pressor response with dose dependent manner in WKY, but such increase was depressed in SHR. However, KL(2-16 KU) produsued a marked increase of the blood pressure in SHR comparing with WKY. Further, the response in male WKY was more sensitive for BK than in the female. But that for KL was vice versa. On the otherhand, KL produced more enhancement of the pressor response in male SHR than in the female. But, the response by BK in female SHR was higher than in the male rat.

Above results suggest that the central kinin may participate in regulation of blood pressure.

221.9

PROTEIN SYNTHESIS INHIBITION ALTERS THE CHARACTERISTICS OF FEVER INDUCED BY INTRACEREBROVENTRICULAR INTERLEUKIN-1β. W. M. Zawada, J. Clarke and W. D. Ruwe. Department of Physiology & Biophysics, University of Arkansas for Medical Sciences, Little Rock, AR 72205.

Pretreatment with a protein synthesis inhibitor, anisomycin (ANI), suppresses fevers normally evoked by endotoxin or crude endogenous pyrogen, EP [Ruwe, W. D. and Myers, R. D. Brain Res. Bull. 4:741-745, 1979]. The effect of ANI on hyperthermias evoked by prostaglandins of the E series (PGE) is less clear. Moreover, recent reports suggest that ANI may significantly prolong fevers induced by EP or PGE_2 [Morimoto, A. et al., Pflügers Arch. 408:414-416, 1987]. A series of experiments was conducted to further evaluate the importance of protein synthesis in the generation of fever. Stainless steel guide tubes were implanted above the lateral cerebral ventricle (LCV) of male Long Evans (280-325 gm). Injections of either human recombinant IL-1β, 5 ng/20μl, or PGE_2, 250 ng/10μl, were made 30 min after an LCV infusion of artificial cerebrospinal fluid (aCSF), 10μl, or ANI, 80 μg/10μl. ANI significantly altered the febrile response to IL-1β, within 45-90 min after injection of the peptide. The response to PGE_2 was unaffected by the inhibitor. These results are consistent with a role for an unknown peptide/protein mediator in the neurochemical pathway to fever. [Supported by a grant (#NS26045) from NINDS to WDR.]

221.10

CENTRALLY ADMINISTERED NEUROPEPTIDE Y ENHANCES THE HYPOTHERMIA INDUCED BY PERIPHERAL ADMINISTRATION OF ADRENOCEPTOR ANTAGONISTS. M.C. Ruiz de Elvira* and C.W. Coen (Spon: Brain Research Association, UK). Division of Biomedical Sciences, King's College, London W2R 2LS, UK.

The distribution of neuropeptide Y in the brain includes extensive coexistence within adrenaline- and noradrenaline-containing neurons and many of its actions are often associated with adrenergic systems. Since neuropeptide Y immunoreactivity is particularly intense in the preoptic area, one of the principal sites for thermoregulation, we have tested the effects of neuropeptide Y on core temperature in normothermic rats, and rats rendered hypothermic by systemic treatment with adrenergic antagonists. In the normothermic rat, intracerebroventricular administration of 1 μg of neuropeptide Y did not have a significant effect on core temperature. Intraperitoneal treatment with the α_1-adrenoceptor antagonist, prazosin, or the β-adrenoceptor antagonist, propranolol, caused an immediate and significant hypothermia; the intracerebroventricular administration of 1 μg of neuropeptide Y, 10 minutes after these drugs, strongly potentiated their hypothermic effect. Although intraperitoneal treatment with the α_2-adrenoceptor antagonist, idazoxan, had no hypothermic effect *per se*, the intracerebroventricular administration of NPY 10 minutes after this antagonist led to a significant decrease in core temperature. The site at which these interactions take place, and their nature, remain to be established.

221.11

BOMBESIN INFUSION INTO THE PREOPTIC AREA ALTERS PLASMA METABOLIC FUELS AND PRODUCES HYPOTHERMIA IN FOOD-DEPRIVED AND INSULIN-PRETREATED RATS. A.M. Babcock[1*], M.W. Cunion[2], M.J. Rosenthal[2*], P. Lomax[3] and C. Barton[1*]. [1]Dept. of Psych., Univ. of S. AL, Mobile, AL 36688; [2]GRECC, Sepulveda VAMC, Sepulveda, CA 91343; [3]Dept. of Pharm., UCLA School of Medicine, Los Angeles, CA 90024.

Bombesin (BN; 0, 50 ng/.25 µl) was infused into the preoptic area (POA) through previously implanted unilateral guide cannulae. Rats (n=8) were tested under conditions of food satiation, food deprivation (18hrs), and insulin pretreatment (Regular Iletin I; 10U/kg im). Blood samples (120µl) were collected from the tail tip at 0, 60, and 120 min postinfusion. Changes in rectal temperature and food intake were also measured. BN increased plasma glucose in food-deprived rats and attenuated insulin-induced hypoglycemia. BN elevated free fatty acids in food-sated rats and prevented a reduction in food-deprived rats. Corticosterone was increased following BN under all conditions. As previously reported, BN produced hypothermia and hypophagia in food-deprived and insulin-treated rats. These results suggest that the POA is an important site for BN action on thermoregulation and metabolic fuel regulation. In addition, this action appears dependent on the metabolic state of the test animal. (Supported by USARC funds [AB], NS20660 [MG], AG04793 [MR], and Vet. Adm. research funds [MG,MR].)

221.12

VASOPRESSIN RELEASE WITHIN THE RAT BRAIN DURING DRUG-INDUCED ANTIPYRESIS. M.F. Wilkinson* and N.W. Kasting. Dept. of Physiology, Univ. of British Columbia, Vancouver, B.C., Canada.

Arginine vasopressin (AVP) is an endogenous antipyretic within the ventral septal area (VSA) of the rat brain. Experiments were conducted in conscious, unrestrained rats to measure VSA AVP release, using push-pull perfusion (ppp), before and after antipyretic treatment with indomethacin (Indo) or acetaminophen (Aceta). Surgical preparation consisted of cannulas directed toward a lateral cerebral ventricle (LCV) and the VSA and an ip transmitter for monitoring of body temperature. Fever was induced by E.coli endotoxin injected into a LCV. Ppp was initiated 120min post-endotoxin. Following the first ppp period (30min) Indo or Aceta was injected ip. In the first ppp period following Indo, but not Aceta, AVP in VSA perfusates increased 188% (0.85±0.11 to 1.6±0.22 pg/sample, $p<0.05$). This corresponded to the greatest decrease in body temperature. Neither ip Tris buffer (febrile rats) nor Indo (non-febrile rats) evoked significant changes in VSA AVP release. These data support a role for VSA AVP in Indo-induced antipyresis. Supported by MRC of Canada.

221.13

The effect of delta sleep-inducing peptide(DSIP) on the body temperature changed by dopamine(DA) and 5-hydroxytryptamine in rat.
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DSIP,a putative endogenious sleep-promoting substance,had an enhancing effect of hypothermia induced by i.p. injection of apomorphine at an ambient temperature(24±1°C).A minimal effective dose of this peptide was 10ng and the dose response relationship exhibited an inverted bell-shape with maximal effective dose of 1mcg.The action of this peptide was antagonized by haloperidol.By the pretreatment of anti-DSIP,this enhancing effect was totally abolished.As it is well-known that both DA and 5-HT are involved in the thermoregulation,the relationship between DSIP and 5-HT was further examined.Rats were injected i.p. with 5-MeODMT and 8-OH-DPAT, serotonergic agonists,and then DSIP(1mcg) was injected icv. DSIP had an enhancing effect on hypothermia induced by these substances at an ambient temperature.It is suggested that DSIP may have a physiological role in the thermoregulatory mechanismsby enhancing the action of DA and 5-HT1a receptors.

221.14

VASOPRESSIN IS INVOLVED IN CENTRAL AMYGDALOID REGULATION OF STRESS-RELATED CHANGES. B.Roozendaal*, G.H.M.Schoorlemmer*, J.M.Koolhaas* and B.Bohus*. (SPON: European Brain and Behaviour Society) University of Groningen, Dept. of Animal Physiology, P.O. Box 14, 9750 AA Haren, The Netherlands.

The central nucleus of the amygdala (CEA) has been implicated in the control of autonomic and behavioural correlates of conditioned stress. Central arginine-8-vasopressin (AVP) elicits cardiovascular, neuroendocrine and behavioural changes. The CEA contains high densities of vasopressin V1 and oxytocin receptors through which AVP may be effective. A nine-minute infusion of 200pg AVP (dissolved in 1 µl artificial CSF) into the CEA of conscious male Wistar rats under resting conditions, led to an increase in heart rate during the infusion period, but neither noradrenaline (NA), nor adrenaline (A) were elevated. This suggests that the tachycardia is due to a reduced parasympathetic control rather than a stimulation of the sympathetic outflow. Furthermore, AVP infusion into the CEA resulted in a behavioural activation and a slight increase in plasma corticosterone.

The present studies suggest that regulation of stress-related autonomic and behavioural changes in the CEA involves vasopressinergic mechanisms through parasympathetic and/or adrenal cortical output.

221.15

INTRACISTERNAL INJECTION OF THE ENDOPEPTIDASE INHIBITOR PHOSPHORAMIDON INHIBITS GASTRIC ACID SECRETION IN PYLORUS-LIGATED RATS. **R.L.Stephens, Jr. and P. E. Ward*, Department of Physiology, The Ohio State Univ., Columbus, Ohio 43210.**

Endopeptidase 3.4.24.11 (Endo 24.11) is thought to contribute to the metabolism of endogenous CNS peptides such as opioids, substance P and bombesin. Phosphoramidon (P) is a specific inhibitor of Endo 24.11. In 2 hour pylorus-ligated rats, intracisternal (ic) injection of phosphoramidon (1-100nmol) produced a marked dose-dependent inhibition in gastric acid output and concentration. In contrast to the effect of ic phosphoramidon, ic injection of the aminopeptidase inhibitor amastatin (A) (100nmol), was ineffective in altering gastric acid output.

Treatment (nmol)	N	volume (ml/2 hr)	acid output (µEq/ 2 hr)	acid conc (µEq/ ml)	
vehicle	10	3.6 ± 0.6	343 ± 81	89 ± 6	
P(100)	6	2.1 ± 0.2*	61 ± 14*	33 ± 10	**
P(10)	6	2.1 ± 0.3*	88 ± 25*	39 ± 8	**
P(1)	4	3.1 + 0.9	213 + 84	65 + 7	*
A(100)	4	2.9 ± 0.7	260 ± 82	84 ± 7	

(: $p< 0.01$; *: $p<0.05$; significantly different from vehicle treated rats). The data suggest that an endogenous Endo 24.11-sensitive peptide in the brain may act as an inhibitor of gastric acid secretion and concentration. The specific brain site of action of this effect remains to be elucidated.** **Supported by OSU Seed Grant 221192 and NIH-DK 28184.**

221.16

NEUROTOXIC EFFECTS OF THYROTROPIN RELEASING HORMONE (TRH) ON FETAL RAT HIPPOCAMPAL CULTURES. J. Phillips, A. Winokur, and J.R. Buchhalter. Depts. of Psychiatry, Pharmacology, and Neurology, U. of PA School of Medicine, Philadelphia, PA 19104

TRH is widely distributed in the CNS and has been implicated as a facilitatory neuromodulator and a trophic factor. TRH enhanced both the number and size of cell processes in cultured ventral horn neurons derived from rat embryo. Additionally, there have been several reports of clinical benefit to amyotrophic lateral sclerosis patients treated with TRH. In light of these findings, we examined the effect of TRH on the development and survival of cultured hippocampal neurons.

Dissociated hippocampal neurons from fetal day 18-19 rats were plated at a density of 100,000 viable cells in Petri dishes containing poly-lysine coated glass coverslips. Cells were incubated in serum supplemented Dulbecco's modified Eagle's medium for 24 hours,and then transferred to 24 well plates containing media with 10^{-2} to 10^{-12}M TRH. Cells were incubated for 1 week, fixed, and neurons counted.

A concentration dependent TRH toxicity was noted. By 24 hours all neurons were dead with a dose of 10^{-2} M TRH. At 1 week, 10^{-14}M TRH,the lowest concentration tested, reduced the survival of neurons by 51% as compared to controls. TRH-OH, a TRH metabolite, was 50% less toxic than TRH at comparable concentrations. The glutamate receptor antagonist 2-amino-phosphonovalerate did not inhibit the effects of TRH on neuronal death. These results indicate that TRH produces profound neurotoxic effects on fetal rat hippocampal cultures, but that these effects are not being exerted through glutaminergic mechanisms (via a glutamate metabolite of TRH). Further studies are required to elucidate the mechanism of action of TRH on hippocampal neurons.

222.1

CORTICOTROPIN-RELEASING FACTOR-IMMUNOREACTIVE (CRF-IR) NEURONS ARE LOCALIZED IN NUCLEI WHICH PROJECT TO THE LOCUS COERULEUS (LC). R.J. Valentino, E. J. Van Bockstaele, and G. Aston-Jones, Department of Mental Health Sciences, Division of Behavioral Neurobiology, Hahnemann University, Philadelphia, PA 19102.

CRF-IR fibers have been visualized in the noradrenergic nucleus, LC (Swanson et al., 1983; Sakanaka et al., 1987), and physiologic studies suggest that CRF may serve as a neurotransmitter in the LC during stress (Valentino, 1989). To further test the hypothesis that CRF acts as a neurotransmitter in the LC, the origin of CRF-IR fibers in LC was investigated using combined immunohistochemical and retrograde tracing techniques. CRF-IR cells were found in two medullary nuclei which are the primary sources of LC afferents, the nucleus paragigantocellularis (PGi) and the nucleus prepositus hypoglossi (PrH), in colchicine-treated rats. Additionally, CRF-IR cells were found in the pericoerulear region, particularly along the lateral border of the LC and in Barrington's nucleus. Preliminary double labeling studies suggest that the pericoerulear CRF-IR cells are not noradrenergic neurons. Microinjection of the retrograde tracer, cholera toxin B subunit coupled to 7 nm colloidal gold particles (CT-Gold), into the LC resulted in labelling that was similar to that produced by iontophoretic injection of the retrograde tracer, Fluoro-Gold, into LC. Cells in PGi, PrH, and the dorsal cap of the paraventricular nuc. of the hypothalamus were labelled with the retrograde tracer. A few cells in PGi and the hypothalamus exhibited double-labelling for CRF-IR and CT-Gold. In the PrH most CRF-IR cells were lateral to cells containing the retrograde label. These results suggest that a small population of CRF cells in PGi and the hypothalamus project to the LC and that CRF-IR fibers in the LC may also be derived from local pericoerulear circuits. This work was supported by PHS Grants MH 40008, MH 42796, NS 24698 and DA 06214.

222.2

DISTRIBUTION OF NEUROPEPTIDE Y-LIKE IMMUNOREACTIVITY (NPY) IN THE FELINE PONS AND MIDBRAIN. P.J. Gatti, Y. Tizabi, and V.J. Massari. Dept. Pharmacol., Howard Univ. Coll. of Med., Washington, D.C. 20059.

We have examined the distribution of NPY perikarya and terminals in the pons and midbrain of colchicinized cats. Frozen sections were processed for immunohistochemistry using an avidin-biotin based method, and a rabbit anti-NPY antibody diluted 1:1000 (Peninsula). In the pons, hundreds of NPY perikarya were noted scattered throughout the inferior colliculus. A large group of densely packed NPY cells was found in the dorsal third of the N. cuneiformis. Substantially fewer labeled cells were seen in the dorsal tegmental nucleus, locus coeruleus, the medial and lateral tegmental field, and the periaqueductal gray. Fewer NPY perikarya were found in the midbrain than in the pons. They were observed in the central tegmental field, the posterior pretectal nucleus, the ventral periaqueductal gray, substantia nigra, and the ventral tegmental area. Very few NPY cells were seen in the superior colliculus. Tyrosine hydroxylase immunoreactive cells in the locus coeruleus, substantia nigra, and ventral tegmental area were vastly more numerous than NPY cells in these nuclei. Therefore, although co-localization of NPY and catecholamines may occur, the vast majority of NPY cells in the pons and midbrain do not contain catecholamines. Supported by A.H.A./N.C.A.

222.3

CYTOCHEMICAL LOCALIZATION OF INSULIN-LIKE IMMUNOREACTIVITY IN THE RAT SPINAL CORD. S.S.W. Tay and W.C. Wong, Department of Anatomy, National University of Singapore, Kent Ridge, Singapore 0511.

Insulin-like immunoreactivity has been localized in the cervical, thoracic, lumbar and sacral segments of the spinal cord. Neurons in both the dorsal and ventral horns showed positive staining. Under the light microscope, staining appeared to be localized mainly in the cell nucleus but not its nucleolus. Normal serum and preabsorbed controls showed an absence of staining. With the electron microscope, fine grains of the reaction product were distributed within the nucleoplasm as well as on the chromatin materials and nuclear membranes. Only small traces of the reaction product were found in the cytoplasm of the neuronal cell bodies. Dendrites were labelled and the reaction product was localized mainly alongside the parallel arrays of neurotubules. Labelled dendrites were mostly distal dendrites, being postsynaptic to non-immunoreactive axon terminals. A few labelled dendrodendritic contacts were present. Axons and axon terminals appeared to be non-immunoreactive. The present results suggest that insulin-like substance(s) in the spinal cord may be modulating neuronal activity in the cell nucleus as well as at the synapse.

222.4

SOME SPINOTHALAMIC NEURONS ARE IMMUNOREACTIVE FOR SUBSTANCE P.

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The undecapeptide Substance P(SP) has been shown to act as a neuromediator in the transmission of sensory stimuli, possibly of noxions origin, from small primary sensory neurons to the superficial laminae of the spinal cord. The presence of SP in neurons relaying sensory imputs from the spinal cord to the thalamus has been investigated in the present study, by means of immunocytochemistry and tracing experiments in the rat and the cat. The present experiments reveal the existence of SP-positive neurons in cervical spinal laminae known to contain spinothalamic neurons, and the presence of SP-positive fibers and nerve terminals in thalamic nuclei known to receive spinothalamic afferents. Lesion experiments reveal an evident depletion of SP fibers in some thalamic nuclei after spinal hemisection at high cervical levels. Tracing experiments demonstrate the presence in the spinal cord of SP-immumoreactive neurons retrogradely labeled after injection of the tracer in the thalamus. These data, taken together, indicate that a fraction of spinothalamic neurons do contain high levels of SP. SP may thus act as a synaptic mediator also in second order sensory neurons, possibly involved in nociception.

222.5

ACUTE C-FIBER STIMULATION CAUSES A DECREASE IN SUBSTANCE P-, GALANIN- AND DYNORPHIN- IMMUNOREACTIVITY IN THE RAT DORSAL HORN. C.M. Klein, L.J. Sorkin, S.M. Carlton, K.N. Westlund and R.E. Coggeshall. Dept. of Anat., MBI, Univ. of Texas Med. Br., Galveston, TX 77550.

In this study, we measured changes in immunoreactive staining in the L4-L5 dorsal horn following acute stimulation of C-fiber afferents in the sciatic nerve. Specifically, we were interested in changes in 3 peptides, all of which have been found in primary afferent fibers and/or intrinsic systems--substance P (SP), galanin (GAL) and dynorphin (DYN)--and have been shown to increase in the dorsal horn following various peripheral manipulations. The sciatic nerve of adult Sprague-Dawley rats was electrically stimulated (1 Hz, 0.2ms) for 20 mins.; the compound action potential (including both A and C fiber volleys) was monitored and did not diminish during the entire stimulation period. Control animals received A fiber stimulation only for the same length of time. Immediately after stimulation, the rats were perfused with 3% paraformaldehyde, 3% glutaraldehyde, 0.1% picric acid in cacodylate buffer. The L4-L5 spinal cord segments were removed and processed for immunocytochemistry by the PAP method. Immunoreactive staining was decreased (SP-53%, GAL-58%, DYN-41%) in the medial part of the superficial dorsal horn as compared to the unoperated side. These findings are interesting in that electrical stimulation of A and C fibers (but not A fibers alone) causes a decrease in staining for these 3 peptides. Supported by grants: NS10161, NS11255 and NS07185.

222.6

RELATIONSHIP OF IMMUNOREACTIVE (IR) NEUROKININ A (NKA) AND SUBSTANCE P (SP) IN VENTRAL MEDULLARY (VM) NEURONS THAT PROJECT TO THE INTERMEDIOLATERAL CELL COLUMN (IML) AND IN IML FIBERS. C. Sasek, M. Haxhiu & C. Helke, Dept. of Pharmacol. Uniformed Services Univ., Bethesda MD 20814.

Multiple mammalian tachykinins have been identified. It has previously been impossible to immunohistochemically distinguish and compare the distributions of these peptides due to the unavailability of specific antisera. The present study uses extensively characterized, specific antisera to compare the distributions of SP and NKA in VM IML-projecting neurons and in their termination site, the IML.

Rats received 40nl injections of rhodamine beads into the T3 IML (Sasek, 1989). Tissue was sectioned and processed for immunofluorescence. Anti-NKA (S. Leeman) and SP (Incstar) were characterized using absorption controls and immunoblotting. To verify that neither antiserum was cross-reacting with neurokinin B (NKB), sections were stained with a specific antiserum to NKB (J. Krause) and the distribution of NKB compared to that of NKA and SP.

NKA and SP were similarly distributed in VM IML-projecting neurons and in fibers in the IML. No NKB IR was identified in the IML, suggesting that the SP and NKA IR in VM IML-projecting neurons was not due to cross reactivity with NKB. The results of these studies demonstrate: 1) the presence of NKA in VM IML-projecting neurons, & 2) the presence of several distinguishable tachykinin systems in the CNS. (NS 24876)

222.7

SUBSTANCE P- AND CALCITONIN GENE-RELATED PEPTIDE-IMMUNOREACTIVITY IN NERVES OF THE RAT UTERUS: LOCALIZATION, COLOCALIZATION AND EFFECTS ON UTERINE CONTRACTILITY. R.L. Shew and R. E. Papka. Dept. Anatomical Sciences, Univ. Oklahoma, Hlth. Sci. Ctr., Oklahoma City, OK 73190.

Immunoreactivity to the neuropeptides substance P (SP) and calcitonin gene-related peptide (CGRP) were examined in nerves in the rat uterus as a prelude to studying their effects on uterine contractility. SP-immunoreactivity (SP-I) and CGRP-I were localized in myometrial nerves throughout the uterine horns. Double labeling revealed SP-and CGRP-I coexist in a subpopulation of uterine nerve fibers, e.g., SP-I was not present in all CGRP-I nerves. Effects of these neuropeptides on uterine contractility was examined in vitro on uterine horns from diethylstilbestrol-treated rats (50ug/rat, i.p., 14-16 hrs prior to study). SP (10^{-4} to 10^{-7}M) stimulated uterine contraction in a dose-related manner. While CGRP has no effect on baseline uterine tension, it (10^{-7}M) reduced 10^{-5}M SP-stimulated uterine contraction by 56%. These results demonstrate that SP- and CGRP-I are present in, and coexist in some, uterine nerves, presumably afferent nerves. Coexisting SP and CGRP could be co-released from the afferent fibers in an "efferent fashion" and affect uterine contractility; SP having a contractile effect and CGRP having a relaxing effect. (Supported by NIH grant NS 22526, Alumni Grant from OUHSC and Presbyt.Health Fdn.)

222.8

DORSAL ROOT GANGLIA ORIGIN OF A SUBPOPULATION OF SYNAPTIC TERMINALS IN THE FEMALE RAT PELVIC PARACERVICAL AUTONOMIC GANGLIA AND VARICOSITIES IN THE UTERUS CONTAINING CGRP. R. E. Papka. Dept. Anatomical Sciences, Univ. Oklahoma, Hlth. Sci. Ctr., Oklahoma City, OK 73190.

Immunohistochemistry and retrograde axonal tracing with fluorogold was used to examine the origin of a subpopulation of synaptic terminals, containing calcitonin gene-related peptide (CGRP), in the pelvic paracervical ganglia (PG) and varicosities in the uterine cervix. Antiserum against synapsin I (SYN) revealed synaptic endings, while antiserum against CGRP revealed a subpopulation of nerves and terminals. Double labeling revealed the existence of CGRP-immunoreactivity (CGRP-ir) in SYN-ir terminals. SYN-ir was present in punctate granules abutting PG neuron somata and in varicose nerve fibers of cervical myometrium, vasculature and epithelium. CGRP-ir existed in subpopulation of SYN-ir terminals in PG and nerves in the cervix. Fluorogold injection of PG labeled neurons in dorsal root ganglia (DRG) and spinal cord; however, fluorogold-labeled neurons containing CGRP-ir were visualized only in DRG. Fluorogold injection of uterine cervix labeled neurons in PG and DRG; but, only those in DRG contained CGRP-ir. Co-containment of CGRP and SYN in a subpopulation of terminals in the PG suggests that CGRP is present in synaptic terminals and not merely in fibers of passage. Also, CGRP-ir terminals in the PG and uterine fibers originate from DRG. (Supported by NIH Grant NS 22526, OU Alumni Grant and Presbyt. Hlth. Fdn.)

222.9

PEPTIDERGIC NEURONS OF POSTNATAL RAT SPINAL CORD AND DORSAL ROOT GANGLION ARE MAINTAINED IN ORGANOTYPIC CULTURES

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Dept. Veterinary Anatomy, Iowa State University, Ames, Iowa 50011.

The aim of the present study was to determine weather culturing of the neurons or injury due to tissue extirpation may evoke a out of place synthesis of the peptides in cultured neurons. The neuronal distribution of calcitonin gene-related peptide (CGRP), galanin, Met-enkephalin (Met-enk), and substance P (SP) in cultures obtained from postnatal (P) rats of different age (P1 to P18) and cultured for different periods of time were studied. Explants of spinal cord (SC) and dorsal root ganglion (DRG) were dissected and cultured on chicken plasma-coated slides in Petri dishes or in rotating test tubes. By using immunocytochemistry we were able to identify peptidergic neurons and observe their distribution and relationship with the total population of cellular elements. CGRP-immunoreactivity (IR) was observed in DRG, dorsal horn (DH) neurons and in motoneurons of the spinal cord. The mean diameter of DH CGRP-IR neurons varied from 9.4 to 12.15μm while ventral horn neurons were from 18.98 to 25.91μm. The number of CGRP-IR neurons per explant was greater in dorsal than in ventral horn. Size of DH neurons IR to galanin, Met- enk, and SP were similar to CGRP-IR neurons (10.76 to 11.34μm). The distribution of Met-enk-IR neurons of DH resembled very much the distribution of enkephalinergic neurons in freshly sectioned spinal cord. The number of CGRP, SP and galanin-IR cell bodies in DRG explants varied from preparation to preparation. These result demonstrate that number of peptidergic neurons from DRG and spinal cord survive in organotypic culture in arrangements similar to that in vivo. Only the CGRP-IR neurons distribution was found different from that found in freshly sectioned SC. Work was supported by NIH grant NS27751 and USDA grant PL95-113..

222.10

ORIGIN OF BRAIN NATRIURETIC PEPTIDE - LIKE IMMUNOREACTIVE INNERVATION OF THE CEREBRAL ARTERIES. S.E. Spencer, M. Kibbe, K.M. Hurley and C.B. Saper, Department of Neurology, Washington Univ., St. Louis, MO 63110 and Depts. of Pharm. & Physiol. Sci. and Neurology, Univ. of Chicago, Chicago, IL 60637

We recently reported that the cerebrovascular tree in the rat is richly innervated by porcine brain natriuretic peptide-like immunoreactive (pBNPir) axons. To determine the origin of these fibers, we examined the trigeminal (TRG), pterygopalatine (PTG), superior cervical (SCG), otic and geniculate ganglia for neurons retrogradely labeled with Fast Blue dye transported from the surgically exposed middle cerebral artery, and for cells stained with antiserum against pBNP. On the ipsilateral side, 54% of retrogradely labeled cells were found in the SCG, 28% in the TRG and 19% in the PTG. Of these, 31% in the SCG, 74% in the TRG and 24% in the PTG were pBNPir. Similar staining patterns were seen on the contralateral side, where 14% of retrogradely labeled neurons were found. Unilateral extirpation of the SCG and the contents of the ethmoidal foramen (including the nasociliary nerve) reduced the circumferential pBNPir fiber density on selected cerebral artery segments ipsilaterally by 33% compared to the contralateral side. We conclude that substantial pBNPir innervation of the cerebral arteries arises from the ipsilateral SCG, TRG and PTG, but that alternative sources such as the carotid miniganglion must make an important contribution.

222.11

CHARACTERIZATION OF TARGET SPECIFIC NEURONS IN THE RAT SUPERIOR CERVICAL GANGLION. J. I. Luebke and L.L. Wright. Dept. Anatomy, Boston Univ. Sch. Med., Boston, MA 02118

The present studies investigated several aspects of the organization and development of target specific neurons in the rat sympathetic superior cervical ganglion (SCG). A series of retrograde tracer, immunocytochemical, and double-labeling studies revealed significant differences in the size, number, localization and neuropeptide content of SCG neurons that project to the submandibular glands (SMGs), the eyes or the pineal gland. The mean areas of the cell bodies of SMG, eye and pineal projecting neurons are 928, 434, and 794 um^2, respectively. More neurons are found to project to the SMG than to the eye or the pineal gland (a total of 1704, 90, 116 respectively). A greater percentage of SMG than of pineal projecting neurons display vasoactive intestinal peptide- like immunoreactivity (VIP-LI), but there are no differences in the percentage of neurons displaying neuropeptide Y (NPY)-LI or somatostatin (SS)-LI between the target specific groups. In the entire SCG, more neurons displaying NPY-LI are found than VIP-LI, while differences between the numbers of neurons displaying NPY- and SS-, and SS- and VIP-LI are not significant. No topographic localization of immunoreactive neurons is observed in the SCG. Neonatal deafferentation does not result in changes in the size of neurons, number of neurons, or number of immunoreactive neurons in these target specific populations, or in the total number of immunoreactive neurons in the SCG. In conclusion, these studies describe differences and similarities among target specific populations of SCG neurons, and provide further evidence for the heterogeneous nature of SCG neurons.
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222.12

SUBSTANCE P AND CALCITONIN-GENE-RELATED PEPTIDES IN THE SYNOVIAL MEMBRANE AND JOINT FLUID IN NORMAL AND PATHOLOGICAL CONDITIONS. R. M. Bowker, Dept. Anat., J. P. Caron*, Dept. LCS, Michigan State Univ., East Lansing, MI 48824 and R. H. Abhold, Dept. Biol., California State Univ. at Fresno, Fresno, CA 93740.

Recent evidence has suggested that neural factors may be involved in the mediation of arthritic disease. Experimentally, the peptide substance P (SP) is released from nerve terminals in joints (Yaksh, 1988) and may contribute to the severity and symmetry of arthritis (Levine et al., 1985). In spontaneous arthritis in man SP content of synovial fluid from the stifle joint appears to vary. In the present study we sought to investigate these peptides in different joints under both normal and pathological conditions. Samples of the equine synovial membrane and fluid were obtained and prepared for routine immunocytochemistry (ICC) and radioimmunoassay (RIA). The synovia and fluid samples obtained from the radiocarpal, intercarpal, tibiotarsal and phalangeal joints. The sectioned synovia were incubated in antisera raised to SP and CGRP while the fluid samples were processed for RIA. The ICC demonstrated that SP and CGRP were present in the synovium in association with the vasculature and the intimal layer. The RIA samples revealed the presence of these peptides normally, but differences between joints as to their peptidergic content were observed. These differences in peptides may be related to the clinical incidence of arthritic disease in theh different joints.

222.13

ORGANIZATION OF PEPTIDE-CONTAINING NERVES OF THE PANCREAS. C. Sternini, R. De Giorgio , K. Anderson, V. L. W. Go , and N. Brecha. Departments of Medicine and Anatomy & Cell Biology and CURE, UCLA School of Medicine, Los Angeles, CA 90024.

The pattern and origin of peptide-containing nerves of the adult rat pancreas were analyzed by immunohistochemistry to gain further insights into the functional organization of peptides that affect pancreatic secretions. In the islets, varicose and thin processes immunoreactive (IR) for bombesin or gastrin releasing peptide (BBS/GRP) and vasoactive intestinal peptide (VIP) are abundant, those IR for calcitonin gene-related peptide (CGRP) and neuropeptide Y (NPY) are moderate, and those IR for substance P or related tachykinins (SP/TK) are sparse. The density of peptidergic nerves in the stroma and acini is: CGRP, NPY and VIP > BBS/GRP > SP/TK. The vasculature receives prominent NPY, CGRP, and SP/TK innervations. Within the intrapancreatic ganglia, IR fibers are: BBS/GRP and CGRP > NPY and VIP > > SP/TK. BBS/GRP-, VIP- and NPY-IR also label cell bodies. Superior abdominal ganglionectomy, but not vagotomy, results in a loss of CGRP- and SP/TK-IR nerves and a reduction of NPY- (with loss of the perivascular NPY) and BBS/GRP-IR nerves. The VIP innervation is not affected. CGRP- and SP/TK-IR colocalize extensively. A few CGRP fibers appear to contain BBS/GRP- or VIP-IR. These findings are consistent with a neural input from intrinsic and extrinsic peptidergic neurons in the control of the endocrine and exocrine pancreas.

Supported by NIH grant DK37852.

222.14

IMMUNOCYTOCHEMICAL LOCALIZATION OF VASOPRESSIN AND VASOPRESSIN-ASSOCIATED NEUROPHYSIN IN HUMAN JEJUNUM. A.S. FRIEDMANN, V.A. MEMOLI*, S.W.T. CHENG*, AND W.G. NORTH. Departments of Physiology and Pathology, Dartmouth Medical School, Hanover, NH 03756.

While the neuropeptide vasopressin (VP) is classically recognized to be a product of hypothalamic neurons, it is now known to be produced by cells of the adrenal medulla and ovaries. Recent studies have reported the localization of this peptide in neuronal fibers of the myenteric plexus and muscularis externa of rat duodenum and throughout the small and large intestine of the cat. In the present study we carried out immunocytochemical examination of human jejunum for the presence of VP and vasopressin-associated human neurophysin (VP-HNP) using a mouse monoclonal antibody to VP and a rabbit polyclonal antibody to VP-HNP. Both of these antibodies were produced in these laboratories and shown to exhibit a high degree of antigen specificity. Acetone-fixed human intestine was embedded in paraffin. Sections of 0.4 microns were incubated overnight with primary antibody and stained using the avidin-Biotin complex technique. Visualization was achieved by the conversion of 3,3' diaminobenzidine to an insoluble, brown precipitate by peroxidase, following which the slides were counterstained with hematoxylin, dehydrated and coverslipped. VP and VP-HNP immunoreactivity was found to be colocalized in neuroendocrine cells at the base of jejunal crypts. This colocalization suggests that VP production by these cells is similar to that occurring in neurons and that VP may perform an endocrine function in the human gastrointestinal tract.

INTERACTIONS BETWEEN NEUROTRANSMITTERS II

223.1

PROGESTERONE PROMOTES GLUTAMATE-INDUCED RELEASE OF GABA FROM PREOPTIC AREA SYNAPTOSOMES. A. Fleischmann, A.M. Etgen and M.H. Makman. Depts. Psychiat., Biochem., Mol. Pharmacol. & Neurosci., Albert Einstein College of Medicine, Bronx, NY 10461.

Glutamate-induced release of newly-synthesized GABA was measured in preoptic area (POA) synaptoneurosomes from ovariectomized rats injected with estrogen alone (2 μg of estradiol benzoate, EB, 24 and 48 hr before sacrifice) or with EB plus progesterone (P, 500 μg, 3-4 hr before sacrifice). Glutamate had little effect on GABA release in synaptosomes from EB-treated animals. However, glutamate induced a robust, concentration-dependent release of GABA when POA synaptosomes were prepared from animals injected with both EB plus P. P affected neither the rate of conversion of ^{3}H-glutamic acid to GABA nor glutamate-induced glutamate release. Aspartate and several excitatory amino acid analogs also released GABA from POA synaptosomes from EB+P-treated rats. Glutamate did not influence synaptosomal GABA uptake, nor did a glutamate uptake inhibitor prevent glutamate-stimulated GABA release. Glutamate-dependent GABA release was calcium-independent and was not blocked by excitatory amino acid receptor antagonists. Thus P may promote a novel, calcium-independent release of GABA by excitatory amino acids in a brain region of importance for the regulation of female reproductive physiology and behavior. Supported by MH41414 and by a fellowship from the Camp David Institute (A.F.).

223.2

EFFECTS OF ADRENAL STEROIDS ON MONOAMINE-INDUCED CELLULAR RESPONSES IN RAT HIPPOCAMPUS. M. Joëls* and E.R. de Kloet. Rudolf Magnus Institute, Utrecht, The Netherlands.

Adrenal steroid hormones can enter the brain and bind to two types of intracellular receptors: The mineralocorticoid receptor (MR) and the glucocorticoid receptor (GR). Pyramidal cells in the rat CA1 hippocampal area contain both MR and GR. In previous electrophysiological studies we have shown that the steroids (perfused for 20 min.) do not change passive membrane properties of CA1 pyramidal cells, but do affect the accommodation and slow afterhyperpolarization (acc/AHP) associated with a short depolarizing pulse. Thus, activation of the MR decreases the acc/AHP with a delay of at least 15 min. In contrast, occupation of the GR increases the acc/AHP. While the GR-effect is slower in onset, it gradually overrides the MR-effect. The acc/AHP is under control of many transmitters in the hippocampus; e.g. norepinephrine (NE) decreases the acc/AHP, probably through the cAMP-linked β-receptor, while serotonin (5HT) decreases the AHP through a pharmacologically undefined receptor not linked to cAMP. In the present study we observed that the NE-evoked decrease in acc/AHP is (in comparison to slices from ADX rats) diminished both in slices from sham-operated rats and in slices treated with corticosterone (1 μM) or the glucocorticoid RU 28362. In contrast, 5HT-induced reductions of the acc/AHP observed in slices from ADX rats were not changed in sham-controls or in slices treated with GR-agonists, indicating that the interaction between NE and the steroids is not at the level of the small Ca-dependent K-channels. 5HT-induced hyperpolarizations which are presumably mediated by the 5HT1a-receptor, were markedly reduced by MR-agonists. The results suggest that MR- and GR-activation in hippocampal cells affects both intrinsic properties and cellular responses to transmitters, such that MR-agonists increase and glucocorticoids decrease cellular excitability.

223.3

CRF AND ALPHA-ADRENERGIC AGONISTS MODULATE EXCITATORY AMINO ACID RESPONSES IN CULTURED CEREBELLAR PURKINJE NEURONS. Edward A. Fox, Nadine M. Di Julio*, and Donna L. Gruol. Res. Inst. of the Scripps Clinic, La Jolla, CA, 92037.

Inputs to cerebellar Purkinje neurons (PNs) include adrenergic fibers arising from the locus coeruleus and climbing fibers originating from the inferior olive. Corticotropin-releasing factor (CRF) and glutamate are colocalized in the latter. Potential interactions between CRF or monoamines and glutamate at these synapses were investigated using extracellular recordings of cultured cerebellar PNs.

Quisqualic acid (Quis), a glutamate receptor agonist, evoked a complex response in PNs which typically consisted of an initial abrupt increase in firing rate, followed by a smaller maintained increase, then a decrease of firing below baseline rate and finally a gradual recovery to baseline.

CRF had either no effect on PN spontaneous activity or decreased it. However, when CRF was applied 3-5sec prior to Quis, components of the PN response to Quis were often altered in a dose-dependent manner. Additionally, such CRF exposures prevented trains of doublet or triplet bursts of action potentials which characterized the Quis response of some PNs. The respective a1- and a2-adrenergic agonists phenylepherine and clonidine similarly altered components of the PN response to Quis including the bursting.

The mechanisms of these modulatory actions are being investigated with intracellular recordings and calcium imaging. Supported by NS21777 and AA07456.

223.4

EXCITATORY AMINO ACID ANTAGONISTS BLOCK AMPHETAMINE-INDUCED ORAL STEREOTYPIES FOLLOWING MICROINJECTION INTO THE VENTROLATERAL STRIATUM. J.M. Delfs and A.E. Kelley. Dept. of Psychology, Harvard University, Cambridge, MA 02138.

Microinjection of amphetamine (AMPH) into the ventrolateral striatum (VLS) elicits intense oral stereotypy (licking,biting,self-gnawing) in rats. The striatum receives glutamatergic projections from cortical regions which may interact with dopaminergic nerve terminals. The present experiments examined the role of excitatory amino acid (EAA) receptors in AMPH-induced oral stereotypies. EAA receptors are classified as NMDA, kainate or quisqualate (QUIS) subtypes. All EAA antagonists were administered as intra-VLS pretreatments immediately prior to AMPH microinjection (20 ug) into the VLS. Kynurenic acid (0, 0.05, 0.5, 5.0 ug), an NMDA antagonist, markedly reduced oral stereotypy induced by amphetamine. In contrast, KYN failed to block mouth movements induced by cholinergic stimulation of this region. The NMDA antagonist, AP-5 (0, 0.3, 1.0, 3.0 ug) completely blocked stereotypy at all doses. DNQX (0, 0.05, 0.25, 0.5 ug), a QUIS antagonist, significantly reduced stereotypy while GDEE (0, 10, 20, 30 ug), a short-acting QUIS antagonist, failed to block stereotypy at any dose. The time course of GDEE may account for its ineffectiveness in blocking stereotypy. These results suggest that glutamatergic inputs may modulate the expression of amphetamine-induced oral stereotypies.

223.5

CCK_B RECEPTOR ACTIVATION CAUSES INCREASED STRIATAL ASPARTATE RELEASE IN VIVO. S Barnes*, F Marshall*, J C Hunter, G N Woodruff and J Hughes Parke-Davis Research Unit, Cambridge, England.

In vitro studies have demonstrated that CCK_B receptor stimulation causes an increase in aspartate, glycine and GABA release. In this study, the technique of in vivo microdialysis was used to determine whether this action of CCK also occurred in the whole animal. Male Wistar rats of 200-280g were maintained under chloral hydrate anaesthesia and microdialysis probes were stereotaxically implanted into the striatum. Basal amino acid release was stimulated by high potassium aCSF and reduced by TTX and ω-conotoxin.

10^{-5} M CCK-8S, when administered down the microdialysis probe, caused a significant increase in aspartate release (333% ± 23) and this could be blocked by prior i.p. injection of the CCK_B selective antagonist L-365,260 (1mg/kg). ^{B}L-364,718, at doses selective for the CCK_A receptor, had no effect on CCK-8S evoked aspartate release.

These data suggest that CCK_B receptor activation in the striatum of the rat results in increased aspartate release.

223.6

DOPAMINERGIC (D2) MODULATION OF STRIATAL GLUTAMATE RELEASE: PHARMACOLOGICAL EVALUATION IN-VIVO. S.J. DAVY and B.K. YAMAMOTO. DEPT. OF PHARMACOLOGY, N.E. OH. Univ. Col. of Med., Rootstown, OH, 44272 and Dept. of Psychiatry, Case Western Reserve Univ., Cleveland, OH., 44106.

Corticostriatal glutamate release appears to be under presynaptic control via D2-type dopamine receptors. The mechanism of this interaction was studied using in-vivo microdialysis to measure potassium-induced glutamate release in awake, behaving rats. Male rats (350-450 g) were chronically implanted with guide cannulae. Forty-eight hours later a dialysis probe was lowered into the caudate. Dialysates were collected every 20 min. and analyzed for glutamate and other amino acids by HPLC with electrochemical detection. Local perfusions of high (80 mM) potassium buffer containing the D2 agonist LY171555 were found to block potassium-induced glutamate release in a dose-dependent manner. Perfusions with high potassium plus the D2 antagonist (-)sulpuride significantly ($p<.05$) increased the potassium-induced release of glutamate. These studies support the theory that striatal glutamate release is modulated by the D2 receptor.

223.7

N-METHYL-D-ASPARTATE EVOKES DOPAMINE RELEASE FROM RAT STRIATUM VIA DOPAMINE UPTAKE SYSTEM. *G. Lonart and M.J. Zigmond. Department of Behavioral Neuroscience and Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260*

Glutamate releases dopamine (DA) from striatal slices via an NMDA receptor. Since NMDA receptors are inhibited by Mg^{2+}, they usually are examined in the absence of this ion. Mg^{2+} can affect DA release at multiple sites. Thus, to investigate this phenomenon we have employed a physiological concentration of Mg^{2+} (1.20 mM), adding excess K^+ (10 mM) to reduce the presence of Mg^{2+} in the NMDA-modulated channel.

	DA overflow (% basal control)		
	300 uM NMDA	10 mM K^+	10 mM K^+ +300 uM NMDA
Control	2 ± 1%	66 ± 6%	293± 23%
Nomifensine		59 ± 8%	68 ± 8%

Whereas NMDA increased DA efflux only at very high concentrations (3-10 mM) under standard conditions, excess K^+ reduced to 300 uM or less the dose of NMDA needed to elicite this response. Nomifensine (10 uM), an inhibitor of high affinity DA uptake, had no effect on the response to K^+ alone but reduced the response to K^+ plus NMDA by 77%. These data suggest that glutamate may initiate the release of DA in striatum via an NMDA-mediated reversal of the high affinity DA transporter. (Supported in part by USPHS grants NS19608, MH43947, and MH00058.)

223.8

IN VIVO EVIDENCE THAT BASAL DOPAMINE EFFLUX IN STRIATUM IS NOT REGULATED BY ENDOGENOUS EXCITATORY AMINO ACIDS. *K.A. Keefe, M.J. Zigmond, & E.D. Abercrombie, Depts. of Behavioral Neuroscience and Psychiatry and the Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260.*

A number of *in vitro* and *in vivo* studies have shown that exogenous excitatory amino acids (EAAs) or their agonists can increase dopamine (DA) release in striatum. Using *in vivo* microdialysis, we examined the extent to which endogenous EAAs are able to regulate the resting level of DA in extracellular fluid (ECF) in striatum of unanesthetized rats. The EAA antagonists kynurenate (KYN), 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), and DL-2-amino-5-phosphonovalerate (APV) were administered into striatum directly via the dialysis probe. Depending on the concentration applied, these compounds produced either no change or an increase in the DA level in ECF.

Antagonist (mM)	Basal DA (pg/20ul)	Drug DA (pg/20ul)
CNQX (0.1; n=3)	12.4 ± 1.8	13.3 ± 1.7
(1; n=3)	38.5 ± 9.9	87.4 ± 28.1
APV (0.1; n=3)	24.7 ± 5.4	27.8 ± 6.1
(0.75; n=3)	32.4 ± 0.8	46.9 ± 8.8
KYN (1; n=6)	17.3 ± 3.4	20.7 ± 4.3
(10; n=4)	23.7 ± 2.3	50.8 ± 12.9

To determine whether these antagonists completely and specifically blocked EAA receptors, the EAA agonists kainate (KA; 0.1 mM) and N-methyl-D-aspartate (NMDA; 1 mM) were administered into striatum with or without co-infusion of the antagonists. KA and NMDA elevated DA in dialysate by 424±78% (n=4) and 249±122% (n=3), respectively. The kainate-induced increase was blocked by co-infusion of CNQX (0.1 & 1 mM; n=3&4) and the NMDA-induced increase was blocked by coinfusion of APV (0.75 mM; n=3) but not CNQX (1 mM; n=2). Thus, although the application of exogenous EAAs or their agonists can increase DA release, the present findings using EAA antagonists suggest that endogenous EAAs do not exert a tonic excitatory effect on basal DA release in striatum (Supported by grants NS 19608 and MH 18273).

223.9

GLYCINE STIMULATES GABA EFFLUX FROM RAT STRIATAL SLICES *S. Bernath, E.S. Vizi and M.J. Zigmond, Dept. of Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15260; Institute of Experimental Medicine 1083 Budapest, Hungary*

We have investigated the effects of glutamatergic and glycinergic drugs on spontaneous and electrically evoked release of GABA from striatum. Tissue slices (350 um) prepared from adult rat striatum were incubated in the presence of [^{3}H]GABA, then superfused (250 ul/min) with Krebs bicarbonate buffer and stimulated twice by electrical field depolarization (20 mA, 10 Hz, 9 sec). Drugs were administered 20 min before the second stimulation and total tritium efflux was used as a measure of GABA release. Resting GABA efflux was elevated by kainic acid (100 uM, +38%); quisqualic acid (10 uM) also increased GABA efflux but this effect rapidly decayed; NMDA (100 uM) had no impact. None of these drugs had any significant impact on electrically evoked GABA overflow. The effect of kainate and quisqualate on resting GABA efflux was not antagonized by CNQX (10 mM). Under our standard conditions glycine (1 mM) had a variable impact on GABA efflux; however, at a higher perfusion rate (1 ml/min) glycine elevated both spontaneous (+35%) and electrically evoked (+138%) GABA efflux. Although neither 7-chlorokynurenic acid (10 uM) nor MK-801 (1 uM) modified the impact of glycine on resting GABA efflux, both of these drugs antagonized the effect of glycine on evoked GABA overflow. These results are consistent with the hypothesis that depolarization-evoked GABA release is modulated by glycine through a mechanism that may involve the NMDA receptor complex. (Supported in part by USPHS grants NS19608, MH43946, and MH45146.)

223.10

NOREPINEPHRINE POTENTIATES GABA-INDUCED CURRENTS IN CEREBELLAR PURKINJE CELLS. I.E. Cheun and H.H. Yeh Dept. Neurobiology and Anatomy, Univ. Rochester Med. Ctr., Rochester, NY 14642.

Extracellular single unit recording studies in the rat cerebellum have shown that norepinephrine (NE) facilitates GABA-induced suppression of Purkinje cell spike activity. Although various lines of indirect evidence suggest that NE may exert its effects by directly influencing Purkinje cell responsiveness to GABA, unequivocal demonstration of such a postsynaptic site of NE action in a preparation which is amenable to further studies of the underlying membrane and intracellular mechanisms has been lacking. Here, using whole-cell patch-clamp recording procedures, we examined under voltage-clamp the effect of NE on GABA-activated currents (I_{GABA}) in Purkinje cells acutely dissociated from the postnatal rat cerebellum.

Immunoreactivity to a Purkinje cell marker, the PEP-19 monoclonal antibody, aided morphological identification following acute dissociation. I_{GABA} was found to be mediated exclusively by the $GABA_A$ receptor, being associated with an increase in chloride conductance, mimicked by muscimol and potentiated by diazepam. Baclofen (100 uM) produced no conductance change. In 26 Purkinje cells, the amplitude of I_{GABA} elicited in response to brief (150-250 ms) pressure pulses of GABA (10-20 uM) was compared before, during and after a period of continuous NE (≤500 uM) application. In all but two cases (24 of 26), exposure to NE resulted in a reversible, fractional increase in I_{GABA}. Importantly, at the concentrations tested, NE by itself had no detectable effects on membrane conductance. Norepinephrine potentiated I_{GABA} at all holding potentials tested but did not alter GABA reversal potential. Consistent with an interaction with $GABA_A$ receptor-mediated activity, both muscimol-induced and GABA-activated chloride currents were potentiated by NE. Thus, our results establish definitively the presence of a postsynaptic mechanism by which NE may modulate $GABA_A$ receptor function in cerebellar Purkinje cells.

Supported by PHS grants NS24830 and NS01340.

223.11

CYCLIC AMP AS AN INTRACELLULAR MODULATOR OF $GABA_A$ RECEPTOR FUNCTION IN CEREBELLAR PURKINJE CELLS. H.H. Yeh and J.E. Cheun, Dept. Neurobiology & Anatomy, Univ. Rochester Med. Ctr., 601 Elmwood Ave., Rochester, NY 14642.

Based on results of the preceding study that GABA-induced currents are potentiated by norepinephrine (NE) and on reports that ligand-gated ion channels can be modified by cyclic nucleotides, we are testing the hypothesis that NE alters $GABA_A$ receptor function in cerebellar Purkinje cells via the beta-receptor/cyclic AMP-dependent protein kinase pathway.

Purkinje cells were identified morphologically following acute papain dissociation from the postnatal rat cerebellum. Conventional whole-cell patch-clamp techniques were used to record under voltage-clamp their current responses to brief (50 - 200 ms) pressure pulses of GABA (5 - 20 uM). In separate series of experiments, isoproterenol (500 uM) or forskolin (200 uM) was used to stimulate directly the beta-receptor or adenylate cyclase, respectively. In all cells tested, continuous application of either agents between GABA test pulses resulted in a fractional increase in GABA-induced current similar to that observed with NE. In addition, exposure of Purkinje cells to the membrane permeable analog, 8-bromo-cyclic AMP ($\lesssim$500 uM), increased the amplitude of the GABA-induced current. This effect was reversible and was observed in 6 of 7 cases. Ongoing experiments are focusing on examining other steps along the beta-receptor/cyclic AMP-dependent protein kinase cascade to provide further insight into determining whether a phosphorylation of the $GABA_A$ receptor by the cyclic AMP-dependent kinase may be involved. Thus far, our results clearly indicate that an elevated intracellular level of cyclic AMP can lead to a modification of $GABA_A$ receptor function.

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223.12

GABAERGIC INNERVATION IN CEREBRAL ARTERIES: A MORPHOLOGICAL AND PHARMACOLOGICAL STUDY. T. OKUNO[1], T. J-F. LEE[1], H. IMAI[*,2], T. ITAKURA[*,2], M. UENO[2], Y. NAKA[2], K. NAKAI[2], S. HAYASHI[*,2] and N. KOMAI[*,2]. Department of Pharmacology, Southern Illinois University School of Medicine, Springfield, IL 62794, USA.[1], Department of Neurological Surgery, Wakayama Medical College, Wakayama 640, JAPAN[2].

Our recent studies have demonstrated that cerebral blood vessels receive dense GABAergic innervation (Imai et al., 1990). It has been suggested that neuronal GABA may act as a modulator altering the release of transmitters from the neighboring nerve terminals. The morphological relationship between GABAergic and other autonomic innervations and the effect of GABA on transmural nerve stimulation (TNS)- and drug-induced vasoreactions were examined in cerebral artery. GAD-immunoreactive (I) nerve fibers were found to overlap with most choline acetyltransferase (ChAT)-I, 62% of calcitonin gene-related peptide (CGRP)-I and 42% of vasoactive intestinal polypeptide (VIP)-I fibers in the cat cerebral artery. Exogenously applied GABA (10^{-5}-10^{-3}M) inhibited TNS-induced contraction of rabbit basilar and ear arteries, and TNS-induced relaxation of pig basilar artery. GABA also inhibited NE- and 5HT-induced contractions, and isoproterenol-induced relaxation. These results indicate that in cerebral arteries GABAergic innervation has close morphological relationship with other autonomic innervations, and further suggest that the neuronally released GABA may act postsynaptically to modulate vasomotor response induced by other transmitters. (Supported by NIH HL27763, AHA/IHA and Southern Illinois University School of Medicine)

223.13

POTENTIATION OF THE BEHAVIORAL EFFECTS OF BUSPIRONE BY THE α_1-ADRENERGIC ANTAGONIST PRAZOSIN. S. Gleeson and J.E. Barrett, Dept. of Psychiatry, Uniformed Services University of the Health Sciences, 4301 Jones Bridge Rd., Bethesda, MD 20814-4799.

The novel anxiolytic buspirone generally is thought to exert its therapeutic effects by decreasing serotonin neurotransmission. However, buspirone has substantial effects on noradrenergic neurotransmission that probably involve α-adrenoceptors.

Pigeons were trained to keypeck under a multiple schedule of reinforcement in which responding during one component also was suppressed by punishment. Unpunished responding was not significantly affected by buspirone except at higher doses which decreased responding. Low rates of responding suppressed by punishment were increased substantially by doses of 0.1 to 5.6 mg/kg buspirone. Prazosin (1 mg/kg) had no effect on unpunished responding, and only slightly increased punished responding. However, a combination of prazosin (0.1 to 3 mg/kg) and buspirone (0.1 to 5.6 mg/kg) produced increases in punished responding that were greater than the increases produced by buspirone alone.

In a second experiment, pigeons were trained to discriminate 1 mg/kg buspirone from saline using standard drug discrimination procedures. Generalization gradients obtained for buspirone showed little or no drug-key responding when low doses of buspirone were administered, although higher doses produced full generalization. Prazosin (1 to 10 mg/kg) produced predominantly saline-key responding in most birds, but these doses in combination with 0.1 or 0.3 mg/kg buspirone resulted in a dose-dependent increase in the proportion of drug-key responses, and in some cases produced complete generalization.

Although the mechanism by which buspirone's effects are potentiated by prazosin remains obscure, the present results suggest that at least some of the effects of buspirone may involve the noradrenergic as well as the serotonergic neurotransmitter system. Supported by DA 02873.

223.14

THE EFFECTS OF $5HT_2$ RECEPTOR ANTAGONISM ON DOPAMINE AND 5HT LEVELS IN RAT BRAIN TISSUE AND DIALYSATE. L. L. DEVAUD and E. B. HOLLINGSWORTH, Division of Pharmacology, Burroughs Wellcome Co., Research Triangle Park, NC 27709.

Ritanserin, a specific serotonin$_2$ ($5HT_2$) receptor antagonist, was employed to investigate interactions between dopamine (DA) and 5HT neurotransmission in brain areas relevant to psychosis. After a single dose of 0.63 mg/kg ritanserin, ip, rats were sacrificed at either 2 hours (acute) or 4 days. Acute ritanserin elicited a 13.6 $\pm$ 6.5% increase in DA levels and a 7.1 $\pm$ 3.0% increase in 5HT levels in the caudate. An increase of 8.64 $\pm$ 3.7% in 5HT levels was observed in the cortex. In the nucleus accumbens (n.a.), both DA and 5HT levels were decreased by 14.6 $\pm$ 6.5 and 11.5 $\pm$ 5.3%, respectively, with increased turnover. Microdialysis studies of the n.a. showed increases in both DA and 5HT levels by two hours after ritanserin treatment, with no changes in metabolite levels. At four days after the single dose of ritanserin, DA levels were now decreased 10.4 $\pm$ 2.4% and 5HT levels decreased 22.0 $\pm$ 4.7% in the caudate. A 12.9 $\pm$ 2.1% decrease in 5HT levels was seen in the cortex. The acute decreases in both DA and 5HT levels noted in the n.a. persisted (14.2 + 4.0 and 23.9 $\pm$ 8.2%, respectively) to the four day time point. These results suggest that $5HT_2$ receptor antagonism modulates both DA and 5HT transmission in a complex, site-selective fashion which varies over time.

223.15

STIMULATION OF DORSAL RAPHE NUCLEUS NEURONS ENHANCES DOPAMINE AND SEROTONIN RELEASE IN THE NUCLEUS ACCUMBENS. K. Yoshimoto, W.J. McBride, L. Lumeng*, and T.-K. Li*. Psychiatric Res. & Regenstrief Insts., Indiana Univ. Sch. Med. & VAMC, Indianapolis, IN 46202.

The effects of microinfusion of glutamate (GLU), muscimol (MUS) and serotonin agonists into the dorsal raphe nucleus (DRN) on dopamine (DA) and serotonin (5-HT) release in the ipsilateral nucleus accumbens (ACC) were studied in freely moving adult, male Wistar rats using brain microdialysis. In all experiments, a 0.5 ul volume was injected over a 5-min period. Within 15 to 60 min after the microinfusion of 0.75 ug GLU, the amounts of DA and 5-HT in the ACC dialysates increased over the baseline values 150 to 200% (N=3, P<0.05). Microinfusion of 0.5 ug MUS did not alter the amounts of either DA or 5-HT in the dialysates although there was a tendency for a reduction of 5-HT levels 45-60 min after infusion. Microinfusion of 2.0 ug 5-HT or 1.0 ug 8-OH DPAT ($5\text{-}HT_{1A}$ agonist) into the DRN decreased the levels of 5-HT 20-50% in the dialysates. There was also a tendency for lower levels of DA in the dialysates following administration of 8-OH DPAT into the DRN. These findings suggest that (a) activation of GLU receptors can stimulate DRN 5-HT neurons; (b) activation of DRN $5\text{-}HT_{1A}$ cell body autoreceptors decreases terminal 5-HT release; and (c) DRN 5-HT projections stimulate VTA DA neurons. (AA 03243, AA 07611)

223.16

PRESYNAPTIC MODULATION OF DOPAMINE EFFLUX BY 5-HT_3 RECEPTOR-MEDIATED MECHANISMS IN RAT NUCLEUS ACCUMBENS: IN VIVO BRAIN MICRODIALYSIS STUDIES. J. Chen, H.M. van Praag*, and E.L. Gardner. Departments of Neuroscience and Psychiatry, Albert Einstein College of Medicine, New York, NY 10461.

CNS serotonergic (5HT) mechanisms are known to innervate and modulate the mesolimbic dopamine (DA) system (de Belleroche & Bradford, *J. Neurochem.* 35:1227, 1980), suggesting novel pharmacological approaches for psychosis and drug addiction (Tricklebank, *Trends Pharmacol.Sci.* 10:127, 1989). To further study these 5HT-DA interactions, we examined DA efflux in the rat nucleus accumbens by in vivo microdialysis and the effect on such efflux of the selective $5HT_3$ agonist 1-phenylbiguanide. At concentrations of 0.01-1.0 mM in the perfusate, 1-phenylbiguanide caused a robust dose-dependent enhancement of presynaptic DA efflux in nucleus accumbens of awake, freely moving rats. This agonist effect by 1-phenylbiguanide was antagonized by co-perfusion of the selective $5HT_3$ antagonists GR38032F or zacopride at concentrations of 1 mM in the perfusate. Similar effects were observed in 5HT-denervated rats. These findings suggest 1) that there is a potent modulation of presynaptic DA efflux in the mesolimbic DA system mediated via $5HT_3$ receptors; and 2) the modulating $5HT_3$ receptors appear not to be located on 5HT afferents to the accumbens but may be located presynaptically on DA terminals of the mesolimbic DA pathway.

223.17

SUBREGIONAL EFFECTS OF COCAINE IN THE NUCLEUS ACCUMBENS. Y.L Hurd, H.C. Fibiger[1] and C. Gerfen. Clinical Neuroscience Branch and Lab. of Cell Biology, NIMH, Bethesda, MD 20892 and Psychiatry Dept., Univ. British Columbia, Vancouver, Canada[1].

The mesolimbic dopamine (DA) system is considered the site of action for the reinforcing properties of drugs of abuse. In the present study subregional alteration of DA transmission within the nucleus accumbens (NAS) was assessed following exposure to cocaine. Cocaine, a potent DA uptake inhibitor, potentiated DA overflow to a greater extent in rostral areas of the NAS, as measured by in vivo microdialysis. This subregional effect of cocaine might be linked to the fact that the density of DA transport sites (labelled by mazindol ligand-binding autoradiography) was higher in rostral NAS. Furthermore, DA transport sites were virtually absent from the most dorsomedial area of the caudal "shell" region of the NAS. Other transmitter systems under the regulation of DA were also monitored by in situ hybridization histochemistry. The mRNA expression for e.g., dynorphin, enkephalin and substance P presented a heterogeneous distribution within the NAS and was affected by exposure to cocaine.

223.18

INTERACTION OF AZIDO-Δ^8-THC WITH THE CANNABINOID RECEPTOR IN BRAIN. A.C. Howlett, D.M. Evans, D. Houston, G. H. Wilken*, A. Charalambous* and A. Makriyannis*. Dept. of Pharmacology, St. Louis University, St. Louis MO 63104 and School of Pharmacy and Institute of Materials Science, University of Connecticut, Storrs, CT 06268

The cannabinoid receptor in rat brain has been described using the agonist ligand [^{3}H]CP-55940 and has been characterized for its ability to inhibit cyclic AMP accumulation through a Gi mechanism (Bidaut-Russell et al, J. Neurochem. '90). Ligands for several classes of neuroreceptors fail to displace binding of [^{3}H]CP-55940, including opioid, sigma, benzodiazepine, GABA, serotoninergic, nicotinic, muscarinic, catecholaminergic and certain peptide ligands.

Further characterization of the cannabinoid receptor has used azido derivatives. Azido-Δ^8-THC and iodo-azido-Δ^8-THC displace [^{3}H]CP-55940 binding with IC_{50} values of 10 and 100 nM, respectively. Following equilibration and irradiation, azido-Δ^8-THC prevents [^{3}H]CP-55940 binding to the cannabinoid receptor. Thus, these compounds can be used as covalent modifiers of the cannabinoid receptor.

Supported in part by R01-DA03690, R01-DA06312 and R01-DA03801.

CATECHOLAMINES II

224.1

BINDING OF [^{3}H]-NISOXETINE TO RAT BRAIN: A NEW RADIOLIGAND FOR NOREPINEPHRINE UPTAKE SITES. **S.M. Tejani-Butt, M. Bauer*, D.J. Brunswick and A. Frazer. Dept. Psychiatry, Univ. of Pa Sch. of Med. & Dept. Vet. Affairs Med. Ctr., Phila., PA 19104.**

The uptake site for norepinephrine (NE) in brain has been studied much less than the uptake site for 5-HT, probably due to the absence of an adequate radioligand for this site. This report describes the binding properties of [^{3}H]-nisoxetine, a selective inhibitor of the uptake of NE. In rat cerebral cortical homogenates, the binding of [^{3}H]-nisoxetine is saturable and a single class of binding sites (K_d=1nM) was obtained even at radioligand concentrations up to 20 times the Kd value. At the K_d concentration, about 70% of total [^{3}H[-nisoxetine binding is specific (as defined by 1μM mazindol). The specific binding of [^{3}H[-nisoxetine was sodium dependent. The potencies of drugs to inhibit the uptake of NE correlate highly with their potencies to inhibit [^{3}H]-nisoxetine binding. High concentrations of [^{3}H[-nisoxetine binding sites were measured in the hypothalamus and cerebral cortex with lower concentrations in the corpus striatum. Studies using [^{3}H]-nisoxetine for mapping of sites associated with NE uptake by quantitative autoradiography indicate that the pattern of binding of [^{3}H]-nisoxetine is consistent with the pattern of noradrenergic innervation. Destruction of central NE neurons by i.p. administration of DSP-4 resulted in large decreases in the binding of [^{3}H]-nisoxetine in areas of brain receiving innervation from the locus coeruleus (LC), with little change in binding in the LC itself. [^{3}H]-nisoxetine should be a useful tool to study noradrenergic innervation in discrete anatomical regions in post-mortem samples of brain from patients with neuropsychiatric disorders. **(Supported by research funds from the Department of Veterans Affairs and USPHS grant DA 05137).**

224.2

ANATOMICAL DISTRIBUTION AND PHARMACOLOGY OF THE DOPAMINE UPTAKE COMPLEX IN RODENT AND HUMAN BRAIN. E.K. Richfield and K. Farrell. Neurology Dept, University of Rochester, Rochester, NY 14620

The dopamine uptake complex (DAUC) is responsible for the reuptake of dopamine (DA) after release by neurons. The DAUC is implicated in neurolologic and psychiatric disorders. A quantitative autoradiographic assay using [^{3}H]-GBR 12935 was developed to study the pharmacology and anatomy of the DAUC in rat and human brain.

The pharmacology of the DAUC is similar in rat and human brain. Binding to this site is saturable and reversible. All competitors tested at this complex displace DAUC specific binding with a single affinity except for cocaine and the cocaine congener WIN 35,428 in both rodent and human brain. These two compounds display biphasic displacements and are best modeled as two sites. The rank order of all competitors is in agreement with drugs active at this complex.

The density of the DAUC in rodent brain is very high in the dorsal and ventral striatum, olfactory tubercle, substantia nigra and ventral tegmental area. Areas with moderate density include the basolateral n. of the amygdala, subthalamic nucleus, and hippocampus. Low, but specific binding is also seen in a wide variety of regions including the cerebral cortex, thalamus, brainstem, and spinal cord.

The regional density in man is less than in rat. The density of the DAUC is heterogeneous in caudate n. similar to that of acetylcholinesterase staining. Other regions are less heterogeneous.

These findings support the existence of two sites for cocaine on the DAUC in rat and man. These findings support the widespread distribution of the DA neuron system in rodent brain. Human basal ganglia displays a heterogeneous pattern of the DAUC which may have clinical implications in several disease states including Parkinson's disease and schizophrenia.

224.3

LIGHT AND ELECTRON MICROSCOPIC CHARACTERIZATION OF DOPAMINERGIC PROCESSES IN HUMAN NEOCORTEX. J.F. Smiley, K. Szigeti, S.M. Williams, and P.S. Goldman-Rakic. Sec. of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

While dopaminergic axons have been demonstrated in the neocortex of non-human primates, they have not been directly visualized in the human cortex. In the present study, human neocortex was removed during tumor excision, or during neurosurgical treatment of intractable epilepsy. Tissue used was resected in order to access the pathological locus, and did not appear grossly abnormal. In 5 cases, cortex was from the anterior temporal lobe, and in 1 case from the frontal pole. Tissue was immediately fixed, and later processed for immunocytochemistry with a monoclonal antibody directed against dopamine, or with a polyclonal antibody against tyrosine hydroxylase (TH).

Light microscopic analysis revealed that in all areas examined, dopamine-like immunoreactive (DA) fibers were more abundant in the superficial layers (lower I,II) and in the infragranular layers (V, VI), and less dense in the intermediate cortical layers (III, IV). In all layers, fibers were seen to form numerous varicosities, and to vary in size from thick fibers with long trajectories, to very fine fibers usually having shorter trajectories. Fibers usually were relatively straight, sparsely branched, and were oriented in various planes within the cortex. However, in layer I there were abundant fibers running parallel to the pial surface. TH showed a similar distribution and morphology of fibers, except it also labeled a population of cells in layers V and VI, which were not seen with DA.

This distribution and morphology of DA fibers is similar to that previously described in the monkey neocortex. Electron microscopic analysis also showed similarities to monkey. In human, DA varicosities were vesicle filled, and formed symmetrical synapses. Spines, dendrites, and axons were seen to receive synaptic input from DA processes. Similar spines and dendrites receiving DA input were demonstrated to belong to pyramidal cells in monkey (Goldman-Rakic et al., PNAS, 86:9105, 1989). Supported by MH44866.

224.4

PATTERN AND EXTENT OF DOPAMINE CELL DEATH IN RATS FOLLOWING INTRAVENTRICULAR 6-HYDROXYDOPAMINE, **J.R. Hollerman & A.A. Grace, Depts of Behavioral Neuroscience & Psychiatry, Univ of Pittsburgh, Pittsburgh, PA, 15260.**

Specific destruction of forebrain dopamine (DA) terminals in rats using intracerebroventricular (ICV) administration of 6-hydroxydopamine (6-OHDA) also causes retrograde degeneration of DA cell bodies in the midbrain. Lesions produced in this manner have been used as an animal model of Parkinson's disease. We investigated the relationship between striatal DA depletion (indicative of DA terminal destruction) and DA cell death in the midbrain in rats treated with 75, 100 or 200μg of 6-OHDA ICV, using HPLC to measure striatal DA and SPG-induced histofluorescence to count DA cell bodies. With the two lower doses, we found a sparing of DA cell bodies in the midbrain relative to striatal DA depletion, especially in anterior regions of the midbrain. With the 200μg dose, cell death was equivalent to striatal DA depletions in central and posterior regions, while anterior regions again were spared. We also attempted to determine the influence of DA cell firing rate on susceptibility to 6-OHDA toxicity. Because ICV 6-OHDA exerts its toxic effect upon entering DA terminals via high affinity uptake, changes in 6-OHDA toxicity with alterations in firing rate should reflect any relationship between cell activity and this uptake process. Neither pretreatment with GBL nor with HAL (to decrease or increase DA cell activity, respectively) prior to administration of 150μg 6-OHDA ICV produced appreciable effects on either the extent of striatal DA depletion or DA cell death produced by this dose of 6-OHDA. Overall, the pattern and extent of cell death are consistent with ICV 6-OHDA having a preferential effect on nigrostriatal DA neurons, although increasing doses have greater effects on other DA projections. Our results with GBL and HAL do not support the idea that high affinity uptake and cell activity are interrelated. **(Supported by MH09660, MH62217 & MS19608.)**

224.5

A COMPARATIVE ANALYSIS OF THE DOPAMINERGIC SYSTEM IN THE BRAIN OF AMPHIBIANS. A.Gonzalez*, W.J.A.J.Smeets[1] and G.E.Meredith[1]. Dept.Cell.Biol., Univ.Complutense, Madrid, Spain and [1]Dept.Anat., Vrije Univ. Amsterdam.

The dopaminergic system of an anuran, *Rana ridibunda*, and an urodele, *Pleurodeles waltlii* was investigated immunohistochemically with antibodies against dopamine (DA) and tyrosine hydroxylase (TH). In both species, DA-immunoreactive (DAi) cell bodies are located in the olfactory bulb, the preoptic area, several hypothalamic nuclei, including the nucleus of the periventricular organ (NPv), the pretectal area, the midbrain tegmentum, and around the solitary tract. CSF-contacting cells are present in the caudal rhombencephalon and spinal cord. The TH antiserum revealed additional cells in the olfactory bulb and the isthmal region, but failed to stain the CSF-contacting cells in NPv.

DAi fibers are widespread throughout the brain in both species. Major differences were noted in the forebrain. In *R. ridibunda*, the densest DA innervation occurs in the nucleus accumbens, in contrast to *P. waltlii* where most fibers are found in the striatum. Furthermore, DAi fibers were observed in the pallial areas of the urodele, but not in the corresponding regions of the frog brain. When compared with other vertebrates, it appears that the dopaminergic system of amphibians shares many features not only with other anamniotes, but also with amniotes.

224.6

FURTHER OBSERVATIONS ON THE DOPAMINERGIC CELLS IN THE HYPOTHALAMIC PERIVENTRICULAR ORGAN OF NONMAMMALIAN VERTEBRATES. W.J.A.J.Smeets and A.Gonzalez*. Dept.Anat., Vrije Univ.Amsterdam, The Netherlands.

Recently, it was shown that the hypothalamic periventricular organ (OPH) of reptiles contains liquor (CSF) contacting cells that are immunoreactive to dopamine (DA) antibodies, but do not stain with tyrosine hydoxylase (TH) antisera (Smeets, W.J.A.J. and H.W.M.Steinbusch, J.Chem.Neuroanat. 3:25, 1990).

In the present study, DA- and TH- immunohisto-chemistry was applied to the brains of two amphibians, the anuran *Rana ridibunda* and the urodele *Pleurodeles waltlii*, and the chicken, *Gallus domesticus*. As in reptiles, DA-immunopositive/TH-immunonegative CSF-contacting cells were observed in OPH suggesting that this is a primitive brain character of nonmammalian vertebrates.

Furthermore, 8 lizards (*Gekko gecko*) received intra-peritoneal or intraventricular injections of a-methylpara-tyrosine, a DA synthesis inhibitor. Survival times ranged from 1.5-5 hours. Subsequent staining with DA antiserum revealed an almost unaffected staining in OPH, but a dramatic decrease in the other DA cell groups supporting the notion that the cells in OPH may accumulate DA. It is suggested that the CSF plays an important role in dopamine neurotransmission in nonmammalian vertebrates.

224.7

CONTINUOUS CEREBRAL MICROINJECTION TECHNIQUE AS A SUBSTITUTION OF NEURAL TRANSPLANT FOR NEUROSECRETORY MODULATION. K.Nishino and M.Kowada*,Dept. of Neurosurg.,Akita Univ.Sch.of Med.,Akita 010,Japan.

We studied whether certain molecules diffuse in the brain. Mini-osmotic pumps (model 2001,Alza, USA) were implanted in the intraperitoneal cavities of mongolian gerbils (BW 50-75gm,male). Then PE 60-tubes connected to the pump were stereotactically placed in the brain. First, each of the six animals were injected 50 l of 1% methylene blue(MB) into the ventricles(gr 1) or 1% MB continuously in the brain (gr 2) to determine to what degree the dye diffuses. Second, seven animals were continuously injected 40 l norepinephrine(NE) (gr 3). Two weeks later the four animals in gr 3 were decapitated, and the brains were divided into 2.5 mm thick hemicoronal slices. NE assay was done with HPLC and ED. 11 animals of the grs 2 and 3 were neurologically free except in two cases with the infection. MB diffuses more in gr 2 than in gr 1. The NE level was 5-30 times and twice higher on the injected and non-injected sides than the regular level of 0.27 ng/mg, respectively. Severe brain damage was not associated with the injection even at the ultrastructural level. The data show that a implanted DDS would be one of rational substitutions of neuronal transplant for neuroseeretory modulation.

224.8

ALPHA$_2$-ADRENOCEPTOR AUGMENTATION OF THE AFTER-HYPERPOLARIZATION OF FROG VENTRAL ROOT POTENTIALS INVOLVES A OUABAIN-SENSITIVE PUMP. S.B. Shope, A.M. Levinson*, A.M. Holohean, J.C. Hackman, and R.A. Davidoff. Neurophysiology Lab., VAMC and Dept. of Neurology, Univ. of Miami School of Medicine., Miami, FL 33101.

Spinal cords were dissected from adult *Rana pipiens*, hemisected, and placed in a sucrose gap recording chamber. The 10th dorsal root was tetanically stimulated (supramaximal 1.0 msec pulses, 25 Hz, 10 sec) and the reflex responses were recorded from the 9th ventral root. The ventral root response consisted of a sustained motoneuron depolarization followed by a long-duration (50 sec), large (4.4 mV) after-hyperpolarization (AHP). Norepinephrine (10 μM) caused an augmentation of both the amplitude (188%) and duration (50 %) of the AHP. This effect was mimicked by the α_2 agonist, clonidine (100 μM). Both the norepinephrine- and clonidine-induced AHP increases were blocked by the selective α_2 antagonist, yohimbine (10 μM), but not by the β antagonist, propranolol (100 μM). Addition of ouabain or dihydro-ouabain in a concentration (10 μM) sufficient to block sodium pumping in frog neurons, eliminated the motoneuron AHPs both in normal Ringer's solution or during the application of 10 μM clonidine.

Thus, the AHP produced in spinal motoneurons by tetanization of afferent fibers and its augmentation by activation of α_2 adrenoceptors are ouabain-sensitive. The data suggest an involvement of the Na^+/K^+ pump in the potential. (Supported by VAMC Funds and USPHS grants NS17577 & NS 07044).

224.9

PHARMACOLOGICAL CHARACTERIZATION OF NORADRENERGIC RESPONSES IN RELAY NEURONS FROM RAT LATERAL GENICULATE NUCLEUS. D.A. Coulter, J.R. Huguenard and D.A. Prince, Dept. Neurology, Stanford University Medical Center, Stanford, CA 94305.

The thalamus receives an adrenergic projection from locus coeruleus, and autoradiographic studies have demonstrated α_1-, α_2-, and β-adrenergic binding sites in thalamus. Using whole-cell voltage-clamp recording in 400 μm thalamic slices, we have characterized the response of relay neurons in rat dorsal lateral geniculate nucleus (dLGN) to norepinephrine (NE) and α-adrenergic agonists.

Drop application of NE (500-2000 μM) to the surface of the dLGN elicited an inward current and conductance decrease in all neurons. In addition, in 33% of these cells, an outward current with a conductance increase preceded the inward current, i.e. NE elicited an outward/inward current sequence. The NE-induced inward current could be blocked by the α_1 antagonist prazosin (1 μM bath applied, or 100 μM drop application to the prechamber), but not by the α_2 antagonist yohimbine (1 μM bath, or 100 μM drop) or the β antagonist propranolol (1 μM bath). A similar inward current accompanied by a conductance decrease could be elicited by the α_1 agonist 6-fluoronorepinephrine (1 mM drop), but not by the α_2 agonist clonidine (CL, 200 μM drop), which elicited an outward current accompanied by a conductance increase. In higher concentrations (500-1000 μM drop), CL evoked an outward/inward current sequence. The reversal potentials of both the inward and outward current elicited by NE and/or CL varied in a Nernstian manner with the external $[K^+]_o$. In addition, both the outward and inward currents elicited by CL or NE showed inward rectification at potentials more negative than -80 mV, and outward rectification more positive than -60 mV.

These data suggest that the response to NE in adult rat dLGN cells results from activation of at least two receptors. A portion of the response appears to be elicited by activation of α_2 receptors, turning on an inwardly rectifying potassium current. A second component is mediated by activation of α_1 receptors, which turn off a potassium current with similar rectification. This study demonstrates the feasibility of recording second messenger-mediated responses using whole-cell voltage-clamp in dialyzed neurons from slices of mammalian CNS. Supported by NIH Grants NS06477, NS12151, and NS07280.

224.10

NORADRENERGIC ENHANCEMENT OF GABA-INDUCED MEMBRANE CONDUCTANCE INCREASES IN PYRAMIDAL NEURONS OF RAT SOMATOSENSORY CORTEX. F.M. Sessler, M.L. Kirifides, W. Liu, C.S. Lin and B.D. Waterhouse. Dept. Physiol and Biophys. Hahnemann Univ., Phila., PA 19102

Previous in vivo studies have shown that microiontophoretic application of norepinephrine (NE) and isoproterenol (ISO) can enhance GABA-induced depressant responses of rat somatosensory cortical neurons. In the present investigation we have examined the transmembrane electrophysiological events which are associated with interactions between NE and GABA in layer IV/ V pyramidal neurons of rat somatosensory cortex. Intracellular recordings were made from electrophysiologically identified cells in a submerged tissue slice preparation before, during and after bath application of GABA, NE and ISO, alone or in combination. Superfusion of GABA (0.5 - 3mM) produced small decreases in resting membrane potential associated with a reduction (22%) in membrane input resistance. NE and ISO also produced small membrane depolarizations (1-4mV) but no concomitant changes in membrane conductance. Simultaneous application of NE with GABA potentiated amino acid-induced changes in input resistance in 4 cases and antagonized (n=4) or had no effect (n=3) on GABA associated membrane events in 7 other cases. However, when the alpha blocker, phentolamine, was added to the medium (10-20 uM), NE-induced enhancement of the GABA response was observed in 5 of 6 cases suggesting alpha receptor masking of this noradrenergic potentiating action. Consistent with this interpretation was the finding that the beta agonist, ISO, produced net increases in GABA-induced conductance changes in the majority (92%) of cases tested (n=13). The potentiating effect of NE and ISO was mimicked by the adenyl cyclase activator, forskolin (n=2), and a membrane permeant analog of cyclic AMP, 8-bromo-cyclic AMP (n=1); and could also be demonstrated when the GABA A agonist muscimol (1-5uM) was substituted for GABA. These findings suggest that previous demonstrations of NE potentiating effects on GABA inhibition are mediated by beta- receptor/cyclic AMP linked actions on mechanisms which regulate GABA A receptor-induced membrane conductance changes. (Supported by AFOSR 87-0138 and an award from the Klingenstein Foundation to BDW)

224.11

THE EFFECT OF ALPHA-METHYL-PARA-TYROSINE (AMPT), AN INHIBITOR OF TYROSINE HYDROXYLASE, ON SLEEP LATENCIES OF RESTED AND SLEEP DEPRIVED VOLUNTEERS. U. McCann, D. Penetar, D. Thorne, H. Sing, M. Thomas, A. Schelling, J.C. Gillin, and G. Belenky. Dept of Behavioral Biology, Walter Reed Army Institute of Research, Washington, DC 20307-5100

Pharmacological, anatomical, and electrophysiological evidence suggests that CNS catecholamines play an important role in maintaining wakefulness. The effect of catecholamine depletion on physiological sleepiness was tested in 40 healthy male volunteers, by preventing the synthesis of catecholamines with AMPT, an inhibitor of tyrosine hydroxylase. Subjects were randomized to one of four experimental conditions. Twenty subjects were sleep deprived for 36 hours (SD), and the remaining 20 subjects slept 7.5 hours nightly for the duration of the study (R). In both the SD and R conditions, half of the subjects received oral AMPT at a dose of 750 mg QID, for a total of 7 doses. Physiological sleepiness was assessed using multiple sleep latency tests (MSLTs) during the two days of drug administration and the day following the last drug dose. AMPT treatment was associated with a marked decrease in SLT times in both SD and R groups. Treatment with AMPT in the R condition produced an MSLT profile similar to treatment with sleep deprivation with and without AMPT. These findings are consistent with the hypothesis that catecholamines are important mediators of behavioral arousal and alertness. Further, they suggest that decreased catecholaminergic activity may be responsible for physiological sleepiness in normal humans.

224.12

COMPARATIVE ANALYSIS OF THE EFFECTS OF IONTOPHORETICALLY APPLIED DOPAMINE IN DIFFERENT REGIONS OF THE RAT BRAIN. M. Beauregard, A. Ferron and L. Descarries. Centre de recherche en sciences neurologiques (Dép. physiologie), Univ. Montréal, Montréal, Canada H3C 3J7.

The effects of microiontophoretically applied dopamine (DA) were examined in the anterior cingulate (ACg), prefrontal (PF) and parietal (Par) cortex, neostriatum (NS) and nucleus accumbens (Acb) of urethane-anesthetized rats treated or not with the DA uptake blockers GBR 12909 or Bupropion, the DA synthesis inhibitor α-MPT, or 6-OHDA. In every region and condition tested, a majority of spontaneously firing neurons were inhibited by DA. Responsiveness was quantitatively assessed by the IT50 (estimate of the amount of DA required to obtain a 50% reduction of firing rate) and the RT90 method (measure of the time interval between the offset of application and return to 90% of initial firing). The average IT50 was comparable in every region irrespectively of the treatment, indicating that it was independent of the regional density of the DA receptors or that of the DA innervation. Rather, the constancy of this parameter suggested the availability of a critical number of postsynaptic DA receptors on most of the neurons tested, sufficient to induce 50% inhibitions of their firing rate. In contrast, the average duration of the DA inhibitions (RT90) was considerably greater in the ACg (575 s) than in the PF, Par, NS and Acb of control rats (50-100 s), regions between which the differences were not significant. Moreover, treatment with both DA uptake blockers prolonged the duration of the DA inhibitions in PF, NS and Acb, but reduced it 4- to 9-fold in ACg while remaining without effect in Par. DA depletion (α-MPT) and 6-OHDA denervation similarely induced a marked reduction (9-fold) of the duration of DA inhibitions in ACg without effect in Par. It thus appeared that, in all regions except ACg, a sufficient number of DA uptake sites were present in the vicinity of responsive neurons to inactivate the ejected amounts of DA. In ACg, the data obtained with Bupropion provided further evidence for the possible existence of a presynaptic positive feedback mechanism triggered by DA and favouring the further release of this transmitter upon its reuptake (Beauregard et al., *Experientia*, 45:888, 1989).

224.13

A COMPARISON OF BEHAVIORAL SENSITIZATION IN RESPONSE TO D-AMPHETAMINE (AMPH) IN SEVERAL STRAINS OF MICE. B.A. Sieber and R.E. Heikkila. Dept. of Neurology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854.

AMPH is a potent releaser of dopamine and a powerful CNS stimulant. The acute injection of AMPH is known to cause a heightened behavioral response in experimental animals previously treated with AMPH compared to that observed in vehicle-pretreated or in naive animals. This phenomenon is known as behavioral sensitization. The purpose of this study was to examine the degree of sensitization occurring in response to daily injections of AMPH in several strains of mice (SJL/J, BALB/cByJ, DBA/2J, C57Bl/6J from Jackson; CFW from Charles River). Initially, the capacity of AMPH (at doses from 0.4 to 6.25 mg/kg) to cause increases in locomotor activity was measured. In some strains (e.g. CFW and SJL/2J) there was a large increase in locomotor activity and a steep dose-response curve for AMPH. For example, in CFW mice, increased locomotor activity was first observed at 0.4 mg/kg AMPH and was nearly maximal at 2.5 mg/kg. In other strains (e.g. BALB/cByJ), however, there were minimal to marginal increases in locomotor activity at all doses tested. Based on the results of these experiments, a specific dose was selected with which to pursue sensitization studies in each strain. The animals were injected daily with the chosen dose of AMPH and locomotor activity was monitored on the first day of injection and at various times thereafter (on days 3, 7 and 22-24). An indication of sensitization was seen in each of the strains, and was particularly striking in certain strains (e.g. SJL/J). Experiments done in these strains of mice may well aid in the elucidation of neuronal mechanisms underlying the sensitization phenomenon.

224.14

PREFERENTIAL RELEASE OF DOPAMINE OR NOREPINEPHRINE IN CAT CAROTID BODY EVOKED BY HYPOXIA AND NICOTINE IN VITRO. A. Gomez-Nino, C. Gonzalez, B. Dinger and S. Fidone. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108.

Previous studies in our laboratory with rabbit carotid body demonstrated that nicotine primarily evokes the release of norepinephrine (NE) from chemoreceptor type I (glomus) cells, while hypoxic stimulation results in release profiles dominated by dopamine (DA). However, because the ratio of DA to NE (8.2) in the low-O_2 superfusates approximates their ratio in tissue catecholamines (CA) stores, it was not possible from these studies alone to determine if hypoxia preferentially mobilized NE or DA, respectively. The present experiments evaluated DA and NE release evoked from cat carotid bodies, a species in which these CA are stored at nearly equal concentrations.

^{3}H-CA release (synthesized from ^{3}H-tyrosine) was evaluated in vitro in sequential 10 min control, 5 min stimulus and 10 min post-stimulus collection periods. The tissues were superfused with modified Tyrode's media (37°C) equilibrated with 100% O_2, except during stimulation when the superfusion media contained nicotine (100 μM), or was equilibrated with 10% O_2. Nicotine evoked the preferential release of ^{3}H-NE (^{3}H-NE/^{3}H-DA = 1.33). In contrast, low-O_2 media resulted in a ratio for ^{3}H-NE/^{3}H-DA = 0.37. Calculation of the absolute amounts of DA and NE, released based upon measured specific activities of ^{3}H-NE and ^{3}H-DA, showed that exposure to nicotine and hypoxia resulted in NE/DA ratios of 6.20 and 0.36, respectively. The data confirm our earlier findings with the rabbit carotid body in suggesting that NE and DA stores are preferentially mobilized by nicotine and hypoxic stimulation, respectively. Supported by USPHS Grants NS12636 and NS07938.

224.15

MONOAMINES OF THE COMMON CAROTID ARTERY WITH CHRONICALLY REDUCED LOCAL BLOOD FLOW IN THE SPONTANEOUS HYPERTENSIVE RATS. K.H.Lee and Y.H.Kim.* Dept. of Neurosurgery and Pharmacology*, Hallym Univ. Sch.of Med., Chuncheon, Korea.

It has been known that the arterial size depends on the role of the endothelium on changing local blood flow and the final morphological accommodation of the artery wall may be in the tunica media innervated by sympathetic fibers containing catecholamine as a neurotransmitter. Using high performance liquid chromatography and electrochemical detection system we have measured the monoamines of the common carotid arterial wall with the intact endothelium of the chronically reduced local blood flow by ligation of the ipsilateral external carotid artery and also the monoamines of the chemically denuded common carotid arterial wall with 2% Triton X-100 of the same state and compared them with the monoamines of the each normal opposite common carotid artery in the spontaneous hypertensive rats. Norepinephrine of the common carotid artery in the normal site (n=6) was 2.13 ± 1.197 ng/mg protein. The levels of norepinephrine of the common carotid artery with the intact endothelium in chronically reduced local blood flow (n=4) were higher than the levels of the normal opposite ateries but the levels of the denuded (n=3) lower. Epinephrine and dopamine were infrequently detected. Norepinephrine may play a role in the morphological accomodation of the artery on changing local blood flow chronically.

224.16

ATP-EVOKED RELEASE OF CATECHOLAMINES FROM PHEOCHROMOCYTOMA PC12h TREATED WITH NGF. N.Nakanishi, K.Hirayama, K.Aono* S.Onozawa*, S.Yamada* Dept. of Biochem., Meikai Univ. Sch. Dent., Sakado, Saitama 350-02, Japan.

We found that ectracellular ATP increases cytosolic Ca2+ of PC12 and PC12h cells, a subclone of PC12. Comparison of the effect of adenine nucleotides on the $[Ca2+]_i$ suggests that this ATP effect is mediated by P2 type purinergic receptors. In the present study, we examine the effect of ATP on secretion of catecholamines from PC12h cells and compared with that of KCl. PC12h cells cultured in collagen-coated dishes were treated with NGF for 2 days, and then culture medium was replaced with release medium with 1 mM ATP, 50 mM KCl, or vehicle. At 5, 10, and 20 min after stimulation, catecholamines released into the medium were analyzed by HPLC. ATP and KCl evoked epinephrine (EP) release from the cells with the similar manner: EP released into the medium lineraly increased upto 20 min and similar amounts of EP were detected with ATP and KCl. On the contrary, ATP also evoked the dopamine (DA) release, while KCl had little effect. Furthermore, the DA amount released by ATP showed the maximal value at 5 min after stimulation. These results suggest that although both ATP and KCl increase $[Ca2+]_i$ of PC12h cells, these agents evoke catecholamines by different mechanisms and that release of EP and DA is controled independently from each other.

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224.17

ISOLATED ADRENAL GLAND OF THE RAT CAN BE USED TO STUDY RELEASE OF ACETYLCHOLINE. S.V. Bhave, R. Shukla*, T.D. Wakade* and Arun R. Wakade. Dept. of Pharmacology, Wayne State Univ., School of Medicine, Detroit, MI-48201.

Perfused rat adrenal gland has been used to study secretion of catecholamines (CA). Major advantage of the preparation is that secretion of CA is evoked by stimulation of splanchnic nerves and exogenous acetylcholine (ACh). Although pharmacological and denervation experiments have established the involvement of ACh, release of ACh after stimulation of splanchnic nerves was not shown. Adrenal glands were perfused with Krebs containing ^{3}H-choline chloride (50 μCi/5ml) for 1 hr, excess label was washed with Krebs containing choline chloride (10 mg/ml) for 30 min and then medium was changed to Krebs containing 10 μM eserine. Perfusates were processed to separate ^{3}H-ACh from total tritium. Field stimulation significantly enhanced fractional release of ^{3}H-ACh over the background release, and the evoked-release increased with increase in frequency of stimulation ($4.5\pm0.9\times10^{-2}$ at 1 Hz for 5 min and $7.4\pm1.1\times10^{-2}$ at 10 Hz for 30 sec). Stimulation -evoked release of ^{3}H-ACh was abolished when calcium was omitted from or cadmium plus nickel were added to the perfusion medium. Evoked release of ^{3}H-ACh was not modified by atropine (0.5 μM) and muscarine (100 μM), but greatly potentiated by tetraethylammonium chloride (5 mM). Thus isolated adrenal gland of rat is useful to study secretion of cholinergic, adrenergic and peptidergic substances.

224.18

EFFECT OF INORGANIC THIOPHOSPHATE ON CHROMAFFIN CELL STRUCTURE AND FUNCTION. J.C. Brooks, M. Brooks* and S.W. Carmichael. Marquette Univ. Sch. of Dent., Milwaukee, WI 53233, Mayo Clinic, Rochester, MN 55905.

We have previously suggested that ATPγS irreversibly thiophosphorylates cellular proteins and inhibits chromaffin cell secretion. It might then be expected that incubation of cells with inorganic thiophosphate (TP_i) would result in its incorporation into cellular ATP pools with a deleterious impact on secretion. Chromaffin cells were cultured with phosphate (P_i)-free medium or media containing P_i or TP_i for periods of 1-3 days. Assay of cellular metabolic activity indicated enhanced activity at day 1 of TP_i exposure and decreased activity by day 3. There was a profound reduction in total cellular catecholamine content and secretion by day 3 of culture. There was no change in the DNA content of the cultures, showing that the decreased metabolic activity was not due to cell death. Uptake of the dye neutral red was reduced for cells treated with TP_i, indicating impairment of chromaffin vesicle function. Electron microscopic evaluation of the TP_i treated cells revealed depletion of vesicle contents and disruption of mitochondria. Radiolabelled TP_i was incorporated into a group of high molecular weight proteins, possibly of mitochondrial origin. The data suggest that TP_i damages the secretory system, perhaps secondarily to disruption of mitochondrial energy production. Supported by NIH Grant R15 NS 23101-01A2.

224.19

EFFECTS OF LITHIUM ON THE NICOTINE- AND POTASSIUM-INDUCED CATECHOLAMINE SECRETION FROM BOVINE ADRENAL CHROMAFFIN CELLS. S.Boehm* and S.Huck (SPON: European Neuroscience Association). Department of Neuropharmacology, University of Vienna, Währingerstraße 13a, A-1090 Vienna, Austria

Li^+ has been shown to reduce catecholamine (CA) release from adrenergic nerve endings. This study investigates acute and chronic effects of Li^+ on cultured chromaffin cells, isolated from bovine adrenal glands.

CA release was elicited in a secretion buffer by either 20μM nicotine or by 56mM K^+, which mediates secretion independently of the nicotinic acetylcholine receptor. Chronic Li^+ effects were studied in cultures exposed for 4 days prior to the secretion. For acute effects, Li^+ was just added to the secretion buffer. Li^+ concentrations ranged from 0.25 to 8mM. The CA secretion was measured as the [3H]noradrenaline release from cultures that had been preloaded with [3H]noradrenaline.

The K^+-induced secretion was unaltered by acute Li^+ applications and was even enhanced (by as much as 15%) following chronic treatment of the cultures. The secretion elicited with nicotine, however, decreased in a dose-dependent manner by 13% after acute, and by 20% after chronic Li^+ administrations. Withdrawal of Li^+ from chronically-treated cultures 1 day prior to the secretion caused the CA release to return to control levels.

Our results indicate contrasting effects of Li^+ on basic secretion mechanisms in bovine chromaffin cells.

224.20

LYSOPHOSPHATIDYLCHOLINE INHIBITION OF ION CHANNELS AND CATECHOLAMINE SECRETION IN BOVINE ADRENAL MEDULLA

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In cultured bovine adrenal medullary cells, lysophosphatidylcholine (LysoPC) inhibited carbachol-induced influx of sodium/calcium, and the secretion of catecholamines. The inhibitory effect of LysoPC was reversible. LysoPC did not inhibit the influx of calcium and secretion of catecholamines evoked by high potassium depolarization. LysoPC did not inhibit the veratridine-evoked influx of sodium and secretion of catecholamines. These findings show the preferential inhibition of acetylcholine-receptor associated ion channels by LysoPC. LysoPC which arises from membrane phosphatidylcholine by phospholipase A_2 may be involved in the feedback regulation of catecholamine secretion by receptor stimulation.

224.21

POTASSIUM CHANNEL BLOCKERS DISRUPT INHIBITION OF DOPAMINE RELEASE BY D-2 DOPAMINE, BUT NOT A1 ADENOSINE, RECEPTORS IN RAT STRIATUM. W.A. Cass and N.R. Zahniser. Department of Pharmacology, University of Colorado Health Sciences Center, Denver, CO 80262.

D-2 dopamine (DA) autoreceptors and A1 adenosine heteroreceptors inhibit evoked release of DA from rat striatum. We examined one possible mechanism underlying these presynaptic modulatory actions, activation of a K^+ conductance. Release of endogenous DA was evoked by electrical stimulation (1 Hz, 1 min) from rat striatal slices superfused with Krebs' buffer containing 10 μM nomifensine. Maximally-effective concentrations of the D-2 DA receptor agonist N-0437 (10 nM) and adenosine (50 μM) both caused a 30% inhibition of DA overflow, and their effects were additive. Next, we investigated the effects of three K^+ channel blockers - quinine, tetraethylammonium (TEA), and 4-aminopyridine (4-AP). By itself, quinine (100 nM) significantly increased evoked DA overflow by 17%. When co-perfused with N-0437, quinine blocked the inhibition caused by N-0437 in a dose-dependent manner; 100 nM quinine produced a complete blockade. Similar effects were found with low concentrations of TEA (0.3 mM - 1 mM) and 4-AP (3 μM). Binding experiments confirmed that none of these K^+ channel blockers are direct-acting D-2 DA receptor antagonists. In contrast, the inhibitory modulation produced by adenosine was not affected by quinine (1 μM), TEA (0.3 mM) or 4-AP (3 μM). These results suggest that D-2 DA and A1 adenosine receptors inhibit DA release in the striatum by different mechanisms. D-2 DA autoreceptor action involves a K^+ channel, whereas A1 adenosine receptor action does not. The present results indicate, for the first time, a mechanism that appears to mediate the actions of release-modulating D-2 DA receptors in the brain. Supported by USPHS NS26851 and AA07464.

224.22

COMPARISON OF AXONAL AND SOMATODENDRITIC DOPAMINE RELEASE USING IN VIVO DIALYSIS. P. W. Kalivas, Ph.D. and P. Duffy*. Department of Veterinary Pharmacology, Washington State University, Pullman, WA 99164-6520.

The release of dopamine from the dopamine cell bodies in the A9 and A10 region has been known for over a decade. However, only recently has the release of endogenous dopamine from the A9 and A10 cell groups been measured in vivo. The present studies characterize the release of somatodendritic dopamine from the A10 region and compare this to axonal release using the technique of in vivo dialysis. Dialysis probes were placed into the A10 region or the nucleus accumbens using a removable probe system the night before experimentation. The following morning, 20 min baseline samples were obtained, after which various drugs were administered through the dialysis probe. Removal of calcium and substitution by magnesium markedly decreased the release of dopamine in both the nucleus accumbens and A10 region. When TTX was placed in the buffer, a marked reduction in the nucleus accumbens was produced, and a much smaller reduction was observed in the A10 region. A D_2 agonist inhibited equally axonal and somatodendritic dopamine release. Dopamine release was augmented with the addition of potassium chloride, amphetamine, or the reuptake blocker nomifensin to the buffer. The release induced by these agents was significantly greater in the nucleus accumbens than in the A10 region. The following data indicate that like terminal field release of dopamine, somatodendritic dopamine release is calcium dependent and regulated by D_2 autoreceptors. However, the somatodendritic release does not appear to be as totally dependent on action potential generation, and agents which release dopamine do not appear to do so as effectively in the A10 region as they do in the nucleus accumbens.

224.23

CHARACTERIZATION OF DENDRITIC DOPAMINE RELEASE IN THE SUBSTANTIA NIGRA OF FREELY MOVING RATS BY IN VIVO MICRODIALYSIS. G. Damsma, G.S. Robertson, D. Wenkstern, H.C. Fibiger. Div. of Neurol. Sci., Dept. of Psychiatry, Univ. of British Columbia, Vancouver, BC, V6T 1W5.

Dopamine (DA) is released not only from the terminals of the nigrostriatal pathway that innervate the striatum (ST) but also from the dendrites of these neurons which arborize in the substantia nigra pars reticulata (SNR). Although ST-DA release has been extensively studied by in vivo microdialysis, dendritic DA release in the SNR has not been characterized by this technique. Extracellular DA was monitored simultaneously in the ipsilateral ST and SNR. The nigral probe was implanted on a 45 degree angle permitting 2mm of SNR to be dialysized. Inclusion of 50mM KCl to the SNR perfusion solution produced a 3 fold increase in DA and a 50% reduction in DOPAC in the SNR; in contrast, DA release was decreased by 20% while DOPAC increased by 50% in the ST. Local administration of nomifensine (10μM) in the SNR produced an 8 fold increase in SNR-DA but had no effect on ST-DA. Amphetamine (2mg/kg, s.c.) elevated DA in the SNR and ST 5-7 fold while DOPAC was decreased in both structures by at least 40%. To determine the effect of tetrodotoxin (TTX) on SNR-DA release, basal SNR-DA (0.7 fmol/min) was first elevated 4 fold by inclusion of nomifensine (1μM) in the nigral perfusion solution. Under this condition TTX (1μM) reduced DA release in both the SNR and ST by 90%. These results demonstrate that ST-DA release can be influenced by manipulations which alter SNR-DA release and that dendritic-DA release in the SNR shares many of the characteristics of ST-DA release.

224.24

RELEASE OF DOPAMINE FROM NORADRENERGIC NEURONS AFTER DOPAMINE-BETA-HYDROXYLASE INHIBITION. B. Ghasemzadeh, P. Capella, R.N. Adams. Dept. of Chemistry, University of Kansas, Lawrence KS66045

The last step in the synthesis of NE is the conversion of DA to NE by the enzyme DBH. The tissue content of DA and its major metabolite DOPAC in predominantly NE innervated areas and NE cell body region Locus coeruleus is very low compared to NE. The source of DA and DOPAC in these structures has been traced to the Noradrenergic neurons. We have investigated the effect of DBH inhibition on tissue Catecholamine content in specific areas of rat brain. In NE cell body and terminal fields the levels of NE was significantly reduced as expected but DA and DOPAC were elevated. In addition, DA levels in Caudate nucleus was increased significantly.

Using In-Vivo Electrochemistry, the effect of DBH inhibition on stimulated release of NE in Thlamus was investigated. The evoked response was not changed after FLA-63 although chemical analysis shows 50% reduction in NE.The DA and DOPAC content were increased by several fold. Since our method can not distinguish between NE and DA, it may be that upon inhibition of DBH the NE neurons will utilize either the storage pool of NE or DA as their neurotransmitter for release.

224.25

SIMULTANEOUS MONITORING OF DOPAMINE OVERFLOW AND OXYGEN USE DURING ELECTRICAL STIMULATION IN SLICES OF RAT CAUDATE NUCLEUS USING FAST SCAN CYCLIC VOLTAMMETRY. R. T. Kennedy and R. M. Wightman. Dept. of Chemistry, Univ. of N. Carolina, Chapel Hill, NC 27599-3290.

Nafion-coated carbon-fiber microelectrodes were implanted at a depth of 75μm into slices of rat caudate nucleus. A twisted pair of bipolar stimulating electrodes (tip spacing of 500 μm) were placed on the surface of the slice and within 100 μm of the voltammetric electrode. A potential waveform was applied to the voltammetric electrode which allowed the oxygen and the dopamine concentration to be measured nearly simultaneously (within 10 ms of each other) at 100 ms intervals. After short stimulations (2 pulse, 480 μAmp, biphasic, 2 ms wide and 100 ms apart) the dopamine levels rose to 0.99 $\pm$ 0.07 μM (n=6) and then returned to basal level within 500 ms. During the same stimulations, oxygen levels decreased by 43 $\pm$ 6.9 μM, reaching a minimum between 5 and 20 s after stimulation and not returning to basal level until after 30 s. The response of both of the compounds to the short electrical stimulation was reversibly eliminated after the removal of Ca++ from the medium. These results indicate that it is possible to investi-gate the interaction between neurotransmitter release and oxidative energy metabolism with a time resolution of 100 ms by this method.

224.26

REGULATION OF NORADRENALINE RELEASE IN RAT HIPPOCAMPUS: IN VIVO MICRODIALYSIS. R.B. Holman and D.N. Thomas*. Reckitt & Colman Psychopharm. Unit, The Medical School, Bristol BS8 1TD UK.

Results with the specific alpha$_2$ antagonist idazoxan (IDX) suggest that presynaptic alpha$_2$ adrenoreceptors play a major role in regulating release of cortical noradrenaline (NA) (Dennis, T. et al. 1987 JPET. 241:642). We now show that systemic IDX causes a dose dependent increase in the release of NA from the hippocampus of chloral hydrate anaesthetised rats. The reuptake blocker desipramine (DMI 0-20μM) administered via the dialysis probe also increased basal NA release in a dose dependent manner. Further, the effects of IDX at the lower dose now become apparent in the presence of DMI.

Treatment	NA release (pg/sample)	Treatment (pg/sample)	NA release
control basal	4.0 ± 0.5(n=9)	DMI 20μM basal	30.6 ± 1.7(n=6)
IDX 1mg/kg	5.7 ± 0.8(n=3)	DMI/IDX 1mg/kg	43.2 ± 5.6(n=5)
IDX 10mg/kg	9.6 ± 1.3(n=4)	DMI/IDX 10mg/kg	66.8 ± 8.3(n=6)

These results suggest that under the above conditions, the extracellular content of NA in the hippocampus may be regulated to a greater extent by reuptake of the transmitter than by its interaction with the presynaptic alpha$_2$ autoreceptor.

224.27

THE COMPOUND N-2-CHLOROETHYL-N-ETHYL-BROMOBENZYLAMINE HYDROCHLORIDE (DSP4) ENHANCES THE SPONTANEOUS AND THE POTASSIUM EVOKED RELEASE OF 3H-NOREPINEPHRINE FROM RAT CORTICAL SLICES. M.E.Landa*,G.Dziewczapolski*,L.M.Zieher*, M.C.Rubio* and G.Jaim-Etcheverry. Instituto Investigaciones Farmacológicas, Junin 956, Buenos Aires, Argentina.

In the search for clues about the mechanisms involved in the long-lasting depletion of brain norepinephrine (NE) produced by DSP4 in rodents, we studied in vitro the effects of the compound (10 umol/l) on the release of NE from rat cortical slices preloaded with 3H-NE (0.4 umol/l) Both the spontaneous and the K+-evoked efflux of tritium were markedly enhanced. While coincubation with desipramine (DMI) abolishes the spontaneous release produced by DSP4, it does not modify the enhancement of the K+-evoked release that is reversed by washing. Coincubation of the slices with the opioid receptor antagonist naloxone (0.1 umol/l) does not affect the increase of spontaneous NE outflow caused by DSP4 but prevents the enhancement of the K+-evoked efflux that it produces. Pretreatment of rats with naloxone (2 mg/kg ip) 10 min before the injection of DSP4 (25 mg/kg ip) does not modify the depletion of NE produced by the compound. Since pretreatment with DMI prevents the depletion of NE caused by DSP4 in vivo, this effect seems to be more directly related to the increase of spontaneous NE release produced by the compound under in vitro conditions than to the potentiation of the K+-evoked tritium outflow.

225.1

RU38486 BLOCKS THE INCREASE IN TRYPTOPHAN HYDROXYLASE (TRPH) ACTIVITY TO ACUTE SOUND STRESS (SS) OR INTRACRANIAL INFUSIONS OF CORTICOTROPIN-RELEASING FACTOR (CRF). M.C. Boadle-Biber, V.B. Singh*, K.C. Corley, and T-H. Phan*. Physiol. Dept., Va Commonwealth Univ., Richmond, VA 23298.

The activity of TrpH, the rate-limiting enzyme in serotonin synthesis is enhanced in an alkaline phosphatase reversible manner by an acute SS consisting of a 1-2 h exposure of male rats to randomly presented sound pulses (110 or 120 dB intensity, 100 msec or 2 sec duration, VI 1 min) (Brain Res. (1989) 482 306-316). This increase in enzyme activity requires the presence of glucocorticoid: It is blocked by adrenalectomy 3 days prior to the SS, and is restored by dexamethasone treatment (Brain Res. (1990) 516 66-76). Intracranial infusion of CRF also activates TrpH (Faseb J. (1989) 3 A729; Abstr. Soc. Neurosci. (1989) 15 91.18). Bilateral intraventricular (ICV) infusions of the glucocorticoid antagonist, RU38486 (100 ug in 5 ul vehicle per side over 30 sec) or vehicle alone (95% propylene glycol, 5% ethanol, 0.1 % acetic acid, and 0.01% ascorbic acid, v/v/v/w) were given daily, via chronic indwelling guide cannulae, for 4 days. The increase in TrpH activity in response to acute SS and to ICV CRF (1.5 ug per side; 60 min treatment), were blocked by RU38486, but enzyme activity from sham-stressed and vehicle-infused rats was unchanged. Supported by NS14090 to MCBB. RU38486 was a gift from Roussel Uclaf.

225.2

DYNAMIC SUPPRESSION OF REM SLEEP BY THE SEROTONIN AGONIST ELTOPRAZINE. J.Quattrochi, D.Binder*, A.Mamelak*, J.Williams*, C.Rittenhouse* and J.A.Hobson. Laboratory of Neurophysiology, Harvard Medical School, Boston, MA 02115

We report that parenteral administration of eltoprazine leads to REM sleep suppression via serotonergic inhibition of the brainstem REM generator. We recorded continuously for 15 days from 4 cats prepared with EEG, EMG, EOG, and LGB electrodes. During the control period (days 1-5) mean REM% was 13.0. Eltoprazine administration (days 6-10) at 1.0 mg/kg b.i.d suppressed REM sleep to 0% for the first 6 hours postinjection and 2.7% for 24 hours. Following drug withdrawal (days 11-15) REM% rebounded to nearly twice baseline levels in the first two days and was still elevated throughout the recovery period. Total suppression of PGO waves in all behavioral states during eltoprazine administration was followed by an intense potentiation of PGO activity during the recovery period. We interpret these results based upon the assumption that eltoprazine acts at 5-HT_1 receptors. REM is suppressed via the inhibition of the distributed brainstem generator, while complete suppression of PGO activity may be due to inhibition of the trigger zone in the dorsolateral pons. REM rebound is probably a compound effect of increased excitability of the generator and prolonged suppression of the endogenous 5-HT system.
Supported by NIH grant MH13923 and Duphar, B.V.

225.3

EFFECTS OF 5-HYDROXY-L-TRYPTOPHAN ON BRAINSTEM AMINE METABOLITES AND BLOOD PRESSURE IN SPONTANEOUSLY HYPERTENSIVE RATS. D. Ghosh*, B. O. Anyanwu*, and H. R. Leuchtag. Department of Biology, Texas Southern Univ., Houston, Tex. 77004.

The effects of three doses (50 mg/kg, 25 mg/kg, and 12.5 mg/kg, i.p.) of 5-hydroxy-L-tryptophan (5-HTP) were studied in male spontaneously hypertensive rats (SHRs). All three doses reduced blood pressure at 2 hrs. from the base-line values at the 5% or lower level of significance. The metabolites were assayed employing HPLC/electrochemical detection. The higher the concentrations of 5-hydroxytryptamine (5-HT) and 5-hydroxy indole acetic acid (5-HIAA), the more pronounced were the blood pressure reductions. Concentrations of norepinephrine, dopamine, 3, 4-dihydroxyphenylacetic acid and homovanillic acid were almost identical in these three groups of rats, and as such, these neurochemicals did not exert any effect on blood pressure reduction. Thus, the brainstem conversions, 5-HTP ⟶ 5-HT ⟶ 5-HIAA, appear to play a key role in blood pressure reduction in SHRs. (Supported by NIH grant RR03045).

225.4

EARLY INCREASES OF SEROTONIN TURNOVER IN BRAIN FOLLOWING PORTACAVAL ANASTOMOSIS: RELATION TO ALTERED SLEEP PATTERNS AND DIURNAL RHYTHMS? M. Bergeron[*1], M.S. Swain[*1], T.A. Reader[2], L. Grondin[*2] and R.F. Butterworth[1], [1]Lab. of Neurochemistry, CRC A-V, Hôpital St-Luc, Montreal H2X 3J4, [2]Dept. Physiology, Univ. Montreal, Que., Canada.

Portacaval anastomosis (PCA) in the rat results in increased brain tryptophan and alterations of sleep-waking cycles and diurnal rhythms. Administration of ammonium salts to rats with PCA precipitates severe signs of encephalopathy progressing to coma. In order to assess the relationship between serotonin (5HT) and neurological status in these animals, 5HT and its metabolite 5HIAA were measured in several discrete brain nuclei by HPLC with electrochemical detection at various stages during the development of encephalopathy. Results demonstrate significantly increased 5HIAA/5HT ratios in Raphe Nucleus (127%), Locus Coeruleus (112%) and Caudate/Putamen (70%) respectively following PCA. However, onset of coma was not accompanied by additional changes in 5HIAA/5HT ratios. Increases of 5HIAA/5HT ratios were previously observed in postmortem brain tissue from cirrhotic patients with hepatic encephalopathy (Neurochem. Res. 14 853, 1989). These findings suggest that early signs of encephalopathy, such as altered sleep patterns and diurnal rhythms, following PCA in rats may result from increased 5HT turnover in brain. (Funded by MRC Canada).

225.5

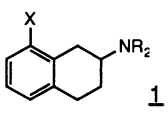
TRYPTAMINE-4,5-DIONE, A SEROTONIN DERIVATIVE PRODUCED BY HYPOXANTHINE AND XANTHINE OXIDASE. J.-C. Chen and L. Volicer. Dept. Pharmacology, Boston Univ. Sch. Med., Boston, MA 02118 and ENRM Vet. Hosp., GRECC., Bedford, MA 01730.

Previously, we have reported a novel neurotoxin, tryptamine-4,5-dione (4,5-DKT) which could be synthesized electrochemically from acidic serotonin (5-HT) solution (JPET 250:141, 1989; Brain Res. 504:247, 1989). 4,5-DKT can also be produced dose-dependently from 5-HT when reacted with hypoxanthine/xanthine oxidase, an oxygen free radical generation system. In the presence of 0.1mM 5-HT, 0.1mM $FeSO_4$, 0.3mM EDTA, 0.1mM hypoxanthine and 0.1U of xanthine oxidase, the yields of 4,5-DKT varied with pH values with maximal response at pH7.0 (7.2μM). At this pH and reaction condition, the rate of 4,5-DKT production was increased linearly up to 60min and gradually decreased during the next 2h. In addition, the 4,5-DKT has high affinity to glutathione which can be monitored by HPLC with 16-channel coulometric detectors. This study suggests that endogeneous indole neurotoxin could be produced by 5-HT and free radicals under condition such as ischemia-reperfusion. (supported by USPHS AG06419 and VA Administration).

225.6

8-SUBSTITUTED-2-AMINOTETRALINS: 5-HT1A AGONIST STRUCTURE/ACTIVITY REATIONSHIP J.M. Schaus, R.D. Titus*, D.L. Huser*, C.S. Hoechstetter*, D.T. Wong, R.D. Marsh*, and R.W. Fuller. Lilly Research Laboratories, Eli Lilly and Co. Indianapolis, IN 46085

8-Hydroxy-2-dipropylaminotetralin (8-OH-DPAT, 1(X=OH, R=Pr)) is recognized as a selective 5-HT_{1a} serotonin agonist. Structure activity studies which have been performed to date have concentrated on studying enantioselectivity, the effects of substituents on the amino group, and the effects of incorporation of a methyl group at positions on the saturated ring. Little work has been reported to date on the effect of varying the substituent at C-8 of the aminotetralin system. We report here the synthesis of a series of twentyfive 8-substituted aminotetralins of the general structure 1. These compounds were evaluated for affinity at the 5-HT_{1a} receptor by ^{3}H-8-OH-DPAT binding studies and compounds with significant affinity were tested for their ability to decrease brain 5-HIAA concentrations. The compound with the highest affinity was LY232067 (1, X=SMe, R=Pr) with IC_{50}=1.23 ± 0.32 nM at the 5-HT_{1a} receptor. This compound had higher affinity than either its 8-OH or 8-OMe analogues (IC50 = 2.73 ± 0.47 and 4.87 ± 0.32 nM, respectively). LY232067 lowered brain 5-HIAA levels indicating that it is a serotonin agonist. The affinity of the 8-halogen substituted aminotetralins decreased in the following order: I > Br ≈ Cl > F.



1

225.7

8-OH-DPAT INCREASES PLASMA GLUCOSE AND INSULIN IN RATS: INVESTIGATION OF THE ROLE OF CNS SEROTONIN IN REGULATING GLUCOSE METABOLISM. A. B. Thiagarajan, K. M. Wozniak, M. J. Durcan, M. Linnoila and R. L. Eskay*. Laboratory of Clinical Studies, NIAAA, National Institutes of Health, Bethesda, MD 20892.

The intravenous (i.v) administration of 8-Hydroxy-2-(di-n-propylamino)tetralin (8-OH-DPAT) has been shown to increase plasma levels of glucose and catecholamines in rats without changing plasma insulin.

Adult, male Sprague-Dawley rats were implanted with lateral ventricle catheters for drug delivery and jugular vein cannulae for blood sampling. Intracerebroventricular (ICV) administration of 0.01 mg/kg of 8-OH-DPAT, a dose which was peripherally inactive, increased plasma insulin levels two fold at fifteen and thirty min post infusion, when compared to saline injected animals. In the same animals plasma glucose increased from 102± 5 mg %(baseline) to 156±10 and 154±8 mg % at 15 and 30 min , respectively. ICV injection of 0.01 mg/kg 8-OH-DPAT in adrenal demedullectomized animals resulted in a three to five fold increase in insulin levels at 5, 15 and 30 minutes without any change in glucose levels. Pretreatment of rats with 5,7 dihydroxytryptamine abolished the increase in plasma insulin and glucose following ICV administration of 0.01 mg/kg 8-OH-DPAT.

These results suggest that the 8-OH-DPAT-induced release of insulin is mediated via 5-HT1A presynaptic autoreceptors in the CNS.

225.8

8-OH-DPAT SUPPRESSES MOTION SICKNESS IN CATS WITH NO EFFECT ON HABITUATION: POSTSYNAPTIC SITES. J.B. Lucot. Dept. Pharmacol., Wright State Univ., Dayton, OH 45435.

These experiments were designed (1) to test for pre-vs postsynaptic sites of action for the antiemetic effects of 8-OH-DPAT (DPAT) and (2) to determine if suppression of motion sickness by DPAT prevents habituation to the motion stimulus. Motion sickness was induced by a device modelled after a Ferris wheel in 29 cats screened for susceptibility to the device. Tests lasted 30 min followed by 1 min observation at rest and were separated by at least 2 wk except in habituation studies. (1) The failure of metergoline (0.1-3 mg/kg) or PCPA (150 mg/kg x 3 D) to suppress motion sickness suggests that postsynaptic sites underlie the antiemetic effects of DPAT. If the relevant sites were presynaptic, then these two treatments also should have suppressed emesis. Further, 3 d of treatment with 0.04 mg/kg DPAT did not decrease the antiemetic effect of DPAT, an effect consistant with a postsynaptic site of action. (2) Habituation was induced by 4 consecutive days of motion testing in 2 series, one with daily saline and one with 3 d of DPAT followed by 1 d of saline. The absence of a difference on the 4th d of motion testing between the two series suggests that suppression of motion sickness by DPAT does not prevent habituation to the stimulus. Thus, stimulation of postsynaptic 5-HT_{1A} sites suppresses motion sickness without interfering with habituation to abnormal vestibular input.

225.9

GEPIRONE BLOCKS THE REVERSIBLE INCREASE IN RAT CORTICAL AND MIDBRAIN TRYPTOPHAN HYDROXYLASE (TrpH) ACTIVITY IN RESPONSE TO INTRACEREBRAL CORTICOTROPIN-RELEASING FACTOR (CRF). K.C. Corley, V.B. Singh*, T-H. Phan*, and M.C. Boadle-Biber. Dept Physiol., Va Commonwealth University, Richmond, VA 23298.

CRF, when infused intracerebroventricularly or bilaterally into the amygdaloid central nucleus (ACE), produces an increase in TrpH activity, in vitro, that can be reversed by alkaline phosphatase (Faseb J. 3: A729, 2977, 1989; Soc. Neurosci. Abstr. 15: 91.18. 1989) and resembles the increase in enzyme activity to acute sound stress (Brain Res. 482: 306-316, 1989; 516: 66-76, 1990). Gepirone, i.p. or infused directly into the dorsal raphe nucleus (DRN) via an indwelling cannula blocks the increase in TrpH activity to sound stress (Faseb J. 4: 3146, 1990). In the present studies, gepirone (or saline vehicle) was given i.p. (10 mg/kg) or by direct infusion into the DRN (14 ug in 2 ul) 15 min before bilateral infusion into ACE of CRF (0.5 ug/2ul per side; in PO_4 buffered saline, pH 6.8, with 0.1% BSA and 0.01 mM ascorbic acid). Rats were killed 30 min after CRF infusion. Gepirone totally blocked the CRF-induced increase in TrpH activity possibly through an action on 5-HT_{1A} receptors in the DRN. (Supported by Bristol-Myers/Squibb and NS 14090-09.)

225.10

CHRONIC INFUSION OF TANDOSPIRONE AND IMIPRAMINE ALTERS 5-HT-MEDIATED BEHAVIORS AND 5-HT RECEPTORS. S. Wieland[1], C. T. Fischette[2] and I. Lucki[1] Dept. Psychiatry[1], Univ. Pennsylvania, Philadelphia, PA 19104 and Pfizer Inc.[2], New York, New York 10017

The 5-HT1A agonist buspirone and related congeners such as tandospirone, have been reported to be useful antidepressant drugs. Because of the involvement of 5-HT in depression, we compared the ability of tandospirone with the antidepressant imipramine to alter behavioral and biochemical measures of 5-HT receptor function following their continuous administration.

Rats (N=10/group) were implanted subcutaneously with Alzet osmotic minipumps (2ML2) set to release either saline, tandospirone, imipramine or 1-PP, a metabolite of tandospirone, at a dose of 20 mg/kg/day. Tandospirone significantly inhibited development of the 5-HT behavioral syndrome by the 5-HT1A selective agonist 8-OH-DPAT (4.0 mg/kg) when tested 24 hr and 14 days after the start of infusion, and 24 hr following the cessation of drug treatment. Imipramine inhibited the syndrome after 14 days of drug treatment and also 24 hr after a drug washout period, but not acutely. Treatment with 1-PP did not alter the 5-HT behavioral syndrome.

The effects of drug treatment were also examined on the head shake response mediated by activation of 5-HT2 receptors with DOB (1 mg/kg). Tandospirone significantly inhibited the head shake response when tested 24 hr and 14 days after pump implantation, and after a 24 hr drug washout period. Similarly, imipramine significantly reduced the number of head shakes when tested at all three time points. 1-PP had no effect on the head shake response.

Continuous administration of tandospirone, imipramine, and 1-PP did not alter 5-HT1A receptor density in hippocampus. However, tandospirone, imipramine, and 1-PP significantly reduced 5-HT2 receptor density in frontal cortex. Imipramine significantly reduced β-adrenergic receptor number in remaining cortex, while tandospirone and 1-PP had no effect. These results suggest certain similarities between continuous infusion of tandospirone and imipramine in rats that may contribute to their effects as antidepressant drugs.

225.11

SUBCHRONIC BUSPIRONE, MESULERGINE, AND ICS-205-930 LACK EFFECTS ON D1 AND D2 BINDING IN THE RAT STRIATUM DURING CHRONIC HALOPERIDOL TREATMENT . P.B. Hicks, R.J. Zavodny* and K.A. Young. Dept. of Psychiatry, Scott and White Hospital/Foundation and Dept. of Pharmacology, Texas A&M College of Medicine, Temple, Texas, 76508.

It has been reported that buspirone reverses neuroleptic-induced dopamine D2 receptor up-regulation (McMillan, B.A., *J. Neural. Transm.* 64: 1-12, 1985). We tested the hypothesis that other serotenergic agents that reverse catalepsy would also reverse D2 receptor up-regulation. We administered buspirone, mesulergine and ICS-205-930 during the last two weeks of a 4 week chronic haloperidol treatment regimen and determined dopamine receptor binding after a 5 day drug washout. As expected, D1 receptor binding was not affected by any treatment. Chronic haloperidol treatment produced a significant increase in the density of D2 receptors for all groups including the groups that were administered serotonergic compounds.

D2 DA Binding: (3H-spiroperidol, sulpiride blank)

4 wk	2 wk(mg/kg/day)	Kd	± SEM	Bmax (fmol/mg)	± SEM
Vehicle	Vehicle	306	16	261	13
HAL	Vehicle	326	12	335*	15
HAL	ICS (2)	338	15	326*	19
HAL	BUS (10)	297	24	338*	14
HAL	MES (0.2)	326	27	326*	16

P<0.05 according to ANOVA with Duncans Multiple Range Test

Our findings suggest that the ability of these drugs to reverse catalepsy does not predict their ability to reverse D2 receptor up-regulation.

225.12

A COMPARISON OF SEROTONIN TURNOVER RATES IN DIFFERENT BRAIN AREAS IN THE FAWN-HOODED AND THE WISTAR RAT-STRAINS. T. I. Tolliver*, C.S. Aulakh*, J.M. Tolliver and D.L. Murphy*. Lab. of Clinical Science, NIMH, Bethesda, MD 20892

We have recently demonstrated that the Fawn-Hooded (FH) rat strain is functionally subsensitive to m-chlorophenylpiperazine (m-CPP, a 5-HT_1, agonist)-induced decreases in food intake (Wang et al 1988) and locomotor activity (Aulakh et al 1989), and increases in plasma prolactin (Aulakh et al 1988) relative to Wistar and Sprague-Dawley rat strains. Other investigators have also demonstrated altered behavioral responses to serotonin agonists in FH rats relative to Sprague-Dawley rats (Gudelsky et al 1985). In the present study, we compared the effects of various doses of m-CPP on plasma growth hormone levels as well as the effects of various doses of ipsapirone (a 5-HT1a agonist) on rectal temperature in FH and Wistar rat strains. Furthermore, we investigated 5-HT turnover rates in different brain areas in FH and Wistar rats. The turnover rates of serotonin were estimated on the basis of the increases in brain 5-HT concentrations as well as the decline of 5-hydroxyindoleacetic acid which followed inhibition of monoamine oxidase after administration of pargyline hydrochloride (75 mg/kg, i.p.). The increases in plasma growth hormone levels following administration of various doses of m-CPP were significantly less in the FH rat strain relative to the Wistar rat strain. Similarly, decreases in rectal temperature following administration of various doses of ipsapirone were also significantly less in the FH rat strain relative to the Wistar rat strain. We will present the data on the 5-HT turnover rates in different brain areas (raphe, hypothalamus, frontal cortex, hippocampus and striatum) in these two rat strains.

225.13

XYLAMIDINE, A PERIPHERAL SEROTONIN ANTAGONIST, ATTENUATES THE PRESSOR RESPONSE TO CENTRAL SEROTONIN. P.E. Pergola and R.H. Alper. The University of Kansas Medical Center, Kansas City, KS 66103.

Recently, we demonstrated that the intracerebroventricular (i.c.v.) injection of serotonin (5-HT) increases mean arterial pressure (MAP) by sympathoexcitation and by stimulating vasopressin release in conscious rats (FASEB J 4:A1064, 1990). In addition the effects of centrally administered 5-HT were abolished by the i.v. injection of the selective 5-HT_2 antagonist LY 53857. The present studies were to determine if the **peripheral** 5-HT_2 antagonist xylamidine, administered i.v., altered the pressor response to centrally infused 5-HT in conscious, unrestrained male Sprague-Dawley rats. It was found that at both 100 or 300 μg/kg, i.v., xylamidine significantly **attenuated** the pressor response to 5-HT (2.5 μg/5μl, i.c.v.) by approximately 27%. In contrast, LY 53857 (100 μg/kg, i.v.), which *in vitro* is equipotent to xylamidine at blocking 5-HT_2 receptors, **abolished** the effect of 5-HT on MAP. A combination of xylamidine with the α_1-antagonist prazosin (100 μg/kg, i.v.) or with a V1-vasopressin antagonist (10 μg/kg, i.v.) did not prevent the increase in MAP produced by 5-HT i.c.v.. In all rats the blood-brain-barrier was determined to be intact since i.v. infused Evans Blue was localized to the circumventricular organs; the ability of xylamidine to attenuate the pressor response to 5-HT was not due to disruption of the blood-brain-barrier. Therefore, it can be suggested that i.c.v. injection of 5-HT elicits a pressor response that is mediated in part by circumventricular organs of the brain to which the peripheral 5-HT_2 antagonist xylamidine gains access. The residual increase in MAP is assumed to be a central action of 5-HT on vasopressin release and sympathetic nerve activity.

[Supported by a Grant-in-Aid from the Kansas Heart Association]

225.14

SEROTONERGIC STIMULATION OF OXYTOCIN SECRETION IN CONSCIOUS MALE RATS: A PHARMACOLOGICAL CHARACTERIZATION. J.A. Saydoff, P.A. Rittenhouse, L.D. Van de Kar, and M.S. Brownfield. Neuroscience Training Program, Sch. Vet. Med., Univ. Wisconsin, Madison, WI 53706, Sch. Med.,Loyola Univ., Maywood, IL 60153.

We studied the role of serotonin (5-HT) in oxytocin (OT) secretion in conscious male rats. Selected 5-HT agonists, antagonists, and releasers were administered to conscious male rats which were later killed by decapitation. Plasma OT concentration was measured by radioimmunoassay.

Both 5-HT releasers, fenfluramine and p-chloroamphetamine, significantly increased plasma OT concentration. D-fenfluramine was more potent than l-fenfluramine with a minimum effective dose of 5.0 mg/kg i.p. This suggests that endogenous 5-HT can stimulate OT secretion.

The 5-$HT_{1\&2}$ agonist, m-CPP (0.5-20 mg/kg i.p.), dose-dependently stimulated OT secretion. In contrast, the 5-HT_{1AB} agonist, RU24969 (5.0 mg/kg i.p.), did not elevate plasma OT concentration. Another 5-$HT_{1\&2}$ agonist, MK212 (10 mg/kg i.p), increased plasma OT and this effect was attenuated by the 5-$HT_{1C\&2}$ antagonist, ritanserin (2.5 mg/kg i.p.). The 5-$HT_{1C\&2}$ agonist, DOI, dose-dependently increased OT secretion with a minimum effective dose of 0.5 mg/kg i.p. Pretreatment with spiperone (5-HT_2 antagonist) or ritanserin (both at .01 or .1 mg/kg i.p.) dose-dependently inhibited the increase in plasma OT DOI dose-response. Intracerebroventricular injection of 200 ug/kg DOI to conscious rats also increased plasma OT concentration.

These studies provide evidence that enhanced serotonergic transmission stimulates OT secretion. Because spiperone has a higher affinity for 5-HT_2 than for 5-HT_{1C} receptors, these data also suggest that the stimulation of OT secretion by DOI is mediated by 5-HT_2 receptors. The site where 5-HT acts to stimulate OT secretion remains to be established. Supported by Univ. Wisc. Grad. Sch. and NIDA DA04865.

225.15

HIPPOCAMPAL SEROTONIN RELEASE IN RATS BRED FOR LEARNED HELPLESSNESS BEHAVIOR. E. Edwards, P.VanHouten, D. Campbell,* and F.A. Henn, Dept. Psychiatry, SUNY, Stony Brook, NY 11794.

We have been able to successfully inbreed learned helpless behavior (LH) into rats. We have previously demonstrated that presynaptic serotonin mechanisms modulate learned helpless behavior in Sprague Dawley rats. In the present experiments we have examined the in vitro release of serotonin in hippocampal slices of rats from the 19th generation of inbred LH and non-learned helpless (NLH) strains.

Spontaneous ^{3}H-5HT release was significantly higher in the hippocampus of rats from the LH strain (+ 55%) as compared to naive controls (NC) and NLH strain rats. K^+ stimulated ^{3}H-5HT release was also significantly increased in the hippocampus of the LH strain rats (% stimulated ^{3}H-5HT release: 7.2 ± 0.87, NC; 6.7 ± 1.0 NLH strain rats; 12.1 ± 0.7 LH strain rats; N=7 separate experiments).

Cumulative dose-response curves to the autoreceptor agonist, 5-HT were constructed (1nM to 1 μM). 5HT caused a concentration dependent inhibition of the K^+ stimulated ^{3}H-5HT release in hippocampal slices from all three groups NC, NLH and LH. In the LH strain, 5-HT caused a greater inhibition of K^+ stimulated ^{3}H-5HT release (at 100nM 5-HT, % inhibition: 75% LH strain vs 47% NC and NLH strain). 5-HT's effect on K^+ stimulated ^{3}H-5HT release was mimicked by TFMPP and CGS 12066, two purported 5-HT_{1b} agonists but not by the 5-HT_{1a} drugs tested.

These experiments demonstrate differences in the regulation of the 5-HT autoreceptor of the LH strain and suggest that the increased stimulation in 5-HT release may be mediated by the 5-HT_{1b} receptor. (Supported by BNS 8614098 to E.E.)

DOPAMINE PHYSIOLOGY II

226.1

SPONTANEOUS ORAL DYSKINESIA IN RESERPINIZED RATS. **J. L. Nelsewander, J. Lucki, and P. McGonigle, Departments of Pharmacology and Psychiatry, University of Pennsylvania School of Medicine, Philadelphia, PA 19104.**

We reported previously that reserpinized rats develop spontaneous oral dyskinesia. To characterize the time course of this response, rats were treated with either reserpine (1 mg/kg, SC) or vehicle once daily for 4 days and then every other day for 6 weeks. Oral dyskinesia was measured by recording the incidence of tongue protrusions (TPs) for 30 min. TPs developed rapidly, reaching a maximal level after only 3 days and then remained steady throughout treatment. TPs persisted at a maximal level for up to 20 days post-treatment, and continued to persist in some animals for at least 60 days post-treatment. Dopamine D1 and D2 receptors were measured 1, 20, and 60 days post-treatment using quantitative autoradiography. D1 receptors were increased in the caudate-putamen (CPu) and nucleus accumbens (NAc) 1 day, but not 20 or 60 days, post-treatment. D2 receptors, however, were increased at all three time points in the CPu, although the magnitude of up-regulation was less at 60 days than at 1 and 20 days. D2 receptors were also increased in the NAc, but only at 1 and 20 days post-treatment. We also examined the effects of dopamine D1 and D2 agonists on reserpine-induced oral dyskinesia. Both SKF-38393 and quinpirole dose-dependently decreased TPs, but only at doses that also produced stereotypy. Therefore, the stereotypy may have masked the TPs. Lastly, we examined whether TPs could be prevented by concurrent administration of SKF-38393. Animals were treated with reserpine as described above, and were given either SKF-38393 (5 mg/kg, SC, twice daily) or vehicle for 7 days. Animals treated with reserpine plus SKF-38393 exhibited fewer spontaneous TPs, but more TPs following acute injection of SKF-38393 (5 mg/kg, SC), than animals treated with reserpine alone. These results may have important implications for understanding and treating tardive dyskinesia. (Supported by USPHS GM 34781, MH 14654, and MH 43821).

226.2

[^{125}I]EPIDEPRIDE BINDING TO DOPAMINE D2 RECEPTORS IN HUMAN BRAIN. J.N. Joyce, A. Janowsk and K.A. Neve. Departmentsof Psychiatry and Pharmacology, University of Pennsylvania School of Medicine, Philadelphia , PA (JNJ) and Veteran's Administration Center, Portland ,OR (AJ, KAN).

[^{125}I]Epidepride is a substituted benzamide with high affinity and selectivity for dopamine (DA) D2 receptors in rat brain (Neve et al, JPET 252:1108-1116.) We examined the distribution and pharmacology of this radioligand in human brain. Scatchard analysis of the binding of this ligand to membranes derived from striatum demonstrated a Kd of 31 pM and a Bmax of 150 fmol/mg P. Binding to frontal and temporal cortex evidenced a significantly higher affinity (18 pM) and lower Bmax (5 and 13 fmol/mg P, respectively). The pharmacological profile of bindng in frontal and temporal cortex was similar to striatum (epidepride> spiperone>butaclamol>flupenthixol>clozapine). Autoradiography also demonstrated the greatestbinding in the striatal complex with D2 receptor density higher by 20-50% in the matrix than striosomal compartments, as demonstrated by comparison to AChE-rich and poor areas in serially adjacent sections. The density declined somewhat at more caudal levels of the striatum, with striosomal/matrix patterns less discernable. The striatum showed 2-fold higher binding than the globus pallidus external which was 50-70% greater in density than the globus pallidus internal. Binding was also dense in a ventral extension of the putamen. Binding in the substantia nigra was 1/8 of that in the striatum, with the A8 and A10 complex exhibiting very low binding. Binding to all cortical areas showed a laminar pattern. Binding in the frontal cortex was densest in the internal layers. Temporal cortex showed higher binding with the order being external layer > internal > middle layers. Within the hippocampal complex binding was low but evident in CA1 and subiculum and almost nonexistent in the presubiclum or other regions of parahippocamapl gyrus. Binding in the occipotemporal gyrus was similar to other regions of temporal cortex. **Funded by MH 43852, MH 43880, MH 45372.**

226.3

[^{125}I]Epidepride Autoradiography and In Situ Hybridization of Extrastriatal Dopamine D2 receptors. A. Janowsky, K.A. Neve, J.M. Kinzie*, T. dePaulis*, G. Higgins, and J. Belknap. VAMC, Portland,OR; Vanderbilt Univ., Nashville, TN; NIA/NIH, MD.

The rank order of potency for the displacement of sodium-dependent [^{125}I]epidepride binding by various drugs suggests that the radioligand is binding to D2 receptors in extrastriatal regions that contain a very low density of binding sites. Autoradiographic analysis of rat brain sections (16um) indicated that [^{125}I]epidepride binding reached equilibrium within 30-45 min in the presence, or within 15 min in the absence of NaCl. K_d values for binding to coronal sections through the caudate or hippocampus were similar to those described for ligand binding to membranes (30-60pM). The B_{max} in sections of caudate nucleus (24 fmol/section) was 15x higher than the binding site density in hippocampal sections. In addition to very high binding in striatum, accumbens, and olfactory tubercle, binding to D2 receptors was observed in cerebellar lobes 9 and 10, in specific layers of the hippocampus, amygdaloid nuclei, and the entorhinal cortex. *In situ* hybridization using probes for $D2_{415}$ and $D2_{444}$ indicated that the long form was more abundant in the olfactory tubercle and entorhinal cortex. The two forms were more evenly distributed in neocortex. The effect of chronic administration of a number of psychotropic drugs, including clozapine, haloperidol, and amphetamine, was also examined. Data suggest that [^{125}I]epidepride is useful for characterizing changes in D2 receptors in brain regions that contain a small density of D2 receptor sites. Supported by NIMH grant MH42894 (A.J.).

226.4

[^{125}I]SCH 23982 BINDING TO DOPAMINE D1 RECEPTORS IN HUMAN BRAIN. A. Murray,* A. Ouyang and J.N. Joyce. Departmentsof Psychiatry , Pharmacology and Medicine (GI section), University of Pennsylvania School of Medicine, Philadelphia , PA .

[^{125}I]SCH 23982 shows high affinity and selectivity for dopamine (DA) D1 receptors in rat brain (Manik et al, 1988; J. Neurochem, 51:391-397) We are examining the distribution and pharmacology of this radioligand in human brain. Autoradiographic analysis also demonstrated the densest binding in the striatal complex with D1 receptor binding 2-fold higher than the globus pallidus external which was 50-70% greater in density than the globus pallidus internal. Binding to all cortical areas showed a laminar pattern. Binding in the frontal cortex was densest in the internal layers. Temporal cortex showed a different pattern with the order being middle> internal = external layers. Insular cortex showed higher binding than other cortical regions with two distinct lamina exhibiting higher binding. Binding in the substantia nigra was low.. -Within the hippocampal complex binding was low but evident. Binding in the occipotemporal gyrus was similar to other regions of temporal cortex. Scatchard analysis of the binding of this ligand to membranes derived from striatum appears similar to that in cortical regions. The pharmacological profile is being currently examined for different regions. Preliminary studies indicate that postmortem tissue derived from cases diagnosed with Parkinson's disease and schizohrenia exhibit reduced density of binding in cortex and limbic regions of striatum. **Funded by MH 43852, MH 43880-**

226.5

EFFECTS OF 6-OHDA AND PERTUSSIS TOXIN LESIONS.ON D_1 AND D_2 RECEPTOR ACTIONS IN THE STRIATUM. Ingrid Strömberg[1] and Paula Bickford-Wimer[2] [1]Dept Histology and Neurobiology, Karolinska Institute, Stockholm, Sweden, and [2]Vet Admin Med Ctr, Denver, CO, USA

The relationship between dopamine D_1 and D_2 receptors was investigated in the rat striatum using a combination of lesioning techniques. Differences in the transduction mechanisms for D_1 & D_2 receptors were exploited to selectively inactive D_2 receptors with pertussis toxin (PT). Rats were injected with 6-OHDA into the medial forebrain bundle and those showing a double peak rotational pattern with apomorphine were selected for further investigations. PT was injected into the DA depleted striata. Two days after the PT lesion extracellular electrophysiological recordings were performed with multibarrel glass micropipettes in urethane anesthetized rats. The D_1 agonist SKF 38393 (1mM) or the D_2 agonist NO437 (1mM) were applied locally by micropressure ejection. There was a significant reduction in response to N0437 from striatal cells in rats with 6-OHDA/PT with 80% of cells demonstrating no response, while 90% of cells responded in the contralateral control stiata. The response to SKF 38393 was also altered in these rats with a reduction in SKF 38393 responses on the lesioned side. Striatal cells from rats with only 6-OHDA lesions showed supersensitivity to NO437 on the lesioned side, however, SKF 38393 was equipotent in both striata. In conclusion, these data demonstrate that a combination of 6-OHDA and PT lesions gives rise to a subsensitivity of D_1 receptors in striatal neurons, which might be due to the inactivated D_2 receptor. (VAMRS and NS09199)

226.6

DOPAMINE D_2 RECEPTORS IN THE NUCLEUS ACCUMBENS AND STRIATUM OF ALCOHOL-FED RATS. M.J. Druse and S. Pellegrino* Department of Biochemistry, Loyola U. of Chicago, Stritch Sch. of Medicine, Maywood, IL 60153.

The present study examined the effects of chronic alcohol consumption on dopamine D_2 receptors in the nucleus accumbens and striatum. Male Fisher 344 rats were pair-fed control or 6.6% (v/v) ethanol-containing liquid diets for 4-5 weeks prior to sacrifice at 3 months of age. Total dopamine D_2 receptors were measured using the antagonist, [^{3}H]-spiperone. The number of high affinity D_2 receptors and their conversion to the low affinity form were determined using [^{3}H]-NPA (propylnor-apomorphine) in the absence and presence of 300 μM GTP.

Following 4-5 weeks of ethanol consumption, rats had blood alcohol levels of ~100 mg/dl during the active feeding period. In the nucleus accumbens and striatum no statistical differences were found in either the Bmax or Kd of total D_2 binding sites. In addition, we did not detect any significant differences in the number of high affinity dopamine D_2 binding sites or in their conversion to the low affinity form. A normal D_2 receptor number differs with our observation of significantly decreased dopamine D_1 receptors in the nucleus accumbens and cortex of similarly treated rats.

226.7

MICRODIALYSIS MEASURES OF DOPA ACCUMULATION AFTER GBL ADMINISTRATION: AN *IN VIVO* MEASURE OF DOPAMINE SYNTHESIS MODULATING AUTORECEPTORS. J.D. Lory, C.W. Bradberry and R.H. Roth. Depts. Pharm. & Psych., Yale Univ.Sch. Med., New Haven, CT 06510.

We developed an *in vivo* model system for determining the presence of synthesis modulating autoreceptors in distinct dopamine projection areas in rat brain. The L-aromatic amino acid decarboxylase inhibitor, NSD 1015 (10^{-5}M), was infused through the dialysis probe and L-DOPA accumulation monitored as an index of tyrosine hydroxylation (TH). Inhibition of impulse flow after the administration of γ-butyrolactone (GBL) produced a 2-3 fold increase in the rate of TH in the caudate. This effect was blocked by addition to the perfusion buffer of a micromolar concentration of the autoreceptor agonist EMD-23488. Since EMD 23488 does not exert direct inhibitory effects on TH activity *in vitro*, the blockage of the GBL induced increase was consistent with an autoreceptor mediated event. In the prefrontal cortex, a brain region believed to lack synthesis modulating autoreceptors, *in vivo* TH was unaffected by blocking impulse flow with GBL. These observations suggest that extracellular DA does not influence prefrontal DA synthesis and that regionally selective changes in DOPA accumulation following GBL administration may be a useful marker of functional synthesis modulating DA autoreceptors. Supported by MH 14092 and the Amer. Heart Assoc.

226.8

SOME TERMINAL RELEASE CHARACTERISTICS OF DOPAMINE NEURONS AFTER CHRONIC HALOPERIDOL TREATMENT. B.S. Bunney and B. Moghaddam, Dept. Psychiatry and Pharmacology, Yale Univ. Sch. Med. New Haven, CT 06510.

Functional consequence of chronic treatment with haloperidol (CHAL, 0.5 mg/kg s.c. for 21-23 days) on terminal release of dopamine (DA) in the striatum was examined in the rat using microdialysis techniques. Basal extracellular DA concentrations were not significantly different in vehicle and CHAL treated animals. Local application of 30 mM K^+ increased release similarly in both sets of animals. A challenge dose of haloperidol two days post treatment increased DA release more in CHAL animals than in controls. Direct application of TTX onto the DA terminals or the medial forebrain bundle completely abolished extracellular DA in both control and CHAL treated animals. These results suggest that DA release after CHAL is impulse-dependent and that depolarization block induced by CHAL does not decrease DA release below basal levels. Furthermore, extracellular DA as measured by microdialysis does not appear to be dependent on afferent striatal inputs (e.g. glutamate) since blockade of axonal impulse flow by TTX completely abolished DA release. Supported in part by MH 28849 and MH 25642.

226.9

TURNOVER RATES OF D1 DOPAMINE (DA) RECEPTORS IN THE STRIATUM (ST), N. ACCUMBENS (NA), S. NIGRA (SN) AND RETINA (RET) OF ADULT AND AGED RATS. O. Giorgi, M.G. Pibiri*, R. Dal Toso 1, G Toffano 1 and G. Biggio. Dept. of Exp. Biology, Chair of Pharmacology, Univ. of Cagliari, and 1 FIDIA Res. Labs, Abano Terme, ITALY.

The present study was designed to investigate the steady state levels and the turnover rates (TR) of D1 DA receptors in the ST, SN, NA, and RET of 4 months old and 23 months old Sprague-Dawley rats by monitoring the increase in the density (Bmax) of 3H-SCH 23390 binding after irreversible inactivation of D1 DA receptors by N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ, 10 mg/kg, s.c.). Significant differences were detected in the TR of D1 DA receptors in the neural tissues analyzed in adult rats, as reflected by the estimated half-lives (T1/2): 36 h, 75 h, 78 h, and 154 h in the RET, ST, NA, and SN, respectively. These differences may be related to the presynaptic (i.e., SN) versus postsynaptic (i.e., ST and RET) localization of the D1 DA receptors. A decrease in the steady state density of D1 DA receptors was observed in the ST (-25%), in the SN (-23%), and in the NA (-28%) of aged rats as compared with their adult counterparts. In contrast, an age-related increase in the density of D1 DA receptors was detected in the RET (+31%). The receptor turnover studies revealed that both the receptor synthesis and degradation rates are reduced in the ST, NA, and SN of aged rats. In contrast, the degradation rate of D1 DA receptors decreases in the RET of aged rats, whereas the synthesis rate remains unchanged. The latter results may account for the age related changes in the density of D1 DA receptors.

226.10

CANDIDATE AFFINITY LIGANDS FOR D_1 AND D_2 DOPAMINE RECEPTORS. J.L. Neumeyer, R.J. Baldessarini, N.S. Kula*, V. Bakthavachalam*, J. Yuan*, N. Baindur* and A. Campbell.* Research Biochemicals, Inc., 1 Strathmore Road, Natick, MA 01776; Neuroscience Program, Harvard Medical School, Mailman Research Center–McLean Hospital, Belmont, MA 02178

Novel derivatives of known D_1 or D_2 selective dopamine agonists and antagonists were prepared and tested for affinity in radioreceptor assays, using rat striatal membranes and ^{3}H-SCH-23390 (D_1) or ^{3}H-YM-09151-2 (D_2) as radioligands. Among D_1 agonist derivatives, 6-halogen-7,8-di-OH-benzazepines (including N-allyl congeners) had high D_1 affinity (IC_{50}=4–10 nM) and D_1/D_2 selectivity (70-900x) as racemates or as their more potent R[+] isomers. Of several D_1 antagonist 1-(p-aminophenyl)-7-Br-8-OH-benzazepines with alkylating substituents on the phenyl moiety, the p-(β-chloroethylamino) derivative was the most potent (IC_{50}=1 nM) and selective (4500x) for D_1 receptors. Of several D_2 agonist N-propyl-N(p-aminophenylethyl)-5-OH-tetralins (PPHTs), those with isothiocyanate, N-ethylfumaryl, or N-bromoacetyl alkylating p-substituents on the phenylethyl moiety were highly potent (IC_{50}=0.2–0.7 nM) and selective (300–1500x) for D_2 over D_1 receptors. Several D_2 antagonist N-p-aminophenylethylspiroperidols (NAPS derivatives) with the same alkylating moieties on the N-phenyl substituent also were highly potent (IC_{50}=0.4–1.9 nM) and D_2 selective (220–450x). Biological activity of compounds with and without alkylating moieties was compared by ability to block radioreceptor binding in vitro or ex vivo, or ability to block behavioral actions of apomorphine, over time. The duration of action of NAPS was similar to that of its presumably alkylating isothiocyanate congener (NIPS). Some of these compounds or other analogs containing alkylating moieties may be suitable for development as selective, irreversible affinity labeling agents for D_1 or D_2 receptors. [Supported by grants from the USPHS (MH-34006, MH-45692, and MH-47370) and the Bruce Anderson Foundation .]

226.11

DOPAMINE DEPOLARIZES RAT SUPRAOPTIC NEURONES BY AN INTRACELLULAR CALCIUM-DEPENDENT CATIONIC CURRENT: AN IONIC SUBSTITUTION STUDY. C.R. Yang, C.W. Bourque and L.P. Renaud. *Centre for Research in Neuroscience, Montreal General Hospital & McGill University, 1650 Cedar Avenue, Montreal, Canada, H3G 1A4.*

Magnocellular neurosecretory neurones (MNCs) of rat supraoptic nucleus (SON) receive a prominent dopaminergic innervation. Activation of post-synaptic dopamine(DA) D2 receptors on MNCs depolarizes the cells by an increase in conductance via TTX-insensitive, voltage- and [Ca^{++}]o-independent mechanisms (Yang et al., Soc. Neurosci. Abst., 15:425, 1989). We now further characterize the ionic basis of the DA-mediated conductance change and evaluate its [Ca^{++}]i-dependence as a possible link in signal transduction.

Intracellular recordings were obtained from MNCs in superfused rat hypothalamic explants. In 10 MNCs recorded in normal ACSF ([Na^+]o= 140mM), DA (20-70μM) induced depolarization with a mean reversal potential (V_R) of -20 ±4mV. Complete substitution of [Na^+]o with equimolar TRIS (TRIZMA, Sigma) resulted in a significant ($p<0.001$) negative shift of V_R to -70±3.7mV, consistently less negative than the equilibrium potential for K^+. Hence, the DA-induced response is largely mediated by Na^+ influx through cationic channels that are also permeable to TRIS. To determine if this DA-induced depolarization was mediated by [Ca^{++}]i, recordings were obtained with microelectrodes containing BAPTA (0.2M in 2M KAc+0.4M KCl), a Ca^{++}-chelator, in 6 MNCs. Intracellular injection of BAPTA (90±5mins) blocked the DA-induced depolarization, as well as the Ca^{++}-dependent post-burst after-hyperpolarization (evoked by 1-2s, 0.2 to 0.7nA pulses) while glutamate-induced depolarization remained unchanged. These data suggest that the depolarizing response of MNCs to DA is associated with an increase of non-selective cationic conductance and requires mobilization of [Ca^{++}]i. (Supported by FCAR, FRSQ & MRC)

226.12

BINDING CHARACTERIZATION OF A NOVEL D_1-RECEPTOR ANTAGONIST, [^{3}H]SCH39166, AND AUTORADIOGRAPHIC COMPARISON WITH [^{3}H]SCH23390. M.E. Alburges, M. Hunt, R.D. McQuade[1] and J.K. Wamsley. Neuropsychiatric Res. Inst., Fargo, ND 58103. [1]Schering-Plough Res., Bloomfield, NJ 07001.

In the search for a more selective D_1-antagonist, Schering-Plough Research synthesized a new benzazepine compound, SCH39166. We have characterized the binding of this D_1-antagonist to the rat brain and compared the distribution of [^{3}H]SCH39166 and [^{3}H]SCH23390 labeled sites. The K_d, determined from dissociation and association rate constants (1.09 nM) was comparable to the K_d value obtained from saturation studies (1.74 nM). [^{3}H]SCH39166 binds in a saturable manner with high affinity and low nonspecific binding to cortical tissue. Inhibition of the [^{3}H]SCH39166 binding by a series of dopaminergic and serotonergic antagonists substantiate the D_1 specificity of this compound.

Autoradiographic localization of D_1-receptors, using [^{3}H]SCH39166, indicates that binding density is high in caudate putamen, nucleus accumbens, olfactory tubercle, substantia nigra, and entopeduncular nucleus. Low binding occurs in lamina IV of cortex and in the choroid plexus (both areas where high receptor binding has been previously reported with [^{3}H]SCH23390). At low concentrations, [^{3}H]SCH39166 is a selective D_1 dopamine receptor antagonist with very little interaction with 5HT receptors in the CNS.

226.13

NIGRAL INJECTIONS OF EEDQ OR PERTUSSIS TOXIN (PT) PREVENT D-2 AGONIST EFFECTS ON SUBSTANTIA NIGRA PARS RETICULATA (SNpr) NEURONS. L.P. Martin, R.F. Cox, and B.L. Waszczak. Pharmacol. Sect., Northeastern Univ., Boston, MA 02115.

Iontophoresis of dopamine (DA) increases the firing rates of SNpr neurons, and it lessens their inhibition by applied GABA. The excitatory effect, which is mimicked by the D-1 agonist SKF 38393, was previously attributed to activation of D-1 receptors on striatonigral terminals. These studies were undertaken to determine if the GABA-modulatory effect, which is reproduced by the D-2 agonist LY 171555 (LY; 0.05 M), might be mediated by D-2 receptors intrinsic to SNpr neurons. Extracellular single unit recordings were made in anesthetized male rats which received: 1) nigral injections of either the receptor inactivator EEDQ (12.5 ug/0.5 ul in 22.5% Molecusol) or the Gi protein inactivator PT (1 ug/ul) 24 hrs prior to recording, or 2) striatal injections of kainic acid (KA; 1 ug/0.5 ul @ 2 sites) 1-2 wks prior. SN injections of both EEDQ and PT prevented the ability of LY to attenuate responses of SNpr cells to GABA (i.e. GABA potencies were reduced by 61±4% at 10 nA LY in control rats, but only by 5±10% in EEDQ- and not at all in PT-treated rats; n=7-17). Similar EEDQ treatments reduced SN D-1 and D-2 receptor densities by >90%. In contrast, striatal KA lesions which destroyed striatonigral inputs did not prevent the GABA-modulatory effect of LY. These data suggest that the attenuation of GABA effects by DA and LY is likely mediated by D-2 receptors located on SNpr cells. Support: NS 23541.

226.14

BHT 920 SELECTIVELY STIMULATES A SUBTYPE OF DOPAMINE D-2 RECEPTOR M.Pizzi*, M.Memo, A.Valerio*, C.Missale,L.Castelletti*,M.O. Carruba*,P.F.Spano. Inst. of Pharmacology and Exper. Ther., Brescia University, Brescia 25124-Italy

We have recently described the existence of two D-2 receptor subtypes named D-2a and D-2b. In particular, studies on mammotrophs (Castelletti L, et al. J.Physiology 410:251, 1989) have evidentiated that dopamine, through a receptor mediated mechanism, stimulates the opening of two different K+ channels, a calcium- and a voltage-dependent channel and that the activation of the former only, is related to a reduction of cAMP formation. In the present study we evaluated the ability of different dopaminergic drugs to selectively activate a single receptor-mediated transduction pathway. We found that quinpirole inhibited basal adenylate cyclase activity (IC-50 = 3uM) and activated both voltage-dependent and calcium-dependent (cAMP sensitive) K+ permeability (measured as 86-Rb efflux) in mammotroph cells. On the contrary, BHT 920 did not modify adenylate cyclase ativity up to 1 mM concentration and it did not activate calcium-dependent 86-Rb efflux. BHT 920 induced a stimulation of voltage-dependent component of K+ flux which was selectively blocked by 1-sulpiride (5 uM). The azepine derivative BHT920 pharmacologically characterizes the D-2 receptor subtype (named D-2b) which is uncoupled to adenylate cyclase and selectively associated to voltage-dependent K+ channels.

226.15

SELECTIVE DOPAMINE D1 AND D2 AGONISTS AFFECT DIFFERENT COMPONENTS OF FREE-RUNNING CIRCADIAN ACTIVITY IN RATS. N. Yamada* and M.T. Martin-Iverson. Neurochemical Research Unit, Dept. of Psychiatry, Univ. Of Alberta, Canada T6G 2B7

We investigated the effects of vehicle (V) or selective D1 (SKF 38393, S) and D2 (PHNO, P) agonists, given alone or together, on circadian rhythms of locomotor activity in rats kept singly housed under constant lighting conditions (continuous light or dark). Male Sprague Dawley rats (N=12 per group) were given subcutaneous infusions of V, P (5μg/h) and/or V or S (336μg/h) sustained for 14 days using Alzet osmotic minipumps. There were 4 independent drug-treated groups in each lighting condition, consisting of V+V, V+P, S+V or S+P. Locomotion was continuously determined by counting interruptions of infrared photobeams transecting the home cages, and reported in blocks of 1 h. Actograms were plotted, and the circadian rhythms were determined by the least squares methods. Parameters of the the rhythms were subjected to analysis of variance to determine significant drug effects. Circadian rhythms in locomotion were found to decompose under constant light, relative to the dark condition, with smaller amplitudes, less discrete onsets of activity, and variability in periods. The D2 agonist increased the amplitude ($F(1,44)=23.1$, $P < 0.001$) and the mesor ($F(1,44)=20.2$, $P < 0.001$) of the activity cycle, but had no effects on the length of the period. On the other hand, the D1 agonist increased the length of the period of the cycle ($F(1,44)=9.13$, $p < 0.005$), but had no effects on the amplitude and the mesor. There were no statistically significant interactions between the D1 and D2 agonists on circadian rhythms. These observations indicate that activation of the different dopamine receptor subtypes affect independent parameters of circadian rhythms of locomotion.

Acknowledgments: These studies were funded by AHFMR and PMHAC.

226.16

DELAYED EFFECTS OF D1 DOPAMINE RECEPTOR BLOCKADE IN THE MEDIAL PREFRONTAL CORTEX ON PRE- AND POSTSYNAPTIC DOPAMINE FUNCTION IN THE NUCLEUS ACCUMBENS.

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Acutely, D1 dopamine (DA) receptor blockade in the medial prefrontal cortex (mPFC) has been shown to potentiate the locomotor activating effects of amphetamine in the nucleus accumbens (N.Acc.). We now report that blockade of D1 DA receptors in the mPFC also produces delayed effects on DA function in the N.Acc. Rats received one intra-mPFC injection of saline (Control animals) or the D1 DA receptor antagonist SCH-23390 (0.25 μg/0.5μl/side). When tested two days post-injection, mPFC-SCH pretreated animals showed lower levels of locomotor activity than Control animals in response to intra-N.Acc. injections of amphetamine (1.5 μg/0.5μl/side). However, these animals also showed higher levels of locomotor activity (+98%) when tested with intra-N.Acc. injections of the D1 DA agonist SKF-38393 (1.0 μg/0.5μl/side) and a 30% increase in maximal DA-sensitive adenylate cyclase activity in comparison to Control animals.

These results demonstrate a delayed action resulting from cortical D1 DA receptor blockade and suggest, in a way consistent with previous findings (Hervé et al., J. Neurosci., 9: 3699, 1989), that mPFC D1 DA receptors are involved in the postsynaptic regulation of D1 DA receptors as well as the presynaptic regulation of DA activity in subcortical DA receptor fields such as the N.Acc.

226.17

METABOLIC CORRELATES OF D1 / D2 DOPAMINE RECEPTOR INTERACTION

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2-deoxyglucose autoradiography was used to characterize patterns of cerebral metabolism associated with D1 and D2 receptor stimulation in rats. In addition to controls treated only with saline, six groups of animals were pretreated with AMPT and then received s.c. injections of vehicle or of the selective D2 dopamine agonist LY171555 (LY) or D1 agonist SKF38393 (SKF) administered alone (3 mg/kg and 32 mg/kg respectively) or in combination: three drug combinations were utilized in which a fixed dose (3mg/kg) of the D2 agonist and escalating doses (2, 8 and 32 mg/kg) of the D1 agonist have been shown to produce increasing levels of behavioral disorganization characterized by intense stereotypy and sensory inattention.

Neither catecholamine depletion or the administration of LY or SKF alone to depleted animals produced significant changes in cortical glucose metabolism. The dose of LY 3mg/kg+SKF 2 mg/kg, which elicited organized motor activity and enhanced responsiveness to exteroceptive stimuli, also failed to produce such changes. However, the dose combinations which resulted in behavioral disorganization were characterized by significant decreases in metabolic activity in anterior and posterior cingulate and concomitant increases in lateral frontal and parietal cortices. These doses produced similar increases in metabolic activity in globus pallidus, subthalamic nucleus, entopeduncular nucleus and ventral-posterior caudate-putamen. In the diencephalon, AMPT pretreatment resulted in decreases in metabolic activity in centromedianum/parafascicular and ventrolateral thalamic nuclei and increases in the lateral habenula which were unaffected by SKF alone but partially reversed by LY alone and LY 3mg/kg+SKF 2 mg/kg. On the other hand, doses which elicit stereotypy and sensory inattention were associated with significant increases in metabolic activity in these thalamic nuclei and decreases in the lateral habenula. Synergistic effects of combined D1/D2 receptor activation were also evident in the dorsal raphe and locus coeruleus where metabolic activity was affected only by the combined administration of D1 and D2 agonists. Analysis of correlations between cortical and subcortical regions of interest revealed distinct patterns of functional coupling associated with organized and disorganized behavioral states. Additionally, administration of LY alone, which paradoxically induces catalepsy in catecholamine depleted animals, was associated with a marked increase in positive correlations between all regions of cortex, basal ganglia and thalamus.

226.18

EFFECTS OF MONOVALENT CATIONS ON DOPAMINE D_2 RECEPTORS LABELED WITH [^{3}H]RACLOPRIDE.

T.A. Reader, S. Boulianne*, E. Molina-Holgado* and K.M. Dewar. CRSN, Département de physiologie, Université de Montréal, Montréal Québec, Canada H3C 3J7.

Specific binding of [^{3}H]raclopride ([^{3}H]RAC) to dopamine (DA) D_2 receptors in the rabbit neostriatum was investigated in the presence of Na^+ and Li^+. Both cations produced dose-dependent elevations in specific [^{3}H]RAC binding; NaCl increased maximal binding two-fold as compared to LiCl. The inhibition of [^{3}H]RAC binding by the antagonist (+)butaclamol was unaffected by the addition of either Na^+ or Li^+ while the potency of DA to compete with [^{3}H]RAC was reduced. Both Na^+ and Li^+ diminished the affinity of DA for the high- and low-affinity states of the D_2 receptor and this effect was more pronounced in the presence of Na^+. The guanine nucleotide derivative 5'-guanylylimidodiphosphate or Gpp(NH)p reduced the potency of DA to compete with [^{3}H]RAC both in the presence and absence of cations; however, this effect of Gpp(NH)p was to shift the D_2 receptors from a high to a lower affinity state. Saturation curves revealed that these ions increased the affinity and the maximum density (B_{max}) of [^{3}H]RAC binding; the presence of Na^+ elicited a significantly greater change in the apparent dissociation constant (K_d) as compared to Li^+. In conclusion, our results show that [^{3}H]RAC binding to rabbit striatal membranes is regulated by cations. Moreover, although Li^+ and Na^+ influence specific [^{3}H]RAC binding in a similar manner, suggesting a similar site of action, there appear to be quantitative differences between the effects of these two ions.

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226.19

LOCOMOTOR ACTIVITY ELICITED BY SKF 38393 IN THE NUCLEUS ACCUMBENS.

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Dopamine D1 receptor agonists such as SKF 38393 have not been reported to elicit locomotor activity when given peripherally; only at high doses have sniffing and grooming been observed. Thus the role of the DA D1 agonist in behavior has been questioned. However, it has recently been shown that SKF 38393 injected directly into the nucleus accumbens (Acb) increased gross activity (Dreher and Jackson, 1989).

We have further examined the effects of SKF 39393 injected bilaterally into the Acb measured every 10 min for 2 hr in an activity monitor. Doses of 0.1, 1.0, and 10 ug/side all induced highly significant increases in locomotor activity relative to the vehicle, but the lowest dose induced the highest level of horizontal, vertical, and stereotyped activity. The effect of the lowest dose peaked at about 100 minutes after injection.

Since SKF 38393 injected directly into the Acb elicits dose- and time-dependent increases in locomotor activity, the role of DA D1 receptors in behavior must be reconsidered.

227.1

MUSCARINIC RECEPTOR MODULATION OF [^{3}H]ACETYLCHOLINE RELEASE IN MECHANICALLY-DISSOCIATED ADULT RAT HIPPOCAMPUS

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We have previously shown that a mechanical sieving method with adult rat brain tissue produces a metabolically active intact cellular preparation with which the study of M1 and M2 receptor coupling to phosphoinositide and cyclic AMP second messenger systems is facilitated (Mol. Pharmacol. 35:39). In the present study we evaluated whether autoreceptor modulation of evoked release of [^{3}H]acetylcholine (Ach) could be observed in this preparation. Adult rat hippocampal tissue was prepared as described and intracellular Ach stores were labeled with [^{3}H]choline in a Krebs buffer under O_2:CO_2 (95.5). In the presence of 100 uM physostigmine, 25 mM K+ was used to evoke [^{3}H]Ach release, which was purified by extraction and quantitated. High K+ stimulation for 15 min evoked 6.5 +/- 4.1 fold [^{3}H]Ach release over basal levels from dissociated hippocampal cells (about 4 mg per vial). Oxotremorine-M (EC_{50}=260 nM) and oxotremorine (EC_{50}=2.5 uM) inhibited the evoked release by 55 +/- 7% and 42 +/- 8%, respectively. Atropine fully reversed the effects of the agonists. These results indicate that this method of tissue preparation may provide a simple system for the evaluation of muscarinic autoreceptor function in brain.

227.2

"M-TOXIN", A NOVEL LIGAND FOR M1 MUSCARINIC RECEPTORS. S.I. Max and L.T. Potter. Department of Pharmacology, University of Miami School of Medicine, Miami, FL 33101.

Mamba venoms contain neurotoxins which act in unusual ways at cholinergic synapses. The venom of the green mamba, *Dendroaspis angusticeps*, contains dendrotoxin, which increases the release of acetylcholine by blocking potassium channels, fasciculin, which causes fasciculations by inhibiting acetylcholinesterase, and several toxins which partially block the binding of quinuclidinyl benzilate (QNB) to muscarinic receptors (Adem et al., Biochim. Biophys. Acta 968, 340). We purified the major antimuscarinic component of this venom, which we call "m-toxin", to assess whether it might be useful for receptor research. Active fractions were assayed for their ability to block the binding of ^{3}H-QNB or ^{3}H-pirenzepine to receptors in membranes from rat and rabbit brains. M-toxin was readily separated from other toxins by gel filtration, since it behaved like a globular protein of about 4000 D unlike other toxins of 6000-9000 D. Purification was completed by cation exchange chromatography. The N-terminal sequence of m-toxin appears unique. M-toxin was active on receptors from fresh or frozen tissue in the presence of EDTA, N-ethylmaleimide, PMSF or digitonin, but was inactivated by heat or SDS. M-toxin fully blocked the binding of 1 nM ^{3}H-pirenzepine to all parts of the rat brain, and reduced the binding of 1 nM ^{3}H-QNB to all M1 receptors as defined by the high affinity binding of pirenzepine. It was inactive on preparations rich in M2 receptors. Binding was effectively irreversible. Radiolabelling of m-toxin and production of antibodies are in progress. Thus, m-toxin appears very promising for studies of M1 receptors.

227.3

EVIDENCE FOR PAIRED M2 MUSCARINIC RECEPTORS.

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Mattera et al (JBC 260, 7410) found Hill coefficients of 1.3-1.5 for the binding of ^{3}H-quinuclidinyl benzilate (QNB) to cardiac M2 receptors exposed to the guanine nucleotide, GppNHp, suggesting bivalent receptors coupled with G-protein. Potter et al (Cell. Mol. Neurobiol. 8, 181) found equal numbers of GppNHp-sensitive high affinity sites (H) and GppNHp-insensitive low affinity sites (L) on hippocampal M1 receptors, suggesting dimeric receptors. Peterson et al (PNAS 81, 4993) found ~50% H sites on pure M2 receptors in solution. The possibility of paired receptors was examined further using M2 receptors from the rat brainstem. Binding assays with ^{3}H-N-methylscopolamine and QNB showed Hill coefficients close to 1.0 in the presence or absence of GppNHp. However competition curves between agonists and ^{3}H-NMS showed equal numbers of GppNHp-sensitive H sites and GppNHp-insensitive L sites for tertiary (e.g. oxotremorine), quaternary (e.g. cis-dioxolane) and alkylating agonists (acetylethylcholine mustard). The 50% H phenomenon persisted at different temperatures, after halving the number of receptors (and thus doubling the remaining G protein: receptor ratio), and after membrane solubilization. Selective occupation of H sites with agonist mustard, or of L sites with irreversible antagonists (in the presence of an agonist on H sites), yielded residual free receptors showing predominant L or H sites, respectively. The results support the concept of paired receptor sites.

227.4

Serotoninergic and Muscarinic Cholinergic Stimulation of Phosphoinositide-specific Phospholipase C in Rat Brain Cortical Membranes. **M.A. Wallace and E. Claro*, Dept. of Biochemistry, University of Tennessee, Memphis, TN 38163.**

Exogenously supplied substrates were used to measure guanine nucleotide dependant stimulation of phosphoinositide-specific phospholipase C (PLC) by muscarinic cholinergic and serotoninergic agonists in rat brain cortical membranes. Serotonin (5-HT), 5-methyltryptamine (5-MT), 5-fluorotryptamine and tryptamine stimulated PLC with EC_{50}'s of 1.7, 29.4, 15.0 and 11.2 μM, respectively. These agonists were all equally efficacious, with maximal PLC stimulation reaching about 30% of that obtained with carbachol. The rank order of potency for the muscarinic agonists was oxotremorine-M > pilocarpine = arecoline > carbachol = bethanecol. Unlike the case when using cortical tissue slices, all these agonists were fully efficacious at stimulators of PLC. Muscarinic agonists significantly lowered the apparent guanine nucleotide requirement for PLC stimulation. Serotoninergic agonists enhanced PLC activity but without any shift in guanine nucleotide requirements. Ketanserin blocked serotoninergic but not cholinergic activation of PLC, while atropine blocked the latter but not the former response. Dopamine inhibited the carbachol response (Wallace, M.A. and Claro, E. *Neurosci Letts* 110: 155, 1990) but not that due to 5-MT. The results indicate that serotoninergic and muscarinic cholinergic agonists stimulate PLC through distinct mechanisms in brain cortex.

227.5

N-ETHYLMALEIMIDE (NEM) AFFECTS [^{3}H]QUINICLINIDIL-BENZILATE ([^{3}H]QNB) BUT NOT [^{3}H]PIRENZEPINE ([^{3}H]PZ) BINDING IN THE PRIMATE CEREBRAL CORTEX.

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It is well established that the sulfhydryl alkylating agent, NEM, modulates the affinity of muscarinic receptors for agonists by uncoupling them from nucleotide regulatory proteins (uncoupling of R-Gi complex). However, the influence of sulfhydryl alkylation on antagonist binding is still a matter of controversy. We have investigated the effect of NEM on the binding of muscarinic antagonists, [^{3}H]QNB and [^{3}H]PZ in the primate cerebral cortex. Temporal cortex of the rhesus monkey (n = 3) was homogenized in 10 mM Na-K–phosphate buffer (pH 7.4) containing 1mM EDTA. Incubation with [^{3}H]QNB and [^{3}H]PZ was carried out in the same buffer in the presence or in the absence of NEM and was terminated by rapid filtration. Nonspecific binding was determined in the presence of 1 µM atropine sulphate. In the absence of NEM, an analysis of the [^{3}H]QNB saturation curve allowed to discern only a single site with K_d = 9.4±1.7 pM (B_{max} = 56.9±8.1 fmol/mg prot.). In the presence of NEM, however, an additional site with K_d = 297.6±96.0 pM (B_{max} = 188.6±36.3 fmol/mg prot.) could be easily detected. The addition to incubation media of 2 µM PZ resulted in the disappearance of the high but not the low affinity sites indicating that the former are likely of M_1 type. The substitution of NEM with 5'-guanyl imidodiphosphate (which along with NEM uncouples R-Gi complex) did not unmask the low affinity sites. Thus, the effect of NEM on [^{3}H]QNB is probably not connected with uncoupling of R-Gi complex. In contrast, we found no influence of sulfhydryl alkylation on specific binding of [^{3}H]PZ. In the temporal cortex, this radioligand binds to a single site with K_d = 3.0±0.4 nM (B_{max} = 65.2±3.7 fmol/mg prot.) in the presence and in the absence of NEM. Thus, although it is widely believed that sulfhydryl alkylation does not affect muscarinic antagonist binding in the central nervous system, our data reveal that sulfhydryl alkylation agent, NEM, can influence the binding of some antagonists in the primate cerebral cortex.

227.6

AN IMMUNOCYTOCHEMICAL AND AUTORADIOGRAPHIC STUDY ON THE CELLULAR LOCALIZATION OF CHOLINERGIC MARKERS IN THE HUMAN CEREBELLUM.

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Although the existence of cerebellar muscarinic receptors and afferent cholinergic projections is well known, the cellular localization for the binding of acetylcholine remains controversial. Several studies have suggested the presence of cholinergic markers in different types of cerebellar neurons (Kan et al, Brain Res 193:165-171; Neustadt et al, Brain Res Bull 20:163-172, 1988). The purpose of this study was to elucidate the cellular localization of muscarinic receptors in the humans cerebellum by using [3H]QNB autoradiography (1 nm, 20 mm Tris, pH 7.4) and choline acetyltransferase (ChAT) immunocytochemistry (1:3 dilution, Boehringer Mannheim). Our results indicate that muscarinic receptors in the cerebellar folias are primarily found in the granule cell layer. [3H]QNB binding was completely abolished when using atropine (1 um) as a blocker but not when using pirenzepine (1 um), suggesting the receptors are of the non-M1 type. Immunocytochemistry for ChAT revealed positive staining fibers surrounding granule cells and occasionally in structures resembling glomeruli. Positive staining varicosities aggregated around stellate and basket cells. Both Golgi and Purkinje cells revealed no signs of ChAT immunoreactivity. In summary, the human cerebellum contains muscarinic (non-M1 type) receptors in the mossy fibers that terminate in the granule cell layer. The prominent ChAT immunoreactivity of terminals in the molecular layer but non surrounding Purkinje cells suggests the presence of cholinergic afferents stemming from a source other than climbing fibers.

227.7

COCAINE INTERACTIONS WITH PRIMARY AND SECONDARY RECOGNITION SITES ON MUSCARINIC RECEPTORS. A.A. Vaishnav, D.C. Mash and D.D. Flynn. Depts. of Pharmacology and Neurology, Univ. of Miami School of Medicine, Miami, FL 33101.

Several lines of evidence have suggested that muscarinic receptors may possess more than one ligand binding site. Muscarinic receptors contain several aspartic acid residues, which are conserved in other members of the large family of membrane-spanning, G-protein coupled receptors. These residues appear to be differentially involved in ligand binding (Fraser et al., Mol.Pharmacol. 36: 840, 1989). In addition, a number of quaternary muscarinic antagonists, most notably gallamine, display both competitive as well as allosteric binding properties at the muscarinic receptor. We have characterized the binding of cocaine to muscarinic receptors in membrane homogenates from post-mortem human brainstem. (-)Cocaine inhibits binding of the tritiated muscarinic antagonists, N-methylscopolamine (NMS) and pirenzepine, to an apparent single class of sites with a K_i of 200-300 μM. (+)Cocaine is 10-fold more potent in inhibiting binding of these ligands. The binding of cocaine is unaffected by guanine nucleotides and is not apparently selective for either the M1 or M2 receptor subtypes. Rosenthal analyses of saturation binding data obtained in the presence of increasing concentrations of cocaine demonstrated that both the K_d and B_{max} values for ^{3}H-NMS are increased. These data suggest both competitive and non-competitive interactions of cocaine with muscarinic receptors. Kinetic data revealed a slowing of the rate of dissociation of ^{3}H-NMS by cocaine by 20-fold, but not of ^{3}H-QNB, ^{3}H-pirenzepine or ^{3}H-oxotremorine-M. This finding is consistent with previous reports that binding of ^{3}H-NMS is more susceptible to allosteric modulation than ^{3}H-QNB or ^{3}H-pirenzepine (Lee and El-Fakahany J.Pharmacol.Exp.Ther. 246: 829, 1988). These results suggest that cocaine may recognize two distinct binding sites on muscarinic receptors.

227.8

DISTRIBUTION OF MUSCARINIC CHOLINERGIC RECEPTORS IN THE DEVELOPING HUMAN BRAINSTEM. H.C. Kinney, V. Greenier*, W.F. White. Children's Hospital, Boston, MA 02115.

Acetylcholine has been implicated in brainstem mechanisms of cardiorespiratory control and arousal. Information about the quantitative distribution of muscarinic receptors in the developing human brainstem is relevant to understanding putative brainstem disorders of cardiorespiration and/or arousal, e.g., apnea of infancy, congenital central hypoventilation syndrome, sudden infant death syndrome. We determined the quantitative distribution of [3H]quinuclidinyl benzilate (QNB) binding to muscarinic receptors with computer-based, tissue section autoradiography. Frozen, serially-sectioned brainstems (postmortem interval 4-24 hrs) were analyzed from 3 fetuses (19-21 wks), 5 infants (1-9 months), 1 child, and 1 adult without neurologic disease. In infants, nuclei with high binding include: nucleus tractus solitarii (NTS), parabrachial complex, griseum pontis, cranial nerve motor nuclei; intermediate binding: reticular formation; low binding: inferior olive. By mid-gestation the relative distribution of muscarinic receptors is established and is similar to that in the infant. Quantitative changes in [3H]QNB binding occur in individual nuclei across the time period studied. The relative distribution also appears to change in the mesencephalic reticular formation from infancy to adulthood. This study provides baseline information for muscarinic receptor analysis in developmental brainstem disorders.

227.9

COMPARATIVE EXPRESSION OF CHOLINERGIC MARKERS DURING BRAIN DEVELOPMENT. I.Aubert, D.Cécyre*, D.M.Araujo, S.Gauthier and R.Quirion. Douglas Hospital Research Centre, McGill University, Dept. Neurology & Neurosurgery, and McGill Centre for Studies in Aging, Montreal, Quebec, Canada H4H 1R3.

The comparative ontogenic profiles of various cholinergic markers were studied in the rat brain using an *in vitro* receptor autoradiographic method. Brain sections from animals at embryonic days 18 and 20 (E18 & E20), postnatal days 1 to 35 (P1 to P35) and adult (3 months) were processed with either [^{3}H]QNB (total population of muscarinic sites), [^{3}H]pirenzepine (M1 sites), [^{3}H]acetylcholine, [^{3}H]AF-DX 116 or [^{3}H]AF-DX 384 (putative M2 sites), [^{3}H]*N*-methylcarbamylcholine (nicotinic sites) and [^{3}H]hemicholinium-3 (HC3) (high affinity choline reuptake sites).

Our results reveal the early appearance of the M1 receptor sites in most brain regions labelled in adult brain, including the striatum (E18) and the hippocampus (E20). In contrast, M2 binding sites, labelled by various ligands, seemed to have developed more slowly. Significant labelling was observed only during the second and third weeks of development (P14-P21), with few possible exceptions such as the anteroventral nucleus of the thalamus which is labelled at E20.

This may suggest that the appearance of M2 sites is delayed (compared to M1 sites) because of their presynaptic location. Another marker of presynaptic cholinergic terminal (HC3) also possessed a slow maturation.

Finally, nicotinic sites are present as early as E18 and E20 in various brain regions suggesting a postsynaptic localization in addition to a possible presynaptic distribution in certain areas (Araujo et al., *J.Neurochem*, 51:292-299, 1988). (MRC, Canada).

227.10

LONG-TERM CHANGES IN PHOSPHOINOSITIDE HYDROLYSIS FOLLOWING COLCHICINE LESIONS OF THE NUCLEUS BASALIS. W. R. Mundy, P. Tandon, S. Barone Jr. and H. A. Tilson. Lab. of Molec. and Integrative Neurosci., NIEHS/NIH, Research Triangle Park, NC 27709.

Long-term changes in the functional integrity of cholinergic muscarinic receptors following lesion-induced cholinergic denervation are not well studied. We examined muscarinic receptor-mediated phosphoinositide (PI) hydrolysis in rat cortical slices up to 14 months after lesions of the nucleus basalis (NB). Rats received bilateral injections of colchicine (1.5 µg/0.5 µl) into the NB and were sacrificed at 1, 3, or 14 months after surgery. Colchicine lesions resulted in a loss of ChAT-immunoreactive cells at 1 month after surgery which was still apparent at 14 months. Choline acetyltransferase activity in the cortex was decreased by 43% one month after lesioning, but returned to control levels by 3 months. The muscarinic agonist carbachol produced a concentration-dependent increase in PI hydrolysis, which was enhanced 3 and 14 months after NB lesions compared to sham-lesioned controls. No change was observed 1 month after lesioning. Norepinephrine and quisqualate-stimulated PI hydrolysis was also enhanced 14 months after NB lesions. These results suggest a slowly developing up-regulation of post-synaptic receptor function following pre-synaptic loss of neurotransmitter.

227.11

RETENTION OF COUPLING BETWEEN M1 MUSCARINIC RECEPTORS AND G-PROTEIN AFTER CHOLINERGIC DENERVATION, WITH AGING, AND IN ALZHEIMER'S DISEASE (AD). B.D. Pearce and L.T. Potter. Deptartment of Pharmacology, University of Miami School of Medicine, Miami, FL 33101

Numerous studies have shown that cerebral M1 receptors are retained after cholinergic denervation and in AD, but few studies have addressed the functional status of these receptors after long-term denervation. We examined the first step in the M1 transduction mechanism, the interaction between receptors and G-protein, one year after unilateral denervation of the rat hippocampus, with aging, and in normal and Alzheimer cerebral cortex. Competition experiments were performed between 1 nM ^{3}H-pirenzepine (which selectively labels the m1 subtype of muscarinic receptors) and oxotremorine-M in the absence and presence of the guanine nucleotide, GppNHp (Potter et al, Cell. Mol. Neurobiol. 8, 181). Retention of a high percentage of high affinity binding sites (% H) and of a substantial loss of these sites in the presence of GppNHp was taken as evidence of receptor: G protein interactions. Normal and denervated hippocampi showed similar % H values (52.3 vs 56.5%) and $3/4^{ths}$ of these sites converted to low affinity with 0.2 mM GppNHp. The cortices from five human controls and seven AD patients also showed similar % H values (37.4 ± 6.4 vs 31.7 ± 4.5%) and $3/4^{ths}$ of these sites were also sensitive to GppNHp. The AD tissue had 68% less choline acetyltransferase activity. There was no correlation between % H and age in rats 1.5-29 months old, or in humans aged 10-85 years. The affinities of high and low affinity binding sites were constant. The data indicate that early steps in receptor transduction remain functional despite prolonged receptor inactivity.

227.12

NEUROPEPTIDE MODULATION OF CARBACHOL-STIMULATED PHOSPHOINOSITIDE METABOLISM IN FRONTAL CORTEX OF AGED RATS. M. A. Rice* and N. W. Pedigo. Dept. Pharmacology, Univ. Kentucky, Lexington, KY 40536.

Several neuropeptides are thought to play an important role in modulating muscarinic transmission in the CNS. Neurotensin (NT), cholecystokinin (CCK), somatostatin (ST), and vasoactive intestinal polypeptide (VIP) were assayed for their ability to modify carbachol-stimulated phosphoinositide (PI) metabolism in cortical slices from young (3 mo) or senescent (27 mo) rats. At least 6 concentrations of carbachol were used in the absence or presence of neuropeptide (1.0 μM final concentration). Results indicated that NT, CCK and VIP were able to potentiate the effects of carbachol in young rats, while somatostatin was without effect. However, only neurotensin significantly enhanced carbachol's effects in senescent animals.

Table 1: ED50 for Carbachol (μM)

Drug	3 mos	27 mos
Control	168 ± 15.5 (10)	146 ± 20.7 (8)
Neurotensin	74 ± 5.8 (6)*	50 ± 8.6 (7)*
CCK	82 ± 16.7 (5)*	87 ± 15.9 (4)
Somatostatin	193 ± 30.9 (5)	154 ± 20.2 (3)
VIP	70 ± 8.9 (5)*	78 ± 17.7 (4)

* $p<.05$ vs age-matched control (Dunnett's test)

These results show the ability of certain neuropeptides to facilitate carbachol-stimulated PI metabolism is not completely retained in cortical tissues of senescent rats.(Supported by an ADRDA grant).

227.13

CHOLINERGIC RECEPTOR ALTERATIONS IN THE CNS OF POST-MORTEM HUMAN ALZHEIMER'S DISEASED TISSUE. X. Ming, L. Tsiokas, Z. Jelisijevic*, S.C. Zhang* and M. Watson. Dept. of Pharmacology, Univ. of Medicine & Dentistry of New Jersey-New Jersey Medical School, Newark, N.J. 07103-2714.

Expression cloning and molecular pharmacologic studies reveal the existence of five muscarinic acetylcholine receptor (mAChR) subtypes. Attention has focused on changes in mAChR subtypes in Alzheimer's Disease (AD). Binding, biochemical and radioautographic studies of human post-mortem brain tissue from AD and control (C) patients have been performed. Our data suggest alterations may occur in mAChR subtypes seen on both pre- and postsynaptic neurons. Assays of [^{3}H](-)quinuclidinylbenzilate ([^{3}H](-)QNB), a highly specific but non-subtype selective antagonist, [^{3}H](+)cis-methyldioxolane ([^{3}H](+)CD), which labels a super high affinity mAChR agonist state, [^{3}H]pirenzepine ([^{3}H]PZ), an M1 selective antagonist, [^{3}H]AF-DX 116, a somewhat M2 selective antagonist and [^{3}H]hemicholinium-3 ([^{3}H]HC-3), an inhibitor of sodium-dependent high affinity choline uptake were done as described. No differences in affinity (Kd) values were seen. Among the alterations in receptor density in AD were in the hippocampal formation where [^{3}H]HC-3 was as low as 18% of C and [^{3}H]PZ was 67% of C. Loss of presynaptic input to the hippocampus and an inability to up-regulate mAChR subtypes may reflect a pathological process in AD. Study of mAChR subtypes via in situ hybridization histochemistry offers an additional means by which such data may be further confirmed. MH43024.

227.14

AGING-INDUCED ALTERATIONS IN CNS MUSCARINIC ACETYLCHOLINE RECEPTOR SUBTYPES AND MRNA LEVELS IN A RODENT MODEL. M. Watson, L. Tsiokas, F. Hameed* and S.C. Zhang*. Dept. of Pharmacology, Univ. of Medicine & Dentistry of New Jersey-N.J. Medical School, Newark, N.J. 07103.

Molecular cloning data show the existence of five muscarinic acetylcholine receptor (mAChR) subtypes previously studied only by data from selective antagonists in binding and functional assays. A senescent rat model was used to study age-related changes in CNS mAChR subtypes. Assays of [^{3}H](-)quinuclidinylbenzilate, a highly specific nonsubtype selective antagonist, [^{3}H](+)cis-methyldioxolane, which labels the highest affinity agonist state, [^{3}H]pirenzepine, a specific M1 antagonist, [^{3}H]AF-DX 116, a somewhat M2 specific antagonist, and [^{3}H]hemicholinium-3 an inhibitor of sodium-dependent high affinity choline uptake were done as described. Quantitative receptor localization and in situ hybridization histochemistry (ISSH) was done with oligonucleotide probe complementary to 4-48/4-51 base sequence of m1-m5 mAChR mRNA by 3'-end labeling via ^{35}S-dATP (specific activity>2.4x10^{9}dpm/ug) by terminal deoxynucleotidyltransferase. Slices were hybridized (25°C; 24h), washed (Tm=55°C;2xSSC), dried, film-apposed (4wk;0-4°C) and quantified. No affinity (Kd) differences were noted. Despite low densities (<80%C) in many areas in 24m vs 3m old rats, many areas had no mRNA changes. Notably, cortex, with a huge loss in mAChRs had higher m3 mRNA levels. One may speculate this implies attempted up-regulation and is linked to lower cognition often seen in aging. MH-43024.

227.15

MUSCARINIC CHOLINERGIC RECEPTOR AGONISTS INHIBIT THE ACTIVITY OF SEROTONIN N-ACETYLTRANSFERASE IN BOVINE GLAND EXPLANTS IN CULTURE. P. Phansuwan-Pujito*, P. Govitrapong*, and M. Ebadi. Lab. of Neurobiol., Mahidol Univ., Salaya, Thailand, and Depts. of Pharmacol. and Neurol., Univ. of Nebraska Coll. of Med., Omaha, NE 68198-6260, U.S.A.

The mammalian pineal gland by synthesizing melatonin and by functioning as a biological clock orchestrates the actions of a plethora of receptors and synchronize the functions of numerous neurotransmitters in the CNS. By using tritiated muscarinic cholinergic receptor antagonist, quinuclidinyl benzilate ([^{3}H]QNB) as a ligand, Govitrapong, Phansuwan-Pujito, and Ebadi (Comp. Biochem. Physiol., 94:159-164, 1989) have identified muscarinic cholinergic receptors in the bovine pineal gland with K_D value of 0.423 ± 0.01 nM and B_{max} value of 69.75 ± 20.91 fmol/mg protein. In addition, a specific choline acetyltransferase has been reported in the bovine pineal gland by Phansuwan-Pujito, Govitrapong, and Ebadi (J. Pineal Res., 7, 1990, in press). In this communication, we report that cholinergic receptor agonists such as metacholine (10 μM), carbachol (10 μM), and oxotremorine (10 μM) inhibited the activity of serotonin N-acetyltransferase (SNAT) in the bovine pineal explants in culture, from a control value of 5.02 ± 0.45 to 1.25 ± 0.25, 1.30 ± 0.15, and 1.22 ± 0.20 pmol/mg protein/min; and this effects were blocked by atropine (20 μM) or QNB (20 μM). The presence of high affinity muscarinic cholinergic binding sites, of a specific choline acetyltransferase, along with dichotomous interaction of muscarinic receptors agonists-antagonists with SNAT, are interpreted to indicate that muscarinic cholinergic fibers modulate the synthesis and actions of pineal melatonin.

SECOND MESSENGERS II

228.1

MODULATION OF PHOSPHOLIPASE D ACTIVITY BY CLONED MUSCARINIC ACETYLCHOLINE RECEPTOR SUBTYPES.
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Human embryonic kidney cells (HEK), stably transfected with expression vectors for either the human muscarinic acetylcholine receptor (mAChR) subtypes Hm2 or Hm3 (Peralta et al., Nature 33: 434-437, 1988), were used to assess changes in the activities of phospholipase D (PLD) and of phosphoinositide-phospholipase C (PI-PLC) resulting from mAChR activation. Cellular phospholipids were labeled by incubating transfected HEK-cells for 24 h in the presence of [^{3}H]inositol and [^{3}H]oleic acid (2.5 uCi and 5 uCi per culture dish, respectively). For assaying PLD we used the transphosphatidylation property of this enzyme which, in the presence of ethanol, results in the production of phosphatidylethanol (PETH; e.g. Kobayashi, Kanfer, J. Neurochem. 48:1597-1603, 1987). When incubated in the presence of lithium chloride (10 mM) and ethanol (400 mM) for 10 min, the muscarinic agonist carbachol (1 mM) elevated [^{3}H]PETH levels by 14770±2015 cpm/mg protein (mean±SD from one representive experiment) in Hm3-expressing HEK-cells. In the same cells, the accumulation of [^{3}H]inositol phosphates was increased 9-fold (from 3180±280 to 27460±2440 cpm/mg protein) by carbachol. In Hm2-expressing cells, [^{3}H]PETH levels were elevated by 5850±1450 cpm/mg protein and [^{3}H]IPs levels increased 4-fold after a 10 min incubation with carbachol. The results show that the Hm3 mAChR subtype strongly activates PLD and PI-PLC whereas the Hm2 subtype stimulates the activities of either phospholipase much less effectively.

(Supported by NIMH grant MH-28783 and by a postdoctoral fellowship of the Deutsche Forschungsgemeinschaft to J.S.)

228.2

PRESENCE OF THE ANF-RECEPTOR COUPLED GUANYLATE CYCLASE IN CNS SYNAPSES. N. G. F. Cooper, A. A. Fedinec, and [1]R. K. Sharma, Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, TN 38163 and [1]Dept. of Brain and Vascular Research, Cleveland Clinic Foundation Res. Institute, Cleveland, OH 44195.

The ANF-receptor coupled guanylate cyclase is a particulate guanylate cyclase which contains a receptor for atrial natriuretic factor (ANF). The peptide, ANF, is reportedly present in the synaptic layers of the retina. ANF and the cyclic nucleotide, cGMP, have both been implicated in synaptic transmission in various regions of the nervous system. The objective of the present study was to determine if the antibody to particulate guanylate cyclase binds to synapses. Rats and chicks were overdosed with anesthetic, and perfusion fixed with 0.1% glutaraldehyde and 4% p-formaldehyde in buffered saline. Vibratome sections of retina and cerebellum were incubated with rabbit primary antibodies for 12 hrs, and then incubated in peroxidase conjugated secondary antibodies for 12 hrs. After peroxidase cytochemistry, sections were processed for electron microscopy, and thin sections were examined for presence of reaction product. Electron micrographs demonstrate the presence of the antigen in retinal synaptic terminals, in association with synaptic vesicles and synaptic terminal plasma membranes. In the molecular layer of the cerebellum, the antigen is present in postsynaptic densities and dendrites. The presence of antigen in these two different loci, strongly support a role for particulate guanylate cyclase in events associated with synaptic transmission, but the mechanism of its activation requires further study. Supported by NIH:NEI-EY2708 (N.G.F.C.), NIH:NS-23744, and NSF:DCB-8800953 (R.K.S.).

228.3

EFFECTS OF (n-3) FATTY ACID DEFICIENCY ON CALCIUM STIMULATION OF 1,2 DAG AND PA FORMATION IN RAT SYNAPTOSOMES.
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We have studied the effect of dietary deprivation of (n-3) fatty acids on the generation of 1,2 DAG and PA in response to Ca^{++} ionophore A23187 in rat cortical synaptosomes. Experiments were conducted in 9-month-old rats raised on semi purified diets containing either safflower oil [(n-3) deficient diet] or soybean oil (control diet). The levels of docosahexaenoic acid [22:6 (n-3)] in synaptosomal phospholipids and PA were significantly decreased in (n-3) deficient rats (25 to 35 % of control levels). This was compensated by a rise in 22:5 (n-6), the total content of polyunsaturated fatty acids remaining approximatively constant. Levels of phospholipids, PA and 1,2 DAG, were not affected by dietary treatment. Addition of the calcium ionophore A23187 (20 μM for 10') caused a two fold increase in the amounts of PA and 1,2 DAG. 1,2 DAG and PA generated in this condition have a different fatty acid compositions, suggesting that increase in intracellular Ca^{++} stimulates both phospholipases C and D pathways. Long term administration of (n-3) fatty acid deficient diet to rats results in a decreased production of 1,2 DAG in response to Ca^{++} ionophore, without modification of PA formation. Theses results indicate that (n-3) fatty acid deficiency can modulate the activation of phospholipase C pathway in response to Ca^{++} in rat synaptosomes independently of modification of PA formation.

228.4

PURIFICATION AND CHARACTERIZATION OF BOVINE BRAIN CYTOSOL PHOSPHOLIPASE A_2
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With the exception of a microsomal enzyme acting on phosphatidylinositol (Gray and Strickland, Lipids 17:91-96, 1982), the purification of phospholipase A_2 from the CNS has not been reported yet. We developed a new assay method for plasmalogen-specific phospholipase A_2 using pyrene-labeled plasmenylethanolamine as the substrate (BBA, in press). Using this assay method, we purified bovine brain cytosol phospholipase A_2. After ammonium sulfate fractionation (40% saturation), gel filtration on AcA54 gave two active peaks. Peak I was eluted in the void volume and Peak II eluted in the position of a 34 kDa protein. Both peaks were purified further with affinity chromatography (PlsEtn-AffiGel 10) and HPLC (MA 7Q). Final purifications for Peaks I and II were 1500 and 1000 fold respectively. With AcA34 gel filtration, Peak I was approximately 110 kDa. Both peaks were nearly pure with SDS-PAGE. The optimal pH for Peaks I and II were 8.0 and 7.4 respectively. Interestingly, both are Ca^{2+} independent. The optimal concentrations of Triton X-100 were 0.05% and 0.1% for Peaks I and II respectively. Peak I is specific to diacylGroPEtn(Pyr) and Peak II is active with both diacylGroPEtn(Pyr) and PlsEtn(Pyr). The Km value of Peak I is 29 μM (diacylGroPEtn). The Km values for Peak II are 70 μM (diacylGroPEtn) and 40 μM (PlsEtn(Pyr)).

228.5

EFFECTS OF 8R AND 8S ISOMERS OF HEPOXILIN A_3 (HxA_3) AND A GLUTATHIONE CONJUGATE (HxA_3-C) AT NANOMOLAR CONCENTRATIONS ON MEMBRANE PROPERTIES OF RAT HIPPOCAMPAL CA1 NEURONS.
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The hepoxilins (Hx) are hydroxy epoxide derivatives of arachidonic acid formed via the 12-lipoxygenase pathway. HxA_3 is metabolized in vitro by the rat hippocampus into a glutathione conjugate (HxA_3-C). We have previously shown that HxA_3 and HxA_3-C display neuromodulatory effects on rat hippocampal CA1 neurons in vitro. We now report that both isomers (8R and 8S) of HxA_3 and HxA_3-C at less than 30 nM concentrations were able to hyperpolarize the resting membrane potential (RMP), increase the amplitude and duration of the post-spike train afterhyperpolarization (AHP), increase the inhibitory postsynaptic potential (IPSP) and decrease the spike threshold with a threshold concentration of 3 nM. These findings suggest that products in the hepoxilin pathway are biologically active and may represent new lipid modulators of neuronal excitability.

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228.6

FATTY ACIDS ENHANCE SYNAPTIC EFFICACY IN GUINEA PIG HIPPOCAMPAL SLICES.
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Release of fatty acids may accompany generation of free radicals in a number of pathological states. The present experiments were initiated to evaluate the role of fatty acids in free radical damage.

Input-output curves were generated by stimulation (0-0.5 mA, 300 μs) of s. radiatum in field CA1. Resultant population spikes (PS) and population synaptic potentials (pPSP) were recorded in s. pyramidale and s. radiatum. Slices were exposed to fatty acids for 30 min and subsequently washed with normal aCSF for at least 30 min.

Capric acid (CA, 250 μM), oleic acid (OA, 100 μM) and arachidonic acid (AA, 50 μM) all had qualitatively similar effects. At these doses, the fatty acids had minimal effects on the pPSP when first applied: The pPSP was slightly decreased by AA, slightly increased by OA and unchanged by CA. Upon washout of fatty acids, pPSPs were consistently potentiated and remained so for the duration of the experiment (30-60 min). In addition, large pPSPs were more effective in generating PSs but small pPSPs were less effective. Higher doses of CA (500 μM) produced a larger inhibition of the pPSP during initial application but subsequent potentiation with washout was not as strong as with the lower dose. These data suggest that fatty acids modulate neuronal excitability through complex mechanisms.

228.7

NADPH-DIAPHORASE SYNTHESIZES A SECOND MESSENGER; YES OR NO.
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The NADPH-diaphorase reaction is used for histochemical studies, however, the function of this enzyme has been completely unknown. The reaction is based on the NADPH-dependent reduction of nitro blue tetrazolium (NBT) to a visible formazan product. We have used a similar method to monitor this activity during purification of this enzyme from whole rat brain using ion exchange followed by affinity chromatography with 2',5'-ADP-agarose. A similar procedure has recently been used to purify the NADPH-dependent enzyme, NO synthetase from rat cerebellum (Bredt & Snyder, '90). This enzyme produces nitric oxide (NO) and citrulline from arginine. NO synthetase activity was monitored by measuring the production of [^{3}H]-citrulline from [^{3}H]-arginine, and was found to copurify with NADPH-diaphorase activity. NO synthetase was inhibited by micromolar concentrations of NBT, the electron accepting substrate of the NADPH-diaphorase reaction. The NO synthetase inhibitor N^G-monomethylarginine did not inhibit NADPH-diaphorase activity.

Immunochemical studies were performed with an antibody (HAN) which recognizes NADPH-diaphorase (Michaels & Knigge, '89). In Western blots of fractions following affinity chromatography, a single 150 kD band was labelled. This is the same size as NO synthetase. Thus NADPH-diaphorase activity is associated with NO synthetase. This histochemical reaction may therefore identify neurons in which the production of NO is associated with the stimulation of soluble guanylyl cyclase.

228.8

AGE-RELATED DEFICITS IN MUSCARINIC AGONIST RESPONSIVENESS MAY OCCUR AT THE LEVEL OF THE RECEPTOR-G-PROTEIN COMPLEX IN SIGNAL TRANSDUCTION.
J.A. Joseph, M. Anson, R. Cutler*, K. Yamagami,* and G.S. Roth*. Gerontology Research Center/NIA, Baltimore, MD 21224.

Cholinergic (ACh) agents used to improve memory in the aged show reduced efficacy that may be the result of defects in the signal transduction process (STP). Using muscarinic (m) (e.g., carbachol) enhancement of K^+-evoked endogenous dopamine release (K^+-ERDA) from perifused striatal tissue, we previously observed defects in K^+-ERDA in the aged Wistar rat. However, no age-related deficits in K^+-ERDA were seen if A23187 or IP_3 were applied to the tissue, suggesting that the decrements following mAChR stimulation may appear early in STP, (e.g., at the level of mAChR-G-protein interface). To investigate this, NaF (5, 10 mM), which has recently been shown to initiate STP through the G-proteins, was used to enhance K^+-ERDA. No age differences were seen (e.g., 5 mM NaF, 6 mo, 146.15$\pm$16.66, 24 mo, 166.70$\pm$ 13.67 p moles DA/mg protein), indicating that the age-decrements in STP may occur at the level of the mAChR-G-protein interface. A subsequent experiment using carbachol displacement of [^{3}H]-QNB in the presence or absence of 1 mM GppNHp (the hydrolysis-resistant analog of GTP) indicated that GppNHP was effective in uncoupling (conversion of hi to lo affinity) mAChR sites from their respective G-proteins in the striata and hippocampi from 6 mo but not 24 mo animals. Thus, loss of efficacy of m agonists may be the result of deficits in mAChR-G-protein uncoupling upon agonist stimulation.

228.9

THE ROLE OF G PROTEINS IN MEDIATING THE SUPERSENSITIVITY OF BIOGENIC AMINES H.Y. Wang, P. Butkerait and E. Friedman, Dept of Psychiatry and Pharmacology, Med. Coll. of Pennsylvania, Philadelphia, PA, 19129

Denervation supersensitivity of biogenic amine systems has been repeatedly reported, but the mechanisms have not yet been fully explored. Since G proteins serve an important function in signal transduction, alteration in G protein activation following changes in neuronal inputs were examined. Activation of surface receptors by selective agonists or neurotransmitters lead to elevation in the Mg^{2+}-dependent GTP binding to specific G proteins. The specific [^{35}S]GTPγS-bound G proteins were immunoprecipitated by antisera against specific Gα subunits. The results indicate that in cortex (C), (1) 5-HT increases [^{35}S]GTP S binding to G_i and G_o, (2)isoproterenol activates G_s exclusively, (3) carbachol elevates [^{35}S]GTPγS labeling of G_i and G_o and (4) in striatum (S), dopaminergic system was found to be linked to G_s and G_i. Following 7-days of repeated reserpine administration, [^{35}S]GTPγS binding to G proteins induced by isoproterenol(C), 5-HT (C) and dopamine (S) were markedly increased. No changes in carbachol responses in C and S were observed. Single injection of reserpine induced no changes in agonist-evoked [^{35}S]GTPγS bindings. These results suggest an important role for membrane G proteins in mediating neuronal plasticity.

228.10

A NOVEL Gs IN THE RAT STRIATUM. S.L. Drinnan, B.T.Hope, S.R.Vincent, Kinsmen Lab. of Neurol. Research, Dept. of Psychiatry, Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

Activation of the dopamine D1 receptor stimulates adenylyl cyclase presumably by coupling with a heterotrimeric GTP-binding protein, Gs. We have used a synthetic oligonucleotide probe to detect the portion of the Gsα mRNA coding for the cholera toxin ADP-ribosylation site. Northern blot analysis of whole rat brain with this probe identified a single band of about 1900 bases. Compared to other rat brain G-protein mRNAs (ie: Gi-2, Go), Gsα was the most abundant. This probe was also used for *in situ* hybridization of rat brain sections. Highest levels of Gsα mRNA were observed in the cortex, hippocampus and cerebellum. Of particular interest was the extremely low signal in the striatum and other areas such as nucleus accumbens and olfactory tubercle which receive a heavy dopaminergic innervation from the midbrain.

Although there appears to be very little Gsα mRNA in the striatum, substantial amounts of a Gsα-like protein are present. Cholera toxin activates Gsα by ADP-ribosylation. In the presence of ^{32}P NAD, cholera toxin ribosylates a major band of 45 and a minor band of 52 kD in striatal membranes. This is opposite to the pattern observed in cortex and cerebellum where the 52 kD band was more strongly labelled. An antibody against the C-terminal decapeptide of Gsα was used for western blotting and immunohistochemistry. Immunoperoxidase staining resulted in heavy labelling in the striatum, nucleus accumbens, olfactory tubercle and substantia nigra pars reticulata. Western blot analysis of striatal homogenates revealed the expected bands of 45 and 52 kD and an additional major band at 46.6 kD. These results indicate that a form of Gsα arising from a distinct mRNA is present in regions receiving heavy dopaminergic input. This novel Gsα may couple the D1 receptor to adenylyl cyclase.

228.11

KINETICS OF G PROTEIN-MEDIATED MODULATION OF M CURRENT. H.S. Lopez. Howard Hughes Medical Institute. Dept. Neurobiology and Behavior, SUNY at Stony Brook, Stony Brook, NY 11794.

The G-protein mediated modulation of the voltage-dependent potassium M current (I_m) was quantitatively examined in whole-cell voltage-clamped B neurons (40-60 μm) of bullfrog ganglia. I_m were recorded by applying 1 s, 20-30 mV hyperpolarizing pulses from a -30 mV holding potential while the cells were internally perfused by exchanging the content of the pipette tip (0.2-1.5 MΩ), via a fine PE tubing (exchanging $[Na]_i$ to 100 mM progressed exponentially with τ=13-40 s (n=5), and could be reversed). Perfusion with control solution alone (in mM): 100 KCl, 4 $MgCl_2$, 1 K_2EGTA, 5 Na_2ATP, 2.5 (Na)Hepes (pH 7.2), or with added GTP (500 μM) had no effect on I_m or on its inhibition by muscarine, while GTPγS/GTP in the perfusate (ratios [analog]:[GTP] 0.5, 1, 2, 5, 10, 20) caused a receptor-independent, usually complete, inhibition of Im, with time courses well fitted by single exponentials, and rates dependent on the ratio GTPγS:GTP (limiting rate= 0.51 min^{-1} ±0.05 SD, n=5, ratio 20). The Hill equation fitted well the data (Vmax=0.52 min^{-1}, $K_{0.5}$=0.75, n=1.02). No recovery of Im occurred over up to 1:40 h following repeated perfusion with control solution or application of muscarine. GppNHp and GppCH$_2$p inhibited I_m less potently than GTPγS (GTPγS>GppNHp>GppCH$_2$p) but with a similar limiting rate (about 0.5 min^{-1}, similar to the rate of GDP dissociation from G proteins). No shifts in the I_m I-V curves was seen in the range -100,0 mV. Fast application of muscarine at subeffective concentrations (10-100 nM) accelerated the rates of GTPγS:GTP(=20)-induced Im inhibition, with time courses also well fit by single exponentials, and an hyperbolic dependence on concentration with midpoint 0.43 μM, and plateau at about 20 min^{-1} (1 μM muscarine). I_m recovered with a rate 3.5-7 min^{-1} upon fast washout of muscarine (1 μM) with 1-10 μM atropine (normal solution in the pipette). These data suggest that the kinetics of G protein activation-deactivation could account for the transient and steady-state aspects of I_m modulation by agonists.

228.12

DIFFERENTIAL REGULATION OF G-PROTEINS IN THE RAT BRAIN BY CHRONIC ETHANOL ADMINISTRATION: T.Akompong,R.L.Spencer and B.S.McEwen. Lab. of Neuroendocrinology, Rockefeller Univ. New York, New York 10021.

Ethanol is thought to produce most of its toxic effects by directly fluidizing membranes. Some of the physiological effects of chronic alcohol may be produced by modulating specific signal transduction systems, such as the adenylate cyclase system. Whereas chronic ethanol affects the production of cAMP both in vivo and in vitro it is not known which component(s) in the adenylate cyclase cascade is modified. In this study chronic ethanol(2.5g/Kg,BW 15% v/v in 0.9% NaCl) was administered i.p two times a day for 6 days to ovariectomized female rats. The G_s and G_i(1&2) content in 5 brain regions were determined by western blot with polyclonal antibodies raised against the carboxyl terminal of G_S and G_i(1&2) (donated by Drs A. Spiegel & C. Unson). The amounts of G_S and G_i(1&2) in the caudate were significantly reduced in the alcohol treated rats compared to the saline treated controls. The amounts of G_s and G_i(1&2) did not change in the cortex, cerebellum, hippocampus and hypothalamus. These results suggest a differential modulation of G_s and G_i in different regions of the rat brain. In areas like the cortex and the hippocampus where chronic alcohol has been shown to cause changes in cAMP, other components of the adenylate cyclase system, rather than the G-proteins, may be targets for ethanol modifications.

228.13

COUPLING PATHWAY OF THE CLONED α_1 ADRENERGIC RECEPTOR IN XENOPUS OOCYTES. G.Omri,S.Cotecchia*,M.G.Caron,R.J.Lefkowitz*, R.Iyengar* and E.M.Landau. Departments of Psychiatry and Pharmacology Mount Sinai School of Medicine and the Bronx VAMC N.Y. and Howard Hughes Institute, Duke Univ.Med.Ctr.,Durham,N.C.

The cloned α_1 adrenergic receptor was expressed in oocytes of Xenopus laevis. Superfusion with norepinephrine resulted in a calcium mediated chloride current which was due to phospholipase C activation by the activated receptor. The Kact was 50 nM and the response was completely inhibited by 10^{-8} M prazosin. The response was insensitive to overnight treatment with pertussis toxin (PTX) the average response after PTX being 91±8% (n=5). This contrasts with the marked sensitivity of the native muscarinic response to PTX (Moriarty et al.,Proc.Nat.Acad. Sci.U.S.A.,**85**,8865;1988). These results indicate that the oocyte contains two different G protein pathways to couple receptors to the phosphoinositide pathway. Supported by a merit VA grant to E.M.L.

228.14

PEPTIDES DERIVED FROM G-PROTEIN SEQUENCES AFFECT TRANSMITTER-GATED CURRENTS IN RAT HIPPOCAMPAL NEURONS AND XENOPUS OOCYTES. O. Gil, G. Omri, R.D. Blitzer, A. Buku*, R. Iyengar*, M.G. Caron, S. Cotecchia*, R.J. Lefkowitz* and E.M. Landau. Psychiatry Svce., Bronx VAMC, Bronx NY 10468

G-proteins mediate numerous transmitter-gated responses in excitable cells. A promising strategy for the study of G-protein coupling is the intracellular application of peptides with sequences specific to various G-proteins, which might mimic, block, or enhance the effect of the transmitter. We have employed this technique in CA1 pyramidal cells (using the 5HT-activated K^+ current) and Xenopus oocytes (using the expressed α_1 adrenergic-activated Cl^- current). Decapeptides were synthesized using the COOH terminus sequence of G_o, G_i, G_s, and G_z (referred to as P_o, etc.). In both preparations, P_o increased the current induced by the transmitter and P_s reduced the current, while P_i and P_z had little effect. In no case did the peptide itself evoke the currents under study. The enhancing effect of P_o may reflect a cooperative interaction of P_o/G_o with the receptor, so that P_o, although devoid of intrinsic signalling activity, improves the coupling of G_o with the receptor. Supported by NIA Grant AG02219 and the VA Merit Program.

229.1

A NEUROTOXIC PEPTIDE FROM CONE SNAIL BLOCKS NMDA-INDUCED CURRENTS IN *XENOPUS* OOCYTES INJECTED WITH mRNA FROM CHICK BRAIN. L.G. Hammerland, B.M. Olivera, and D. Yoshikami. Dept. of Biology, University of Utah, Salt Lake, City UT 84112.

Marine snails of the genus *Conus* have venoms with neurotoxins targeted at a variety of ion channels. Recently a family of conus peptides, called conantokins, has been identified as glutamate antagonists (Haack et al. (1990) J. Biol. Chem. 265, 6025-6029). We report here the effect of these toxins on glutamate receptors expressed in *Xenopus* oocytes injected with mRNA from chick brain.

Glutamate receptor agonists, NMDA, quisqualate, and kainate, were applied separately in boluses to a voltage-clamped oocyte in a bath perfused with control saline, or saline containing a glutamate antagonist (either AP5 or CNQX) or conantokin-G (a 17 amino acid peptide). The currents induced by NMDA were specifically blocked by 10 μM AP5, whereas those induced by kainate and quisqualate were specifically blocked by 10 μM CNQX. Conantokin-G, at concentrations near 1 μM, blocked 50% of the NMDA-induced current. In contrast, quisqualate- or kainate-induced currents were unaffected by the toxin, even at concentrations of 10 μM. These results provide a direct demonstration that the toxin is a selective antagonist of the NMDA receptor.

229.2

CONANTOKINS AND GLUTAMATE RECEPTOR SUBTYPES. J.A. Haack, G.J. Rose, T.N. Parks+, B. Olivera. Dept. of Biology, Univ. of Utah and +Natural Product Sciences, Salt Lake City, Utah.

The conantokins are peptides from the venoms of fish-hunting Conus snails which elicit age-dependent behaviors upon intracerebral injection into mice (Haack et. al.,1990, JBC. 256:6025-6029, L.Cruz this meeting). Earlier experimental evidence indicated that these peptides were antagonists for a subset of glutamate receptors; with specificity for the NMDA receptor subtype. We have examined the effects of these peptides on glutamate receptor activity using three separate phylogenetic systems-electric fish, xenopus oocytes (L. Hammerland, this meeting), and mammalian central nervous system neurons.

The specificity of conantokins for particular glutamate receptor subtypes was tested using the weakly electric fish, Eigenmannia. In their social communication, these fish produce gradual or abrupt modulations of the frequency of their electric organ discharges (EODs). These two types of communication signals can be independently elicited by iontophoresis of L-glutamate (1.0 M, 50-100 nA negative current) within particular subregions of a pretectal region known as the prepacemaker nucleus. Iontophoresis of the conantokins into this region strongly attenuated the glutamate-induced gradual modulations of the EOD frequency, but failed to attenuate the abrupt form of modulations evoked by glutamate.

The effects of the conantokins on NMDA receptor-mediated influx of calcium in dissociated rat central nervous system neurons, were monitored by fura-2 fluorescence. The conantokins produce a dose-dependent antagonism of NMDA induced increases in cytosolic free calcium concentration in both cultured cerebellar granule and cortical cells, with half maximal inhibition occurring in the low micromolar range. Thus, experimental evidence from several systems indicate that the conantokins are the first peptides targeted to specific subclasses of glutamate receptors.

Supported by grants from NSF and Sloan Foundation to G.J.R. and NIH22737 to B.M.O.

229.3

NMDA RECEPTORS AND THE DOPAMINERGIC NEUROTOXIC ACTION OF METHAMPHETAMINE. W. D. Sullivan*, A. L. Martello, G. Hatzidimitriou* and G. A. Ricaurte. Dept. of Neurology, Johns Hopkins School of Medicine, Baltimore, MD 21224.

N-methyl-D-aspartate (NMDA) receptor antagonists block the dopamine-depleting effects of methamphetamine in the rat striatum (Sonsalla et al., Science 243: 398, 1989). This interesting observation has implicated excitatory amino acids (EAAs) in the dopaminergic neurotoxic action of methamphetamine. The goal of this study was to further evaluate the role of EAAs and NMDA receptor stimulation in the long-term dopamine-depleting effects of methamphetamine. Two experiments were performed. In the first, the influence of cortical ablation on methamphetamine-induced dopamine depletion was investigated, since a major source of EAA input to the striatum comes from the cerebral cortex. In a second experiment, the effect of direct intrastriatal administration of NMDA on nigrostriatal dopaminergic nerve terminals was evaluated. Both experiments were performed in male albino Sprague-Dawley rats weighing approximately 200 grams at the time of the experiment. Unilateral cortical ablation performed two weeks prior to methamphetamine administration did not alter the dopamine-depleting effects of methamphetamine in the ipsilateral striatum, even though it markedly reduced the in vitro striatal uptake of 3H-glutamate. Direct administration of NMDA (100, 200 or 400 ug in 10 ul vehicle) into the striatum did not cause a lasting depletion of striatal dopamine. By contrast, comparable doses of intraventricular 6-hydroxydopamine (6-OHDA) caused profound reductions of striatal dopamine. These findings suggest that while EAAs and NMDA receptor activation may be involved in the dopaminergic neurotoxic action of methamphetamine, the nature of this involvement is complex and will require further elucidation.

229.4

REGULATION OF 3H-NOREPINEPHRINE RELEASE BY N-METHYL-D-ASPARTATE IN SLICES FROM DENTATE GYRUS AND CA1-CA3 AREA OF THE RAT HIPPOCAMPUS. K. Gysling, M.E. Andrés* and G. Bustos. Lab. of Biochemical Pharmacology, Faculty or Biological Sciences, Catholic University of Chile, Chile.

The hippocampus presents the highest density of N-methyl-D-aspartate (NMDA) type receptors for excitatory amino acids in the CNS. The purpose of this study was to investigate possible functional relations of NMDA-mediated excitatory amino acid neuronal systems and norepinephrine (NE) nerve terminals in two hippocampal regions: the dentate gyrus (DG) and CA1-CA3 area.

Minislices from DG and CA1-CA3 area were superfused to study the effects of ligands of the NMDA-type receptors upon the release of recently taken up 3H-NE. NMDA (10-50 uM) induced a calcium dependent release of 3H-NE from DG and CA1-CA3 slices in the absence of Mg+2. The effect was significantly higher in the DG than in the CA1-CA3 area and significantly inhibited by the selective NMDA type receptor antagonist, amino-phosphonovaleric acid (APV). In addition, the effect of NMDA was suppressed by 60% in the presence of tetrodotoxin. Finally, NMDA potentiated the release of 3H-NE evoked by 25 mM K+ in the DG but not in the CA1-CA3 area.

The results suggest that the excitatory amino acids differentially regulate NE release from DG and CA1-CA3 area of the hippocampus.

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229.5

REGULATION OF TRANSCRIPTION FACTOR PROTEINS BY SYNAPTIC NMDA RECEPTOR ACTIVATION IN CULTURED CORTICAL NEURONS. T.H. Murphy, Y. Nakabeppu*, P.F. Worley and J.M. Baraban. Depts. of Neuroscience and Molecular Biology and Genetics, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

Synaptic activation of transcription factor genes may elicit long-term effects of neuronal stimulation. We have examined the regulation of Jun-B and c-Jun, components of the AP-1 transcription factor complex, in primary cortical cultures immunohistochemically with affinity purified antisera. For the first two weeks in culture (prepared on E17), less than 10% of neurons display immunostaining with these antisera, however, after 3 weeks approximately 30% of neurons are stained by each of these antisera. To determine whether these basal levels are induced by ongoing synaptic stimulation, we examined the effect of tetrodotoxin, and the NMDA receptor antagonists APV or MK-801. After 6 h exposure to these agents, the number of Jun-B immunoreactive neurons fell by over 60%, while c-Jun staining was unaffected. In contrast, cycloheximide reduces both c-Jun and Jun-B immunostaining to a similar extent. Blockade of inhibitory activity with picrotoxin doubles the number of Jun-B positive neurons. This rise is also blocked by tetrodotoxin or MK-801. Whole cell recordings from neurons maintained in vitro for 3 weeks, demonstrate large, 300 pA, spontaneous synaptic currents that are suppressed by APV. Accordingly, these NMDA receptor mediated synaptic currents may induce expression of Jun-B.

229.6

POLYAMINE BINDING SITES ON SYNAPTOSOMAL MEMBRANES C.R. Mantione, S. Demirgören, and E. D. London. Neuropharmacology Laboratory, NIDA Addiction Research Ctr., Baltimore, MD. 21224.

The polyamines, putrescine, spermidine, and spermine, are intracellular diamines, triamines, and tetramines, respectfully. Spermine stimulates the binding of [^{3}H]MK-801 to the cationic channel of the N-methyl-D-aspartate (NMDA) receptor in neuronal tissue (Ransom, R.W. and Stec, N.L., J. Neurochem., 51:850, 1988). This finding suggests the presence of polyamine recognition sites in brain membranes. We have described the binding of [^{3}H]spermidine to rat whole brain membranes (Mantione, C.R. et al., Eur. J. Pharmacol., in press, 1990). Binding is maximal at pH 8, is destroyed by freeze/thawing, and occurs at 25°C but not at 4°C. It has the following parameters: Kd = 564 ± 97.7 μM, Bmax = 63.4 ± 5.89 nmoles/mg protein (mean ± S.E.). The rank order of potency for polyamines to inhibit [^{3}H]spermidine binding is as follows: spermine > spermidine > putrescine. The only other compounds with significant activity observed at this site are poly-L-arginine (IC50 = 7.7 μM) and poly-L-lysine (IC50 = 207 μM). Basic amino acids are inactive. Cations such as Na+ (100 mM), Li+ (50 mM), and Ca++ (2 mM) produce as much as 66-70% inhibition of binding. We now report that synaptosomal preparation of the tissue results in a 200-fold increase in the binding affinity for [^{3}H]spermidine (Kd = 2.7 μM) and a 100-fold lower Bmax (593 pmoles/mg protein). The characterization of a synaptosomal membrane binding site for [^{3}H]spermidine is an important step in elucidating the molecular role of polyamines in the nervous system.

229.7

IN VIVO LABELLING OF SIGMA SITES IN THE MOUSE BRAIN WITH ^{3}H-IFENPRODIL.
J. Benavides, B. Peny*, H. Schoemaker* and B. Scatton. Synthélabo Recherche (L.E.R.S.), 31, av. Paul Vaillant-Couturier, 92200 Bagneux (France).

The anti-ischaemic drugs ifenprodil and SL 82.0715 interact in vitro with two different binding sites : a polyamine sensitive site associated with the NMDA receptor complex (Carter et al, Europ. J. Pharmacol. 164, 611, 1989) and the sigma site (Schoemaker et al, British Pharmacol. J., in press). We here demonstrate that, in vivo, ^{3}H-ifenprodil selectively labels sigma sites in the mouse brain. I.v. injection of 5 μCi ^{3}H-ifenprodil resulted in an accumulation of brain radioactivity which was maximal at 5 min post-injection and decreased in a biphasic fashion. Under standard experimental conditions (radioactivity measured 2 h after ^{3}H-ifenprodil administration) more than 65 % of the incorporated label was displaced by ip administration (30 min before the radiotracer) of SL 82.0715 (10 mg/kg). At this time, most of the radioactivity (80 %) present in the brain corresponded to authentic ^{3}H-ifenprodil. When administered 30 min before the radiotracer, several ligands with affinity for sigma sites inhibited ^{3}H-ifenprodil accumulation (ID_{50}'s mg/kg ip) : haloperidol (0.23) > ifenprodil (0.83) > SL 82.0715 (1.37) > BMY 14802 (5.5) > DTG (18). (+)3-PPP, phencyclidine, TCP and MK-801 were inactive at 10 mg/kg ip. The potency of ifenprodil, SL 82.0715 and haloperidol as inhibitors of in vivo ^{3}H-ifenprodil or ^{3}H-(+)-3-PPP binding was similar. The present results demonstrate that ^{3}H-ifenprodil is a suitable ligand for labelling mouse brain sigma sites in vivo.

229.8

NMDA-INDUCED CURRENTS IN HIPPOCAMPAL NEURONS CAN BE MODULATED BY THE POLYAMINE SPERMINE.
V.L. Dawson[1], K. Williams[2], C. Romano[2], P.B. Molinoff[2], and M.A. Dichter[1,3]. Dept. Neurology[1] and Pharmacology[2], University of Pennsylvania, Dept. Neurology[3], Graduate Hospital, Philadelphia, PA.

NMDA receptor activity can be regulated by a number of endogenous modulators including Mg^{2+}, Zn^{2+}, H^+, and glycine. Recently radioligand binding studies have suggested a role for polyamines as another endogenous modulator of the NMDA receptor. Electrophysiological studies were performed on cultured rat hippocampal neurons using whole cell patch-clamp to examine this possibility. The dose-response curve of the spermine effect on NMDA-induced currents was biphasic. Low concentrations of spermine enhanced NMDA currents with maximal enhancement of 84% occurring at 1 µM spermine. Higher concentrations of spermine inhibited NMDA currents with 70% inhibition observed at 100 µM spermine. Both effects of spermine were blocked by the specific polyamine antagonist, diethylenetriamine (DET). The voltage dependence of the spermine effect at both low and high concentrations was examined and neither the enhancement nor the inhibition of NMDA currents were voltage dependent. Additionally, in the presence of Mg^{2+} in the extracellular bath 1 µM spermine did not shift the voltage dependence, but did elicit enhancement at all membrane potentials that NMDA induced currents. Spermine did not alter currents induced by other amino acid neurotransmitters, quisqualate, kainate or GABA. In addition to exogenously applied NMDA, spermine modulated responses to endogenously released excitatory neurotransmitter in culture. The amplitude of spontaneous postsynaptic currents is increased by 20 µM spermine and decreased by 50 µM DET (DET in the absence of spermine has no effect on NMDA currents), suggesting that there may be an endogenous spermine "tone" in the cultured neurons. These data suggest that spermine may have a physiological role as a modulator of excitatory neurotransmisstion in the mammalian CNS.

229.9

EFFECTS OF POLYAMINES ON [^{3}H]MK-801 BINDING IN DIFFERENT REGIONS OF THE RAT BRAIN.
S. Subramaniam and P. McGonigle. Department of Pharmacology, Univ. of Pennsylvania School of Medicine, Philadelphia, PA 19104.

Polyamines have been shown to modulate the NMDA receptor channel complex through a distinct receptor site. Assays using brain membranes have suggested the possible existence of more than one polyamine receptor. We have studied the effects of a number of polyamines on [^{3}H]MK-801 binding in anatomically discrete brain regions using quantitative autoradiography. The polyamine agonists spermidine and spermine enhanced [^{3}H]MK-801 binding in all the regions studied. Pre-washing the sections in buffer at room temperature for 1 hr increased the magnitude of this effect. The antagonist diethylenetriamine significantly reduced binding in unwashed sections suggesting the presence of residual endogenous polyamines. This effect was attenuated but not eliminated after washing. The inverse agonist diaminodecane significantly inhibited [^{3}H]MK-801 binding in washed sections. There was considerable regional heterogeneity in the magnitude of the effects of the polyamines. In washed sections of the striatum a maximally effective concentration of spermidine enhanced [^{3}H]MK-801 binding to a greater extent in the medial as compared to the lateral striatum. There was an inverse correlation between the enhancement produced by spermidine and the inhibition produced by diethylenetriamine in all the regions studied suggesting that regional variations in polyamine effects might be due to differences in levels of endogenous polyamines. The effects of polyamines on [^{3}H]MK-801 binding have been further characterized in synaptic plasma membranes made from different brain regions. (Supported by USPHS GM 34781 and the Pew Charitable Trusts)

229.10

WITHDRAWN

229.11

MECHANISMS UNDERLYING THE INTERACTION OF POLYAMINES WITH THE NMDA RECEPTOR.
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The polyamines (PA) spermidine and spermine increase [^{3}H] MK801 binding at physiological concentrations (Ransom & Stec, J.Neurochem. 51:830, 1988) suggesting a possible role as endogenous modulators of the NMDA receptor. I have investigated the mechanisms by which PAs modify the NMDA receptor by monitoring [^{3}H] MK801 binding and NMDA-induced $[Ca^{2+}]_i$ changes in cultured forebrain neurons.

Spermidine-induced increases in [^{3}H] MK801 binding were competitively reduced by arcaine (IC_{50} 1.5μM). Arcaine also reduced binding in the absence of added PAs which implies that agonist occupation of the PA site is obligatory for [^{3}H] MK801 binding. Arcaine blocked Sr^{2+}-, Ca^{2+}- and Ba^{2+}-induced increases in [^{3}H] MK801 binding, suggesting that these cations act at the PA site. Spermidine increased the affinity of [^{3}H] MK801 at equilibrium (≥ 4hr +100μM Glu and 30μM Gly) in an arcaine-reversible fashion. However, PA-induced changes in ligand dissociation were arcaine insensitive. Thus, PAs apparently do not increase activation of the NMDA receptor but instead increase ligand affinity at the phencyclidine binding site. This is distinct from the mechanism of action of NMDA and glycine.

NMDA (30μM) and glycine (1μM) increase $[Ca^{2+}]_i$ in individual forebrain neurons measured using fura-2 microspectrofluorimetry. Spermine and spermidine (0.5-500μM) and arcaine (0.3-30μM) had no effect on NMDA/Gly-induced $[Ca^{2+}]_i$ changes. Arcaine (300μM) did not affect kainate or KCl-induced $[Ca^{2+}]_i$ increases. Thus, although PAs may profoundly alter the function of the NMDA receptor monitored with [^{3}H] MK801 binding, these drugs may not gain access to their site of action in intact cells.

230.1

PURIFICATION OF CPP BINDING PROTEINS FROM IBOTENATE-TRISACRYL MATRICES AND CHARACTERIZATION OF PROTEIN INTERACTIONS WITH OTHER MATRICES. K.T. Eggeman and E.K. Michaelis, Department of Biochemistry, University of Kansas, Lawrence, Kansas 66045

We have previously isolated a fraction from rat synaptic membranes enriched in 58-60kDa proteins that specifically interact with CPP, a selective antagonist of the NMDA type of excitatory amino acid receptor. These proteins also interact strongly with 2-AP5, 2-AP7 and CNQX, suggesting that they may contain the antagonist binding site [Cunningham & Michaelis, J. Biol. Chem, in press]. The ioslation of these proteins was achieved through affinity chromotography on ibotenate-agarose columns. The agonist-binding proteins were retained on the column by specific and non-specific interactions with the agorose matrix and could be eluted with high salt. Recently we have shown that proteins with similar activities can be enriched by using an ethanolamine -blocked affigel column alone, suggesting that interactions of these proteins with hydroxyl residues of the matrix may play a significant role in these purifications. The same CPP binding proteins can be isolated together with other proteins that may form the NMDA receptor complex through chromotography on ibotenate-trisacryl matrices. This procedure is being pursued to obtain proteins for sequencing and production of polyclonal antibodies.
(Supported by grant AA04732 and DAAL 03-88-K0017).

230.2

IN SITU HYBRIDIZATION OF THE mRNA FOR KAINATE RECEPTORS IN RAT BRAIN USING THREE DISTINCT PROBES. Y. Fujiwara[1], K. Akiyama[1], I. Sora[*1], H. Tomita[*1], S. Otsuki[*1], M. Tyler[*2] and H.I. Yamamura[3] [1]Dept. of Neuropsychiatry, Okayama Univ. Med. Sch. Okayama 700, Japan, [2]Medical Products Dept. E.I. Du Pont De Nemours & Co. Boston, MA 02118, [3]Dept. of Pharmacol. Univ. of Arizona, Tucson, AZ 85724

It was reported that the cDNA clone for the kainate receptor was isolated (Hollman, M. et al. Nature 342:643-648, 1989). To investigate the regional distribution of mRNA encoding the kainate receptor, the present study conducted in situ hybridization histochemistry in the rat brain using three distinct probes, each of which consists of separate 45 consecutive sequence ('4-48, 262-306, 1951-1995, according to Hollman, M. et al., 1989). Each 45 base probe was 3'-end labeled using terminal deoxy nucleotidyl transferase with [^{35}S]dATP. In situ hybridization was conducted with a probe concentration of 1x10^{6} dpm/100 µl of buffer [1xSSC, 50% formamide, 1x Denhardt's solution, salmon sperm DNA, 100mM DTT, and 10% dextran sulfate] per brain slice at 37°C for 18-20 h in a humid chamber. Slices were washed four times for 15 min at 55°C in 1xSSC and for 60 min at room temperature also in 1xSSC. Slides were dried and exposed to x-ray film for 1 week. The autoradiograph showed that mRNAs were present at high level in the pyramidal cells of the hippocampus. Identical patterns of labeling were obtained using the three distinct probes and a mixture of three probes.

230.3

cDNA CLONING OF A KAINATE BINDING PROTEIN FROM PIGEON CEREBELLUM. H.P. Ottiger, B. Niederöst, and P. Streit
Brain Research Institute, University of Zürich, August-Forel-Str. 1, CH-8029 Zürich, Switzerland.

In the vertebrate central nervous system, the excitatory amino acid (EAA) glutamate plays a role as major neurotransmitter via various types of pharmacologically defined receptors and its action is thought to be terminated by neuronal and glial uptake. Litle is known, however, about the molecular characteristics of these receptors, of certain pharmacologically related binding proteins and carrier systems. A characterization at the molecular level, on the other hand, should yield a better understanding of EAA function, not the least by facilitating the development of important tools.

Binding sites pharmacologically related to EAA receptors are highly enriched in the molecular layer of the pigeon cerebellum as shown autoradiographically using tritiated kainate as tracer. A similar labelling pattern is obtained by means of a monoclonal antibody which precipitates a 50 kD kainate binding protein from detergent extracts of pigeon cerebellum. The relationship between this kainate binding protein and functional kainate receptors remains to be defined.

As a step to meet this goal, a further characterization of the protein is being conducted by analysing cDNA clones identified through screening of pigeon brain cDNA libraries. We are currently investigating the extent of cross-species expression, chromosomal origin, and developmental onset of expression. The results on the identification and characterization of these clones will be presented.
Supported by Swiss National Foundation grant 3.118.88.

230.4

TRANSCRIPTS FROM A SINGLE cDNA INDUCE IN *XENOPUS* OOCYTES A RECEPTOR ACTIVATED BY KAINATE AND QUISQUALATE. L. Prado de Carvalho[*1], P. Curutchet[*1], J. Stinnakre[*2], P. Bregestovski[*2], B. Lambolez[*1], J. Rossier[1]. [1]Laboratoire de Physiologie Nerveuse and [2]Laboratoire de Neurobiologie Cellulaire et Moléculaire, CNRS, 91198 Gif sur Yvette, CEDEX, France.

Recently, a cDNA clone (GluR-K1) encoding a member of the glutamate receptor family was isolated from a rat brain cDNA library (Hollmann, M. *et al.*, *Nature* 42: 643, 1989). Injection of GluR-K1 transcripts into *Xenopus* oocytes induces the expression of functional receptor-channel complex activated by kainic acid (KA), domoic acid (DA) and glutamic acid (GA, *ibid).* The same receptor-channel complex is now shown to respond to both KA and quisqualic acid (QA). The apparent affinity (EC_{50}) of QA (0.1 µM) is higher than that of KA (50 µM). However, the maximal response induced by QA corresponds to only 1/10 of the KA maximal response. QA and GA competitively inhibit KA induced current. The "non-NMDA" receptor antagonist 6,7-dinitroquinoxaline-2,3 dione (DNQX) blocks the effects of both KA and QA. Currents induced by KA, QA and DA in oocytes expressing GluR-K1 show the same voltage sensitivity: no outward current and exponential increase upon hyperpolarization. These data provide direct evidence that transcripts of a single cDNA direct synthesis of ligand gated channels activated by both KA and QA agonists, suggesting that these two agonists act at the same receptor.

230.5

SOLUBILIZED RAT BRAIN AMPA RECEPTOR BINDING TO LECTIN COLUMNS. W. McNeel, J. Mills*, P. Crowley*, and A.B. Young. Neuroscience Program and Dept. of Neurology, University of Michigan, Ann Arbor, MI 48109.

AMPA, (α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid), is an agonist at a subtype of excitatory amino acid receptor which is a glycoprotein, and can be solubilized in Triton X-100. Specific [^{3}H]AMPA binding in solubilized tissue was determined by a filter binding assay usinig 0.3% PEI (polyethylenimine) treated filters and 0.1 mM glutamate as a blank. Sugar residues of this glycoprotein can be determined by binding to lectin columns. The AMPA receptor bound to Con A (Canavalia ensiformis), WGA (Triticum vulgaris), and RCA-1 (Ricinus communis) columns, indicating the presence of mannose and/or glucose, N-acetylglucosamine, and galactose residues, respectively, in its carbohydrate structure. The AMPA receptor did not bind to the PNA lectin, suggesting that it contains a terminal sialic acid. Treatment of the AMPA receptor with sialidase permitted some binding to the PNA column. The SNA lectin has an affinity for β-D-Gal (1->4)-D-Glc, which is increased if there is a terminal sialic acid. Some, but not all of the solubized AMPA receptor bound to the SNA column, suggesting the possibility of multiple AMPA receptor subtypes with the differing carbohydrate structures. Treatment of the AMPA receptor with sialidase prevented all binding to the SNA column. The use of lectin columns may differentiate AMPA receptor subtypes and aid in their purification.
Supported by the Markey Foundation.

230.6

Phosphorylation of A Kainic Acid Binding Protein. **D.R. Hampson and M.F. Hullebroeck. Faculty of Pharmacy, University of Toronto, Toronto, Ontario Canada M5S 2S2**

Kainic acid receptors or binding proteins have been purified and/or cloned from frog, chicken, and rat brain. The proteins from frog and chicken brain are approximately 50,000 daltons while the rat protein is 99,000 daltons. All 3 proteins have a high degree of homology, particularly the frog and chicken protein. In each case there appears to be 4 membrane spanning domains and a large intracellular loop. In each species, this intracellular loop contains a number of consensus sequences for potential protein kinase-mediated phosphorylation. We have examined the cyclic AMP-dependent protein kinase phosphorylation of the proteins from frog and chicken CNS.

Kainic acid binding proteins were solubilized and then purified from frog whole brain and chicken cerebellum using ion exchange chromatography and domoic acid affinity chromatography. Cyclic AMP-dependent protein kinase was from bovine heart. In vitro experiments using purified kainate proteins and purified kinase demonstrated that the protein from chicken CNS was phosphorylated by protein kinase A. The frog protein did not appear to be phosphorylated under the same conditions. The phosphorylation of the chicken receptor was very rapid; detectable P-32 incorporation was observed within the first 1 minute of incubation at 30°C. These results support the findings by others [e.g. see Liman, Knapp, and Dowling; Brain Res. 481:399(1989)] that protein kinase A modulates kainate induced currents and suggest that phosphorylation may be a physiologically important mode of regulation for this class of receptors. Supported by the NSERC and the MRC of Canada.

230.7

SOLUBILIZATION AND RECONSTITUTION OF ACTIVE NMDA RECEPTORS FROM RAT BRAIN. D.E. Pellegrini-Giampietro, R. Haring* and R.S. Zukin. Department of Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

The NMDA receptor complex, defined by specific binding of [^{3}H]TCP, was solubilized from rat forebrain membranes with the zwitterionic detergent CHAPS. A nearly complete (> 80%) restoration of binding activity was achieved upon incorporation of solubilized membrane proteins into lipid vesicles prepared by addition of a total brain lipid extract and 60-fold dilution of CHAPS.

Proteoliposomes displayed saturable [^{3}H]TCP binding. Scatchard analysis showed the recovery of an apparent single high affinity binding site (K_D=20nM; Bmax=5.1 pmol/mg protein). Six-fold purification of binding sites was achieved. The rank order of potency of drugs binding to the PCP site was retained (MK-801 > TCP, dexoxadrol >> levoxadrol, haloperidol). In proteoliposomes prepared from well-washed membranes, glutamate (0.1-100 μM) dose-dependently increased [^{3}H]TCP binding; potentiation of binding by glutamate was enhanced in the presence of glycine (1-10 μM). The competitive NMDA antagonist APV inhibited [^{3}H]TCP binding (K_i=100 nM). In photoaffinity labeling experiments, [^{3}H]azido-PCP labeled two polypeptides, M_r 59,000 and 98,000, as was observed for rat brain membranes.

These results indicate the successful reconstitution into liposomes of TCP binding sites associated with the NMDA receptor/ion channel complex. This preparation may be helpful in the purification of active receptors and in electrophysiological studies on artificial lipid bilayers.

230.8

DEVELOPMENTAL REGULATION OF mRNAs ENCODING RAT BRAIN GLUTAMATE RECEPTORS. G.M. Durand, R. Roginski, M.V.L. Bennett and R.S. Zukin. Dept. Neuroscience, Albert Einstein College of Medicine, Bronx, NY 10461.

The developmental regulation of kainate receptor gene expression in embryonic and neonatal rat brain has been examined. We used the ploymerase chain reaction to clone a cDNA with a sequence apparently identical to that reported by Hollmann et al. (*Nature* 342: 643-648, '89). Messenger RNA translated from this cDNA and injected into *Xenopus* oocytes directed the translation of functional kainate channels. Northern blot analysis carried out with a riboprobe made to the full length kainate receptor cDNA under conditions of high stringency revealed the presence of large amounts of a RNA species, 5.2 kb in size, in poly(A)$^+$RNA from adult rat brain. Additionally, a minor band corresponding to RNA 3.8 kb in size was observed. In contrast, mRNA from a 7-day-old rat brain showed the 5.2 kb RNA and two minor bands at about 2.0 and 1.6 kb. These findings indicate developmental regulation of the expression of the smaller mRNA species. When RNA samples from the brain tissue of embryonic E-18, neonatal days 1, 4, 7 and 11, and adult rat brain were analyzed by Northern blot, the density of the 5.2 kb species (expressed as a ratio to the density of 18s rRNA, which does not appear to change during development), increased monotonically until neonatal day 11, then decreased by about 16% in adult. These findings indicate changes in both quantity and species of kainate receptor mRNA expressed at different stages of brain development.

230.9

CHARACTERIZATION OF SOLUBILIZED AMPA BINDING SITES WITH POLYCLONAL AND MONOCLONAL ANTIBODIES C.Hunter and R.J.Wenthold. Laboratory of Molecular Otology, NIDCD, NIH, Bethesda, MD 20892.

In previous work, we have solubilized and partially purified an AMPA binding protein (ABP) from rat brain (J. Neurochem 54,118 1990) using Triton-X 100. We have now obtained a highly purified preparation of ABP from bovine brain. SDS-PAGE of the purified ABP gives one band which migrates at M_r=114,000. Scatchard analysis of purified ABP in the presence of KSCN resolves the binding into both a high and low affinity component similar to other reports. In competition studies quisqualate and AMPA are the most potent displacers of ^{3}H AMPA binding while kainic acid is 150 fold less effective. To further study the ABP, monclonal and polyclonal antibodies were made to the purified preparation. Polyclonal anti-ABP immunoprecipitated greater than 60% of AMPA binding activity solubilized from frog, chick, rat, guinea pig or bovine brain. Further, antisera tested with solubilized membranes from rat and frog immunoprecipitated high affinity ^{3}H AMPA binding while ^{3}H kainate binding at 5nM was unaffected. These data suggest that the AMPA and kainic acid binding proteins are distinct in the rat and frog brain. Monoclonal antibodies which selectively immunoprecipitate quisqualate and kainic acid receptors will be useful in disinguishing between these two receptor subtypes.

230.10

INTERSPECIES DIFFERENCES IN THE POPULATION OF MULTIPLE GLUTAMATE RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES AFTER INJECTION OF RODENT FOREBRAIN MESSENGER RNAS. S. Kaneko*, M. Sugimura* and M. Satoh. Dept. of Pharmacol., Fac. of Pharm. Sci., Kyoto Univ., Kyoto 606, Japan.

Expression of multiple responses to glutamatergic agonists was compared in *Xenopus* oocytes several days after injection of poly(A)$^+$RNAs from guinea pig, mouse and rat forebrains. No difference was observed in the pharmacological characteristics of the electrical responses of oocytes to *N*-methyl-D-aspartate (NMDA), kainate (KA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA), or quisqualate (QA) among the oocytes injected with mRNA from the different species. However, KA responses produced by guinea pig brain mRNA or mouse brain mRNA was about half the magnitude of that by rat brain mRNA. Moreover, the peak amplitude of oscillatory QA responses by guinea pig mRNA was 1/6 times of that by rat brain mRNA, or 1/4 times of that by mouse mRNA. The amplitude of NMDA responses were equivalent among these mRNA preparations. These results may reflect that the proportion of metabotropic QA receptor subtype in the guinea pig forebrain is much lower than that in the rat or mouse brain, and that the non-NMDA/NMDA ratio of ionotropic receptor density is higher in the rat brain than in the guinea pig or mouse brain.

230.11

LOCALIZATION OF mRNA FOR KAINATE RECEPTORS IN THE RAT BRAIN BY *IN SITU* HYBRIDIZATION HYSTOCHEMISTRY. **P. McGonigle, S. Subramaniam, and D. Brousseau. Department of Pharmacology, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania, 19104.**

Three [^{35}S]-labelled oligonucleotides derived from unique portions of the coding region of the rat kainate receptor cDNA have been used as probes for the mRNA for this receptor. Cells in the rat brain containing mRNA coding for the kainate receptor were localized using *in situ* hybridization hystochemistry. A 48 base oligonucleotide of random sequence served as the control. Two of the oligonucleotides produced a heterogeneous and comparable distribution of labelling and combination of these probes improved the signal to noise ratio. The highest level of hybridization was observed in the CA_1, CA_2 and CA_3 fields of the hippocampus as well as the dentate gyrus. High mRNA content was observed in the anterior olfactory nuclei and the pyramidal layer of the olfactory tubercle, and the olfactory nerve layer. Lower levels were seen in the outer layers of the frontal and orbital cortex and the amygdaloid nuclei. In most of these areas, the distribution was similar to the distribution of kainate receptor labelled with [^{3}H]-kainate. However, in the hippocampus, the level of hybridization was comparable in all of the CA fields, whereas [^{3}H]-kainate preferentially labels the CA3 field. In some areas, like the striatum, kainate receptors have been visualized but no significant hybridization signal could be detected. The mRNA coding for these receptors in these areas could be contained in cells outside of the brain area, be different from the one recognized by our probes or be present at levels below the detection limits of our procedure. (Supported by USPHS GM 34781 and the Pew Charitable Trusts)

230.12

FUNCTIONAL CHARACTERIZATION OF THREE GLUTAMATE RECEPTOR cDNA CLONES OF THE KAINATE SUBTYPE SUGGESTS A HETERO-OLIGOMERIC STRUCTURE FOR THE NATIVE RECEPTOR. M. Hollmann*, J. Boulter*, M.E. Hartley*, A. O'Shea-Greenfield* and S. Heinemann. Mol. Neurobiol. Lab., Salk Institute, La Jolla, CA 92037.

Using GluR-K1 (K1), a glutamate receptor cDNA clone of the kainate subtype (Hollmann, M. et al, *Nature*, 243:643, 1989) as a probe in low-stringency hybridization experiments, we have identified two additional clones that show high amino acid sequence identity (around 65%) to GluR-K1 (K1). Upon injection into *Xenopus* oocytes of RNA synthesized *in vitro* from clones GluR-K2 (K2) and GluR-K3 (K3) we observed functional kainate receptors. This proves that each of the proteins encoded by these cDNAs is sufficient to form functional receptors, probably by forming homo-oligomers in the oocyte membrane. K3 RNA elicited responses to kainate receptor agonists similar in size to those seen with K1, while K2 gave significantly smaller responses. The pharmacological characteristics of the three receptors, though being very similar in general, differ slightly. Notably, dose-response curves reveal different apparent K_d's for receptor agonists. Pairwise injections of K1, K2 and K3 transcripts into single oocytes led to a ~5-fold increase in responses over the sum of the responses detected with the individual transcripts. Combining all three transcripts in one oocyte yielded no further stimulation of the response. This finding suggests that the interaction of different receptor subunits, probably through formation of hetero-oligomers, is responsible for the observed potentiation of the receptor response. Also, responses seen with total polyA(+) RNA are more similar to responses elicited by combinations of subunits than to responses seen with individual subunits. This indicates that native receptors most probably are hetero-oligomers consisting of at least two different subunits.
Supported by the Deutsche Forschungsgemeinschaft (M.H.), and NINCDS

230.13

MOLECULAR STRUCTURE AND EXPRESSION OF RAT GLUTAMATE RECEPTORS.

B.Bettler*, J.Boulter*, E.Deneris*, I.Hermanns-Borgmeyer*, A.O'Shea-Greenfield*, M. Hollmann*, M. Hartley*, C.Moll*, R.Papke and S.Heinemann Molecular Neurobiology Laboratory, The Salk Institute, San Diego, CA 92138.

Recently our laboratory has characterized a cDNA clone encoding a functional glutamate receptor of the kainic acid subtype (GluR-K1; Hollmann et al., *Nature*, 342:643, 1989). Low stringency screening of cDNA libraries constructed from rat central nervous system RNAs has resulted in the isolation of four additional cDNAs with similarity to GluR-K1. Two of these cDNAs (GluR-K2/GluR-K3) encode kainate-sensitive ion channels (see abstract by Hollmann et al.), the functional properties of the two other isolates are under investigation. We compare the primary structure of all five cDNA clones isolated and present an *in situ* hybridization analysis in the rat and developing mouse nervous systems.

Supported by the Swiss Science Foundation (B.B) and the NINCDS.

230.14

THE KAINATE SUBTYPE OF THE GLUTAMATE RECEPTOR FAMILY: BIOCHEMICAL CHARACTERIZATION. G. Gasic, M. Hollmann*, T. Hughes, S.W. Rogers and S. Heinemann. Molecular Neurobiology Lab., The Salk Inst., LaJolla, CA 92037.

This laboratory has used a molecular approach to identify cDNAs which encode members of the glutamate-gated ion channel receptor family. One cDNA encodes for a functional glutamate receptor of the kainic acid subtype and was named GLUR-K1 (Hollmann, et al., Nature 342:643, 1989). We have made rabbit polyclonal antibodies to a portion of the putative extracellular and intracellular domains using the trpE bacterial overproduction method to aquire antigen. These antibodies reveal on western analysis a 105 kD glycoprotein which is predominantly found in crude membrane extracts of the rat hippocampus and cerebellum and to a lesser extent in the cortex and midbrain. Antigen is not observed in the brain stem or liver. Further fractionation of these membrane preparations demonstrates that this protein is enriched in synaptic plasma membranes and that it co-localizes with post-synaptic densities suggesting that the GLUR-K1 is a post-synaptic ligand-gated ion channel. (Supported by NICDS and HHMI)

230.15

EXCITOTOXICITY IN RAT 2 FIBROBLASTS STABLY EXPRESSING A cDNA ENCODING A MEMBER OF THE GLUTAMATE RECEPTOR FAMILY. S.W. Rogers and S. Heinemann. Molecular Neurobiology Lab., The Salk Inst., LaJolla, CA 92037.

This laboratory has used a molecular approach to identify cDNAs which encode members of the glutamate-gated ion channel family. One cDNA encodes for a functional glutamate receptor of the kainic acid subtype and was named GLUR-K1 (Hollmann, et al., Nature 342:643, 1989). We have stably transfected cultured Rat 2 fibroblasts with this cDNA to produce cell lines which express functional receptors of the GLUR-K1 type. Employing the methods of molecular biology and immunology, we are evaluating in detail the pharmacology and cell physiology of kainic acid induced excitotoxicity. Preliminary analyses of these cell lines indicate that cell survival in glutamate-containing culture medium is enhanced by the presence of kynurenic acid. In addition, GLUR-K1 expressing cells die in response to prolonged exposure to kainic acid. This approach offers the advantage of studying the contribution of individual excitatory amino acid ligand-gated channel subtypes to the more general process of excitotoxic cell death. (Supported by NICDS and a NIH post-doctoral fellowship to SWR)

230.16

THE KAINATE SUBTYPE OF THE GLUTAMATE RECEPTOR FAMILY: IMMUNOHISTOCHEMICAL LOCALIZATION IN THE RAT BRAIN.

T.E. Hughes, S.W. Rogers, G. Gasic, M. Hollmann, S. Heinemann. Dept. of Neurosciences, UCSD & Molecular Neurobiology Lab., The Salk Institute for Biological Studies, La Jolla CA 92037.

The kainate subtype of the glutamate receptors is thought to mediate excitatory neurotransmission at many synapses, and has been implicated in excitotoxic cell death, but little is known about the cellular distribution of this receptor. Rabbit antisera against a putative extracellular portion of GluR-K1 (see Gasic et al. abstract) provides an immunohistochemical means to study the receptor's distribution. The antiserum labels many neurons in the forebrain; there is a dense labeling of the cytoplasm within their somata and a fine granular labeling of their dendrites and axons. The majority of the labeled neurons are in the hippocampus, amygdala, lateral septum, and the bed nuclei of the stria terminalis. In the hippocampus, the pyramidal cells throughout Ammon's horn are labeled, as well as somata in the dentate gyrus, taenia tecta and indusium griseum. The lateral septum, which receives a large input from the cells of Ammon's horn, contains many large, labeled multipolar cells. Within the amygdala, there is a dense plexus of labeled processes and medium-sized round somata in the central nucleus. From this nucleus a path of labeled fibers and cells can be followed through the stria terminalis into the oval and juxtacapsular bed nuclei (Ju & Swanson, *J.Comp.Neurol.*, 280:587, 1989) which contain many tightly packed round to oval receptor-positive cells. These findings provide an initial glimpse of the cellular distribution of the receptor. With electron microscopy, it should be possible to further define the distribution of the receptor and it's relationship to the synapse. Supported by the NEI & NINCDS.

230.17

IMMUNOCHEMICAL PURIFICATION AND CHARACTERIZATION OF BRAIN SYNAPTIC MEMBRANE GLUTAMATE-BINDING PROTEINS.

K.N.Kumar, M.J. Eaton, K.T. Eggeman and E.K. Michaelis, Depts. of Pharmacology & Toxicology, Biochemistry and Center for Biomedical Research, University of Kansas, Lawrence, Kansas 66045

Two glutamate binding proteins (GBP's) were previously purified from rat brain synaptic membranes. [J. Biol. Chem 263, 417 - 426, 1988]. The ligand binding characteristics of two proteins were suggestive of a role as glutamate receptors in synaptic membranes. Polyclonal antibodies were raised against the denatured 71 and 63 kDa GBP's in rabbit, including sets of antibodies against each of the 71 and 63 kDa proteins. The antibodies recognized the denatured form of the proteins in Western blots and the native state of the proteins in ELISA assays. The two proteins were closely related immunologically but the reactivity on Western blots differed between the two proteins. An immunoaffinity purification procedure was developed to purify the two proteins. This method involved linking of the antibodies to a Trisacryl matrix. The proteins purified by this procedure had molecular sizes of 71 and 63 kDa on SDS-PAGE. The molecular size of native proteins estimated by sucrose density centrifugation was 75 and 135 kDa, i.e., indicative of monomer and dimer formations. Immune extraction of the 71 and 63 kDa proteins from solubilized synaptic membrane proteins led to a 60% decrease in L-[^{3}H] glutamate binding activity suggesting that the71 and 63 kDa proteins are the major group of glutamate binding entities in synaptic membranes.

(Supported by grants AA 04732 and DAAL 03-88-K0017)

230.18

IMMUNOLOCALIZATION OF GLUTAMATE-BINDING PROTEINS (GBP's) IN CULTURED PRIMARY BRAIN NEURONS.

M.J.Eaton, S.Schuler *, M.L.Michaelis and E.K. Michaelis, Dpt of Biochem & Ctr for BioMed Res, Univ of Kansas, Lawrence, KS 66045.

Polyclonal antiserum against the 63/71 kDa GBP's from rat brain synaptic membranes was used in immunolocalization studies with cultured primary neurons. Neonatal rat brain cortex, cerebellum, and hippocampus were non-enzymatically dissociated and cultures were grown in serum-free supplemented DME/F12 media. Mature neurons had L-(^{3}H)glutamate binding sites with a Kd equal to 0.5uM. L-Glutamate binding to these sites was inhibited by metal ligands and quisqualate, ligands that inhibit binding to purified GBP's. The antibody used is selective for brain synaptic membrane GBP's and inhibits most of the specific L-(^{3}H) glutamate binding to synaptic membranes. GBP-like immune reactivity was localized in mature cultured neurons by means of silver-enhanced immuno-gold reaction. This reactivity was seen on membranes of cell bodies and neuronal processes. Image analysis of immune reaction sites in developing cortical neurons revealed that the highest concentration of label was in immature and the lowest in mature neurons. The distribution of labeling changed from an exclusively somal location in immature cells to one of distal membranes of neuronal processes in mature cells. Much of the latter reactivity was localized to apparent synaptic contracts. GBP immune reactivity was also demonstrated by Western blot analysis of proteins from cultured neurons. Based on these observations it is proposed that some of the glutamate-binding sites in cultured neurons are identical to the 63/71 kDa GBP's.

(Supported by grants AA04732 and DAAL 03-88-K0017).

230.19

IMMUNOLOCALIZATION OF GLUTAMATE-BINDING PROTEINS (GBP'S) IN GLUTAMATERGIC PATHWAYS IN RAT BRAIN.
E.K. Michaelis and M.J. Eaton, Dpt of Biochem, Univ of Kansas, Lawrence, Kansas 66045

A polyclonal antiserum against the 63/71 kDa GBP's from rat brain synaptic membranes was used for immuno-gold localization of the protein in rat brain sections and synaptosomes. Silver-enhanced immuno-gold GBP like immunoreactivity was visualized in sections of cerebral cortex, hippocampus, and cerebellum by light microscopy. Electron microscopy was used to visualize colloidal gold reactivity in whole brain synaptosomes and dendritic areas of the different brain regions. Somal membranes and terminal dendritic fields of the perforant path, mossy fiber and Schaffer collateral paths of the hippocampus were well labeled for GBP-like immunoreactivity at the light microscopic level. Post-synaptic membranes and post-synaptic densities contained the immuno-gold reaction product in these areas at the electron microscopic level. Immunoreactivity was localized also to the granule cell membrane and dendrites and the parallel fiber synapses in the cerebellum and in neuronal membranes and dendrites in the sensory cortex. In synaptosomes from whole brain, reactivity was localized exclusively to post-synaptic membranes and post-synaptic densities. Immune labeling with a polyclonal antibody directed against phosphate activated glutaminase (PAG) localized to the pre-synaptic terminals in many of the GBP-containing synaptosomes. In brain sections, the most intensely double-labeled synapses were the mossy fiber terminals in the hippocampus and the parallel fiber synapses in the cerebellum. Most terminals that contained PAG immunoreactivity also contained GBP immunoreactivity. (Supported by grants AA 04732; DAAL 03-88-K0017).

230.20

CHARACTERIZATION OF EXCITATORY AMINO ACID STIMULATED TURNOVER OF PHOSPHOINOSITIDES IN NEONATAL HIPPOCAMPAL TISSUE.
S.D. Flagg and M.B. Robinson, Children's Seashore House, Depts. Ped. and Pharm., Univ. of Pennsylvania, Philadelphia, PA 19104.

It has been suggested that there are two pharmacologically distinct excitatory amino acid (EAA) receptors that are coupled to increased turnover of phosphoinositides (pI) (For review see Sladezek at al., 1986), one of which is blocked by phosphonate-containing analogs of glutamate. Using hippocampal tissue prepared from neonatal (5-11 days) rats, the EAA stimulated production of inositol phosphates was characterized. Compounds tested for stimulation were quisqualate (Quis), ibotenate (Ibo), and trans-1-amino-cyclopentane-1,3-dicarboxylic acid (trans-ACDP) with EC50's of 0.56, 45, and 70 µM, respectively. N-Methyl-D-aspartate was inactive. DL-2-amino-3-phosphonopropionate (DL-AP3), an inhibitor of EAA stimulated turnover of pI (Schoepp, J. Neurochem, 53 (1989) 273), blocked all three agonists with similar potency. The IC50's were as follows: Ibo (IC50 = 500 µM), Quis (IC50 = 833 µM) and trans-ACDP (IC50 = 684 µM). All three of these inhibition curves were consistent with a single site. The concentration dependence of the Quis stimulated turnover of pI was determined in the presence of 300 µM and 1000 µM DL-AP3. In preliminary studies, DL-AP3 decreased the maximum response induced by Quis with no effect on the EC50. These studies suggest that DL-AP3 may non-competitively inhibit the EAA receptors that are coupled to increased turnover of phosphoinositides. Further, the present data are consistent with a single EAA receptor coupled to pI hydrolysis in hippocampal tissue prepared from neonates.

EXCITATORY AMINO ACIDS: RECEPTORS V

231.1

POLYAMINE SPIDER TOXINS ARE POTENT UN-COMPETITIVE ANTAGONISTS OF NMDA AND KAINATE RECEPTORS.
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We examined the effect of argiopine and argiopinine 3, low molecular weight polyamine components of the spider *Argiope lobata* venom [1], on rat brain EAA receptors expressed in *Xenopus* oocytes. Using a single intracellular microelectrode, oocytes injected with rat brain poly A+ RNA were voltage clamped at -60mV while drugs were applied to the cell via a flowing stream of Mg^{++} free Barth's medium. Responses to 100µM NMDA with 10µM glycine were potently blocked by both polyamine toxins in a dose dependent manner; argiopine was slightly more potent than argiopinine 3. Both compounds had similar potencies against 100µM KA but were unable to block more than 50% of the response, possibly suggesting the existence of more than one subtype of KA receptor. Another KA antagonist, BOAA, was able to block all of the KA response [2]. Oscillatory responses to 2µM QA were unaffected by either polyamine toxin. Dose response curves for NMDA/glycine and KA both with and without the polyamine toxins demonstrated that the antagonism against these two sites was not competitive; increasing agonist concentrations were unable to overcome the antagonism by either spider toxin. We were able to demonstrate a use-dependent phenomenon similar to that of PCP; neither polyamine toxin affected the NMDA or KA response without the presence of the respective agonist. The work presented here suggests that these compounds may prove useful in distinguishing among the different classes of glutamate receptors, particularly among QA and KA receptors.

[1] Grishin, E.V. *et. al.*, (1988) Bioorganicheskaya Khimiya, 14 (7) 883-892.
[2] Watson, G.B. *et. al.*, (1990) *in preparation.*

231.2

DEVELOPMENTAL CHARACTERISTICS OF L-ASPARTATE BINDING SITES IN NEURONS AND GLIA FROM THE RETINA.
F.Somohano, M.Romo de Vivar* and A.M.López-Colomé. Instituto de Fisiología Celular, UNAM. Apdo. Postal 70-600, 04510 México, D.F., México.

Previous studies in the whole chick retina have demonstrated that receptors which bind L-Asp and NMDA are present in very low concentrations and rise markedly between days 14 and 18 of embryonic development. Since these receptors could be of neuronal or glial origin, we studied ^{3}H-L-Asp binding to membranes from neuronal (N) and glial (G) cultures from chick retina at DIV 1,5,8 and 12. ^{3}H-Asp binding to N and G decreased 50% from day 1 to day 12 IV. Scatchard analysis of saturation curves in N was biphasic at all ages with K_{B1}=35 nM and K_{B2}=400 nM, B_{max} decreasing from 0.4 to 0.2 pmol/mg protein from day 1 to day 8 IV. The most potent displacers of ^{3}H-L-Asp at 1 DIV were L-Asp=NMDLA= L-Glu>AMPA=APV, whereas at 8 DIV, AMPA no longer displaces L-Asp. This is in agreement with the increase in specificity observed in ED of whole retina. In G, kinetics of L-Asp binding revealed three sites: K_{B1}=30 nM, K_{B2}=400 nM and K_{B3}=1.5 µM; while K_{B1} and K_{B2} coexist at 1 DIV, K_{B2} and K_{B3} are present at 12 DIV. In these membranes (G), while inclusion of Na^+ in the medium yielded the pharmacological profile reported for high affinity uptake systems, in the absence of this ion, pharmacology is in line with that described for QA metabotropic receptors in astrocytes.

Supported by grant P228CCOX891617 from CONACyT.

231.3

ARE NMDA RECEPTORS IN THE RETINA EQUIVALENT TO THOSE IN THE BRAIN? ^{3}H-CPP BINDING STUDIES.
A.M. López-Colomé and F. Somohano. Instituto de Fisiología Celular, UNAM. Apdo. Postal 70-600, 04510 México, D.F., México.

CPP (3-(RS)-2 carboxypiperazin-4-yl) propyl-1-phosphonic acid has been known for some years as one of the most selective antagonists at the NMDA receptor in the brain. We have measured ^{3}H-CPP binding to membranes from chick retina in the presence and absence of Mg^{++}. Saturation curve and Scatchard analysis revealed a single site in the absence of Mg^{++}, with K_D=400 nM and B_{max}=9.5 pmol/mg protein. In the presence of Mg^{++} however, a second, high affinity component of binding was evident, with K_D=59 nM and B_{max}=2.2 pmol/mg protein. Bound CPP was preferentially displaced from the high affinity site by CPP=AMPA=APH but not by L-Asp; at the low affinity site, the most efficient displacers were also CPP=AMPA=APH, L-Asp being as potent. Reciprocal experiments binding ^{3}H-AMPA showed that APV and APH were potent displacers at this site. Our results suggest that either ^{3}H-CPP is not such a specific ligand for the NMDA recognition site, or alternatively AMPA sites are not restricted to QA receptors in the retina.

This work was supported in part by grant P228CCOX891617 from CONACyT.

231.4

DECREASED 3H-AMPA BUT NOT 3H-TCP BINDING IN HIPPOCAMPUS AFTER KAINIC ACID ADMINISTRATION.
K.K. Devgan, G. Tocco, S. Hauge*, J. Najm, M. Baudry, & R.F. Thompson. Program in Neuroscience, University of Southern California, Los Angeles, CA 90089-2520.

Systemic or intracerebral administration of kainic acid (KA) in rats induces limbic seizures and extensive neuronal damage throughout the limbic system. We were interested in evaluating changes in different subtypes of glutamate receptors that might be involved in determining seizure susceptibility.

Sprague Dawley rats received KA (10 mg/ Kg, i.p.) and were sacrificed 12-15 hours after drug administration. Quantitative ligand binding autoradiography was performed on brain sections with either 3H-AMPA, a ligand specific for the ionotrophic quisqualate receptor, or 3H-TCP, a ligand which binds inside the NMDA receptor channel. 3H-AMPA binding was significantly reduced in different regions of the brain with the highest reduction in stratum radiatum of hippocampal field CA1. 3H-TCP binding, however, was not decreased in any structure, suggesting that the decreased AMPA binding is not likely to reflect neuronal cell death. These results indicate that AMPA/quisqualate receptors may be down-regulated as a result of intense physiological activity. It is possible that the activity- dependent regulation of the AMPA/quisqualate receptor may participate in long term changes in hippocampal function.

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231.5

DESENSITIZATION OF THE IONOTROPIC QUISQUALATE RESPONSE IN XENOPUS OOCYTES IS DUE TO AN AUTOINHIBITION. B.Lambolez*, M.Geoffroy*, J.Rossier and R.T.Kado. Centre d'Etudes de Systême Nerveux, C.N.R.S. Gif sur Yvette, 91198 FRANCE

The responses of Xenopus oocytes injected with mRNA from rat cerebral cortex to Quisqualate (QA) , a-amino-3-hydroxy-5-methyl-isoxazole-4-proprionate (AMPA) and glutamate (GLU) have been studied using standard two-electrode voltage clamp techniques. QA doses >2x10^{-6}M ([QA]$_H$) applied to mRNA injected oocytes held at -60mV, produced rapid initial current responses which quickly decreased to a steady level. At wash-out, the current initially increased making a "hump" before returning to the holding level. The wash-out hump was suppressed by returning to [QA] <10^{-6}M or if [DNQX] <1/10 [QA] was applied, both increased the currents but wash-out did not produce a hump. The hump indicated that the steady current was an inhibited state of the response. [QA]$_H$ doses applied after starting at doses <10^{-6}M did not produce the initial peak but did reduce the current. Risetimes for the initial peaks at [QA]$_H$ doses were markedly slower than for lower concentrations. These results showed that the QA response was rapidly inhibited by [QA]$_H$ doses and passed through a disinhibited state on wash-out before complete agonist removal. The effect was more pronounced with QA than with AMPA or GLU. This receptor may therefore have a low affinity inhibitory site which can be occupied by QA at sufficient doses.

231.6

FUROSEMIDE, A LOOP DIURETIC, DIFFERENTIALLY AFFECTS GLUTAMATE RECEPTORS IN CULTURED SPINAL CORD NEURONS. J. Lerma* and R. Martín del Río†, *Instituto Cajal, C.S.I.C. and †Dept. Investigación, Hospital "Ramón y Cajal". Madrid, Spain.

Glutamate receptors, in particular of the NMDA type, have been implicated in the pathophysiology of epilepsy, stroke and neurodegenerative disorders. With the probable exception of dissociative anesthetics and some σ-opioids ligands, clinical useful drugs acting on excitatory amino acid receptors are not currently available. Preliminary experiments in our laboratory have shown that furosemide, a well characterized loop diuretic widely used for edema treatment, blocks the response to locally applied NMDA in the "in vivo" rat hippocampus. To determine the action of furosemide on glutamate receptors, we have used the patch-clamp technique in the whole-cell configuration and a fast perfusion system to apply excitatory amino acids to spinal cord neurons maintained in culture. Furosemide inhibited the NMDA-induced current in a dose-dependent manner. IC_{50} value was calculated from dose-inhibition curves to be 1.2 mM. Hill coefficient was close to unity. Block was non-competitive, since IC_{50} values obtained with 30 and 300 μM NMDA were similar. Inhibition of NMDA-induced responses by furosemide developed fast and was fully reversible upon antagonist withdrawal and independent of voltage (-80 to +30 mV). In a few cells furosemide (5 mM) induced a slight inhibition (10%) of kainate-evoked current while in others no effect was observed. In contrast peak and steady responses to AMPA (100 μM) were both slightly potentiated by furosemide. Degree and time course of desensitization remained unaltered. This result provides evidence supporting K and Q receptors as separate structures. We suggest that furosemide may be a good starting structure for designing specific, non-competitive, voltage-independent NMDA antagonists as well as for synthesis of new compounds to pharmacologically separate K and Q receptors.

231.7

QUISQUALATE-EVOKED RELEASE OF VASOPRESSIN FROM ISOLATED SUPRAOPTIC NUCLEI OF THE RAT C.R. Jarvis, B. Hu, and L.P. Renaud. Centre for Research in Neuroscience, Montreal General Hospital & McGill University, Montreal, Canada H3G 1A4; Neuroscience Unit, Ottawa Civic Hospital, Ottawa, Canada K1Y 4E9.

The release of vasopressin (VP) from hypothalamic magnocellular nuclei in vitro has been demonstrated under conditions which increase neuronal activity. Glutamate is known to have potent excitatory actions on magnocellular supraoptic neurosecretory neurons via activation of postsynaptic receptors. The present study examines the ability of the non-NMDA glutamate agonist, quisqualate, to release (VP) from isolated supraoptic nuclei maintained in perifusion chambers supplied with oxygenated (95% O_2 5% CO_2) artificial cerebrospinal fluid and maintained at 37°C. Perfusate samples were collected over 10 minute intervals and assayed for VP by radioimmunoassay. Following an initial incubation period (45-60 mins.) perifusion with quisqualate (up to 100 uM) induced a dose-dependent increase (up to 10-20 fold) in the basal levels of VP. These results support the proposal that VP can be released locally (e.g. from somas and dendrites) as well as distally (e.g from axon terminals) in response to stimulation by neurotransmitters. Supported by FCAR, FRSQ, and MRC.

231.8

MAGNESIUM DIFFERENTIALLY MODULATES EXCITATORY AMINO ACID EFFECTS ON THE AXOLOTL VESTIBULAR SYSTEM AFFERENTS. M.E. Pérez*, R. Vega* C. Erósteguí* and E. Soto, Univ. Autónoma de Puebla - ICUAP, P.O. Box 406, Puebla, México.

Numerous reports have indicated that divalent cations play a major modulatory role upon the activity of excitatory amino acid (EAA) agonists. The aim of this study was to evaluate the effects of Mg^{++} on the vestibular afferent fibers response to EAA agonists.

Multiunit spike activity of the axolotl (Ambystoma tigrinum) semicircular canal afferents was extracellularly recorded with a suction electrode. Kainate (KA, 0.1 to 100 uM), quisqualate (QA, 0.01 to 100 uM) and NMDA (1 uM to 10 mM), were pressure ejected from a pipette in volumes of 20 ul in a 10 ml bath. Preparation was perfused with Mg^{++} free, 1, 3 and 5 mM Mg^{++} Ringer. In Mg^{++} free Ringer, EAA agonists tested induce a dose dependent excitatory effect. Increasing Mg^{++} in the bath solution to 1, 3, and 5 mM, increments the duration and amplitude of the QA action up to 3 times control, diminish the NMDA effect to 0.1 times control, and apparently does not affect KA dose response.

These results indicate that extracellular Mg^{++} concentration changes might be a valuable tool to discern between NMDA, KA and QA receptor evoked responses.

231.9

FUNCTIONAL CORTICAL KAINATE RECEPTORS IN THE RAT ARE EXPRESSED LATER THAN SODIUM CHANNELS AND GABA RECEPTORS IN EMBRYOGENESIS. N.S. Nadi, V.Smallwood*, S.V. Smith* and J.L. Barker, Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD.

The development of functional glutamate receptors (Glu-R), sodium channels (Na^+ Ch) and GABA receptors (GABA-R) was investigated in acutely dissociated cortical neurons of the rat from embryonic day (E) 13 to postnatal day (PN) 13 using tetraphenylphosphonium (TPP^+) method and fluorescence activated cell sorter (FACS) analysis using cells stained with the voltage sensitive dye, oxonol . The TPP^+ analysis revealed an average membrane potential (MP) of -30 mV in early embryonic cells (E13-E16). The MP became more hyperpolarized and reached a level -60 mV at PN3. Depolarization (DPL), induced by Glu and kainate appeared at E17. CNQX partially blocked this effect at E19. Glu DPL became more pronounced in the early PN reaching a maximum at PN13. Throughout this period only a partial block with CNQX could be shown. AP-5 had no effect on Glu DPL. The Na^+ Ch, as demonstrated by veratridine DPL, developed at E13 and was blocked by tetrodotoxin. GABA and muscimol caused DPL at E15, which was blocked entirely by bicuculline and partially by picrotoxin. The DPL induced by GABA decreased in later embryogenesis. FACS analysis of the cells of the same population was complementary to the TPP^+ findings While being more qualitative in nature, FACS allowed visualization of several cell populations (based on size and fluorescence) with different responses to Glu, kainate, veratridine and GABA. The results demonstrate that functional Glu-R develop later than the GABA-R and Na^+ and that the first subtype to emerge is the kainate response.

231.10

SENSITIVITY OF QUISQUALATE AND KAINATE CHANNELS TO D-2-AMINO-5-PHOSPHONOVALERATE (APV) IN CULTURED RAT CEREBELLAR GRANULE NEURONS. C.E.Herron* and S.G.Cull-Candy, Dept. Pharmacology, University College London, London WC1E 6BT.

NMDA, kainate and quisqualate activate channels with conductance levels ranging from 6 to 50pS in cultured cerebellar granule neurons. The 50pS channel however is preferentially operated by NMDA. We have therefore examined the effect of the competitive NMDA antagonist APV on channels activated by quisqualate and kainate to determine if large conductance openings correspond to those activated by NMDA. Current amplitude distributions where fitted with 1-4 Gaussian components and only completely resolved channel openings where used (duration> 2.5τ). In all patches quisqualate (10-30μM) produced large conductance openings (~48pS comprising 58% of openings n=5) with measurements ranging from 7 to 56pS. Kainate (10-30μM) also activated channels of either large or small conductance; in 4 out of 6 patches 90% of openings had an amplitude of 46pS while in two patches 80% of openings where 7pS. APV (20-50μM) reversibly abolished or reduced the frequency of opening of the large (~ 50pS) channels activated by both agonists and had no apparent effect on the frequency or amplitude of the small (7-20pS) conductance openings. This suggests that the large conductance kainate and quisqualate channels originate from activation of the NMDA type receptor in granule neurons. *Present address: Wyeth Research (UK), Huntercombe Lane South, Taplow, BERKS SL6 OPH.

231.11

EFFECT OF WHEAT GERM AGGLUTININ ON QUISQUALATE CHANNELS IN CULTURED POSTNATAL RAT HIPPOCAMPAL NEURONS. L.L. Thio, D.B. Clifford, and C.F. Zorumski. Dept. of Psychiatry, Washington Univ. School of Medicine, St. Louis, MO 63110.

The lectin wheat germ agglutinin (WGA) markedly inhibits rapid quisqualate receptor desensitization in cultured postnatal rat hippocampal neurons. We have examined the effect of WGA on quisqualate channels in outside-out patches to determine whether quisqualate desensitization results from a decrease in the number of channels, the channel open probability, or the single channel conductance. Quisqualate (2.5-1000μM) in the presence of 1mM D,L-2-amino-5-phosphonovalerate (APV) evokes bursts of openings having a primary single channel conductance of 8.6pS. The bursts are well-defined as the closed time histogram is described by three exponentials having time constants of <1, 1-5, and 30-500ms. If closures belonging to the two fastest components are defined to be within a burst, the burst length histogram is described by one or two exponentials and gives an arithmetic mean burst length of 8.3 ± 0.6ms (mean ± SEM, n=24) for all concentrations of quisqualate examined. WGA (25μg/ml) does not alter the single channel conductance (8.4pS) and tends to increase the mean burst length (17 ± 3.5ms, n=15). These results suggest that WGA inhibits quisqualate receptor desensitization by increasing the channel open probability.

231.12

THE MODULATION OF KAINATE RECEPTOR CHANNELS BY Ca IONS IN ISOLATED TRIGEMINAL NEURONS OF RAT. Y.-P. Gu and L.-Y.M.Huang, Marine Biomed. Inst., Univ. of TX Med. Branch, Galveston, TX 77550.

The effects of divalent cations, especially Ca^{++}, on N-methyl-D-aspartate (NMDA) responses have been studied in many preparations. In contrast, the role of Ca^{++} in non-NMDA responses is not well described. We have studied the permeation of Ca^{++} and its blockade of kainate receptor channels in isolated trigeminal neurons using the patch clamp method. Under whole cell recording conditions, kainate responses were blocked substantially when 10-50 mM Ca^{++} were included in the external solution. The equilibrium dissociation constant (Kd) for the Ca block of kainate channels was 30 mM. The blocking effect of Ca was slightly voltage dependent. The Kd increased e-fold per 127 mV depolarization. The electrical distance of the binding sites, δ, was 0.1. The affinity of kainate to its receptor, on the other hand, did not change in different Ca medium. With an increase in external Ca concentration, the reversal potential of kainate response shifted in the depolarizing direction. The permeability ratio P_{Ca}/P_K calculated from the shift was 0.20. These results suggested that kainate receptor channels were moderately permeable to Ca^{++}, and the binding site for Ca was located near the extracellular surface of the kainate receptor channels. Supported by NS01050, NS23061, John Sealy Mem. Endow. Fund.

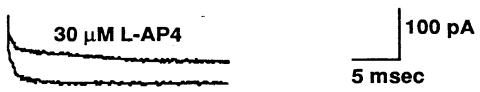
231.13

L-AP4 REDUCES HIGH THRESHOLD CALCIUM CURRENTS IN OLFACTORY BULB NEURONS. P.Trombley †, and G.Westbrook. †Dept. Biology, Univ. Oregon & Vollum Inst., Oregon Hlth. Sci. Univ., Portland, OR.

The glutamate analogue L-AP4 acts presynaptically to reduce transmitter release at excitatory nerve terminals in the CNS, although the mechanism is unclear. L-AP4 also reduces EPSPs evoked by stimulation of cultured mitral/tufted cells (Trombley & Westbrook, J. Neurophysiol.,1990). We tested whether L-AP4 inhibits voltage-gated calcium currents as a possible mechanism of presynaptic inhibition.

Acutely dissociated olfactory bulbs neurons from PN 5-7 rat pups were whole-cell voltage clamped at -60 mV. L-AP4 (3-300 µM) was delivered by a multibarrelled flow pipe in solutions containing (mM): Ba 10, Na 145, K 2.5, Mg 1, glycine 0, Hepes 10, Glucose 10; patch pipettes contained (mM): Cs 140, ATP 5, GTP 0.5, Ca 0.1 , EGTA 11, Hepes 10.

High (HT) threshold calcium currents were activated by 50 ms jumps to 0 mV. HT currents were reduced by L-AP4 (30/38 cells) as well as by NE (34/38 cells). Peak current was reduced 25% (range 11-53, n=30) by 30 µM L-AP4 compared to 34% by 30 µM NE. However HT currents in neurons cultured from PN 1 pups were not inhibited by L-AP4 or NE, although evoked EPSPs were reduced by L-AP4. Inhibition by L-AP4 was irreversible after intracellular perfusion with GTPγS (100 µM), suggesting coupling of the AP4 receptor via a G protein.



Supported by the McKnight Foundation,PHS grant NS26494 (GW) and a predoctoral NIH traning grant (PT).

231.14

EFFECTS OF 2-AMINO-4-PHOSPHONOBUTYRATE (AP4) ON GUINEA PIG HIPPOCAMPAL SLICE PHOSPHOINOSITIDE (PI) TURNOVER. S.J.Gibbons*[1], P.J.Roberts[1], A.C.Foster[2]. [1]Dept. Physiology and Pharmacology, University of Southampton, U.K., [2]Merck, Sharp and Dohme Neuroscience Research Centre, Harlow, Essex. U.K.

AP4 has both inhibitory and stimulatory effects on inositol monophosphate formation in guinea pig hippocampal slices (Gibbons and Roberts, Br.J. Pharm. in press). Some of its analogues possessed the same mixed effects as L-AP4 e.g. 1mM L-serine-O-phosphate (inhibition v ibotenate (ibo) 37.4% P<0.001 n=4, effect alone 148±7% n=6 P<0.05), other compounds were more potent inhibitors of PI turnover than L-AP4 but had no stimulatory effects (e.g. 1mM 2-amino-4-arsenobutyrate: inhibition vs ibo 50% P<0.001 n=4, effect alone 101±3% n=4) whilst the ω-phosphino analogues (Fagg and Lanthorn, Br.J. Pharm., 86:743, 1985) only had stimulatory effects (5mM 134±8% n=4). The replacement of chloride ions by equimolar acetate reduced the stimulation of PI turnover by 1mM ibo (63% p<0.001), quis (68% p<0.001) and 3mM carbachol (56% p<0.001) but not 10mM L-AP4. From these results we conclude that AP4 has a weak non-specific stimulatory effect on guinea pig hippocampal PI turnover which is distinct from its inhibitory effect. Neither of these effects is comparable with the inhibition of synaptic transmission or chloride dependent glutamate binding by AP4 and its analogues. This work was supported by the SERC and MSD. The ω-phosphino analogues were a gift of Dr.G.E.Fagg.

231.15

EFFECT OF POSTNATAL D,L-2-AMINO-3-PHOSPHONOPROPIONATE (D,L-AP3) TREATMENT ON PHYSICAL DEVELOPMENT, BEHAVIOR, AND PHOSPHOINOSITIDE (PIn) HYDROLYSIS IN THE RAT. J.P. Tizzano, R.L. Bailey*, J.A. Engelhardt*, and D.D. Schoepp. Lilly Research Laboratories, Eli Lilly and Company, Greenfield, Indiana 46140.

Previous studies have shown that D,L-AP3 is a preferential inhibitor of metabotropic (PIn hydrolysis-coupled) excitatory amino acid (EAA) receptors as opposed to ionotropic EAA (NMDA, AMPA, kainate) receptors. Metabotropic EAA receptor coupling is greatly enhanced during the early period of neonatal development in the rat, suggesting that these receptors play a role in brain development. This study evaluated the postnatal treatment effects of D,L-AP3 on 1) EAA-stimulated PIn hydrolysis in brain slices 2) various behavioral tests including locomotor activity, auditory and tactile startle, and passive avoidance, and 3) gross pathology of the brain and visual system. Fifteen litters of CD rats were dosed i.p. (1 pup/sex/litter) on postnatal days 3-12 with 0, 125, 250, or 500 mg/kg/day D,L-AP3. Hippocampal slices from rats at different ages during and after D,L-AP3 treatment were examined for *trans*-(±)1-amino-1,3-cyclopentanedicarboxylic acid (ACPD)-stimulated PIn hydrolysis. At 7 and 15 days of age, in the 250 and 500 mg/kg dose groups ACPD-stimulated PIn hydrolysis was greatly inhibited. However, in adult rats at all doses ACPD-stimulated PIn hydrolysis was not different from control. Auditory and tactile startle were increased in the 250 and 500 mg/kg D,L-AP3 groups at 19 and 38 days of age. In 21 and 30 day old rats, the 500 mg/kg D,L-AP3 treatment impaired passive avoidance learning and increased motor activity, respectively. Retinal and optic nerve dysplasia was found in the 250 and 500 mg/kg D,L-AP3 dose groups, with severe atrophy of the optic nerve and chiasm at the higher dose. These studies show that dosing with D,L-AP3 during postnatal development inhibits the metabotropic EAA receptor in a reversible manner. This is associated with deficits in behavior and profound degeneration of the rat visual system.

231.16

BETA-METHYL-AMINO-L-ALANINE (BMAA) ACTIVATES METABOLOTROPIC GLUTAMATE RECEPTORS IN BRAIN SLICES AND NEURONAL CULTURES. A. Copani*, P.L. Canonico, M.V. Catania*, V. Bruno, E. Ratti#*, G. Gaviraghi#* and F. Nicoletti*, Institute of Pharmacology, Catania University School of Medicine, Catania, and #GLAXO Research Laboratories, Verona, Italy.

Although the effects of BMAA have been ascribed to activation of NMDA receptors, the toxin was much more potent in inhibiting [^{3}H]glutamate binding than the binding of the selective NMDA ligand, [^{3}H]CPP in rat brain membranes. This suggests the existence of a non-NMDA receptor component in the action of BMAA. Accordingly, BMAA potently increased inositol phospholipid (PPI) hydrolysis in both hippocampal slices and cultured cerebellar neurons. BMAA stimulated [^{3}H]inositol-monophosphate formation to a much greater extent in slices from newborn than from adult animals and its action was antagonized by L-AP4 but not by D-AP5. BMAA was more potent and efficacious than glutamate and nearly as effective as ibotenate or trans-ACPD. In cultured cerebellar neurons, stimulation of PPI hydrolysis by BMAA was largely insensitive to D-AP5 and was not reduced by Mg^{2+} ions. We conclude that BMAA acts as a potent agonist of metabolotropic glutamate receptors in the brain.

231.17

DESENSITIZATION OF METABOLOTROPIC GLUTAMATE RECEPTORS IN NEURONAL CULTURES. M.V. Catania*, E. Aronica*, A. Copani*, F. Nicoletti*, M.A. Sortino and P.L. Canonico. Institute of Pharmacology, University of Catania School of Medicine, Italy.

Pretreatment of cerebellar, cortical or hypothalamic neurons with glutamate reduced the stimulation of inositol phospholipid hydrolysis induced by subsequent addition of glutamate without affecting the response to carbamylcholine. Desensitization of metabolotropic glutamate receptors developed rapidly and persisted for a long time (24-48 hours) after removal of glutamate from the incubation medium. NMDA and quisqualate did not induce cross-desensitization, supporting the existence of two distinct metabolotropic glutamate receptor subtypes. Desensitization induced by a 30-min (but not 6-hour) preexposure to glutamate was attenuated by putative protein kinase C inhibitors, including the gangliosides GM_1 and GT_{1b}, sphingosine and polymyxin B, but not by inhibitors of arachidonic acid metabolism, by the calpain inhibitor, leupeptin, or by the lectin, concanavalin A. These results suggest that desensitization of metabolotropic glutamate receptors involves, at least in its rapid component, activation of protein kinase C.

231.18

ENHANCEMENT OF SYNAPTIC RESPONSES IN AREA CA_1 OF THE HIPPOCAMPUS BY SELECTIVE ACTIVATION OF THE ACPD SUBTYPE OF EXCITATORY AMINO ACID RECEPTOR. M.A. Desai and P.J. Conn. Dept. of Pharmacology and Div. of Neuroscience, Emory Univ. Atlanta, GA 30322.

Evidence suggests that an excitatory amino acid (EAA) receptor subtype exists that employs phosphoinositide hydrolysis for signal transduction. This receptor is clearly distinct from NMDA, kainate, and quisqualate receptors and has been named the ACPD receptor (Monaghan et al. (1989) *Ann. Rev. Pharmacol. Toxicol.* 29:365). This is based on the finding that the glutamate analogue *trans*-ACPD (*trans*-1-amino-1,3-cyclopentanedicarboxylic acid) is a selective agonist at this receptor subtype (Desai and Conn (1990) *Neurosci. Lett.* 109: 157; Palmer et al. (1990) *Eur. J. Pharmacol.* 166: 585). To investigate the physiological role of this receptor, we measured the effects of *trans*-ACPD on extracellular field potentials in area CA_1 of rat hippocampal slices. *trans*-ACPD induced a transient increase in the amplitude of population spikes in area CA_1 in response to stimulation of the Schaffer collateral. Concentrations of *trans*-ACPD that are effective in stimulating phosphoinositide hydrolysis increased population spike amplitude in 21 of 22 slices investigated (mean increase = 74.4 $\pm$ 11.9%; range = 14.3% - 172%). In addition, *trans*-ACPD induced formation of a second population spike in 15 of 22 slices. In 3 of these slices, 3 or more population spikes were observed. These effects occurred in the absence of a change in the initial slope of field EPSPs measured in the stratum radiatum. These data suggest that activation of the ACPD receptor enhances responses at the Schaffer collateral-CA_1 synapse. It is likely that this effect is not mediated by an increase in glutamate release but involves changes in pyramidal cell excitability or blockade of recurrent inhibition. Supported by P.M.A. Foundation, Emory Univ. Research Committee, and N.I.H. (NS28405-01 and BRSG S07 RR05364).

231.19

AP3 AND L-BMAA DISPLACE [^{3}H]GLUTAMATE BINDING TO THE METABOTROPIC RECEPTOR. J.J. Cha, R.L. Makowiec, J.B. Penney, and A.B. Young. Neuroscience Program and Department of Neurology, University of Michigan, Ann Arbor, MI 48104-1687.

A novel excitatory amino acid (EAA) receptor linked to phosphoinositide (PI) metabolism has been described. This "metabotropic" EAA receptor is activated by quisqualate, ibotenate, glutamate, and *trans*-1-amino-cyclopentyl-1,3-dicarboxylic acid (*trans*-ACPD). We have described an autoradiographic binding assay for the metabotropic receptor using [^{3}H]glutamate as a ligand (Cha et al., Neurosci. Lett., in press). Binding to this receptor was distinguished by its low affinity for (R,S)-α-amino-3-hydroxy-5-methyl-4-isoxazole-propionic acid (AMPA) but high affinity for quisqualate, glutamate, ibotenate, and *trans*-ACPD.

D,L-2-amino-3-phosphonopropionate (AP3) has been reported to be a relatively selective inhibitor of EAA stimulated PI hydrolysis (Schoepp and Johnson, J. Neurochem., 53:273-278, 1989). AP3 inhibited [^{3}H]glutamate binding to the metabotropic receptor with an IC_{50} = 190±30 μM. This IC_{50} is consistent with reports of AP3 inhibition of EAA stimulated PI hydrolysis and suggests AP3 may be a useful pharmacological tool in studying the metabotropic receptor.

The plant neurotoxin β-N-methylamino-L-alanine (L-BMAA) has been implicated in the pathogenesis of amyotrophic lateral sclerosis-Parkinsonism-dementia of Guam. L-BMAA has recently been found to stimulate PI hydrolysis via the metabotropic receptor (Nicoletti et al., Eur. J. Pharmacol., in press). L-BMAA inhibited binding to the metabotropic receptor with an IC_{50} = 21±3 μM. These data support the idea that L-BMAA can activate metabotropic receptors and raises the possibility that metabotropic receptors may participate in neurodegenerative processes.

Supported by NIH NRSA 5T32 GM 07863 and USPHS Grant NS 19613.

TRANSMITTERS IN INVERTEBRATES II

232.1

RELEASE OF A MODULATORY NEUROPEPTIDE FROM CULTURED *APLYSIA* NEURONS. J.D. Hall and P.E. Lloyd. Dept. Pharm. Physiol. Sci., Univ. of Chicago, Chicago, IL 60637.

Pedal peptide (Pep) is a prevalent neuropeptide in *Aplysia*. It is found throughout the CNS, but is predominantly localized to a band of more than 60 neurons on the dorsal surface of each pedal ganglion. We have previously shown that Pep has a modulatory effect on foot muscle during *Aplysia* locomotion. Specifically, it increases the amplitude and relaxation rate of foot muscle contraction.

Examination of Pep release upon stimulation of pedal nerves has proven to be difficult due to the proteolytic activity of the foot tissue and the hemolymph. In an attempt to circumvent these problems we have set up a system for the primary culture of individual Pep-neurons isolated from the pedal ganglia dorsal cluster. This allows an *in vitro* analysis of the release of pedal peptide. After several days in culture these cells regenerated neurites which exhibited extensive branching and contained many large varicosities and growth cones. Both the neurites and the growth cones exhibited Pep-immunoreactive staining. Neurons were labeled for 48 hr in a medium containing ^{35}S-methionine. Liquid scintillation counting of HPLC fractions of these neurons has shown that radiolabeled Pep was synthesized in these cells and was transported into the regenerated neurites. In addition, following intracellular stimulation of cultured Pep-neurons, radiolabeled Pep was detected in the releasate. Thus, we have seen synthesis, transport, and release of pedal peptide by cultured Pep-neurons. Supported by NS 23569.

232.2

IDENTIFICATION OF *APLYSIA* MOTOR NEURONS THAT EXPRESS MODULATORY PEPTIDES. P. J. Church and P. E. Lloyd. Committee on Neurobiology, The University of Chicago, Chicago, Il 60637.

Neuropeptide synthesis was determined for individual identified ventral cluster neurons in buccal ganglia of *Aplysia*. These cells were motor neurons (MNs) which innervate buccal muscles that generate biting and swallowing movements during feeding. Individual neurons were identified by a battery of physiological criteria, stained with intracellular injection of fast green, and the ganglia incubated in ^{35}S-methionine for 24 hr. Peptide synthesis was determined by measuring the appearance of labeled peptides by HPLC of extracts from individual neurons. Five identified peptides were observed to be synthesized by individual neurons including buccalin, FMRFamide, myomodulin, and the 2 small cardioactive peptides (SCPs). Each of these neuropeptides has been shown to modulate responses of the muscles of the buccal mass. Identified neurons consistently synthesized the same peptide(s), however, MNs which innervate the same muscle often expressed different peptides. Neurons which synthesized SCPs also contained SCP-like activity as determined by snail heart bioassay. Our results indicate that every MN synthesized a subset of the 5 methionine-containing peptides. In addition, several neurons consistently synthesized multiple peptides likely to be processed from separate precursors. Supported by NS 23569.

232.3

THE SEROTONERGIC HEART EXCITER NEURON, RB_{HE} OF *APLYSIA CALIFORNICA* SYNTHESIZES R15α2 PEPTIDE. M.E. Skelton, K.R. Weiss & J. Koester. Center for Neurobiology and Behavior, Columbia University, 722 W 168 St., New York, N.Y. 10032.

The neuron R15 in the abdominal ganglion of *Aplysia* expresses an mRNA that is translated into R15α1 neuropeptide. The precursor for this mRNA is spliced differently in other neurons to encode R15α2 peptide (Buck et al., 1987). This study aims to determine the identity and function of neurons that express R15α2 peptide.

Karagogeos et al. (1989) raised 2 antibodies that recognize R15α1 and R15α2 peptides. Antibody I binds only to R15α1 peptide; antibody I/II binds to both peptides. Alevizos (1989) found that both antibodies label processes in the heart and both peptides are cardioexcitatory. R15 has been shown to innervate the heart, so some cardiac immunoreactivity is likely to be present in R15's processes due to R15α1 peptide. We attempted to determine whether other neurons also contribute to the immunoreactivity in the heart.

One neuron that bound antibody I/II appeared from its location and size to be RB_{HE}, the serotonergic heart exciter. We confirmed this by identifying RB_{HE} physiologically and labeling it with lucifer yellow before double-labeling it with antibody I/II. We assumed that the antibody labeled R15α2 peptide in this case, because antibody I does not bind to RB_{HE} (Alevizos, 1989). To test this hypothesis, ganglia were incubated in the presence of ^{35}S-methionine, RB_{HE}s were dissected, pooled and ran on 3 different reverse phase HPLC gradients. In each case a peak of radioactivity coeluted with synthetic R15α2 peptide, confirming that RB_{HE} synthesizes R15α2 peptide. Thus, R15 may use 5-HT and R15α2 peptide as cotransmitters.

These results suggest that the heart contains R15α1 peptide derived from R15 and R15α2 peptide derived from RB_{HE} innervation. The effects of R15α1 and R15α2 peptides on the heart are being studied to determine how the peptides interact with each other and with 5-HT, and whether the peptides, like serotonin, employ cAMP as a second messenger in cardiac tissue.

232.4

THE DISTRIBUTION OF IMMUNOREACTIVE FMRFAMIDE IN NEURONAL FIBERS, VARICOSITIES, AND SOMATA IN THE HEART OF THE SEA HARE, APLYSIA CALIFORNICA. L. L. Harris* and J. K. Ono. Dept. of Biological Science, California State University, Fullerton, CA 92634.

The tetrapeptide FMRFamide, first isolated using a molluscan heart as a bioassay, has been shown to be present in the CNS of the sea hare, Aplysia californica. Whether this peptide functions as a neurotransmitter in the Aplysia heart is being investigated. Immunohistochemical localization of the peptide indicates that immunoreactive (IR) nerve fibers, varicosities, and neuronal somata occur in the Aplysia heart. Despite earlier studies indicating that FMRFamide affects the mechanical activity of the ventricle, the most dense innervation appears to occur in the auricle and especially in the AV and aortic valves. FMRFamide-IR neuronal somata in the areas near the valves indicate that the heart also contains intrinsic neurons that may play a role in modulating cardiac activity. (Supported by D.A.C. grants to L.L. Harris)

232.5

DISTRIBUTION OF GABA IN THE PLEURAL-PEDAL GANGLIA OF *APLYSIA*. L.J. Cleary and T. Li*. Dept. of Neurobiology and Anatomy, Univ. Texas Med. School, Houston, TX 77225.

Sensory neurons mediating the tail-siphon withdrawal reflex in *Aplysia* receive no spontaneous synaptic input. They are hyperpolarized, however, when a stimulus is applied outside their receptive fields. GABA is one transmitter that could elicit this hyperpolarization. As a first step towards investigating a role for GABAergic neurons in tail-siphon withdrawal, we examined the distribution of GABA-like immunoreactivity in the pleural and pedal ganglia. Adult ganglia were sectioned serially with a cryostat and stained with conventional immunocytochemical techniques. In addition to immunoreactivity in these two ganglia, we found GABAergic processes in the cerebral, buccal and abdominal ganglia.

In the pleural ganglion, there are a small number of immunoreactive neurons scattered irregularly through the medial cluster. In the neuropil, the majority of immunoreactive processes are located at the root of the pleural-abdominal connective. The cell bodies of the tail sensory neurons do not receive axosomatic input from GABA-containing neurons. Thus, the distribution of GABA differs markedly from the modulatory transmitters 5-HT, SCP_B and FMRFamide. In the pedal ganglion, there are several clusters of immunoreactive cell bodies. Although variable, these clusters are generally located on the surface of the neuropil at the base of pedal nerves, the pedal commissure and the cerebral-pedal connective. Immunoreactive processes within the neuropil project toward peripheral nerves, but end in dense arborizations that may extend 200-700 μm into the proximal segment of the nerve. Fibers exit the ganglion primarily through the pedal commissure or the cerebral-pedal connective. These results suggest that GABAergic neurons in the pleural-pedal ganglia are primarily interneurons. While they do not appear to synapse on tail sensory neurons within the pleural ganglion, GABAergic neurons could make synapses as the sensory neurons traverse or exit the pedal ganglion.

232.6

THE DISTRIBUTION OF CARDIOACTIVE TRANSMITTERS IN THE HEART AND NERVOUS SYSTEM OF THE NUDIBRANCH, Archidoris montereyensis. B.L. Wiens and P.H. Brownell. Oregon State University, Corvallis, OR. 97331.

The heart of the nudibranch mollusc *Archidoris montereyensis* is regulated by at least five excitatory and inhibitory motor neurons located in the right pleural and visceral ganglia. We are currently interested in determining the transmitters that mediate the actions of these neurons on the heart. A number of common molluscan cardioactive transmitters are active when bioassayed on an isolated preparation of the *Archidoris* heart. The aminergic transmitters serotonin and dopamine (thresholds = 10^{-8}-10^{-6} M), and the peptides SCP_B (10^{-8}-10^{-7} M) and FMRF-NH_2 (10^{-6}-10^{-5} M) excite the heart, while acetylcholine (10^{-9}-10^{-8} M) inhibits the heart. Using whole-mount immunocytochemical techniques we have mapped the distribution of serotonin, FMRF-NH_2, and SCP_B in the heart and CNS. The pericardial nerve, which innervates the heart and other visceral organs, contains processes immunoreactive (-ir) for all 3 transmitters. However, only serotonin-ir and FMRF-NH_2-ir processes are present in cardiac tissues. Nerves containing serotonin-ir appear to terminate diffusely throughout the ventricle and atrium with a concentration of terminals on the atrio-ventricular valve. FMRF-NH_2-ir processes densely innervate the anterior aorta, the aortic and atrio-ventricular valves, and the efferent gill vein, but not ventricular or atrial muscle. Preliminary studies in the CNS indicate that the right pleural ganglion contains neurons immunoreactive for all 3 transmitters, but only FMRF-NH_2 antisera appears to label cells in the region of the heart motor neurons. Additionally, each of the antibodies labeled processes, but not neurons, within the visceral ganglia. In summary, the differences in the pattern of serotonin and FMRF-NH_2 innervation in the heart and the higher threshold of FMRF-NH_2 actions suggest that these transmitters have different modes of action. Future studies will be directed at identifying the cells of origin for the serotonin-ir and FMRF-NH_2-ir processes and physiological studies of the respective actions of these transmitters. (NIH fellowship #MH09818-02, Sigma Xi)

232.7

THE SEARCH FOR A CLASSICAL TRANSMITTER IN A PEPTIDERGIC INTERNEURON. K. McKenney, N.I. Syed, A.G.M. Bulloch. Department of Medical Physiology, University of Calgary, 3330 Hospital Dr. N.W., Calgary, Alberta, T2N 4N1, Canada.

The goal of this research project is to determine the identity of colocalized transmitters. We have chosen for study an identified interneuron in the snail, Lymnaea stagnalis, that plays a role in coordinating many rhythmic behaviors. VD4 makes predictable excitatory, inhibitory or biphasic synaptic connections with more than 30 identifiable follower neurons. VD4 was previously shown to contain the neuropeptide FMRFamide and related peptides using immunohistochemical and radioimmunoassay techniques. When FMRFamide (10^{-6} M) is bath applied over isolated brain preparations in a low Ca^{++} (0.1 mM)/high Mg^{++} or normal saline many of VD4's follower neurons are hyperpolarized. In none of the cells tested, including neurons which are strongly excited by VD4, was FMRFamide excitatory.

The above indicates that the excitatory actions of VD4 are brought about by a second transmitter. To test whether the excitatory actions of VD4 are mediated by a classical transmitter, various putative transmitters were bath perfused over isolated brain preparations while recording intracellularly from follower neurons that are excited by VD4. Of the transmitters tested, which include acetylcholine, glutamate and glycine, the amines (octopamine, serotonin and dopamine) were best able to mimic the excitatory actions of VD4. However, none of these putative transmitters could mimic all of the excitatory actions of VD4.

The results from these initial experiments suggest that VD4 may use more than one classical neurotransmitter, in addition to FMRFamide. Alternatively, VD4 is using an excitatory neuropeptide which has yet to be determined. Either way, VD4 promises to be an excellent neuron for studying cotransmission at a neuro-neuronal synapse.

Supported by MRC (Canada), AHFMR and NSERC.

232.8

NOVEL NEUROPEPTIDES REVEALED BY cDNAs CLONED FROM *Helix aspersa* NERVOUS SYSTEM. E.M. Lutz*, W. Lesser, M. Macdonald* and J. Sommerville*. Dept. of Biology, Univ. of St. Andrews, Fife KY16 9TS, Scotland; Whitney Lab, St. Augustine, FL 32086-8623.

Two tetrapeptides (FMRFamide and FLRFamide) and five heptapeptides (pQDPFLRFamide, NDPFLRFamide, NDPYLRFamide, SDPFLRFamide and SEPYLRFamide) have been sequenced from the snail *Helix aspersa*. All seven FMRFamide analogues are cardioactive in *Helix*, though they have different potencies. These peptides are also active on other *Helix* muscles and neurones. Evidence from immunohistochemistry, HPLC and RIA suggests that the tetrapeptides and heptapeptides have different distributions within *Helix* tissues.

To determine how these analogues are organized on the precursors, we have isolated and sequenced a series of cDNA clones derived from mRNA of the *Helix* CNS. Clone HF1 (1.9 kb) encodes 10 copies of FMRFamide and 1 copy of FLRFamide, each flanked by basic amino acids (potential cleavage sites). The peptides are spaced out over a total length of ~150 amino acids by acid-rich regions. Clone HF4 encodes only N-terminally extended forms of FLRFamide. In addition to the heptapeptides previously described, 3 novel neuropeptides are found: pQDPFLRIamide (2 copies) and putative peptides of 9 and 16 residues. The extended forms all follow on immediately from a hydrophobic signal sequence and are tightly organized with no acidic spacers. Hybridization experiments indicate that the two clones are derived from different mRNA molecules and that the mRNAs are transcribed from separate genes.

The 9 and 16 residue peptides are also cardioexcitatory in *Helix*, with their apparent potencies in line with those of the other peptides; pQDPFLRIamide is currently being synthesized.

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232.9

EFFECT OF NEUROTRANSMITTERS ON CELL F1 OF THE LAND SNAIL *HELIX ASPERSA* IN PRIMARY CELL CULTURE. Seema Tiwari* and Michael L.Woodruff. Department of Chemistry and Biochemistry, Southern Illinois University, Carbondale, IL 62901-4409.

The giant F1 cell of *Helix aspersa* circumoesophageal ganglia exhibits endogenous oscillatory activity. Bursts of action potentials oscillate with interburst hyperpolarizations. The frequency and pattern of the bursts can be modulated by different neurotransmitters and analogues of cyclic GMP and cyclic AMP.

Primary cell culture of F1 cells from adult organisms has been difficult. In this study cells were mechanically dissociated from ganglia without the aid of enzyme digestion and plated onto polylysine coated coverslips in "co-culture" with *Helix* ganglia (1 ganglion/ml of solution) in conditioned *Helix* media. The F1 grow processes (3 to 5) and have resting potentials between -70 mV and -80 mV. Cells can be recorded from multiple times and used for several days (placed back in culture overnight) before they show deterioration. When the F1 are exposed to 5-HT and forskolin they depolarize to between -40 mV and -50mV and fire action potentials spontaneously (beating). After the addition of both 5-HT and dopamine the F1 show "oscillatory" activity with small bursts of actions potentials (2 or 3) alternating with hyperpolarizations.

232.10

SEASONAL VARIATIONS OF IR-MET AND IR-LEU ENKEPHALIN CONTENT IN THE PERIOESOPHAGEAL GANGLIA OF HELIX ASPERSA. R.Gutiérrez and M.Asai*, Lab. Neurophysiol. Neurosci. Div. Instituto Mexicano de Psiquiatría, México 14370.

Increasing body of evidence suggests the presence of opioid mechanisms in invertebrates. In molluscs, it has been proven that opioid agonists and antagonists exert a number of behavioral and electrophysiological responses, though there are some reports on the lack of effects of opioids at both levels. This lack of effects can be explained if one considers the high dependence of these animals on environmentally precipitated and endogenous rhythmic physiological changes. In order to determine the presence of opioid peptides and their possible seasonal variation in the terrestrial snail Helix aspersa, we carried out radioimmunoassays with specific antisera risen for mammalian Met- and Leu-enkephalins. A pool of 20 perioesophageal ganglia were monthly dissected from snails kept in laboratory conditions. They were divided into three regions: ventral, dorsal, and cerebroid ganglia. IR-Met and IR-Leu enkephalin contents were determined for each pool and the results were expressed in IR-pmol/g tissue. Our results show: A) A clear variation in both IR-Met and IR-Leu enkephalin contents, being January, May, and August the months where peak concentrations are reached. March, June and December have the lowest contents. B) A higher content of Leu-Enkephalin than that of Met-enkephalin is evident in all the CNS regions throughout the year. C) Met-enkephalin contents are higher in the ventral ganglia and no clear regional differences were observed in regard to Leu-Enkephalin. These results provide evidence of the presence of Met- and Leu-Enkephalins in the CNS of Helix aspersa. Moreover, these peptides appear to vary throughout the year showing seasonal peaks. It is noteworthy to point out a striking resemblance of this pattern of variation found in opioid binding studies in other mollusc species (Stefano and Leung, CRC Handbook Com. Opioid. Vol I, 1986).

232.11

THE GENITAL GANGLION OF THE SNAIL MELAMPUS BIDENTATUS CONTAINS NEURONS IMMUNOREACTIVE TO THE PEPTIDES aBCP, SCP_B, BUCCALIN, AND CARP. S. B. Moffett. Dept. of Zoology, Washington State Univ., Pullman, WA 99164-4236.

The family of peptides associated with egg-laying in Aplysia and Lymnaea has been studied in bag cells/caudodorsal cells in the CNS as well as in nonneural tissue. When an antibody to alpha bag cell peptide (aBCP) was used to map the Melampus CNS and reproductive organs, the genital ganglion was discovered. A pair of large neurons, a bilaterally symmetrical cluster and an unpaired right cluster are in the cerebral ganglia. Neurons probably homologous to bag cells/caudodorsal cells are in pleural and visceral ganglia and along nerves innervating reproductive organs, but many more aBCP immunoreactive neurons are in the genital ganglion. It is strategically located for control of gamete release at the juncture of the genital nerve with the common duct; it fans out in the region of the hermaphroditic duct, mucus gland and albumen gland and innervates spermoviduct, ovotestis, oviduct and vas deferens. The ganglionic nature of the genital ganglion is evidenced by the presence of neurons with other transmitter immunoreactivities, including SCP_B, buccalin, catch relaxing peptide (CARP) and by projections into the ganglion of 5-HT and FMRFamide immunoreactive axons. The genital ganglion may be the site of neural coordination of gamete release. (Supported in part by NS27514 to J.E. Blankenship and NS22896 to SBM.)

232.12

IDENTIFICATION OF A FMRFAMIDE-LIKE PEPTIDE BINDING PROTEIN IN SQUID NERVOUS TISSUE. K.Payza, D.A.Price#, M.J.Greenberg# and G.J.Chin*. Laboratory of Developmental Neurobiology, NIH, Bethesda, MD 20892 and #C.V.Whitney Laboratory, University of Florida, St. Augustine, FL 32086.

The neuropeptide FMRFamide and FMRFamide-like peptides have been identified in many animals. As a first step towards molecular characterization of the molluscan FMRFamide receptor, we undertook to identify a binding protein in squid (*Loligo pealii*) optic lobes, which have been shown to contain immunoreactive FMRFamide that co-eluted with the authentic peptide on reverse-phase HPLC. A photoreactive analogue of FMRFamide was synthesized, radio-iodinated and purified by HPLC. This ligand was incubated at 4°C with a crude membrane fraction and irradiated to effect covalent attachment. The membranes were washed and analyzed by SDS gel electrophoresis and autoradiography. Incorporation of radioactivity into a 52,400 dalton protein was reduced by incubation in the presence of 100 µM acFnLRFamide.

232.13

ISOLATION AND IDENTIFICATION OF SCPs IN BIVALVED MOLLUSCS. A. Candelario*, D.A. Price, K.E. Doble* and M.J. Greenberg. The Whitney Lab, Univ. of Florida, St. Augustine, FL 32086-8623.

Using an RIA with an antiserum raised to SCP_B, a neuropeptide from *Aplysia*, we have purified related peptides from a clam, *Mercenaria mercenaria*, and have characterized them by FAB mass spectroscopy and by sequencing. At least two SCP-like peptides occur in the clam: YFAFPRQa (927 da) and IAMSFYFPRMa (1277 da); another peptide, AMSFYFPRMa (1148 da), may be a fragment formed during purification. The 1277 da decapeptide has the same C-terminal tetrapeptide amide as that of the gastropod SCPs; but YFAFPRQa has a Gln substituted for the terminal Met residue and is quite distinct, notwithstanding its clear homology to SCP_B (MNYLAFPRMamide). The same three peptides have been detected in each of several clam tissues examined separately, so there is no evidence of tissue specific expression or processing. Immunohistochemical staining with a monoclonal antibody raised to SCP_B revealed conspicuous staining in cell bodies and fibers in the visceral ganglion, and less so in the cerebral and pedal. Nerve fibers innervating muscle in the rectum were also stained. Low doses of synthetic clam SCPs relaxed the rectum (threshold, $<10^{-9}$ M), but higher concentrations ($>10^{-8}$ M) contracted the tissue or produced a biphasic response. These actions, and the immunohistochemical localization, are reminiscent of the putative role of the SCPs as modulators of feeding in gastropods. The clam peptides had virtually no effect, at moderate doses, on cardiac or somatic muscles of *Mercenaria*. Funded by NIH grant HL28440.

232.14

OCTOPAMINE-IMMUNOREACTIVE NEURONS IN INVERTEBRATE NERVOUS SYSTEM. T. Karhunen* and P. Panula. Department of Anatomy, University of Helsinki, Siltavuorenpenger 20 A, Helsinki, Finland.

Octopamine acts as a neurotransmitter in invertebrate nervous system, but the anatomy of the octopamine-containing neuron systems is poorly known. Antisera against octopamine conjugated to hemocyanin with carbodiimide were produced in rabbits, and the specificity of the antisera was established with dot-blot tests on nitrocellulose membranes.

For immunocytochemistry, specimens of two molluscan species, Mytilus edulis (Bivalvia) and Macoma balthica (Bivalvia) and common earthworm (Oligochaeta) were fixed by immersion in 4% carbodiimide overnight and washed in buffer overnight. In cryostat sections, octopamine-immunoreactive neurons were found in the ganglia of all species studied. The distribution of octopamine in the pedal ganglion of Macoma differed from that of histamine, GABA and taurine. Adsorption studies indicated that GABA and histamine did not affect the staining, while octopamine completely abolished all immunoreactivity. The results suggest that the method enables detailed studies on the organization of octopamine-containing systems.

232.15

EXPRESSION OF OCTOPAMINE RECEPTORS IN *DROSOPHILA*. L. M. Hall, F. Hannan*, J. Pursey*, and D. Urquhart. Dept of Biochem. Pharmacol., SUNY AT BUFFALO, BUFFALO, NY 14260.

Octopamine is a neurotransmitter, neuromodulator, and neurohormone in invertebrates. Three pharmacologically distinct receptor subtypes (octopamine 1, 2A & 2B) have been defined in locust using adrenergic agonists and antagonists (Evans, P.D., *J. Physiol.*, 318:99, 1981). An octopamine-1 receptor cDNA (OctoR1) has recently been cloned and sequenced from *Drosophila* (Arakawa, S. *et al.*, *Neuron*, 2:343, 1990). This cDNA hybridizes at high stringency to four distinct poly A^+ mRNA species. The expression of two of these (3.7 kb and 4.2 kb) is greatly enriched in heads and is also developmentally regulated. Expression of the 3.7 and 4.2 messages is first detected during nervous system formation in 11 to 22 hour old embryos. Expression is seen throughout the larval stages. It decreases at the time of initial pupae formation but rises after 24 hours and is seen throughout the pupal phase of the life cycle. A 2.7 kb mRNA is enriched in bodies and a 1.4 kb mRNA is present in all tissues. PCR technology has been used to amplify DNA across the intracellular loop between transmembrane segments III and VI using either genomic or cDNA as a template. The expected product from this reaction using OctoR1 as a template is 1 kb. With wild-type genomic DNA as the template, 7 distinct DNA fragments ranging in size from 0.4 kb to 1.7 kb were produced. Only the 1.7 kb fragment hybridized at high stringency to OctoR1, suggesting the OctoR1 gene contains an intron in this region. Some of the other products may correspond to other members of the octopamine receptor family. Wild-type cDNA prepared from head mRNA produced a major fragment of 1 kb corresponding to OctoR1 and a minor product of 0.8 kb. None of the products hybridized to a *Drosophila* muscarinic receptor probe under high stringency conditions. The major products will be analyzed using asymmetric PCR and fluorescence based sequencing and localized to the chromosome using *in situ* hybridization to determine which are receptor genes and where they map in the genome.

232.16

RECEPTORS FOR EXCITATORY AMINO ACIDS IN THE INSECT CENTRAL NERVOUS SYSTEM K.A. Wafford, M-I. Sepulveda* & D.B. Sattelle, AFRC Lab. of Molecular Signalling, Dept. of Zoology, Univ. of Cambridge, Cambridge, CB2 3EJ, U.K.

An identified neuronal cell body in the cockroach (Periplaneta americana) has been used to examine the effects of a number of excitatory amino acids. On this cell at resting membrane potential (-60 to -80mV), glutamate elicited a fast hyperpolarization, sometimes followed by a longer, smaller depolarization, which was more apparent at high concentrations (1.0mM). Reversal at the chloride equilibrium potential and dependence on external chloride ions indicated a glutamate-activated chloride channel. Cross-desensitization experiments and antagonist studies showed that glutamate activated a receptor distinct from that operated by GABA. Ibotenate and aspartate mimicked the chloride-mediated response, while quisqualate and kainate application produced small, slow depolarizations. Other agonists such as NMDA and homocysteate, which are active on vertebrate preparations were inactive on this insect neurone. A number of non-NMDA type receptor antagonists were tested and found to be active only at concentrations higher than 100uM. Thus there are at least two distinct glutamate receptor subtypes in the insect central nervous system, that do not readily correlate with those described in vertebrates, and in this case are co-localized on the same cell.

232.17

CHARACTERIZATION OF MUSCARINIC BINDING SITES IN THE COCKROACH BRAIN. G.L. Orr, N. Orr and R.M. Hollingworth*. Pest. Res. Ctr., M.S.U. (Neurosci. Prog.) E. Lansing, MI, 48824

Muscarinic acetylcholine-receptors in cockroach brain were characterized using the muscarinic antagonist, ^{3}H-quinuclidinyl benzilate (QNB). QNB bound saturably with low levels of non-specific binding (defined with atropine). Scatchard and Hill analysis indicated a single population of non-interacting receptor sites (Kd=0.25 nM, Bmax=604 fmoles/mg, Hill coeff.=0.97). The kd values calculated from kinetic data indicated a high affinity (kd=0.02 nM) and a lower affinity (kd=0.23 nM) population of binding sites. Displacement studies defined the muscarinic nature of the QNB binding site(s) and compounds selective for vertebrate muscarinic receptor subtypes displayed an order of effectiveness similar to vertebrate M1 receptors but the absolute affinities of these compounds were at least an order of magnitude lower than expected for M1 receptors. Analysis of these displacement curves provided no evidence for multiple binding sites for the selective compounds. Displacement of QNB by carbachol was modulated by Mg^{2+} and GppNHp indicating the presence of a G protein associated with this receptor site. Autoradiographic analysis of brain slices showed QNB-binding sites to be heavily concentrated in the calyces but to be absent from other neuropile areas in the brain. QNB-binding was also visualized in the neuropile of the sixth abdominal ganglion.

232.18

VENTRAL GIANT INTERNEURON SYNAPSES TO THORACIC INTERNEURONS IN THE COCKROACH ARE CHEMICAL AND CHOLINERGIC. J.L. Casagrand and R.E. Ritzmann. Dept. of Biology, Case Western Reserve University, Cleveland, OH 44106.

We are interested in how populations of neurons are organized to generate a defined behavior. We have been investigating several thoracic interneurons (TIs) in the cockroach which receive inputs from ventral giant interneurons (vGIs). Type A TIs follow the vGIs with short latency at high frequencies (up to 200 Hz). Previous morphological and developmental studies indicated that this input is direct. These connections are important in the integration of directional wind information involved in determining the escape response. In an attempt to learn more about the integration process, we have begun to investigate the biochemical nature of these connections.

We report here on several experiments that provide evidence that the vGI to TI connections are chemical and that the transmitter involved is acetylcholine (ACh). Injection of hyperpolarizing or depolarizing current into the postsynaptic TIs resulted in an appropriate alteration in PSP amplitude. In addition, bathing cells in zero calcium saline resulted in a gradual decrement of the PSP, and ultimately block of synaptic transmission. The response recovered when calcium was replaced. To identify the actual transmitter used at these synapses, we tested the actions of various cholinergic drugs on vGI input to type A TIs. ACh is a major excitatory neurotransmitter in insects, and is known to be the transmitter at the synapses between sensory neurons and vGIs. We found that cholinergic antagonists reversibly blocked transmission at concentrations similar to those previously reported for nicotinic synapses in insects indicating that the transmitter released by vGIs at these synapses is also ACh.

This work was supported by NIH grant NS 17411 to R.E.R.

232.19

CHARACTERIZATION OF AN INSECT CELL LINE AS A MODEL FOR STUDYING OCTOPAMINE RECEPTORS. N. Orr, G.L. Orr and R.M. Hollingworth*. Neuroscience Prog./Dept. of Zoology and Pesticide Research Center, Michigan State University East Lansing, MI 48824

The Sf9 cell line (American Type Culture Collection, MD) derived from Spodoptera frugiperda ovarian tissue is readily grown in culture. In order to evaluate it as a ready source of octopamine (OA) receptors, its responses to biogenic amines were characterized. A radioimmunoassay was used to measure changes in cyclic AMP (cAMP) levels. In intact cells, 100 uM OA increased cAMP levels 3X over control, whereas dopamine and serotonin were without effect. This OA-mediated production of cAMP was blocked in the presence of 10 uM phentolamine. The diterpene forskolin (10uM), enhanced cAMP production 4.4X over control.

Washed-membrane preparations of Sf9 cells exhibited dose-dependent stimulation of cAMP in the presence of forskolin, sodium fluoride and Gpp(NH)p. The OA-mediated cAMP production (Ka=0.3 uM) was GTP-dependent and enhanced in the presence of 0.5 uM forskolin. It is apparent from the absence of interfering amine-sensitive adenylate cyclases and the pharmacology of the OA-mediated response, that the Sf9 cell line may be a good candidate for reliably and easily screening the adenylate cyclase coupled OA-receptor and for further studies to characterize the receptor and its signal transducation mechanisms.

233.1

USE OF FLUORESCENT TRACERS TO IDENTIFY LIVING PREGANGLIONIC CARDIAC MOTORNEURONS IN THE MEDULLA. D. Mendelowitz and D.L. Kunze. Dept. of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.

Neurophysiological studies of preganglionic cardiac motorneurons (PCMs) have generally relied upon antidromic stimulation techniques to identify these neurons in the medulla. These *in vivo* preparations have been limited in that only extracellular recordings are typically possible. To characterize the spontaneous and synaptically evoked ionic currents in these neurons, and identify the neurotransmitters that influence PCM activity, either a slice or dissociated preparation is necessary. As a preliminary step to studying dissociated PCMs we have examined various fluorescent dyes to determine which can be taken up by the PCM terminals in the heart, transported to the soma in the medulla and retained in the neuron during slicing and/or dissociation.

Rats were anesthetized with pentobarbital, respirated and a right thoracotomy was performed to expose the heart. Fluorescent dyes were applied to the epicardial surface of the atrial muscle. After a 3-5 day survival period the animals were sacrificed, perfused with fixative and the medulla was examined for labeled PCMs. Rhodamine (XRITC, Molecular Probes) was very effective, labeling 80-120 PCMs in the nucleus ambiguus and 5-15 PCMs in the vagal nucleus per rat. In contrast, fluorescent dextrans (10,000 and 70,000 mol. wt.) were ineffective. Rhodamine (XRITC) has also been used to identify labeled PCMs in living slices 150 microns thick. These results demonstrate that fluorescent dyes can be used to select identified PCMs for study in either a "thin-slice" or dissociated preparation.

233.2

RESPONSES OF VENTROLATERAL MEDULLARY NEURONS WITH CARDIORESPIRATORY RELATED DISCHARGE TO HYPOXIA AND HYPERCAPNIA. P.C. Nolan* and T.G. Waldrop. Dept. of Physiology, Univ. of Illinois, Urbana, IL 61801.

A previous study from this laboratory demonstrated that microinjection of an excitatory amino acid antagonist into the ventrolateral medulla (VLM) produces a large augmentation of the respiratory response to hypoxia with a smaller effect on the hypercapnic response. The purpose of the present study was to determine if individual neurons in the VLM are affected by both hypoxia and hypercapnia. Single unit responses were recorded during inhalation of hypoxic (10% O_2) or hypercapnic gas (5% CO_2) in anesthetized, spontaneously breathing rats. Hypoxia produced a stimulation in 70% of the neurons and a depression in 20% of the neurons. Hypercapnia elicited increases in the discharge frequency in 40% of the neurons and depressed the activity in 15% of the neurons. The discharge frequency of fifty percent of the neurons was affected by both hypoxia and hypercapnia. The resting discharge of most neurons studied was related to the cardiac (45%) and/or the respiratory cycle (50%); however, the observed responses to hypoxia and hypercapnia were not caused by baroreceptor input. In conclusion, these results suggest that both hypoxia and hypercapnia can affect the same VLM neurons and that the respiratory responses to these stimuli may be modulated by neurons in this area. (Supported by NIH 38726; American Heart Assoc.)

233.3

ROSTRAL VENTROLATERAL MEDULLARY (RVL) LESIONS AFFECT PLASMA CATECHOLAMINES IN THE CONSCIOUS DOG **K.J. Dormer, R.D. Stith*, R.E. Papka and A.F.Sved. Depts. Physiol. and Anat. Sci., Oklahoma Univ.,Okla. City, OK 73190 and Dept. Behav. Neurosci., Univ. Pittsburgh, Pittsburgh, PA 15260.**

To further identify the role of RVL as a vasomotor center in regulation of arterial pressure (AP) 6 chronically instrumented dogs were observed before and after partial excitotoxin (kainic acid, 75-100 nl, 100 mM) lesions were made in RVL. Resting AP and heart rate (HR) were obtained from aortic solid-state pressure transducers while norepinephrine (NE) and epinephrine (E) were determined by radioenzymatic assay of venous plasma samples. Subsequent immunocytochemical staining for phenylethanolamine N-methyl transferase documented the extent of RVL lesions. Mean ± S.E. results include:

	NE (pg/ml)	E (pg/ml)	AP (mmHg)	HR (bpm)
pre	228±102	47±27	100±5	89±8
post	276±71	33±13	88±4	101±9

Two way analysis of variance with repeated measures indicated the means were probably different (F=2.99), there was a significant difference between the pre- and post lesion states (F=0.017) and there was significant interaction between variables (F=0.10). HR always increased when AP decreased after lesions. Postlesion E was also lower than 15 normal dogs (45±15 pg/ml) tested by the same protocol. Thus, it appears RVL lesions affect adrenal medullary function, contributing to the hypotension in conscious dogs. Supported by NIH grant HL 39105.

233.4

SERIAL TRANSECTIONS THROUGH MID-MEDULLA AFFECT SYMPATHETIC NERVE POWER AND COHERENCE BUT NOT RAPID RHYTHM GENERATION. M.J. Kenney, S. Zhong and G.L. Gebber. Dept. of Pharmacol., Michigan State Univ., E. Lansing, MI 48824.

We studied the effects produced by serial brain stem transections on 1) total power in inferior cardiac and renal postganglionic sympathetic nerve discharge (SND), 2) the 2- to 6-Hz rhythms in these nerves and 3) the coherence of inferior cardiac and renal SND in baroreceptor-denervated cats anesthetized with chloralose. Methods used included autospectral and coherence analyses. Before transection, most of the power in SND was between 2 and 6 Hz and the discharges of the inferior cardiac and renal nerves were correlated between 0.2 and 12 Hz (peak coherence value, 0.64±0.16). Midbrain transection at stereotaxic plane A3 reduced total power in SND to 76% of control without affecting the 2- to 6-Hz rhythm (i.e., shape of autospectrum of SND was unchanged) or the strength of coherence of inferior cardiac and renal SND. Serial transections at rostral and mid-medullary levels progressively decreased total power and the coherence of inferior cardiac and renal SND. This occurred without a change in shape of the autospectra of SND. In some experiments, the coherence of inferior cardiac and renal SND between 2 and 6 Hz was eliminated by rostral medullary transection. The power in SND was reduced to ~17% of control after mid-medullary transection. High spinal transection essentially eliminated SND. These results suggest that 1) the 2- to 6-Hz rhythm in SND arises from multiple brain stem sources, 2) these sources form a distributed system since they are normally coupled, and 3) coupling involves rostro-caudal connections. (Supported by NIH grant HL13187.)

233.5

SYNAPTIC CONNECTIONS BETWEEN CARDIOVASCULAR REGIONS OF THE NUCLEUS TRACTUS SOLITARIUS (NTS) AND NEURONES IN THE VENTRO LATERAL MEDULLA OF THE CAT. J. Deuchars*, K.M. Spyer, and P.N. Izzo*. Department of Physiology, Royal Free Hospital Sch. Med., Rowland Hill Street, London NW3 2PF, U.K.

Unilateral ionophoretic injections of the anterograde tracer biocytin were made into discrete regions of the NTS, in some cats at sites identified by recording evoked potentials to stimulation of the carotid sinus nerve. Sites of injection were characterised by a core of labelled neurons, from which labelled axons coursed bilaterally through the NTS, dorsal vagal motor nuclei (DVM), hypoglossal motor nuclei and reticular formation. Labelled axons in the reticular formation were observed mainly in a band extending from the NTS to the lateral reticular nucleus (LRN). At the light microscopic level these fibres could be distinguished as either myelinated axons coursing in fibre tracts or fine varicose fibres. In some animals anterogradely labelled axons in both the DVM and nucleus ambiguus (NA) were observed in the vicinity of vagal preganglionic neurons identified by the injection of cholera toxin-HRP into either the cervical vagus or the myocardium. Ultrastructural examination of labelled axons in the NA and LRN revealed a number of them to be in synaptic contact with dendritic shafts and occasionally cell bodies. Identification of the postsynaptic neurons awaits further investigation.

233.6

CENTRAL PATHWAY INVOLVED IN THE SYMPATHOLYTIC RESPONSE TO STIMULATION OF THE SUPERIOR LARYNGEAL NERVE. D. Huangfu* and P.G. Guyenet. Dept. of Pharmacology, Univ. of Virginia, Charlottesville, VA 22908.

The central pathway involved in the hypotensive and sympatholytic response to stimulation of the superior laryngeal nerve (SLN) was studied in halothane-anesthetized and paralyzed rats. Stimulation of SLN evoked a stimulating intensity, frequency and pulse duration-dependent hypotensive response as well as inhibition of lumbar sympathetic nerve discharge (LSND) (onset latency to single pulse stimulation: 120.2±2.3 ms).

The hypotensive response and LSND inhibition were markedly attenuated by bilateral microinjection of 100 nl of 45 mM kynurenic acid (glutamate receptor-antagonist) into caudal ventrolateral medulla (CVL), or by bilateral administration of 50 nl of 4.5 mM bicuculline methiodide (GABA-receptor antagonist) into rostral ventrolateral medulla (RVL). In most cases the baroreceptor reflex (to aortic constriction) was also blocked. Injection of these drugs elsewhere in the medullary reticular formation did not affect the hypotensive response and LSND inhibition to SLN stimulation. Out of 25 RVL reticulospinal barosensitive neurons recorded, the activity of 18 neurons was inhibited by SLN stimulation (onset latency: 45.5±1.4 ms). This inhibition was attenuated by iontophoretic application of bicuculline (n=5). The remaining 7 RVL reticulospinal barosensitive neurons were unaffected by SLN stimulation.

In conclusion, glutamate in CVL and GABA in RVL are probable transmitters involved in the sympathoinhibition produced by stimulation of SLN.

233.7

CHANGES IN ARTERIAL BLOOD PRESSURE INDUCED BY LOCAL INJECTION OF ATRIAL NATRIURETIC FACTOR AND ARGININE VASOPRESSIN INTO THE ROSTRAL VENTROLATERAL MEDULLA OF THE RAT. D. BHASKARAN and C.R. FREED. Depts. of Med. and Pharm., Univ. of Colo. Health Sci. Ctr., Denver, CO 80262.

We have reported changes in extracellular fluid neurotransmitter levels that occur after drug-induced alterations in arterial blood pressure (JPET 249: 660-666, 1989). We have now studied the cardiovascular effect of atrial natriuretic factor (ANF) and arginine vasopressin (AVP) locally injected into the rostral ventrolateral medulla (RVLM). Urethane anesthetized male Sprague-Dawley rats (250-300 g) were artificially ventilated and the femoral artery was catheterized to measure the blood pressure (BP). Doses of ANF of 2 to 40 pmols and AVP 10 to 1000 pmols were injected into the RVLM through a 32 gauge stainless tubing stereotaxically implanted into the RVLM. Phosphate buffered saline served as a control. Nucleus tractus solitarius and medial ventral medulla were studied as control sites. Local injection of ANF into RVLM produced a dose-dependent increase in BP. AVP produced a biphasic effect. Low doses of AVP reduced BP whereas the 1000 pmol dose increased BP. ANF injected into other brain regions did not show a hypertensive effect. Since ANF increased BP and low doses of AVP reduced BP when given into the RVLM, these compounds may be activating specific receptors involved in regulating sympathetic tone.

233.8

INTRACISTERNAL INJECTION OF HEMICHOLINIUM-3 DOES NOT LOWER BLOOD PRESSURE IN THE ADULT SPONTANEOUSLY HYPERTENSIVE RAT. H.M. VARGAS and B. RINGDAHL*. Department of Pharmacology, UCLA-School of Medicine, Los Angeles, CA 90024-1735.

Brain cholinergic mechanisms are known to be linked to the expression of hypertension in the spontaneously hypertensive rat (SHR). Evidence suggests cholinergic processes in the hypothalamus and brainstem may be involved. Our study assessed the role of brainstem cholinergic neurons in the maintenance of hypertension in SHR. Mean arterial pressure (MAP) was recorded from freely-moving, unanesthetized SHR (18 week; basal MAP: 190 ± 5 mmHg) which were previously implanted with a chronic indwelling cannula aimed at the cisterna magna (icm) or lateral ventricle (icv). After infusion of saline or HC-3 (10 μg/5 μl), MAP was followed for 2 hours. Treatment with icv HC-3 produced a maximal fall in blood pressure (-25 mmHg; at 30 min) which recovered to baseline levels by 120 min. In contrast, icm HC-3 or saline did not cause a hypotensive effect in the SHR throughout the treatment period. Two hours after treatment, however, the iv physostigmine pressor response was significantly inhibited (>80%) by both HC-3 treatments when compared to the saline group (ΔMAP: +34 ± 4 mmHg). These results suggest that brainstem cholinergic neurons may not be tonically active or only secondarily related to the maintenance of hypertension in adult SHR. (Supported by GM37816 to B.R.)

233.9

EFFECT OF CENTRAL ADMINISTRATION OF ANGIOTENSIN II ON THE NEURONAL ACTIVITIES IN ROSTRAL VENTROLATERAL MEDULLA AND BLOOD PRESSURE OF SPONTANEOUSLY HYPERTENSIVE AND NORMOTENSIVE RATS. R.K.W. Chan*, Y.S. Chan and T.M. Wong*. Dept. of Physiology, University of Hong Kong, Sassoon Road, Hong Kong.

To determine whether rostral ventrolateral medulla (RVL) is a site of action of angiotensin II (Ang II), which had been shown to increase arterial blood pressure (BP) and sympathetic nerve activities, the effects of central administration of Ang II on the spontaneous neuronal activities of RVL and BP changes in pentobarbital anesthetized normotensive Wistar Kyoto rats (WKY) and spontaneously hypertensive rats (SHR) were studied. Extracellular activities of RVL neurons were recorded either by a tungsten electrode in experiments involving intracerebroventricular (i.c.v.) administration or by a double barrel glass pipette in experiments involving iontophoretic application to RVL. In WKY and SHR, both i.c.v. and iontophoretic administration of Ang II increased the firing rates of single and double discharge RVL units dose-dependently. Following i.c.v. or iontophoretic administration, the increase in firing rates was greater and the duration of the responses was longer in SHR than in WKY. With iontophoretic administration, RVL units also showed lower threshold current and longer duration of response in SHR than in WKY. The BP elevation accompanying the increase in firing rate of RVL neurons following i.c.v. administration of Ang II was significantly greater and lasted longer in SHR than in WKY; BP change following iontophoresis was too small for statistical analysis. The effects of i.c.v. and iontophoretic administration of Ang II on BP and RVL neuronal activities were completely abolished by co-administration of saralasin, an Ang II antagonist. These findings suggest that the enhanced neural and pressor responses to brain Ang II probably act via RVL neurons, leading to the genesis of hypertension. (Supported by grants from Lee Wing Tat Fund, Sun Yat Sen Fund, C.R.C.G. of H.K.U., and Croucher Foundation.)

233.10

DIFFERENTIAL LOCALIZATION OF ANGIOTENSIN II (AII) AND MU OPIOID BINDING SITES IN THE AREA POSTREMA (AP) OF SPONTANEOUSLY HYPERTENSIVE RATS (SHR). C.O. Andrews, C.F. Phelix, R.L. Tackett; D.K. Hartle. Dept. Pharmacology and Toxicology, College of Pharmacy, UGA, Athens, GA 30602

Previous reports from our laboratory have described anatomical subdivisions of the rat AP based upon immunohistochemical analysis of aminergic and peptidergic neuronal elements, and glutamate-sensitive neurons in SHR and Wistar Kyoto rats. For the present study we used autoradiography to map in situ peptide binding sites within these AP subdivisions. Fresh frozen coronal sections (30 μm) of the medulla oblongata were incubated with ^{125}I-[Sar^1,Ile^8]-AII or ^{125}I-DAGO. Autoradiograms (film and emulsion) were examined or quantitated with a Zeiss IM-3000 Real Time Image Analysis System or by dark field microscopy. Three major subdivisions were established: the rostral level or APr (150 μm), middle level or APm (210 μm), caudal level or APc (150 μm). These AP levels were further subdivided into dorsal and ventral, midline or lateral regions using two parasagittal and one horizontal reference planes. Both AII and DAGO overall binding was greatest in the APm with the APc showing the next highest binding density. In APr binding density decreased significantly more for AII than DAGO. Binding density for DAGO was lowest in midline and highest in dorsolateral AP; lowest in dorsolateral and highest in ventral midline AP for AII. In conclusion, both AII and DAGO binding densities exhibit non-homogeneous distributions in SHR AP. Correlation of peptide binding maps with histochemically-identified neuron and afferent fiber localization may yield useful information about diverse functions of peptides within the AP. HL37705 to DKH.

233.11

EFFECTS OF 5-HT$_{1A}$ AND 5-HT$_2$ AGONISTS ON AUTONOMIC OUTFLOW IN CATS. S.L. Shepheard* D. Jordan[1] & A.G. Ramage, Dept. Pharmacology and [1]Physiology, Royal Free Hospital School of Medicine, London, U.K.

Administration of the 5-HT$_{1A}$ agonist 8-OH-DPAT i.v. to anaesthetised cats causes sympatho-inhibition, hypotension and increased vagal tone (Ramage & Fozard, Eur. J. Pharmacol., 138: 179, 1987), whilst the 5-HT$_2$ agonist DOI causes a potent sympathoexcitation and hypertension (McCall & Harris, Eur. J. Pharmacol., 151: 113, 1988). The central site/s at which these agonists produce these effects remain to be determined.

In α-chloralose anaesthetised cats recordings were made of renal, splanchnic, inferior cardiac and phrenic nerve activity, heart rate, femoral flow, arterial, tracheal and intragastric pressures. Drugs were administered cumulatively into the IVth ventricle and their effects on autonomic outflow compared. Compounds with 5-HT$_{1A}$ agonist properties; 8-OH-DPAT, 5-CT, n-dipropyl-5-CT and sumatriptan produced hypotension, a potent inhibition of renal nerve activity and varying degrees of inhibition in the other sympathetic nerves. Only 8-OH-DPAT caused an increase in cardiac and gastric vagal tone. 5-HT had variable effects but in the presence of the 5-HT$_2$ antagonist cinanserin produced effects similar to the 5-HT$_{1A}$ agonists but no increase in vagal tone. DOI produced a weak hypertension, only a slight generalised sympatho-excitation but a large decrease in femoral arterial conductance.

These results indicate that renal sympathetic outflow is the most sensitive to the sympatho-inhibitory action of 5-HT$_{1A}$ agonists, and the tryptamine-related 5-HT$_{1A}$ agonists failed to increase vagal drive when given into the IVth ventricle. 5-HT$_2$ receptor activation at this level of the brain causes only a surprisingly weak sympatho-excitation.

This work was supported by the Wellcome trust.

233.12

FURTHER CHARACTERIZATION OF SEROTONERGIC MEDULLARY RAPHE NEURONS. **M.E. Clement,* R.B. McCall, and C.J. Hudson, Cardiovascular Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.**

The midline medulla is believed to play a role in the cardiovascular regulation. We recently showed that this area contains spinally projecting serotonergic neurons with similar electrophysiological and pharmacological properties as 5-HT neurons in the dorsal raphe (DR). We have now investigated further the pharmacologic characteristics of serotonergic neurons in the raphe pallidus and obscurus.

We examined the sensitivity of medullary 5-HT neurons to noradrenergic input using the α_1 antagonist prazosin. I.V. prazosin decreased unit activity with a maximum inhibition of 75%. We also found that iontophoretic norepinephrine had variable effects on firing rates, usually producing excitation at lower ejection currents and inhibition at higher currents. These results reflect similar data obtained in the DR.

The effects of two 5-HT agonists, 5-MeOHDMT and DOI, were examined. Iontophoretic 5-MeOHDMT inhibited unit firing for extended periods of time. In addition, i.v. 5-MeOHDMT exhibited a dose-dependent decrease of unit activity with complete inhibition by 100 μg/kg. Conversely, DOI, a selective 5-HT$_2$ agonist, had no effect on medullary 5-HT activity given iontophoretically or in i.v. doses up to 1 mg/kg. These results indicate that medullary 5-HT neurons are similar to but not identical to DR 5-HT neurons.

233.13

CARDIOVASCULAR ACTIONS OF CENTRAL AND PERIPHERAL ADMINISTRATION OF A SEROTONIN 5-HT$_2$ RECEPTOR AGONIST. A. Dedeoglu* and L.A. Fisher. Department of Pharmacology, College of Medicine, University of Arizona Health Sciences Center, Tucson, AZ 85724.

Serotonin (5-HT) receptors comprise several distinct subtypes and are distributed nonuniformly throughout the central nervous system (CNS) and periphery. While it is well known that vascular smooth muscle 5-HT$_2$ receptors mediate pressor responses, several studies suggest that stimulation of CNS 5-HT$_2$ receptors also activates sympathoadrenal outflow and produces pressor responses. The present experiments were performed to compare the effects of intracerebroventricular (icv) and intravenous (iv) administration of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT$_2$ receptor subtype-selective agonist that crosses the blood-brain barrier. All studies utilized conscious unrestrained male Sprague-Dawley rats fitted with indwelling icv cannulae and iliac arterial and venous catheters. Iv injections of DOI (10-300 nmol/kg) produced dose-related elevations of diastolic pressure (DP), systolic pressure (SP), mean arterial pressure (MAP), and pulse pressure (PP) whereas no consistent changes in heart rate (HR) occurred. DP, SP, MAP, and PP were increased maximally by 1 min postinjection and returned to control levels by 30 min except after treatment with the highest dose. Icv administration of DOI (3-100 nmol) produced dose-related elevations of DP, SP, MAP and PP and transient increases of HR; moderate reductions of HR followed the transient tachycardia after icv injection of 100 nmol DOI. In general, DOI-induced elevations of DP, SP, MAP and PP were 20-30% greater after iv administration versus icv administration. Moreover, in contrast to the short latency between iv injections and maximal responses, cardiovascular changes did not reach peak magnitudes until 10 min after icv injections of DOI. These data suggest that a significant component of the cardiovascular responses to icv injection of DOI may result from leakage of DOI from the CNS to the periphery.

233.14

KETANSERIN SYMPATHO-INHIBITION MEDIATED BY A CNS α_2-ADRENOCEPTOR MECHANISM. M.C. Koss. Dept. Pharmacology, Univ. Okla. Hlth. Sci. Ctr., Oklahoma City, OK 73190.

Experiments were undertaken to determine if sympatho-inhibition produced by ketanserin is due to central antagonism of α_1-adrenoceptors rather than CNS 5-HT$_2$ receptors and if (like prazosin) it produces sympatho-inhibition indirectly via a CNS α_2-adrenoceptor mechanism. Intravenous administration of ketanserin (0.1-3.0 mg/kg) caused a dose-related depression of amplitude of sympathetic-cholinergic electrodermal responses (EDRs) evoked by electrical stimulation of the posterior hypothalamus in pentobarbital anesthetized cats. No effect of ketanserin was observed when EDRs were evoked by preganglionic sympathetic nerve stimulation. Pretreatment with α_2-adrenoceptor antagonists yohimbine, idazoxan, or rauwolscine significantly blocked ketanserin-induced sympatho-inhibition. Depletion of CNS monoamines totally prevented ketanserin-induced sympatho-inhibition although clonidine (30 µg/kg) continued to be effective. These results suggest that ketanserin acts in the CNS to reduce sympathetic reactivity by blocking α_1-adrenoceptors and not 5-HT$_2$ receptors. In this regard, ketanserin appears to act in a manner similar to other α_1-adrenoceptor antagonists (e.g. prazosin and indoramin) by an apparent "prejunctional" facilitation of α_2-adrenoceptor mediated tonic inhibition descending from the lower brainstem.

233.15

CLONIDINE FAILS TO INHIBIT THE CARDIOVASCULAR DEFENCE REACTION IN ANESTHETIZED RATS. L. L. Watkins and W. Maixner. Dental Research Center and Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, N.C. 27514.

The cardiovascular defence reaction (CDR) is an adaptive response characterized by an increase in blood pressure, heart rate, respiration, hindlimb blood flow and sympathetic tone. Electrical stimulation of the dorsal periaqueductal grey region (50 uAmp, 100 Hz, 10 sec) produces the CDR in pentobarbital anesthetized rats. Increases in blood pressure (39.5 ± 5 mmHg), heart rate (32 ± 6 bpm), and hindlimb blood flow (200%) were observed during electrical stimulation of the periaqueductal grey region. The increase in blood pressure was transient (1 sec) and was followed by a transient drop after stimulation. Clonidine did not alter the peak increase in blood pressure or heart rate but prolonged the duration of the pressor response and eliminated the post-stimulation decrease in blood pressure. Clonidine significantly inhibited the increase in hindlimb blood flow. These results suggest that clonidine does not lower blood pressure by inhibiting the CDR. Supported by DE08013 and RR05333.

233.16

INTEGRATION OF CARDIOVASCULAR AND VESTIBULAR INPUTS OCCURS IN THE CAUDAL MEDULLARY PARAMEDIAN RETICULAR FORMATION OF THE CAT. B.J. Yates and Y. Yamagata*. Lab. Neurophysiol., The Rockefeller University, New York, NY 10021.

There is considerable evidence to suggest that inputs from the vestibular system influence sympathetic outflow and blood pressure. Electric stimulation of the vestibular nerve (VN) elicits reflexes recordable from sympathetic nerves (Uchino et al., Brain Res. 22). In addition, VN transection compromises the ability of a cat to compensate for hypotension produced by 60° tilt (Doba and Reis, Circ. Res. 34). However, little is known about the neural circuitry which underlies vestibulosympathetic reflexes.

In this study we considered whether the paramedian reticular formation (PRF) mediates vestibular inputs to spinal autonomic neurons. We made extracellular recordings from 47 neurons in this region which were driven by electric stimulation of the VN, in chloralose/urethane-anesthetized cats. 11 were excited by VN stimulation at latencies LE 2 msec, and thus apparently received disynaptic inputs from the labyrinth. 20/47 of the cells in the PRF which responded to VN stimulation also received convergent cardiovascular inputs elicited by electric stimulation. However, only a small fraction (2/13) was shown to be reticulospinal by antidromic stimulation. The PRF may participate in vestibulosympathetic reflexes indirectly, by relaying inputs from the VN to reticulospinal neurons located elsewhere. Supported by NIH grants NS02619 and DC00693.

233.17

CARDIOVASCULAR AND AMINO ACID LEVEL CHANGES AFTER MICRODIALYSIS OF THE FASTIGIAL NUCLEUS "PRESSOR REGION" WITH KAINIC ACID IN CONSCIOUS RATS. T. J. Parry and J. G. McElligott. Dept. of Pharmacology, Temple Univ. Sch. of Med., Philadelphia, PA 19140.

Previous work with microinjected kainic acid (KA) in the rostral fastigial nucleus (RFN) of anesthetized rats has indicated neurons intrinsic to the nucleus modulate cardiovascular function. In this study, changes in cardiovascular parameters as well as extracellular amino acid (AA) levels were examined in response to KA microdialysis in the RFN of conscious male rats. A microdialysis probe equipped with an intramembrane stimulating electrode was stereotaxically positioned within the RFN of previously adapted awake head restrained rats. Electrical stimulation (50 µA), used to locate the pressor region, evoked a rise in mean arterial pressure (MAP; 40 mmHg) and heart rate (HR; 90 bpm). After 30 min, the probe was perfused with an isosmotic buffered solution (1 µl/min) for 90 min followed by 30 min perfusions with 0.5 and 1 mM KA. After KA administration, a control perfusion (60 min) using the isosmotic solution was also performed. Dialysates were collected every 10 min and analyzed for AA levels using HPLC with fluorimetric detection. MAP and HR remained unchanged during the 90 min predrug control period. During KA perfusion, a steady dose dependent increase in MAP and HR was seen (maximum response 25 mmHg and 100 bpm, respectively). In addition, phasic increases in MAP (75 mmHg) and HR (100 bpm) were seen at two minute intervals with the 1 mM dose. After KA washout, the phasic responses decreased in frequency and magnitude, HR decreased to normal predrug values, but MAP remained slightly elevated (12.5 mmHg). KA caused large increases over baseline controls in glutamate (2.3X), taurine (2.7X), and alanine (2.1X) while aspartate, threonine, glycine, and GABA increased slightly (1.3X). The KA induced increase in extracellular glutamate indicates the possible involvement of glutamate in the production cardiovascular changes by the RFN. This glutamate increase supports previous studies which show the need for intact glutaminergic innervation for KA induced excitation. The role of taurine is unknown; however, it is known to be inhibitory and may be released in response to excessive excitation.

233.18

CENTRAL SITE OF THE HYPOTENSIVE RESPONSE TO INTRACEREBROVENTRICULAR ANGIOTENSIN II IN THE ANESTHETIZED RAT. D.M. Campos* and F.M.A. Corrêa. Dept. Pharmacology, Sch. Med. Rib. Preto-USP, SP, Brazil, 14049.

ICV AII caused biphasic responses in urethane-anesthetized rats, characterized by short lasting pressor followed by long lasting depressor components. To identify CNS regions involved in the hypotensive response, we compared dose-effect curves for AII administered into the lateral and the fourth ventricles and the effect of occlusions of ventricular spaces on the response to AII. Male Wistar rats (240-280g) were used. Cannulas were stereotaxically implanted into the lateral or fourth ventricles for dose-effect experiments. For occlusions, cannulas were simultaneously implanted into the lateral ventricle and the aqueduct or the fourth ventricle. ED50 for AII in the fourth ventricle (0.06nmoles) was significantly lower than in lateral ventricle (0.7nmoles). Ventricle occlusions were performed with Nivea cream. A 50% reduction in the response to AII was observed after the occlusion of the aqueduct (-23 ± 3mmHg to -12 ± 2mmHg, n=9), whereas occlusion of the fourth ventricle did not modify the response (-23 ± 3mmHg to -20±3mmHg, n=12). Our results indicate the involvement of two sites in the response to AII, one anterior to the aqueduct and the other in the fourth ventricle floor.

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233.19

ONTOGENY AND DISTRIBUTION OF GABA RECEPTORS IN THE RAT BRAINSTEM AND HIGHER BRAIN AREAS. Y. Xia and G.G. Haddad. Dept. of Pediatrics, Section of Respiratory Medicine, Yale Univ. Sch. Med., New Haven, CT 06510.

Considerable evidence suggests that GABA is involved in regulating cardiorespiratory function under physiologic and pathophysiologic conditions such as 02 deprivation. Recently, we have shown that newborn brainstem neurons respond differently to hypoxia than those in the adult. This age-related difference may be due to differences in neurotransmitter release or in the activity of GABA receptors in the brain. However, little is known about the development of the GABA receptor system, especially in the brainstem. The purpose of this study is to investigate GABA receptor distribution in the rat brainstem as well as in higher brain areas. We used autoradiographic methods and rats at ages of postnatal 1 (P1), 5 (P5),10 (P10),21 (P21) and 120 (adult) days. Sections (10 um) from various levels of the brain were incubated in Tris-citrate buffer with 50 nM 3H-muscimol. We found that 1) in the adult, 3H-muscimol binding was much less in the brainstem than in higher brain, 2) in most brainstem nuclei, the density of 3H-muscimol sites were much higher in the newborn than in the adult with a maximum at P5 or P10, and 3) in higher brain areas (e.g. cortex), 3H-muscimol binding density increased with age and reached the highest density at P21 or adult. Our results demonstrate that GABA receptors develop earlier in the brainstem than in higher brain areas and may play an important role in brainstem function in early life.

233.20

EFFECTS OF ENDOTHELIN-1 (ET) ON MEDULLARY CARDIOVASCULAR NEURAL SUBSTRATES. M.A. Hashim* and A.S. Tadepalli. Division of Pharmacology, Wellcome Research Labs., Research Triangle Park, NC 27709.

Centrally mediated cardiovascular effects of ET were examined in anesthetized, ventilated rats. ET was applied to the exposed 4th cerebral ventricle (4CV) or microinjected into discrete sites within the nucleus of the solitary tract (NTS). ET (1,3,10 pmol) applied to the 4CV elicited, within 0.5 min, dose-dependent decreases in mean arterial pressure (12-77%), heart rate (4-37%) and renal blood flow (RBF; 33-50%) which lasted 30 to 180 min. ET produced similar effects when administered into the 3rd or lateral CV, but the latency was long (2-5 min). Similar responses occurred when ET (1-30 fmol) was microinjected at discrete loci within a 500 μm long dorsal strip and the commissural subnucleus of the NTS. However, at glutamate-responsive deglutitive solitarial sites, ET was ineffective. An ET-antiserum, but not normal serum, applied to 4CV prevented the action of ET. Vehicle microinjections into NTS, or equal doses of ET given i.v. were without effect. These data indicate that ET evokes hypotension, bradycardia and decreases RBF by a specific action on medullary cardiovascular substrates which include the NTS.

233.21

PHYSIOLOGICAL ANATOMY OF THE PARABRACHIAL NUCLEUS IN THE RAT. N.L. Chamberlin and C.B. Saper. Dept. Pharm. & Physiol. Sci., Univ. of Chicago, Chicago IL 60637.

Electrical stimulation of different subregions of the parabrachial nucleus (PB) can produce an increase or decrease in blood pressure and apnea or tachypnea. The goal of this study was to use small injections of glutamate to determine which PB subnuclei and projections mediate each type of response.

Glutamate (25 nl; 1-10 mM) was injected from one barrel of a triple-barrel micropipette while changes in blood pressure and heart rate were measured with an arterial catheter connected to a pressure transducer and respiratory rate was determined with a diaphramatic electromyograph. Saline was injected from an adjacent barrel to control for pressure artifacts. Phaseolus vulgaris leukoagglutinin (PHA-L) was iontophoresed from the third barrel of the micropipette to label the injection site as well as its efferent pathways.

Apnea, associated with a small depressor response, or tachypnea, often accompanied by an increase in blood pressure, was produced by injections of glutamate near the ventrolateral tip of the superior cerebellar peduncle. In these cases PHA-L labeled terminal fields were located in the lateral facial nucleus, periambiguous area, the rostral ventrolateral medulla, the nucleus tractus solitarious (NTS), the hypoglossal nucleus, and the phrenic motor column of the spinal cord. Projections from sites where glutamate caused a decrease in blood pressure without a respiratory change terminated mainly in forebrain areas including the hypothalamus, amygdala and basal forebrain. Since the PB is the main target of NTS efferents, dissection of the PB into functional subregions should shed some light on its role in processing visceral sensory information.

233.22

ASCENDING INPUTS TO THE NUCLEUS OF THE DIAGONAL BAND FROM THE A1 NORADRENERGIC GROUP AND PARABRACHIAL NUCLEUS. W.B. Mathieson, Q.J. Pittman and W.L.Veale. Dept.Med. Physiol., U. Calgary, Alberta, Canada.

Neurons in the diagonal band of Broca (DBB) are responsive to increases in arterial blood pressure and regulate the activity of supraoptic neurosecretory neurons (Jhamandas & Renaud, Can. J. Neurol. Sci.14:17, 1987). To investigate ascending pathways that may relay this baroreceptor input, injections of Fluorogold (FG) were made into the DBB and the distribution of retrogradely labeled neurons within brainstem cardiovascular centres was recorded. FG-labeled cells were located in parabrachial nuclei, rostral and caudal ventrolateral medulla and nucleus of solitary tract (NTS). Immunohistochemical staining revealed that 28% of retrogradely labeled neurons in the caudal VLM were tyrosine hydroxylase-immunoreactive whereas FG-labeled parabrachial and solitary tract neurons were non-catecholaminergic. The results suggest that A1 noradrenergic cells, parabrachial neurons and possibly non-catecholaminergic cells in the NTS may relay ascending baroreflex input via the DBB.

233.23

INFLUENCE OF NUCLEUS OF TRACTUS SOLITARIUS STIMULATION AND BARORECEPTOR ACTIVATION ON RAT PARABRACHIAL NUCLEUS NEURONS. J.H. Jhamandas & K.H. Harris*, Dept. of Medicine (Neurology) & Div.of Neuroscience, University of Alberta, Edmonton, Alberta, Canada T6G 2B7.

The parabrachial nucleus (PBN) within the dorsolateral pons is a major recipient of autonomic-related inputs from more caudal levels of the neuraxis, and in particular the nucleus of the solitary tract (NTS). Although projections from the NTS to the PBN are well-characterized anatomically, the type of cardiovascular information that is transmitted within such pathways is less clear. The present study examined, in urethane-anesthetized rats, the influence of baroreceptor activation on PBN neurons that responded to electrical stimulation within the NTS. Extracellular recordings revealed 60 PBN neurons that increased their firing frequency in response to NTS stimulation and of these, 6 of 14 cells also increased their firing rate consequent to baroreceptor activation, achieved by brief metaraminol-induced hypertension. Thirty-seven PBN cells decreased their activity following NTS stimulation and 7 of 9 such cells displayed a similar depressant response to baroreceptor activation. These observations reveal a population of neurons within the PBN that display a striking similarity of responses both to the activation of synaptic inputs originating in the NTS as well as peripheral arterial baroreceptors.

Supported by the MRC of Canada & AHFMR

233.24

CHARACTERIZATION OF A PROJECTION FROM THE PARABRACHIAL NUCLEUS TO THE HYPOTHALAMIC SUPRAOPTIC NUCLEUS IN THE RAT. T.L. Krukoff, K. Harris* and J.H. Jhamandas. Depts. of Anatomy & Cell Biology, and Medicine (Neurology), Univ. of Alberta, Edmonton, Alberta, Canada T6G 2E1.

While the pontine parabrachial nucleus (PBN) has been implicated in central cardiovascular control and neuroendocrine responses, including the release of vasopressin (VP), the pathways mediating the latter are uncertain. This study, using anatomical and electrophysiological methods, describes a projection from the lateral PBN towards the hypothalamic supraoptic nucleus (SON). Rats received iontophoretic injections of the anterograde tracer, PHA-L, in the PBN region. After 14-18 days, brains of perfused animals were processed for immunofluorescence to visualize projections to the SON region. PHA-L labelled terminals were found primarily in a region dorsal to the SON. Extracellular recordings from 36 antidromically-identified SON neurons revealed a set of complex synaptic responses after electrical stimulation in the PBN. Excitatory (31/36) and inhibitory (5/36) responses were found; a few cells showed excitation with durations of over 100 msec. These results suggest that the PBN projection to the SON is indirect via the adjacent dorsal perinuclear zone. The variety of synaptic influences on SON neurons may help to explain recent conflicting reports about VP release after stimulation in the PBN region.

Supported by the MRC of Canada and AHFMR.

234.1

NICOTINE ALTERS HYPOTHALAMIC GABAERGIC MECHANISMS IN HYPERTENSIVE RATS. R.W. Stremel, A.E. Jimenez and J.C. Passmore*. Dept. of Physiol. & Biophys., School of Medicine, Univ. of Louisville, Louisville, KY 40292.

Chronic nicotine exposure exacerbates the blood pressure increase in salt sensitive hypertension. GABA related mechanisms within the posterior hypothalamus (PH) are known to be altered in some forms of hypertension and the PH also contains nicotinic receptors. Therefore, we studied the cardiovascular and respiratory responses to PH microinjection of the GABA antagonist, bicuculline methiodide (BMI, 5 ng/nl, 25-75 nl) or the GABA agonist, muscimol (5 ng/nl), in chloralose-urethane anesthetized rats. Deoxycorticosterone acetate - salt (DOCA) hypertensive rats, with chronic nicotine infusion (2.4 mg/kg/day; DOCA-NIC) or vehicle (DOCA-VEH), and normotensive controls were studied. In DOCA-VEH and normotensive controls, BMI increased blood pressure (BP), heart rate (HR) and diaphragmatic activity (DA). These effects were reversed by muscimol. BMI in DOCA-NIC rats elicited a similar BP increase (40-60 mm Hg) but produced a dramatic fall in HR (-100 to -150 b/min); DA in response to BMI was attenuated in the DOCA-NIC group. These data suggest that nicotine alters the posterior hypothalamic GABAergic influence over cardiovascular and respiratory control mechanisms and thus nicotine may act at this site to influence salt sensitive hypertension. Support - KY THRI.

234.2

DIFFERENTIAL INNERVATION OF FOREBRAIN STRUCTURES BY THE DORSAL AND VENTRAL MEDIAN PREOPTIC NUCLEUS IN THE RAT: A PHA-L STUDY. A.M. Zardetto-Smith, T.G. Beltz*, R.J. Johnson, and A.K. Johnson. Depts. of Psychology and Pharmacology and The Cardiovascular Center, Univ. of Iowa, Iowa City, IA 52242.

The periventricular region surrounding the anteroventral third ventricular (AV3V) region has been demonstrated to play a critical role in body fluid homeostasis and cardiovascular regulation. The median preoptic nucleus (MnPO), an important component of this region, is comprised of a dorsal (dMnPO) and ventral (vMnPO) subdivision, which functional data suggest may play varying roles in the drinking response to AII. As part of a study comparing the cytoarchitecture and connectivity of dMnPO and vMnPO, the efferent projections of dMnPO and vMnPO within the forebrain were examined using the anterograde tracer Phaseolus vulgaris leucoagglutinin (PHA-L). Small deposits of PHA-L were iontophoretically placed within the dMnPO or vMnPO of male Long-Evans rats. PHA-L fibers and terminals were visualized using the avidin-biotin immunoperoxidase technique. Injections confined to dMnPO produced dense labeling in the organum vasculosum of the lamina terminalis (OVLT) and subfornical organ. The paraventricular, supraoptic and perifornical/lateral hypothalamic nuclei, and ventral bed nucleus of the stria terminalis received moderate innervation. Sparse label was also noted within the central and basomedial amygdaloid nuclei and septal nuclei. In comparison to dMnPO injections, PHA-L deposits confined to vMnPO produced sparse, fine innervation of the periventricular and paraventricular hypothalamic nuclei, and OVLT. These preliminary results suggest that efferents to the forebrain from MnPO may arise primarily from its dorsal subdivision. (Supported by NHLBI.)

234.3

AV3V LESION REDUCES THE PRESSOR, DIPSOGENIC, NATRIURETIC AND KALIURETIC RESPONSES INDUCED BY CHOLINERGIC STIMULATION OF THE MEDIAL HYPOTHALAMUS AND SEPTAL AREA IN RATS. J.V. Menani*; A.S. Valladão*; E. Colombari*; W.A. Saad; L.A.A. Camargo*; A. Renzi*; L.A. De Luca Jr.* and W.A. Saad*. Department of Physiology, Dentistry School, UNESP, Araraquara 14800 SP and Department of Surgery, School of Medicine, USP, São Paulo 01000 SP, Brazil.

Injection of the cholinergic agonist carbachol into several areas of the rat forebrain induces pressor, dipsogenic, natriuretic and kaliuretic responses. The anteroventral third ventricle (AV3V) region is important for the cardiovascular and electrolytic balance in rats. In the present study we investigated the effect of AV3V lesion on the pressor, dipsogenic, natriuretic and kaliuretic responses produced by carbachol (2 nmol) injection into the medial septal area (MSA) and into the ventromedial hypothalamic nucleus (VHN) in rats. These responses were almost abolished in the AV3V-lesioned rats. The decrease in all responses was 50 to 95% in AV3V-lesioned rats. These results show that the AV3V region is essential for the pressor, dipsogenic, natriuretic and kaliuretic responses induced by cholinergic stimulation of the MSA and VHN in rats.
(Research supported by FAPESP: 88/0293-4 and 88/0188-8)

234.4

PRESSOR RESPONSES TO COMMON CAROTID OCCLUSION IN CONSCIOUS RATS WITH COMBINED LESIONS OF THE ANTEROVENTRAL THIRD REGION AND THE MEDIAL FOREBRAIN BUNDLE. M.T.B.Bedran de Castro*, J.V.Menani* and J.C.Bedran de Castro. Dept. of Physiology, UNESP, Araçatuba SP 16015 and Dept. of Physiology, UNESP, Araraquara, SP 14800, Brazil.

In conscious rats, the antero ventral third ventricle region (AV3V) lesion depresses both components of the pressor response to common carotid occlusion (cco), especially the initial peak (IP) which is of carotid reflex origin. Furthermore, medial forebrain bundle (MFB) lesion depresses more intensely the maintained response (MR) which is probably of central ischemic origin. In the present study we investigated the effects of combined AV3V and MFB lesions on the pressor responses to cco in conscious rats. Short-term (6h) and long-term (2 and 6 d) lesions greatly depressed the pressor responses to 60 seconds of cco. The IP was reduced by 52% (from 44±2 to 21±3 mm Hg), and the MR was reduced by 54% (from 24±1 to 11±1 mm Hg). Aortic denervation performed eight days before, in lesioned rats, potentiated the pressor responses to cco with no difference between the IP and MR (40±6 and 35±7 mm Hg, respectively). These data indicate that combined lesions of AV3V and MFB produce an overall depression of the development of the pressor response to cco but maintain almost unchanged the inhibitory action of the aortic baroreceptors on the responses.

234.5

LOCAL EXCITATORY AMINO ACID (EAA) RECEPTORS MEDIATE THE CARDIOVASCULAR RESPONSE TO MICROINJECTON OF THE $GABA_A$ ANTAGONIST BICUCULLINE METHIODIDE (BMI) INTO THE DORSOMEDIAL HYPOTHALAMUS (DMH) IN RATS. R.P. Soltis and J.A. DiMicco. Dept. of Pharmacol. and Toxicol., Indiana Univ. School of Medicine, Indianapolis, IN 46202-5120.

Microinjection of either $GABA_A$ antagonists or EAAs into the DMH of anesthetized rats produces marked increases in heart rate (HR) and modest increases in blood pressure (BP). Here, we examined the role of local EAA receptors in mediating these effects caused by injection of BMI 20 pmol. All injections were made into the DMH in 50 nL of saline. Below are mean maximal increases ± SEM in HR (beats/min) and BP (mmHg) caused by injections of BMI alone and (1) with the non-selective EAA antagonist kynurenate (KYN, Group I, n=3), or (2) with 50 pmol AP5 (an NMDA receptor antagonist), 25 pmol CNQX (a dose previously shown to selectively block non-NMDA EAA receptors), and both AP5 and CNQX (Group II; n=5).

Group I	HR	BP	Group II	HR	BP
BMI alone	+117±11	+16±4	BMI alone	+119±7	+10±1
BMI+KYN 0.5 nmol	+88±22	+4±2	BMI+AP5	+93±9	+4±1
BMI+KYN 2.0 nmol	+58±12	+3±2	BMI+CNQX	+83±11	+6±2
BMI+KYN 5.0 nmol	+20±8	+3±1	BMI+AP5+CNQX	+47±5	+3±1

These results suggest that the cardiovascular response caused by blockade of $GABA_A$ receptors in the DMH is dependent upon activity at local NMDA and non-NMDA EAA receptors. (Supported by USPHS NS 19883 and American Heart Association, Indiana Affiliate)

234.6

FUNCTIONAL AND ANATOMICAL ORGANIZATION OF POSTERIOR HYPOTHALAMIC CARDIOVASCULAR PRESSOR AND DEPRESSOR SITES. G.V. Allen and D.F. Cechetto. Robarts Research Inst., Univ. of Western Ontario, Canada.

The present study describes the anatomical organization of projections from functionally defined cell groups of the lateral hypothalamic area (LHA). In anesthetized rats, cardiovascular pressor and depressor sites were identified following microinjection (5-30 nl) of D,L-Homocysteic acid (0.1-1.0M) and the efferent projections of these sites were mapped following injections of *phaseolus vulgaris* leucoagglutinin or WGA-HRP. Decreases in blood pressure (5-40 mmHg) and heart rate (20-70 beats/sec) were elicited from tuberal and posterior regions of the LHA. Depressor cell groups project to the central gray, pedunculopontine region, parabrachial nucleus, laterodorsal tegmental nucleus, locus coeruleus, nucleus of the solitary tract and rostrocaudal ventral lateral medulla. In contrast, increases in blood pressure (5-15 nl) and heart rate (20-60 beats/sec) were elicited from neurons in the perifornical area. These pressor cells project to the parventricular hypothalamic nucleus and lateral preoptic area as well as to the central gray, laterodorsal tegmental nucleus and locus coeruleus. Both pressor and depressor cells have direct projections to cervical and thoracic levels of the spinal cord. The results demonstrate that functionally distinct cell groups in the posterior hypothalamus have differential pathways to forebrain and brain stem autonomic structures.

234.7

IMMUNOCYTOCHEMISTRY (ICC) AND ELECTRICAL STIMULATION (ES) OF BED NUCLEUS OF STRIA TERMINALIS (BST): CONNECTIONS WITH MEDULLARY CARDIOVASCULAR (CV) REGULATORY CENTERS (CRCs) IN SPONTANEOUSLY HYPERTENSIVE (SHR) AND NORMOTENSIVE RATS. C.F. Phelix, W.K. Paull; D.K. Hartle. Cardiovascular Res. Laboratories, College of Pharmacy, UGA, Athens, GA 30602.

This study implicate corticotropin releasing factor (CRF) as a neuromessenger in BST-paraventricular nucleus of hypothalamus (PVH)-CRC circuits involved in stress-related activation of the CV system that is exaggerated in SHRs vs Wistar Kyoto (WKY) and Sprague Dawley (SD) normotensive rats. ICC was used to study CRF neurons or axons in BST, PVH and CRCs (nucleus tractus solitarius, NTS, dorsal motor - vagus, DMNX; rostral ventrolateral medulla, RVLM) in SHR and SDs. CRF axons contacted catecholamine (CA)- or acetylcholine-synthesizing neurons in the NTS, RVLM or DMNX. CRF neurons in BST were more numerous in SHR. A tract tracing study using injected lectin or anitbody in BST showed synaptic connections with PVH. BST efferents contacted CRF PVH and CA NTS neurons; both were CRF or CA afferent neurons of BST. In both SHR and WKY, ES of BST produced a decreased heart rate (HR) and decreased blood pressure (BP) with pentobarbital anesthesia, decreased HR and increased BP with urethane anesthesia, and increased HR and BP in conscious SHR. The magnitude of response was greater in SHR ($p < .05$-$.01$). Thus the BST is part of a limbic-hypothalamic-brainstem circuit involved in an augmented CV responsivity in rats with hereditary spontaneous hypertension. Support by Georgia Heart Association to CFP and NIH-NS19266 to WKP.

234.8

MECHANISMS INVOLVED IN THE PRESSOR RESPONSE TO CARBACHOL (CARB) INJECTED INTO THE POSTERIOR HYPOTHALAMIC NUCLEUS (PHN). J. R. Martin, Dept. of Pharmacology, Kirksville College of Osteopathic Medicine, Kirksville, MO 63501.

Microinjection of CARB into the PHN of conscious rats increases arterial pressure (AP) partly due to sympathoexcitation. The present study further evaluated the mechanisms of this CARB-induced pressor effect. Sprague-Dawley rats were instrumented for arterial pressure (AP) measurement, systemic drug administration, and microinjection of 5.5 nmol of CARB into the left PHN. Pretreatment of rats with prazosin (PRAZ; 1 mg/kg, i.v.), methyl-atropine (ATR; 2 mg/kg, i.v.), or a vasopressin V_1 receptor antagonist (V_1 ANT; 20 µg/kg, i.v.) attenuated the CARB-induced rise in AP. Coadministration of PRAZ and ATR produced an additive blockade while the combination of PRAZ, ATR, and V_1 ANT reversed the CARB-induced pressor effect to a modest, transient depressor response. Propranolol (PRO; 2 mg/kg, i.v.) pretreatment potentiated the CARB-induced increase in AP. This potentiation was blocked by administration of the V_1 ANT resulting in a change in AP identical to that evoked by CARB alone. Furthermore, the V_1 ANT blocked a CARB-induced suppression of the baroreceptor reflex. These results suggest that the pressor response to PHN injection of CARB is complex and involves, in addition to sympathoexcitation: 1) the sympathoadrenal axis; 2) circulating vasopressin; and 3) a cholinergic pressor mechanism. (Supported by AHA MO Affiliate grant.)

234.9

RESPONSES OF HYPOTHALAMIC CARDIOVASCULAR NEURONS TO HYPERCAPNIA AND HYPOXIA IN BARODENERVATED CATS. G.H. Dillon and T.G. Waldrop. Dept. of Physiology and Biophysics, University of Illinois, Urbana, IL 61801.

Our laboratory has shown recently that the majority of hypothalamic neurons stimulated by hypercapnia or hypoxia in baroreceptor intact cats have a discharge related to cardiovascular activity (cardiac cycle and/or sympathetic activity). The purpose of this study was to determine if this relationship was dependent upon input from baroreceptors and peripheral chemoreceptors. Experiments were performed on anesthetized, barodenervated, artificially ventilated cats. Single unit recordings from hypothalamic neurons were performed; computer averaging techniques were used to determine if neuronal discharge was related to cardiovascular activity. Neuronal responses to inhalation of a hypercapnic (5% CO_2) and a hypoxic (10% O_2) gas were recorded. Hypercapnia activated 38% of hypothalamic neurons studied in barodenervated cats; 75% of those activated had a discharge related to cardiovascular activity. Forty-six percent of the neurons were stimulated by hypoxia; a cardiovascular-related discharge was observed in 67% of these. The above results are similar to those obtained in cats with intact baroreceptors. Thus, these results indicate that baroreceptor and peripheral chemoreceptor afferents are not required for: 1) stimulation of hypothalamic neurons by hypercapnia or hypoxia or 2) neuronal discharge related to cardiovascular activity.

234.10

INTRACEREBROVENTRICULAR CAPTOPRIL LOWERS PRESSURES IN DAHL SALT-SENSITIVE RATS. L. A. Lark and J. A. Weyhenmeyer. Neuroscience Program and College of Medicine, University of Illinois, Urbana, IL 61801.

In the present study we assessed the ability of intracerebroventricular (i.c.v.) injection of the angiotensin converting enzyme (ACE) inhibitor captopril to lower mean arterial pressure (m.a.p.) in conscious, freely moving inbred Dahl salt-sensitive (DS/JR) rats and their normotensive control inbred Dahl salt-resistant (DR/JR) rats. Four week-old rats were placed on a maintenance 8% salt diet. At 7 weeks of age, a cannula was implanted in the lateral ventricle. One week later a catheter was inserted into the femoral artery and exteriorized between the scapulae. Animals were allowed to recover overnight. Captopril (10 µg/4 µl) or vehicle (4 µl) were administered i.c.v. while m.a.p. was constantly monitored. DS/JR rats had significantly elevated m.a.p.'s as compared to DR/JR rats (188 ± 9 vs. 99 ± 1 mm Hg, $p < .01$). A significant depressor response with a maximum of -17.6 ± 4.1 mm Hg lasting for several hours duration was observed in DS/JR rats after i.c.v. captopril administration. Captopril had no effect upon the m.a.p. of DR/JR rats. Both strains were unaffected by i.c.v. injection of vehicle. Further studies will be necessary to elucidate the antihypertensive mechanism of centrally administered captopril in DS/JR rats.

Supported by NSF BNS 17117 and NIH HD 07333-03.

234.11

EXPOSURE OF BORDERLINE HYPERTENSIVE RATS TO A HIGH NaCl DIET DURING WEANING ALTERS CONTROL OF ADULT BLOOD PRESSURE. R. Hunt and D.C. Tucker. Dept. of Psychology, Univ. of Alabama at Birmingham, Birmingham, AL 35294

Borderline hypertensive rats (BHR) show an increase in adult BP when exposed via the maternal diet to 3% NaCl from conception to weaning (Domino et al., 1990). We tested the hypotheses that exposure to 8% NaCl during the weaning period (14-28 days of age) would elevate adult BP and alter the BP response of BHR to 8% NaCl in adulthood. All BHR were fed 1% NaCl from 4-8 wks of age. At 8 wks, half of the males from within a litter were placed on 8% NaCl. BP measured by femoral artery cannula at 17 wks did not differ between rats fed 1% and 8% NaCl during weaning. BHR fed 8% NaCl during weaning showed attenuated depressor responses to administration of chlorisondamine (2.5 mg/kg, i.v.), suggesting decreased sympathetic control of adult BP. In ganglion-blocked rats, responses to saralasin (100 µg/kg, i.v.) suggested that the contribution of angiotensin II to BP is attenuated in BHR fed 8% NaCl in adulthood. Administration of the arginine vasopressin antagonist Manning compound (100 µg/kg, i.v.) to BHR pretreated with chlorisondamine and saralasin suggested increased AVP influence on BP in rats fed 8% NaCl during adulthood. The data suggest that while ingestion of 8% NaCl during the weaning period is not sufficient to increase adult BP in BHR, it does affect sympathetic control of BP and may alter other controls of BP as well.

234.12

STIMULATION OF HISTAMINERGIC NEURONS RESULTS IN INCREASED PLASMA NOREPINEPHRINE. V. Akins and S.L. Bealer, Dept. Physiol. and Biophysics. Univ. Tenn., Memphis, TN 38163.

Neurons containing histamine (HA) are found in the tuberomammillary nucleus (TMN) in the posterior hypothalamus of the rat brain and may function in cardiovascular regulation and fluid balance. To test the effects of stimulated HA release on plasma vasopressin (PAVP) and norepinephrine (PNE) levels, male Sprague-Dawley rats were instrumented with a chronic bipolar electrode in the TMN, a microdialysis probe near the supraoptic nucleus (SON) to measure changes in HA release from nerve terminals, and femoral venous and arterial catheters. The SON of conscious rats was dialyzed with saline at 2 µl/min and 4 30-minute collections of the effusate were made. Three blood samples for measurement of PNE and PAVP were taken. During the third 30-minute collection period, the TMN was stimulated with a 1 minute train of 0.5 msec pulses of 1-10V and 100 HZ every 5 minutes. Increases in extracellular HA concentrations near the SON during TMN stimulation (+88%) were correlated with significant increases in PNE (+52%). Both values returned to baseline levels during the recovery period. No changes in PAVP were measured. Increases in PNE were abolished when the H1 antagonist, pyrilamine, was continuously administered iv throughout the protocol. These results support the conclusion that brain HA mediates activation of the peripheral sympathetic nervous system through central H1 receptors. (Supported by NIH and AHA.)

234.13

REGIONAL HEMODYNAMIC CHANGES PRODUCED BY ACTIVATION OF THE CORONARY VASOCONSTRICTOR PATHWAY. L.F. Jones, D.D. Gutterman and M.J. Brody. Depts. Pharmacol., Int. Med. & Cardiovasc. Ctr., Univ. of Iowa, Iowa City, IA 52242.

Hemodynamic characteristics following electrical activation of the coronary vasoconstrictor pathway in the anterior hypothalamus (AHA) were studied to determine if activation of the pathway produces specific profiles of regional hemodynamic changes. Coronary, femoral, renal and mesenteric blood flow velocities (BFV) were recorded in cats. Following vagotomy and propranolol, electrical stimulation of the AHA produced a decrease in CBFV associated with the defense reaction, an integrated response including a decrease in hindquarter vascular resistance (HqVR) and an increase in renal vascular resistance (RVR) and mesenteric vascular resistance (MVR). In a second area of the AHA, the defense reaction was elicited without a decrease in CBFV, while a third region produced an increase in both HqVR and MVR. The decrease in CBFV and FVR was also produced by electrical stimulation in the parabrachial nucleus, in an area close to the ventral surface of the pons lateral to the pyramidal tract, and in rostral ventrolateral medulla. These results support the existence of different patterns of hemodynamic responses between coronary vasoconstrictor regions and other sites. These data also support the hypothesis that coronary vasoconstriction is a component of the defense reaction.

234.14

RELEASE OF PARAVENTRICULAR NUCLEUS (PVN) NOREPINEPHRINE DURING HYPOTENSION IN DIABETIC RATS. S.L. Bealer, Dept. Physiol., Univ. Tennessee, Memphis, TN 38163.

Reductions in blood pressure increase extracellular norepinephrine concentration in the PVN (PVNne) in normal animals. Furthermore, studies have shown that central noradrenergic systems are altered in diabetes mellitus. The present studies used in vivo microdialysis to evaluate PVNne in control rats (Cont) and animals made diabetic (DM) with streptozotocin during hemorrhage. Two-three wks after treatment animals were prepared with arterial catheters and microdialysis probes adjacent to the PVN. Brain dialysate was collected before, during, and following hemorrhage which lowered blood pressure to 75 mmHg. Blood glucose was elevated in DM (338± 39 mg/dl) compared to Cont animals (106± 7 mg/dl). Prior to hemorrhage, there were no differences in blood pressure or PVNne between Cont (105±5 mmHg; 99±16 pg/ml) and DM rats (110±4 mmHg; 74±10 pg/ml). However, during hemorrhage, PVNne increased significantly more in DM (312 ±40%) than in Cont animals (140±15%). These results indicate an enhanced PVN noradrenergic nerve activity in DM animals during hypovolemic hypotension compared to normal animals. (Sponsored by grants from the National Institutes of Health and the American Heart Association).

234.15

ENHANCED PRESSOR RESPONSES TO INTRACEREBROVENTRICULAR (ICV) VASOPRESSIN AFTER PRETREATMENT WITH ICV OXYTOCIN. Y. Takahashi*, A. Komulainen*, P. Poulin, Q.J. Pittman, Neuroscience Research Group, U of Calgary, Calgary, Can.

Arginine vasopressin (AVP) given ICV causes motor disturbances in rats. The severity increases and the threshold dose decreases following prior sensitization with oxytocin (OXY). As ICV AVP also increases heart rate (HR) and blood pressure (BP), we have asked if these cardiovascular responses could also be sensitized. Rats were given ICV saline (5 µl) or were sensitized with 1 pmole OXY (ICV). 24 h later rats anaesthetized with urethane received ICV vehicle which was without effect on BP or HR. AVP (1 pmole) increased BP (4.2 ± 1.8 mm Hg) and HR (19 ± 11 bpm) less in control (n=8) than in sensitized rats (8.4 ± 2.6 mm Hg; 43 ± 8 bpm; n=6); 2 other sensitized rats died after AVP. In sensitized rats, 5 pmole AVP caused 50% mortality; survivors (n=6) displayed increases in BP (19.0 ± 4.5 mm Hg) and HR (81 ± 22 bpm) significantly greater than those of controls (8.1 ± 1.6 mm Hg; 38 ± 7 bpm; n=13). 10 pmole AVP increased BP (10.3 ± 2.2 mm Hg) and HR (39 ± 5 bpm) in controls but sensitized rats all died following wide oscillations in BP and HR. These responses to AVP (and no mortality) were also observed when rats were paralyzed and ventilated. We conclude that prior exposure to OXY can sensitize brain AVP receptors involved in central cardiovascular control.

234.16

CENTRAL CARDIOVASCULAR REGULATION OF TACHYKININS: INVOLVEMENT OF THE SYMPATHETIC NERVOUS SYSTEM AND VASOPRESSIN RELEASE. H.Kamiya*, A.Nagashima*, T. Hagio*, R.Saito* and Y.Takano. Dept. of Pharmacol., Fac. of Pharmaceutical Sci., Fukuoka Univ., Fukuoka 814-01, Japan.

Five mammalian tachykinins have been so far isolated, substance P(SP), neurokinin A (NKA), neurokinin B(NKB), neuropeptide K and neuropeptide γ(NPγ). Here we report studies on the mechanisms of central cardiovascular regulation of tachykinins. Intracerebroventricular (i.c.v.) injections of tachykinins caused dose-dependent increases in the blood pressure and heart rate. The pressor responses to SP, NKA and NPγ were blocked by sympathetic blocking agents. In contrast, the pressor responses to an NKB analogue senktide (10 µg, i.c.v.) was not blocked by the ganglionic blocker or adrenalectomy. The senktide-induced pressor response was inhibited by pretreatment with a vasopressin antagonist (10 µg/kg, i.v.), and senktide caused an increase in plasma vasopressin level. However, the vasopressin antagonist did not influence the SP- and NKA-induced pressor responses. These results suggest that central SP and NKA increase the blood pressure and heart rate via sympathetic nerve activity, whereas central NKB increases the blood pressure via release of vasopressin from the hypothalamus.

234.17

THE EFFECTS OF VASOPRESSIN ANTAGONIST ON PHYSOSTIGMINE INDUCED PRESSOR RESPONSES IN CONSCIOUS RABBITS. **M. Hay, Y. Nishida* and V.S. Bishop. Dept. of Pharmacology, Univ. of Texas Health Science Center, San Antonio, TX 78284.**

A number of previous studies have shown that intravenous administration of physostigmine (PHYS) results in an increase in mean arterial pressure (MAP), heart rate (HR) and sympathetic activity. It has been suggested that PHYS may act at central sites to enhance sympathetic outflow and ultimately increase MAP. However, it has also been suggested that the pressor effects of PHYS may be due to a central activation of arginine-vasopressin (AVP) release. The present study was designed to determine the effects of V_1 AVP antagonist d(CH_2)5TYR(ME)AVP (AVPX) on PHYS induced increases in MAP and HR in conscious rabbits. New Zealand rabbits were chronically instrumented with arterial and venous catheters for the monitoring of MAP and HR, and the administration of drugs. Some animals were also instrumented for the recording of renal sympathetic nerve activity (RSNA). Resting MAP and HR were 79 ± 4 mmHg and 248 ± 26 beats/min., respectively. PHYS (75 µg/kg) was infused intravenously (i.v.) and changes in MAP and HR were recorded. Intravenous PHYS resulted in an averaged 22 ± 3 mmHg increase in MAP and an averaged 15 ± 8 beat/min. increase in HR. In 2 animals instrumented for the recording of RSNA, PHYS was found to induce only minimal changes in RSNA possibly due to buffering capabilities of the intact baroreflex. In all animals tested, administration of ganglionic blocking agent hexamethonium (25 mg/kg) did not alter the pressor effects of i.v. PHYS. Intravenous administration of AVPX (10 µg/kg) had no effect on baseline MAP or HR. However, following AVPX administration, i.v. PHYS increased pressure by only 9 ± 6 mmHg and raised HR 31 ± 11 beats/min. These results suggest that, in the conscious rabbit, the pressor effects of i.v. administered PHYS may be the result of increased levels of circulating AVP. (Supported by HL36080 and HL12415).

234.18

VASOPRESSIN (VP) mRNA, BUT NOT OXYTOCIN (OT) mRNA IN THE PARAVENTRICULAR HYPOTHALAMIC NUCLEUS (PVN) IS ASSOCIATED WITH SALT-INDUCED HYPERTENSION. B. H. Hwang and J.-K. Zhang*. Terre Haute Ctr. for Medical education, Indiana Univ. School of Medicine, Terre Haute, IN 47809.

The VP and OT are abundant in several brain regions including the PVN and supraoptic nucleus (SON). However, exactly how VP and OT neurons participate in the development of hypertension remains to be studied. In this study, we used ^{35}S-VP and -OT oligonucleotide probes in conjunction with in situ hybridization to study how VP and OT mRNAs in the PVN and SON are related to salt-induced hypertension. Male Dahl salt-resistant (SR) and salt-sensitive (SS) rats were fed with high salt diet containing 8% NaCl for 4 weeks. SS rats developed hypertension (191 mm Hg) with cardiac hypertrophy, whereas SR rats remained normotensive (119 mm Hg). Further, quantitative autoradiography showed that SS rats contained more VP mRNA (310 fmol/mg protein) in the PVN than SR rats (254 fmol/mg protein). OT mRNA in the PVN and SON were not significantly different between SR and SS rats. Results suggest that increased VP gene expression may play a role in pathogenesis of salt-induced hypertension. Supported by NIH grant NS 25087.

234.19

OPIOID μ_1- AND μ_2-RECEPTOR EFFECTS ON HEART RATE IN THE RAT. P. Paakkari, G. Feuerstein[†] and A.-L. Sirén. Dept of Neurology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814, [†]Dept of Pharmacology, SmithKline & Beecham, King of Prussia, PA 19406.

The possibility that μ-opioid-induced tachycardia and bradycardia could be mediated by different subtypes of the μ-receptor was studied in conscious male Sprague-Dawley rats. The selective μ-receptor agonist dermorphin (DM) and its analog TAPS (Tyr-D-Arg-Phe-sarcosine), a proposed μ_1-receptor agonist (Paakkari P, et al, Progr Clin Biol Res 328:385, 1989), were given centrally.

TAPS 0.3, 3 and 30 pmol increased the heart rate (HR) by 71±22, 49±14 and 30±17 beats/min (bpm), the response being inversely correlated to the dose. DM 1 pmol increased HR by 39±14 bpm while DM 10 pmol had no effect. After treatment with the μ_1-selective antagonist naloxonazine (NX), TAPS 30 pmol and DM 1 pmol decreased HR by -22±10 and -24±7 bpm, respectively. The bradycardic effect of 10 nmol DM (-25±8 bpm) was potentiated by NX (-97±22 bpm, $p<0.01$) but abolished by naloxone (non-selective antagonist).

These data suggest that the high affinity μ_1-opioid receptors mediate tachycardic responses and μ_2-receptors mediate bradycardic responses.

234.20

EFFECTS OF CENTRAL INJECTIONS OF DILTIAZEM ON BLOOD PRESSURE. R. de Beaurepaire and S.N. Thornton*. Lab. de Psychopharmacologie Experimental, CHU Côte de Nacre, Faculté de Med., Caen 14032 and Lab. de Neurobiologie des Régulations, Collège de France, 11 Place Marcelin Berthelot, 75231 Paris Cedex 05, France.

The calcium channel inhibitor Diltiazem (DLT) is widely used in the treatment of cardiovascular diseases. It also has been successfully used in the treatment in some psychiatric disorders but this effect is not easy to understand since DLT crosses only poorly the blood brain barrier. Neverthless, the hypothesis of the central action of calcium channel inhibitors has been raised for their psychiatric as well as for their cardiovascular effects.

We tested the effects of DLT on blood pressure (femoral artery catheter) following peripheral and central administration in urethane anesthetized rats. The effective dose of DLT injected intravenously appears between 1 ug/kg (no effect) and 10 ug/kg (significant decrease in blood pressure). With intracerebroventricular injections (3 ul into the lateral ventricle) no effect on blood pressure was observed with 1 ug/kg but a significant decrease in blood pressure appeared with 10 ug/kg. Similar results were obtained with injection of DLT, in very small volumes of vehicle, into several hypothalamic sites; no effect with 1 ug/kg, decrease in blood pressure with higher doses.

If, as has been proposed, DLT acts through the central nervous system to produce its effects, in this case a decrease in blood pressure, we would have presumed that the central doses should have been smaller than the peripheral ones. As this was not the case, our results do no support the hypothesis that DLT acts through the central nervous system to decrease blood pressure.

234.21

ELECTROPHYSIOLOGICAL EVIDENCE FOR HYPOTHALAMIC DEFENSE AREA INPUT TO CELLS IN THE LATERAL TEGMENTAL FIELD OF THE MEDULLA IN RABBITS. R.W. Winters*, P.M. McCabe, E.J. Green, Y-F. Duan*, Y. Huang*, C.G. Markgraf, and N. Schneiderman. Department of Psychology, University of Miami, Coral Gables, FL 33124.

There is considerable evidence that neurons in the lateral tegmental field of the medulla (LTFM) are involved in cardiovascular regulation. Pressor responses are elicited by electrical stimulation of the LTFM, and cells in this region are activated antidromically by stimulation of the rostral ventrolateral medulla, a structure that is essential to the maintenance of vasomotor tone. The present study sought to determine if neurons in the posterior hypothalamic defense area (HYP) make functional connections with cells of the LTFM. Albino rabbits were anesthetized with isofluorane, and a bipolar electrode was implanted into the HYP. This site was verified by the presence of a large tachycardia/pressor response elicited by electrical stimulation. A recording/stimulating electrode was lowered into the LTFM, primarily in the nucleus reticularis parvocellularis, approximately 2.0 to 3.5 mm rostral to obex. Stimulation of LTFM elicited a tachycardia/pressor response. Responses of single cells in LTFM were then recorded during hypothalamic stimulation. Less than one-half of the cells recorded in LTFM changed their firing rate in response to HYP stimulation. Approximately 50% of the cells influenced by HYP stimulation were excited. In some cases this excitation was secondary to an increase in blood pressure. A substantial number of cells that were affected by HYP stimulation showed a change in discharge rate in response to phenylephrine (1µg/kg, i.v.) and/or showed spontaneous activity that was phasically modulated within the cardiac cycle. These findings provide evidence that LTFM receives input from the posterior hypothalamic defense area, and that this input may be important in the mediation of the cardiovascular components of the defense response in rabbits. (Supported by NIH grants HL36588, HL 07426, NS 24874).

234.22

BULBAR ORIGIN OF CATECHOLAMINERGIC PROJECTIONS TO THE CENTRAL AMYGDALOID NUCLEUS IN THE RAT. *B.R. Due & J.S. Schwaber, Neural Computation Group, E.I. duPont Co., Wilmington, DE 19880-0352*

The central amygdaloid nucleus (CeA) is known to modify autonomic function through projections to autonomic centers in the medulla and pons. Norepinephrine (NE) and other catecholaminergic (CA) terminals are present in the CeA, and NE injected into the CeA has potent influences on autonomic function, including heart rate, blood pressure and cardiac rhythm (Leonzio et al., Soc. Neuro. Abstr., 80.1, 1987). CeA NE is known to be of bulbar origin. In order to determine the specific cells groups of origin, rhodamine beads or Fluorogold was injected into the CeA and brainstem sections processed for tyrosine hydroxylase (TH), or alternate sections for TH, phenylethanolamine-n-methyltransferase (PNMT) or dopamine-beta-hydroxylase (DBH). In some cases the lateral bed nucleus of the stria terminalis (BNST) was also injected. Cell groups projecting to the CeA and BNST were largely co-extensive but distinct. The great majority of neurons double-labeled for CA enzyme and CeA projections were located in the medulla in the A1 and A2 regions, plus a distribution of neurons extended through the medulla between the two. On average somewhat more double-labeled cells/section were located ventrolaterally at levels caudal to the obex. A significant proportion (approx. 40%) of NTS neurons that project to CeA, both caudal and rostral to the obex, appear to also be CA neurons. On the other hand, ventrolaterally located neurons in the A1 region were about 25% CA, and only a very few C1 area neurons were CA cells. Unexpectedly, only a relatively few A6 (locus coeruleus) and A7 neurons project to the CeA.

234.23

DISCHARGE DEPENDENCIES OF AMYGDALA CENTRAL NUCLEUS NEURONS TO THE CARDIAC AND RESPIRATORY CYCLE FOLLOWING LOCAL COCAINE ADMINISTRATION. J.X. Zhang, H. Ni, and R.M. Harper. Brain Research Institute and Dept. of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024.

We examined dependencies of amygdala central nucleus (ACE) neuronal discharge to the cardiac and respiratory cycle in freely moving cats following microinjection of cocaine (100 µg in 0.2 µL) into that structure, a nucleus with heavy projections to cardiorespiratory regions of the parabrachial pons and the nucleus of the solitary tract. Cats were instrumented with EEG, ECG, and diaphragmatic EMG electrodes and indwelling ACE cannulae and microwire bundles. Following surgical recovery, each cat was allowed to sleep undisturbed in a quiet chamber and then administered cocaine through the indwelling cannula. A second sleep-wake recording was taken following cocaine microinjection. Cardiac R-R intervals, respiratory breath-to-breath intervals, and interspike ACE neuronal intervals were stored on digital disks, and cross-correlation dependencies were calculated between neuronal discharge and cardiac and respiratory intervals in baseline and post-cocaine administration periods. Cross-correlation dependencies between neuronal discharge and respiratory and cardiac cycles were found in 3 of 15 cells and 8 of 15 cells, respectively, during baseline periods. The dependencies between the cardiac cycle and neuronal discharge vanished or greatly weakened following cocaine administration. One respiratory dependency disappeared while two remained with a phase shift in the respiratory cross-correlation after cocaine administration. We suggest that cocaine alters ACE neuronal phasic discharge with the respiratory and cardiac cycle when administered locally. Supported by R01-DA04913.

234.24

CARDIAC INTERVAL PATTERNING FOLLOWING COCAINE ADMINISTRATION TO THE CENTRAL NUCLEUS OF THE AMYGDALA. R.M. Harper, R.K. Harper, C.A. Richard, R.R. Terreberry and R.C. Frysinger. Brain Research Institute and Dept. of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024.

We examined sequencing of cardiac R-R intervals in freely moving cats following administration of 3 dose levels of cocaine (0.625, 1.25, 2.5 mg) into the central nucleus of the amygdala (ACE), a limbic structure with heavy projections to the parabrachial pons and the nucleus of the solitary tract. Cats were instrumented with EEG, ECG, indwelling ACE cannulae, laryngeal dilator muscle, and diaphragmatic EMG electrodes. Following recovery, each cat was allowed to sleep undisturbed in a quiet chamber and then administered cocaine through the indwelling cannulae. Cardiac R-R intervals were stored on digital disks. Each cardiac interval was plotted successively prior to and following cocaine administration, and the distribution of variation and determination of respiratory and slower sources of variation was assessed from these plots and from measures of interquartile range of intervals. Although ACE cocaine administration elicited a variety of behavioral and respiratory sequelae, the extreme tachycardia with abolition of respiratory and slower variation found following intravenous or intraventricular administration was not a prominent feature. Slow variation from sources yet undetermined was present following ACE administration. We suggest that the extreme tachycardia associated with intraventricular cocaine administration results from other than ACE influences.

Supported by R01-DA04913.

234.25

ANALYSIS OF OVERNIGHT CARDIAC INTERVAL PATTERNS IN PATIENTS WITH COMPLEX PARTIAL SEIZURES.
R.C. Frysinger, M.F. Levesque*, and R.M. Harper. Dept. of Anatomy and Cell Biology, Division of Neurological Surgery, and the Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024.

We have shown that single cell discharge in temporal structures is modulated by the cardiac cycle and by changes in heart rate, and stimulation and lesion studies in animals suggest that mesial temporal structures play a role in modulation of cardiovascular patterning as well as in the genesis and propagation of seizure activity. Patients with epilepsy may thus show alterations in cardiac rate patterns associated directly or indirectly with changes in mesial temporal structures associated with epileptogenesis. We assessed overnight cardiac interval (RRI) records from 19 patients with intractable epilepsy who were candidates for anterior temporal lobectomy. We examined the amplitude and frequency characteristics of RRI variation, and partitioned presumed sources of the variation on the basis of morphology and frequency characteristics.

Two patients showed a dramatic reduction in RR variability, probably resulting from peripheral vagal neuropathy. Of the remainder, nine patients showed high-amplitude rhythmic variation at 4-6/min, with a time constant overlapping that ascribed to the baroreflex. The high relative amplitude of this variability may reflect diminished modulation of the baroreflex by mesial temporal structures such as amygdala.

Supported by NIH Grant NS 02808-27

234.26

ELECTROPHYSIOLOGICAL AND OPTICAL STUDY OF VAGAL AFFERENT PROJECTION IN THE RAT INSULAR CORTEX.
S.Ito, Dept. Physiol., Kumamoto Univ. Med. Sch., Kumamoto, 860, Japan.

Anatomical studies have suggested that the visceral sence is represented in the insular cortex although very little functional evidence was available. To examine this physiologically, responses to vagal stimulation were recorded. SD rats were anesthetized with sodium amobarbital, ventilated artificially, and mounted on a stereotaxic apparatus. Cervical vagus nerves were stimulated and excited fiber components were monitored at the solitary tract nucleus (NTS). No cortical response was detected under the stimulus intensity of the A-fiber range. A surface-negative field potential (FP) appeared bilaterally with 40-60ms latency when the C-fiber response was obtained at NTS. This FP was recorded mainly around the border between the granular and dysgranular subareas anterior to the middle cerebral artery. Topical application of GABA diminished this FP. These results indicate that vagal C-fiber afferents project to the superficial layers to produce EPSPs in the rostral insula. Attempts were made to record this response optically with voltage-sensitive dye. Optimal staining condition differed from that of the somatosensory cortex probably because the sites of origin of the main signal differ.

234.27

PLASMA AND ORGAN CATECHOLAMINE LEVELS FOLLOWING INSULAR CORTEX STIMULATION.
S. Oppenheimer, T. Saleh*, J. Wilson*, D. Cechetto. Robarts Research Institute/Department of Physiology, University of Western Ontario, London, Ontario, Canada.

Phasic microstimulation of the rat insular cortex triggered by the ECG R wave produces heart rate changes independent of blood pressure and respiratory effects. Bradycardia, tachycardia and control responses (no heart rate change) were identified and respectively formed three groups of ketamine anesthetised rats. Blood samples were taken immediately before, and 1 hour after insular stimulation for catecholamine analysis. Hearts and kidneys were removed after 1 hour of stimulation, and immediately frozen in liquid nitrogen for subsequent catecholamine analysis. Heart rate changes significantly correlated with changes in plasma norepinephrine ($p<0.01$). No correlation between intracardiac or intrarenal norepinephrine and heart rate or plasma norepinephrine was seen at bradycardia or control sites; at tachycardia sites, stimulation elevated plasma norepinephrine ($p<0.05$), while intracardiac norepinephrine levels inversely correlated with heart rate change ($p<0.02$). No changes in plasma epinephrine were seen; epinephrine was not detected in organ samples. Stimulation induced tachycardia possibly results from increased cardiac norepinephrine release and is not balanced by increased synthesis.
(Supported by the Heart and Stroke Foundation of Ontario).

SPINAL CORD: ANATOMY AND PHYSIOLOGY

235.1

PROPOFOL ALTERS DORSAL HORN NEURONAL RESPONSES TO LOW INTENSITY RECEPTIVE FIELD STIMULATION.
H. Uchida*, J.G. Collins, K. Kishikawa, H.Hirata. Dept. Anesth. Yale Sch. Med. New Haven. CT 06510

General anesthetics (GAs) have the potential to influence all areas of the neuraxis but anesthesia is often thought of as a supraspinal event. This is one of a series of studies examining the effects of GAs on spinal sensory processing. Tungsten microelectrodes were used to record extracellularly from single spinal dorsal horn neurons in physiologically intact, awake, drug-free cats. Each neuron's low threshold receptive field area and baseline response to brushing and pinching was determined. Propofol (2,6-diisopropylphenol), an I.V. anesthetic agent, was administered (7.5-10.0 mg/kg iv). Drug injection produced a brief period of apnea (15-30 sec.) that was followed by a deep anesthetic state that quickly dissipated with full recovery apparent within 30 to 45 minutes.

Propofol reduced the low threshold receptive field size of 11 of the 18 neurons studied by at least 20%. Five receptive fields were reduced in area by at least 70%. When recovery was studied most receptive fields returned to their baseline size as the animals regained consciousness. Neuronal responses to brush ($n=15$) and non-noxious pinch ($n=15$) were also depressed. Brush responses returned to baseline with recovery, pinch was not studied during recovery. General anesthesia has been defined as the loss of all sensation. We have previously reported that pentobarbital also depresses low intensity stimulation but ketamine does not. The ability of some GAs to reduce low threshold sensory input at the level of the spinal dorsal horn as well as their ability to depress responses to noxious stimulation within the spinal cord may contribute to the reduction in sensory input that ultimately results in the state of sensory impairment known as anesthesia. This possible spinal site of action warrants further study as we begin to appreciate the complex nature of sensory modulation within the spinal dorsal horn. Supported by NIH GM 29065

235.2

PRECISION OF CAT DORSAL HORN SOMATOTOPIC MAP.
P.B. Brown, H.R. Koerber, and G.R. Hobbs. Dept. of Physiol. and Dept. of Stat. and Computer Sci., W. Va. Univ. Health Sciences Ctr., Morgantown, WV 26506; and Dept. of Neurobiol., Anat., and Cell Sci., Univ. Pittsburgh Med. School, Pittsburgh, PA 15261.

Statistical methods were used to estimate pre- and post-fixation, deviations from bilateral symmetry, and precision of the dorsal horn map of the hindlimb skin. Light touch excitatory receptive fields (RFs) of 150 single units were mapped, and recording sites were marked by small electrolytic lesions, in six random-source urethane-anesthetised adult cats.

Sigmoid functions were fitted to plots of RF distance from tips of toes (D) as a function of mediolateral (ML) position in the dorsal horn, combining data across cats, for each segmental level. Data from individual cats were shifted rostral or caudal for best fits with the sigmoids. The average shift magnitude was 0.22 segment, with a range of 0.4 caudal to 0.3 rostral (pre- and post-fixations). The difference between predicted and observed ML for an observed D was assumed to be a combination of interanimal variation, measurement error, modeling error, deviations from bilateral symmetry, and intra-animal variation. Measurement error was estimated as 30 μm. Bilateral symmetry and across-animal errors were both ca. 60 μm, or 7.5% of the dorsal horn width, substantially less than the width of a dendritic tree or the terminal distribution of a single primary afferent. Within-dorsal horn error was ca. 5 μm, 0.6% of the dorsal horn width, less than a soma diameter. Modeling error was 27 μm.

This remarkable precision implies that errors in map organization would contribute an average of only 0.2 mm to errors of stimulus localization along the 30 cm length of the leg. Due to varying map scale, such contributions would be smaller than the average on the toes, and larger on the proximal leg.

This work was supported by USPHS grant NS 12061.

235.3

DEVELOPMENT OF CUTANEOUS AND PROPRIOCEPTIVE AFFERENT PROJECTIONS AND MOTONEURONAL DENDRITES IN CHICK SPINAL CORD. B. Mendelson, H.R. Koerber and E. Frank. Dept. of Neurobiology, Anatomy and Cell Science, University of Pittsburgh, School of Medicine, Pittsburgh, PA 15261.

Muscle and cutaneous nerves were individually labeled with DiI in developing chick embryos fixed at different stages. After allowing 6-14 d (at 37° C) for diffusion, the detailed anatomy of sensory afferent arbors and motoneuronal dendrites was analyzed. At first, cutaneous and muscle afferents exhibited similar morphologies. Between stages 28-29 (E5.5-6) the first small branches began to penetrate the gray matter. Both cutaneous and muscle afferents had processes in the primordium of the dorsal funiculus (the oval bundle of His) extending for at least 2 segments rostral and caudal to their point of entry. At stage 33 (E7.5) both types exhibited collaterals coursing ventrally through the superficial laminae of the dorsal horn (to lamina V). Target-specific differences in projections were first observed shortly thereafter (St 34, E8) when some muscle afferent projections extended unbranched through the dorsal horn to the level of motoneuronal somata. In contrast, cutaneous afferents branched and remained within the dorsal horn. These results suggest that cutaneous and muscle afferent collaterals enter the dorsal horn simultaneously and subsequently grow to appropriate destinations.

At the earliest stages examined, the motoneurons studied (sartorius) extended dendrites both dorsally and medially along the gray-white border. By stage 34 (E8) dendrites arborized extensively in the marginal zone (white matter), but in the gray matter dendrites were restricted to the lateral motor column. Dorso-medially directed dendrites were observed in St 40 (E14) embryos and thus grow into a region already occupied by muscle afferents with which they form synaptic connections.

Supported by NSF (EF) and NS-23725 (HRK)

235.4

SPECIFICITY OF REGENERATION AND EFFECTS ON CENTRAL FUNCTION FOLLOWING PERIPHERAL AXOTOMY. H.R. Koerber, A.W. Seymour and L.M. Mendell. Dept. of Neurobiol., Anat. & Cell Sci. Univ. of Pittsburgh, Pittsburgh, PA 15261 and Dept. of Neurobiol. and Behav., SUNY, Stony Brook, NY 11794.

Primary afferents were impaled in the L7 ganglion of 15% chloralose anesthetized cats 6-17 months after tibial nerve transection. After characterizing their reinnervated receptor, single fibers were stimulated and the resulting cord dorsum potentials (CDPs) averaged. Previous studies have shown slowly (SA) vs rapidly adapting (RA) receptor-dependent functional specializations of central connections (Koerber & Mendell, J.Neurophysiol.'88) that are maintained after faulty reinnervation (Koerber et al., Neurosci. Lett.'89). These criteria were used to evaluate 427 of 543 tibial afferents (79%) that had successfully reinnervated peripheral targets. Many (46%) of these fibers had reinnervated inappropriate tissue or receptors (i.e. center-periphery mismatches). However, cutaneous afferents reinnervating subcutaneous tissue tended to be SA (3:1), and non-cutaneous fibers reinnervating skin favored SA over RA receptors (4:1). SA cutaneous afferents reinnervating skin preferred SA over RA receptors (4:1), and RA afferents also displayed some preference for RA receptors (2.5:1). The fate of regenerating fibers also affected the amplitude of the monosynaptic CDPs produced by their stimulation. Previously cutaneous fibers reinnervating any structure evoked larger CDPs than those that were unsuccessful ($p<.01$). Those reinnervating skin produced larger CDPs than those supplying muscle ($p<.025$). RA afferents reinnervating RA receptors produced larger CDPs than control RA fibers ($p<.005$), but CDPs produced by SA fibers reinnervating SA receptors were similar to controls. In summary, although center-periphery mismatches occur after tibial nerve regeneration, SA and RA fibers exhibit some selectivity in reinnervation. Furthermore, the appropriateness of the reinnervated target can influence the effectiveness of a fiber's central projections which can differ depending on the afferent's original receptor type. Sup. by NS-23725 (HRK) and NS-16996 & NS-14899 (LMM).

235.5

AFFERENT NEURONES INNERVATING A NOVEL TARGET FORM SOMATOTOPICALLY APPROPRIATE CONNECTIONS IN THE DORSAL HORN. By Gary Lewin and Stephen McMahon, Dept. Physiology U.M.D.S. (St Thomas' campus), London SE1 7EH.

We have previously shown that when muscle afferents innervate skin they become capable of exciting dorsal horn neurones (DHN)(Lewin & McMahon, Soc Neurosci Abs. 15: 13.9, 1989). We have now examined the receptive field (RF) properties of DHN's driven by redirected afferents. In adult rats the nerve to the gastrocnemius muscle (GN) and the cutaneous sural nerve (SN) were self- and cross-anastomosed on left and right, respectively. Ten to 12 weeks later, recordings were made from functionally isolated DHN's driven by electrical stimulation of the GN, in urethane anaesthetised animals. When the GN innervated skin, 24 DHN's were found with brush, pinch or brush and pinch RF's including the skin exclusively innervated by the GN. For 76% of these, the RF extended contiguously into adjacent nerve territories. Moreover, DHN's activated by the GN were restricted to areas of the spinal cord normally receiving SN input: DHN's with medial foot RF's ($n=23$) lacked inputs from the GN. Finally, electrical stimulation of the GN innervating muscle failed to activate more than the occasional DHN with a RF inside or outside the SN territory.

Thus when the GN innervates a foreign target it contributes to approximately normal RF's in the dorsal horn, and more remarkably it appears to selectively establish connections with somatotopically appropriate DHN's.

235.6

PROPERTIES OF MUSCLE AFFERENTS CROSS-INNERVATING SKIN IN CATS. H. Nishimura+, R.D. Johnson# and J.B. Munson+, +Departments of Neuroscience, College of Medicine and #Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610.

We are investigating the ability of muscle and cutaneous afferents to innervate foreign tissue and foreign receptors, and the effects of such innervation on the electrical properties and synaptic efficacy of those afferents. Part or all of the medial gastrocnemius (MG) muscle nerve of cats was cross-connected with the caudal cutaneous sural (CCS) nerve, thus giving muscle afferents the opportunity to innervate skin and skin receptors. Individual MG-->CCS afferents were classified physiologically (up to 3 years later) with regard to receptor innervation, response properties, and conduction velocity. Of 18 analyzed afferents, 2 innervated hair follicle G_2 type receptors, 4 innervated SA I type receptors, and 12 innervated SA II type stretch receptors. Thus, like group I muscle afferents, all had static response properties. Conduction velocities, measured distal of the coaptation, ranged from 7-47 m/s. Stroking of the skin normally innervated by CCS evoked synaptic activity in triceps surae motoneurons similar to that elicited by muscle stretch. Electrical stimulation of the CCS nerve cross-innervated by MG produced EPSPs in triceps surae motoneurons like those produced by normal MG Ia afferents. MG nerve axotomized 1 year produced no such PSPs. Thus MG muscle afferents innervate and are activated by cutaneous receptors; this innervation preserves the synaptic efficacy of Ia afferents to motoneurons, which is seemingly sustained equally by innervation of muscle or skin receptors. Supported by NS15913 and NS27511.

235.7

TOPOLOGY OF PRIMARY SOMATOSENSORY CORTICAL (SmI) MODULATION OF CUTANEOUS AFFERENT INPUT TO THE CAT BRACHIAL DORSAL HORN. R. Sonty, Department of Physiology, WV Univ. Med. Ctr., Morgantown, WV 26506.

SmI modulation of cutaneous afferent input to the cat brachial dorsal horn was investigated by observing change in amplitude of the N-1 component of cord dorsum potentials (CDPs) evoked by stimulation of forelimb peripheral cutaneous nerves, before and after application of cortical surface conditioning volleys at a rectangular grid of loci spaced 1mm apart. Topology of cutaneous nerve representation was determined by mapping the somatotopic projection of the nerves to the brachial dorsal horn and SmI using averaged evoked potential techniques. Topology of descending effects was determined by correlating modulation of CDPs by cortical stimulation on a point on the grid. Results showed that (1) SmI effects on afferent input were predominantly inhibitory ($n=204$; $p<.0001$). (2) Maximal inhibition was observed at a 20 ms interval between the cortical conditioning stimulus and the peripheral nerve stimulus ($F=18.63$;$p<.003$).(3) The biphasic, initially negative, slow wave recorded of the cord on cortical stimulation attained a maximal negative value at 20 ms (4) Polysynaptic pathways were preferentially inhibited. ($n=103$; $p<.0001$) (5) A precise topology of SmI effects were seen, with maximal effects at corresponding locations in the two maps. This topology of effects may play a significant role in mechanisms of selective attention, spatial discrimination, and dorsal horn plasticity.

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235.8

BLADDER DISTENSION MODULATES LUMBOSACRAL DORSAL HORN NEURON RESPONSIVENESS. J.W. Downie and D.X. Zhang*. Dept.Pharmacol, Dalhousie Univ, Halifax, Canada, B3H 4H7

Convergent neural inputs onto sacral spinal neurons may account for aberrant reflexes subsequent to spinal injury. We sought to determine whether subthreshold visceral inputs to dorsal horn neurons could influence responsiveness to tactile stimuli.In chloralose-anesthetized cats extracellular unit recordings were made with carbon-filament electrodes. Receptor fields were mapped and responsiveness was assessed, with the bladder empty and distended, using 4 tactile stimuli ranging from innocuous to intensely noxious. Of 13 neurons studied to date 7 responded (>20% change in activity) to bladder distension. Activity increased in 4 units (42-112%) and decreased in 3 (42-71%). The overall responsiveness to tactile stimulation (basal activity subtracted) was decreased during bladder distension in 7 units (17-81%) and increased in 3 (43-106%). In 3/13 neurons bladder distension changed the modality producing maximum response from noxious to innocuous. In one neuron the reverse change was seen and there was no change in the others. The data indicate that bladder distension can modulate the responsiveness of dorsal horn neurons to tactile stimuli. Whether these effects are mediated by a spinal path of vesical afferents or to a pathway relaying at a supraspinal level has not yet been determined.(Supported by CPA and MRC of Canada)

235.9

INHIBITORY ROLE OF MECHANORECEPTIVE AFFERENTS FROM THE DISTAL GLANS PENIS ON SPINAL REFLEXES IN THE RAT. R.D. Johnson. Department of Physiological Sciences, College of Veterinary Medicine, University of Florida, Gainesville, FL 32610.

Mature male Wistar rats were anesthetized with urethane (i.p. induction and i.v. maintenance) and cannulated for vital signs monitoring. The spinal cord was acutely transected at T6-7 and the L5-S1 cord exposed for single cell recording. Stimulating electrodes were placed over the ipsilateral and contralateral dorsal nerve of the penis (DNP). The ipsilateral motor branch (MB) of the pudendal nerve was prepared for whole nerve recording. The glans penis and prepuce were mechanically stimulated with hand held probes or a Chubbuck electromagnetic mechanostimulator.

Reflex discharges in the MB were elicited by electrical stimulation of either the ipsi- or contralateral DNP. These evoked discharges could be completely inhibited by a conditioning stimulus consisting of gentle squeezing or pressure on the inner prepuce, ventral glans raphe or distal glans provided the stimulus was given not more than approximately 50 ms prior to the electrical stimulus. A very small distal glans region (within 2 mm of the tip) was the most effective penile inhibitory site.

Almost all of the Class 2 and 3 spinal interneurons responding to penile input exhibited an excitatory response to gentle squeeze, pressure, and stretch of the distal glans region. These neurons exhibited a short peripheral latent period and their evoked impulse discharge was temporally related to the short inhibitory period (20-40 ms following interneuron burst) for the reflex discharge in MB. These temporal relationships suggest that the inhibitory period may result from activity in these interneurons and is restricted to activity originating in myelinated penile mechanoreceptors.

Supported by NS27511.

235.10

ORGANIZATION OF LOCAL AXONAL CONNECTIONS IN NUCLEUS PROPRIUS OF HAMSTER SPINAL DORSAL HORN IN VITRO. S.P. Schneider. Department of Physiology, Univ. of N. Carolina, Chapel Hill, NC 27599.

As a prelude to study of mechanisms underlying local synaptic transmission in the mammalian dorsal horn, the distribution of axon branches of nucleus proprius (NP) neurons was analyzed in an isolated spinal cord preparation with intact innervation from a patch of hairy skin.

Twenty NP neurons were characterized by their responses to electrical volleys in a cutaneous nerve and to mechanical stimulation of the skin surface and then stained intracellularly with horseradish peroxidase.

Axons of NP neurons terminated in two general patterns, each with a substantial local distribution. One group of neurons had axons that formed dense "patches" of terminals, 100-200 μm in extent, oriented rostrocaudally in laminae II_i, III and IV of a single spinal segment. Axons of a second group were directed ventrally and bifurcated into long rostrocaudal branches that extended into adjacent segments, giving off periodic terminal arborizations in laminae deep to the parent cell body. Sensory input to both groups of neurons was dominated by innocuous mechanoreceptors with myelinated afferent fibers.

The results suggest that the integrative functions of the NP are subserved by at least two different groups of interneurons with distinctive patterns of local axon terminations.

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235.11

NOCICEPTIVE AND NON-NOCICEPTIVE DORSAL HORN NEURONES DIFFER IN SUBSTANCE P AND OTHER SYNAPTIC INPUT: A COMBINED ELECTROPHYSIOLOGICAL AND IMMUNOCYTOCHEMICAL STUDY IN THE CAT. A. Ribeiro-da-Silva, Y. De Koninck, A.C. Cuello and J.L. Henry. Depts. Pharmacology & Therapeutics, Physiology and Anaesthesia Research, McGill Univ., Montréal, Qué., H3G 1Y6

This study focuses on functionally classified dorsal horn neurones and examines the morphological and immunocytochemical characteristics of axonal varicosities contacting these neurones. In anaesthetized cats, intracellular recordings were obtained from dorsal horn neurones at the lumbar level using electrodes filled with HRP. Cells were classified on the basis of their responses to natural stimulation of the receptive field. Subsequently, HRP was injected intracellularly and the cats were perfused with an aldehyde mixture. The relevant tissue was processed for demonstration of HRP and Substance P-like immunoreactivity (SP-LI) at the light and electron microscopic levels, by means of a bi-specific anti-SP monoclonal antibody. With neurones which were exquisitely sensitive to noxious stimuli, up to 40% of the synaptic boutons contacting them contained SP-LI. Distal dendrites had a lower proportion of SP-LI positive boutons. These neurones did not appear to be associated with synaptic glomeruli. In contrast were large lamina III neurones, with axons projecting in the dorsal column and in the dorso-lateral funiculus, and neurones of inner lamina II; they were strongly associated with low threshold hair afferent input and with weak nociceptive responses if any. These cells were found to have a very low proportion of synaptic boutons containing SP-LI. These neurones also had a large number of dendritic spines and their dendrites were strongly associated with synaptic glomeruli in laminae II_i-III but very few in laminae I-II_o. Based on their anatomical localization, these glomeruli are likely to be associated with non-nociceptive low threshold mechanical input. These results suggest that nociceptive and non-nociceptive neurones differ in the chemistry and morphology of their synaptic inputs. (Supported by the NIH, YDK is funded by the FRSQ.)

235.12

TRIGEMINAL AND CERVICAL INPUT ONTO SINGLE NEURONS IN THE C1 SPINAL CORD. E.H. Chudler, C.E. Poletti* and W. Foote*. Lab. of Pain Research, Depts. of Neurosurgery and Psychiatry, MGH and Harvard Med. Sch., Boston, MA 02114.

Previous anatomical studies have shown that trigeminal and cervical afferent nerve fibers project to the upper cervical segments of the spinal cord. To determine the degree of convergence between these pathways, we studied the response properties of C1 dorsal horn neurons to electrical and mechanical stimulation of the face, head and neck in paralyzed cats anesthetized with sodium pentobarbital. Neurons were classified as low threshold mechanoreceptive (LTM), wide-dynamic-range (WDR), nociceptive-specific (NS) or unresponsive based on their responsiveness to graded mechanical stimulation. Extracellular single unit recordings were obtained from 71 neurons electrically excited by cervical (21), trigeminal (15) or both cervical and trigeminal (35) stimulation and from 16 neurons unresponsive to peripheral stimulation. WDR and NS neurons exhibited more convergence, had larger receptive fields and longer first spike latencies than LTM neurons. These data suggest that some upper cervical spinal cord neurons receive convergent input from trigeminal and cervical pathways and may be involved in mediating orofacial and cranial pain.

PAIN MODULATION: ANATOMY AND PHYSIOLOGY IV

236.1

RECIPROCAL PROJECTIONS BETWEEN THE MEDIAL PREOPTIC AREA AND MIDBRAIN PERIAQUEDUCTAL GRAY. T. Rizvi, M. Ennis, M. Behbehani and M. Shipley. Depts. Anatomy & Physiology. Univ. Cincinnati. Coll. Med., Cincinnati, OH 45267.

Previous anatomic studies indicate that the medial preoptic area (MPO) is a source of afferent input to the periaqueductal gray (PAG). Here, we have examined the topography of reciprocal connections between these two structures.

WGA-HRP injections into several rostrocaudal levels of PAG retrogradely labeled large numbers of neurons in MPO which shift mediolateraly along the rostrocaudal axis; rostral MPO contained heavy retrograde labeling restricted to the medial region while in caudal MPO, neurons in the lateral zone are retrogradely labeled. In addition, tracer injections in PAG produced dense anterograde labeling in both medial and lateral MPO.

WGA-HRP injections in MPO resulted in dense anterograde labeling in PAG. In most cases the labeling comprised two discrete longitudinal bands in dorsomedial and lateral PAG. Labeling was heavier in the central than the peripheral part of PAG. In addition, MPO injections produced retrograde labeling of neurons located in dorsomedial and ventral/ventrolateral parts of PAG.

The present findings suggest that connections between PAG and MPO are heavy, reciprocal and spatially organized. This circuit may play important roles in forebrain-brainstem integration of thermoregulation, analgesia, maternal and reproductive behaviours. (Supported by PHS Grants NS20643, NS24698 and HL08097).

236.2

HYBRIDIZATION HISTOCHEMICAL ANALYSIS OF NEUROTENSIN AND ENKEPH ALIN mRNA IN POLYARTHRITIC RATS. F. G. Williams*, G. D. Kaufman* and A. J. Beitz. Dept. of Vet. Biology, University of Minnesota, St. Paul, MN 55108

Chronic polyarthritis was induced in male Sprague-Dawley rats (275 - 300 grams body weight) by injecting 150 µl Freund's Complete adjuvant in the tail base. Control animals received mineral oil. Several parameters were quantitated to monitor the development and progression of the disease, including tail-flick latency, paw size, and tibio-tarsal joint size. Animals were decapitated 4, 8, and 24 days following injection and their brains were rapidly removed and frozen. Fifteen µm cryosections of the midbrain were prepared for hybridization histochemistry by formalin fixation, alkylation, dehydration and delipidation. Hybridization to biotinylated oligonucleotides complementary to pro-neurotensin/neuromedin N (NT) or pro-enkephalin A (Enk) mRNA was carried out as previously described and detected using fluorescein-avidin. The frequency and distribution of fluorescent neurons containing Enk and NT mRNA were mapped and the relative fluorescence intensity of subsets of hybridization-positive neurons was noted.

The onset of polyarthritic symptoms occurred approx. 7 days following injection. By day 24, average tail-flick latencies had decreased 10% to 4.2 seconds ($p=.02$), hind paw circumference increased 13.5% to 2.9 cm ($p<.01$), and tibio-tarsal joint circumference increased 17.1% to 3.1 cm ($p=.02$). The distribution and frequencies of hybridization-positive neurons in the midbrains of 4 and 8 day rats were indistinguishable from those of controls. In the 24 day experimental brains, however, significant changes were observed in both frequencies and distributions of neurons containing mRNA's for Enk and NT. NT mRNA-positive neurons were most frequent in the dorsal raphe (DR) and ventral periaqueductal gray (PAG). Polyarthritis increased the frequencies of positive neurons in these regions, but had the greatest effect lateral to these regions, where numbers of hybridization-positive neurons in the tegmental and cuneiform nuclei were significantly increased. Enk mRNA-positive neurons also responded to polyarthritis. PAG and DR neuron population frequencies did not increase significantly, but signal intensities were increased. The tegmental and cuneiform nuclei of arthritic animals contained increased numbers of Enk mRNA-containing neurons, and the hybridization signal strength in these neurons was also increased. These results demonstrate that chronic polyarthritis causes significant increases in the biosynthetic metabolism of peptidergic neurons within midbrain regions that are known to be activated by nociceptive stimuli. The data suggest that both NT and Enk peptides from these midbrain regions may participate in the modulation of chronic nociception. Supported by NIH grants NS 28016, NS 19208, and DE 06682

236.3

MAPPING OF BRAINSTEM CHOLINERGIC PATHWAYS INVOLVED IN THE MODULATION OF NOCICEPTION. L.F. Fitzgerald and H.K. Proudfit. Dept. of Pharmacology, Univ. of Illinois at Chicago. Chicago, IL 60680.

The pedunculopontine tegmentum (PPT) is a cholinergic cell group in the brainstem that modulates nociception. These studies identified interconnections between PPT neurons and those in the nucleus raphe magnus (NRM), gigantocellular reticular nucleus pars alpha (Giα) and the A7 catecholamine cell group.

An anterograde tracer, phaseolus vulgaris leucoagglutinin (PHA-L), was injected into the PPT and axon terminals were labeled in the NRM and Giα. In a separate experiment, a retrograde tracer (Fluoro-Gold or Fast Blue) was injected into the NRM or Giα, and choline acetyltransferase-reactive PPT neurons were labeled with tracer. This suggests that PPT neurons project to the NRM and Giα.

Retrograde tracer was then injected into the A7 while PHA-L was injected into the PPT. Axon terminals of PPT neurons in the NRM and Giα were adjacent to neurons which project to the A7. This suggests that antinociception produced by stimulation of the PPT may be mediated by neurons in the ventromedial medulla which project to the A7 nucleus.

Supported by USPHS Grant DA03980.

236.4

PHARMACOLOGIC EVIDENCE FOR A PATHWAY BETWEEN THE PEDUNCULOPONTINE TEGMENTAL NUCLEUS (PPTn) AND THE NUCLEUS RAPHE MAGNUS (NRM) MODULATING NOCICEPTION. E.T. IWAMOTO. Dept. of Pharmacology, Coll. of Med., Univ. of Kentucky, Lexington, KY 40536.

Antinociception assessed by the hot-plate and tail-flick assays was induced by subcortical microinjection of nicotine in 0.5 μl of phosphate buffer in unanesthetized male Sprague-Dawley rats implanted with subcortical guide cannulas. Out of the 185 brain sites examined, PPTn and NRM were the most sensitive with median effective doses of nicotine ranging between 1.4 and 2.9 nmole. Parenteral naloxone administration had no effect on PPTn nicotine-induced antinociception. Coadministration of mecamylamine or pirenzepine with nicotine in the NRM competitively antagonized nicotine antinociception. Methoctramine alone into the NRM induced strong antinociception which was blocked by 1 hr pretreatment of the NRM with hemicholinium-3 (HC-3; no effect when injected alone). HC-3 pretreatment of either the PPTn or NRM antagonized the antinociceptive effects induced by nicotine at these sites. HC-3 pretreatment of the NRM also antagonized the antinociceptive effects of a subcutaneous injection of 0.5 mg/kg of nicotine. Ibotenic acid lesions of the PPTn or HC-3 pretreatment of the NRM abolished the antinociception induced by nicotine or (+)-cis-dioxolane microinjections into the PPTn. Neither unilateral ibotenic acid lesions nor bilateral electrolytic lesions of the PPTn alone altered hot-plate or tail-flick nociceptive responding. Procaineamide microinjection into the NRM abolished the antinociception induced by bilateral nicotine microinjections into the PPTn. Bilateral microinjections of procaineamide into the PPTn did not alter the antinociception induced by nicotine injected into the NRM. A cholinergic pathway which modulates nociception originating in the PPTn and terminating in the NRM is proposed. Activation of the cell bodies in the PPTn results in acetylcholine release onto M_1 receptors located in the NRM; the release of acetylcholine is regulated by autoinhibitory M_2 receptors. We propose that stimulation of this PPTn-to-NRM pathway activates known descending pain control systems. (Supported by the KTRB).

236.5

INTERPEDUNCULAR NEURONS ARE UNRESPONSIVE TO PINCH WHILE VENTRAL TEGMENTAL NEURONS CAN BE INHIBITED. J.L. Kim*, L. Gollapudi*, and I.D. Hentall. Univ. of Illinois, College of Medicine, Rockford IL 61107.

The interpeduncular nucleus (IPN) inhibits or excites cells in the nucleus raphe magnus (NRM) which respectively suppress or facilitate nociception. To decide whether this polysynaptic pathway is active during acute pain, we recorded spontaneously active neurons extracellularly in the IPN and adjacent catecholaminergic ventral tegmental area (VTA) and interfascicular nucleus (IF) in rats anesthetized with pentobarbital. Recording micropipettes contained 2M sodium acetate and saturated fast green (which marked recording sites later found histologically). Metal stimulating microelectrodes were placed bilaterally in the medial habenula, and their locations confirmed with electrolytic lesions.

Of cells calculated to be in the IPN, 93% (n=15) did not respond to noxious squeezing of the skin on the hindlimb (one border cell was excited). Habenula stimulation caused synaptic excitation with a long delay (>10ms) in 86% (n=14) IPN cells. In the VTA and IF (n=28), 36% of cells were markedly inhibited by pinch, 14% weakly excited and 50% unaffected. Inhibition in the VTA had a typical onset of 1-2s and recovery of 1-2 min.

We conclude that the IPN modulates nociception via the NRM without reference to levels of pain input. (Supported by NINDS grant NS26116).

236.6

INHIBITION OF PERIAQUEDUCTAL NEURONS BY SEROTONIN IS MEDIATED THROUGH $5HT_{1A}$ RECEPTORS. H. Liu*, S. Chandler, M. Jiang* and M.M. Behbehani. U. of Cincinnati, Dept. of Physiology, Cincinnati OH 45261-0576

The midbrain periaqueductal gray contains a high concentration of 5HT immunoreactive terminals and 5HT receptors. The goals of this study were to determine the effect of 5HT on PAG neurons and to characterize the effect of $5HT_{1A}$ agonist and antagonist on these cells. Rats were used for both in vivo and in vitro experiments. In the in vitro studies, extracellular recordings were made from neurons in 400 μ thick PAG slices. Drugs were applied by pressure from electrodes placed near the recording site. In vivo experiments were conducted on chloral hydrate anesthetized adult male rats and the effects of iontophoretically applied serotonin, 8-OH-DPAT (DPAT), methysergide (MET) and spiperone (SPIP) were recorded.

In the in vitro experiments, responses of 203 neurons to 5HT and DPAT were tested. 5HT and DPAT inhibited 46% and 60% of the cells respectively. In contrast 30% of the cells were excited by 5HT compared to 14% that were excited by DPAT. The inhibitory effect of 5HT and DPAT could be blocked by SPIP but the excitatory effect of 5HT was more effectively blocked by MET.

In the in vivo experiments recordings were made from 130 neurons In 54 cells DPAT altered the baseline firing rate. The majority of these cells were inhibited (44/54) and a small population were excited (10/44) The effect of DPAT could be blocked by spiperone.

It is concluded that the major effect of 5HT on PAG neurons is inhibition that is mediated by $5HT_{1A}$ receptors. Supported by PHS grant #NS20643.

236.7

INTERACTION BETWEEN THE INSULAR CORTEX (IC) AND THE PERIAQUEDUCTAL GRAY (PAG). M. Jiang and M.M. Behbehani. Dept. of Physiology. U. of Cincinnati. Cincinnati OH 24267-0576

The objectives of the current studies were to determine the physiological interaction between PAG and IC and to examine the roles of glutamic acid and enkephalin in the interaction between these sites. Experiments were conducted on chloral hydrate anesthetized 250-300 gram male rats. Of 403 PAG cells that were recorded, 143 neurons respoded to electrical stimulation of the IC. The responses to electrical simulation of IC at 1 or 0.5Hz could be classified in two major categories. 1)In 50 cells IC stimulation produced excitation with onset latency that ranged between 11 and 34 msec (mean 27.3 ±2.13 (SEM)), average duration of 38.5±3.55 (SEM) followed by inhibition with mean duration of 89±4.7 (SEM) msec. 2) In 83 cells IC stimulation produced inhibition with mean onset latency of 26.18±1.68 (SEM) msec and duration of 108.±8.97 msec. The remaining 10 neurons showed more complex triphasic responses. In 13 out of 29 cells that were excited by IC stimulation local application of kynurenic acid (KA) blocked the excitatory response. In these neurons, KA had very little effect on the inhibition that followed the excitatory phase of the response. Local application of naloxone had no effect on the response of PAG cells to IC stimulation (n=30). It is concluded that: 1) Approximately 30% of PAG cells are responsive to IC stimulation. 2) the interaction between the IC and PAG includes a slow conducting monosynaptic and one or more polysynatic pathways. 3) The excitatory response includes a glutaminergic system and 4) the inhibitory response is not mediated by enkephalin. Supported by PHS grant # NS20643.

236.8

ANALGESIA PRODUCED BY LIDOCAINE INJECTION INTO RAT DENTATE GYRUS. J.E. McKenna and R.Melzack*, Department of Psychology, McGill University, Montreal, Quebec, Canada H3A 1B1.

Previous studies in our laboratory have indicated that injection of a local anesthetic into discrete sites within the rat brain, including the lateral hypothalamus and anterior cingulum bundle, causes a significant long-lasting analgesia during the formalin test.

As a continuation of this work, the present study investigated the analgesic effects of lidocaine injected into regions of the dentate gyrus. Anesthetized Long-Evans rats were stereotaxically implanted with cannulae unilaterally. Eight to ten days after surgery each rat received a 1.0 μl injection of 2% lidocaine or saline. The injection was given either before or after the ipsilateral or contralateral hindpaw received an injection of 50 μl of 2.5% buffered formalin. The strength and time course of analgesia differed according to laterality and time of the microinjection. The implications of these results are discussed. Supported by NSERC grant A7896.

236.9

TOWARD OBJECTIVE MEASURES OF POST-SURGICAL PAIN: NALOXONE AND EEG SPECTRAL ANALYSIS IN RATS. Vahn A. Lewis. Dept. Pharmacology, Dental Branch, U. of Texas, Houston, Tx. 77030.

Computerized EEG (electrocorticographic) recordings have been used measure surgery induced changes in EEG. 11 S-D rats were anesthetized with pentobarbital(PB) and EEG, EMG electrodes and intrajugular catheter implanted. This surgery was the pain stimulus. Recordings of EEG and EMG and body movement were made at 4, 8 and 27 hours post surgery. One week following surgery, animals were again anesthetized with PB and controls recorded at equivalent times to the earlier recordings. Ten minutes prior to all recordings 10 mg/kg naloxone was administered to antagonize endogenous opiate activity. Data were automatically sampled (2 seconds every 30 seconds of run time). The power spectrum (PS) was calculated over 8 samples representing samples 5-9 minutes (E, early samples) and 15-19 minutes (L, late) samples after placing the animals in the recording chamber. At 8 hours following surgery the log of the area under the PS curve was depressed relative to control at E -22%, P=<0.05, and L -46% P=<0.001. At 27 hours the PS was depressed in the L -26% P=<0.05). This research will lead to improved methods for assessing pain after injury in man and animals.

236.10

ROLE OF DESCENDING INHIBITION ON THE RELEASE OF CALCITONIN GENE-RELATED PEPTIDE AND SUBSTANCE P FROM RAT DORSAL LUMBAR SPINAL CORD IN-VITRO. L.D. Aimone and T.L. Yaksh, Dept. of Anesthesiology, UC, San Diego, La Jolla, CA 92093.

The content of norepinephrine (NE, measured by HPLC) in the dorsal lumbar spinal cord was reduced to 35% and 24% of control levels by spinal cord transection (T8, Trans) or the intrathecal administration of 6-OHDA (100 µg) 7 days prior. Calcitonin gene-related peptide (CGRP) and substance P (SP) content (measured by RIA) in the dorsal lumbar spinal cord was only slightly reduced by Trans (11% & 8%,) or 6-OHDA pretreatment (22% & 20%). In-vitro perfusion of dorsal lumbar spinal cord slices revealed that trans or 6-OHDA resulted in a marked increase in the basal release of CGRP and SP. Basal NE release was reduced by 6-OHDA, while Trans actually resulted in a small increase. The K+ (50 mM) evoked release of NE was decreased by either of the pretreatments with only slight changes in the evoked release of either CGRP or SP. (pg/mg)

		CONTROL	TRANS	6-OHDA
NE	basal	5.37±0.54	7.45±1.40	1.43±0.36
	evoked	23.7±2.96	14.4±3.13	5.68±2.14
CGRP	basal	8.08±1.01	15.8±2.55	11.3±0.73
	evoked	50.6±6.38	37.0±4.60	47.8±18.4
SP	basal	0.34±0.04	1.13±0.22	0.90±0.16
	evoked	1.73±0.19	2.43±0.39	1.99±0.42

These observations suggest that basal release is subject to a local control by systems which arise from bulbospinal pathways and 6-OHDA sensitive terminals.

236.11

MAMMALIAN NEURONAL BRADYKININ$_2$ RECEPTORS: DIFFERENTIAL AFFINITY OF THE ANTAGONIST [D-ARG(0), HYP(3),D-PHE(7)]-BRADYKININ. D.J. Dooley, M. Belledin* and K.I. Bär*. Gödecke Research Institute, D-7800 Freiburg, F.R.G.

Pain is one pathophysiologic effect of bradykinin (BK) which reflects activation of BK receptors associated with nociceptors of afferent sensory neurons (Steranka et al., PNAS, 85:3245, 1988). These receptors of four mammalian species (bovine, rat, guinea pig, monkey) were characterized by using spinal cord membranes and selective BK-related drugs in a [^{3}H]-BK binding assay. Across the species, the BK$_1$ receptor agonist [des-Arg(9)]-BK (10 µM) and antagonist [Leu(8), des-Arg(9)]-BK (10 µM) were ineffective to inhibit [^{3}H]-BK binding (0.25 nM). The BK$_2$ receptor agonist [Tyr(Me)(8)]-BK (0.3 nM) inhibited [^{3}H]-BK binding by ~50-70%. The BK$_2$ receptor antagonist [D-Arg(0),Hyp(3), D-Phe(7)]-BK (10 nM), in contrast, differentially inhibited [^{3}H]-BK binding as evidenced by a range of ~10% (guinea pig) to ~70% (bovine). These results suggest that mammalian neuronal BK receptors are of the BK$_2$-subtype, and that the affinity of a BK$_2$ receptor antagonist for these receptors can vary across different species.

236.12

THE FORMALIN TEST IN MICE: INFLUENCE OF THE AMBIENT TEMPERATURE. K.Hole, J.H.Rosland, A.Tjølsen, A.Lund and O.-G.Berge. Dept. of Physiology, Univ. of Bergen, Bergen, N-5009 Norway.

The late phase in the formalin test may be due to inflammation, and it is possible therefore that the response may be influenced by blood flow and tissue temperature. When the test was performed at different ambient temperatures, the licking response in the late phase was weak in a cool environment (20 °C) and increased with increasing ambient temperature up to 28 °C. In the early phase there was no effect of temperature.

Lesioning of descending serotonergic systems with i.th. 5,6-dihydroxytryptamine (5,6-DHT) increased the licking response in the late phase at 20 °C compared to controls, but not at 25 °C. The response in 5,6-DHT treated animals at 20 °C was approximately the same as for animals tested at 25 °C, indicating no effect of ambient temperature in 5,6-DHT lesioned animals. 5,6-DHT lesions caused an increase in paw skin temperature of animals kept at 20 °C, indicating that the increased licking response may be due to a higher blood flow and increased tissue temperature in the paw, facilitating the inflammatory response.

It was concluded that the paw skin temperature may influence the response in the late phase of the formalin test. An apparent hyperalgesia in the late phase after lesions of raphe-spinal 5-HT systems may be due to an increase in blood flow.

PAIN MODULATION: PHARMACOLOGY II

237.1

ANTINOCICEPTION PRODUCED BY A HIGH DOSE OF MEDETOMIDINE DUE TO SPINAL ALPHA-2-ADRENERGIC MECHANISMS. A.Pertovaara, T.Kauppila*, E.Jyväsjärvi* and E.Kalso* Depts.Physiol. and Anesthes. Univ.Helsinki, Helsinki, Finland.

In the current study we determined the effect of a high, anesthetic dose of medetomidine, an alpha-2-adrenoceptor agonist, on nociceptive reflex and sensory neuronal responses in rats. In intact rats, the latencies to the mechanically- and heat-induced tail reflex responses were prolonged by systemic medetomidine (300 µg/kg). This antinociceptive effect was reversed by a high systemic dose (1.5 mg/kg) or a small intrathecal dose (25 µg) of atipamezole, an alpha-2-adrenoceptor antagonist. Also in spinal rats, the latencies to the heat-induced tail flick were prolonged by the high dose of systemic medetomidine in an atipamezole-reversible way. In 50 % of the nociceptive spinal dorsal horn projection neurons systemic medetomidine produced an atipamezole-reversible suppression of peripherally-evoked responses. The results suggest that systemic medetomidine at a high, anesthetic dose can produce antinociception due to segmental spinal alpha-2-adrenergic mechanisms.

237.2

ANTAGONISM OF NALOXONE-INSENSITIVE STRESS-INDUCED ANALGESIA IN MICE BY SPECIFIC NMDA RECEPTOR ANTAGONIST MK-801. P. Marek, G. Page, S. Ben-Eliyahu and J. C. Liebeskind. Department of Psychology, University of California, Los Angeles, CA 90024.

The effect of a specific NMDA receptor antagonist MK-801 on analgesia produced by 3 min forced swimming in 20°C water was studied in CXBK and CXBH mice. Analgesia was measured using the hot-plate test (52°C) with hind paw flick as the criterion pain response. In opiate receptor deficient CXBK mice, swim-induced analgesia was completely insensitive to naloxone (10 mg/kg, i.p.), but was abolished by MK-801 (0.075 mg/kg, i.p.). In CXBH mice, rich in brain opiate receptors, analgesia induced by swim was more pronounced and only partially antagonized by naloxone or MK-801 alone. When administered in combination, MK-801 and naloxone at the same doses almost completely blocked swim-induced analgesia. In CXBH mice, using the same doses of naloxone and MK-801, the former completely antagonized and the latter had no effect on the robust morphine analgesia caused by 10 mg/kg. These results indicate that stress may produce parallel activation of naloxone-sensitive and naloxone-insensitive analgesia mechanisms, the latter of which involves NMDA receptors. Supported by Unrestricted Pain Research Grant from the Bristol-Myers Squibb Company and NIH grant NS 07628.

237.3

SUBSTANCE P (SP) MICROINJECTIONS INTO THE A7 NUCLEUS PRODUCES ANTINOCICEPTION IN THE RAT

D. C. Yeomans and H. K. Proudfit. Dept. of Pharmacology, University of Illinois at Chicago, Chicago, Illinois 60680.

Antinociception evoked by stimulation of the raphe magnus (RMg) may be mediated in part by projections from the SP-containing cells in the RMg to noradrenergic (NA) neurons in the A7 nucleus. SP released onto the A7 cell bodies may activate spinopedal projections and thereby induce antinociception.

To test this hypothesis, hindlimb nociceptive reflexes were tested bilaterally in 24 lightly anaesthetized rats, prior to, during, and following microinjection of 0.5, 5.0, 50 ng of SP or vehicle into the A7 area. A "bell"-shaped dose-response curve was obtained, with the strongest anti-nociceptive effect observed after injection of 5.0 ng, both in terms of increased response latencies and the duration of the effect. The SP-induced antinociceptive effects were more pronounced when the hindlimb ipsilateral to the microinjections site was tested. This observation is consistent with anatomical findings that NA A7 neurons project ipsilaterally to the spinal cord dorsal horn.

The antinociceptive effect of SP appears to be mediated by activation of spinally-projecting NA neurons in the A7, since intrathecal phentolamine completely blocked the antinociceptive effects of SP. (Supported by USPHS Grant DA03980)

237.4

ANTINOCICEPTIVE EFFECT OF INTRATHECAL NEUROPEPTIDE Y ANALOGS ON HOT PLATE AND PAW PRESSURE TESTS.

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Neuropeptide Y (NPY) is found in bulbospinal pathways. We have previously shown (Hua, et al in preparation) that NPY (1-36) administered intrathecally in rats will elevate the hot plate response latency, but not the paw pressure threshold while NPY (18-36) will elevate both the hot plate response latency and paw pressure for withdrawal. In the present studies, we examined a number of truncated analogs given intrathecally in chronically catheterized rats on the 52.5C hot plate (60 sec cut off) and the paw pressure test (0-400 gm). The intrathecal ED_{50} of these agents is presented below. For active agents, maximum effects were observed in the absence of any detectable motor impairment. We also synthesized and tested several truncated and cyclic 26- to 28- peptides that conserved both the N- and C- termini of native NPY. These cyclic analogs were found to have activity profiles similar to that of NPY (18-36) but with significantly greater potencies. These results suggest the possible presence of more than one NPY-sensitive receptor type associated with spinal systems which modulate nociceptive processing for thermal and mechanical stimuli.

INTRATHECAL DRUG	Hot Plate (ED 50) µg	Paw Pressure (ED 50) µg
NPY (1-36)	5	>100
NPY (18-36)	32	51
Des - NH_2-Ala^{18} - NPY (18-36)	>100	>100
NPY (18-32)	>100	>100

237.5

ANALGESIC EFFECT OF SYNTHETIC FRAGMENTS OF VASOACTIVE INTESTINAL PEPTIDE IN THE RAT. B.R. Komisaruk[1], C. Banas*[1], A. Mehta*[2], P. Cash*[2], B. Whipple*[2] and F. Jordan*[2]. Inst. Animal Behavior[1], Dept. Chemistry[2], and Coll. Nursing[3], Rutgers University, Newark, NJ 07102

Vasoactive Intestinal Peptide (VIP 1-28), produces analgesia when administered intrathecally (i.t.) to the spinal cord (Ann. NYAS: 527:650,'88). Incubation of VIP 1-28 with spinal cord homogenate yielded several fragments, including VIP 1-10 and 11-28 (Ann. NYAS: 527:582,'88). In the present study, these were synthesized, injected i.t., and tested on two measures of pain threshold: vocalization threshold to tail shock (VOCT), and tail flick latency to radiant heat (TFL). **Results** (all dosages reported are the molar equivalent of 25 ug VIP 1-28; a lower [1/5] dose of each fragment had little or no effect). VOCT: VIP 11-28 significantly elevated VOCT (50-100+%) from 1-60 min post-injection. At 30 min, this effect was significantly greater than that of 25ug morphine sulfate (i.t.). VIP 1-10 produced a significant but lesser elevation for 5 min. The effect of another fragment, VIP 8-11, did not differ from saline. TFL: VIP 11-28 produced a significant, but shorter-lived elevation (75%, 5 min) than on the VOCT test. VIP 1-10 produced a significant (10-20%) elevation for 20 min, and VIP 8-11 produced a significant elevation (<20%) for 10 min. Thus, native VIP 1-28 can apparently be cleaved by spinal cord enzymes into peptide fragments that are analgesically active. Support: NIH NLS-2 5RO1 NS 22948 and GRS 5 SO6 RR08223 (BRK) and Busch Fdtn (FJ, BW, BRK).

237.6

BRADYKININ MODULATION OF A NOCICEPTIVE REFLEX. M.B. Bauer, S.T. Meller, and G.F. Gebhart. Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242

The objective of the present study was to examine the effect of bradykinin (BK) administered intravenously on the nociceptive tail flick (TF) reflex. BK (12 - 288 µg/kg) produced dose-dependent increases in TF latency and heart rate and a dose-dependent decrease in blood pressure. Maximal TF inhibition was observed 10 sec post administration. To determine if tachyphylaxis developed to the effect of BK on the TF reflex, BK was administered at 5, 10, or 40 min intervals. There was no significant difference in BK-induced alterations in TF latency at the three different dosing intervals. At the doses tested, complete spinal cord transection at a low thoracic level (T10-12) markedly attenuated BK-induced inhibition of the TF reflex. Bilateral vagotomy did not alter BK-induced inhibition of the TF reflex, whereas bilateral stellate ganglion removal shifted the dose response curve to the right. These results suggest that at the doses used, BK does not act directly on the spinal cord to induce a change in the TF latency and is able to modify the TF reflex by engaging descending inhibitory systems at least partially by engaging visceral afferents.

237.7

ANTINOCICEPTIVE CONCEQUENCES OF B-16 MELANOMA CELL TRANSPLANTS IN MOUSE AND RAT SPINAL CORD.

H. Wu, B. R. Lester*, Z. Sun* and G. L. Wilcox. Department of Pharmacology, University of Minnesota, Minneapolis, MN 55455, U.S.A.

It has been observed that α_2 agonists potentiate the antinociceptive effect of morphine in mouse and rat spinal cord. The aim of the current study was to determine whether catecholamine-containing cells transplanted into mouse and rat spinal cord would potentiate the antinociceptive effect of subthreshold doses of morphine. Two B-16 melanoma clones were chosen. The F10-C23 clone was considered to have low catecholamine content while the F1-C29 clone was considered to have high catecholamine content. Recipients were 20-24 g male ICR mice or 75-100 g male Sprague-Dawley rats. Mice were given 50,000 cells in 5µl PBS and rats were given 500,000 cells in 10 µl PBS by intrathecal (i.t.) injection. The control group received the same amount of PBS solution with no cells i.t. After 2, 4 and 7 days, a subthreshold dose of morphine, 0.1 µg for mice and 0.3 µg for rats, was given i.t. The tail flick and hot plate tests were performed before and after drug injection. Antinociception was quantified as the percentage of maximum possible effect (%MPE) which was determined in the usual way. Two days after cell injection, in both mice and rats, the groups that received F1-C29 (high catecholamine content) cells showed significantly higher antinociception than both the groups that received F1-C23 (low catecholamine content) cells and the groups that received no cells. This effect of morphine was blocked by the opioid antagonist naloxone (mice) and by the α_2 adrenergic antagonist idazoxan (mice and rats). Therefore, α_2 adrenergic receptors seem to be involved in manifestation of this antinociception. This result indicates that B16 melanoma cells transplanted into mouse and rat spinal cord may potentiate the antinociceptive effect of morphine, apparent involving activation of α_2 adrenergic receptors. (Supported by NIDA R01 grants 01933 & 04274 to GLW and Leukemia Task Force grant to BRL.)

237.8

FORMALIN-EVOKED FOS EXPRESSION IN RAT SUPERFICIAL DORSAL HORN: REDUCTION BY AN NMDA ANTAGONIST. L.J. Kehl, A.I. Basbaum, K.R. Gogas, C.H. Pollock*, M. Mayes*, G.L. Wilcox. Graduate Program in Neuroscience and Departments of Pharmacology and Psychology, University of Minnesota, Minneapolis, MN 55455, USA and Department of Anatomy, University of California San Francisco, San Francisco, CA 94143, USA.

Various forms of natural stimulation induce the expression of Fos, a nuclear phosphoprotein product of the *c-fos* proto-oncogene. Since endogenous excitatory amino acids (EAA's), which terminate densely in laminae I and II, have been implicated as neurotransmitters of nociresponsive primary afferents, the present experiments were designed to test the involvement of N-methyl-D-aspartate (NMDA) receptors in Fos induction using the non-competitive NMDA antagonist, MK801.

Sprague Dawley rats (100 - 150 gm) from either Bantin & Kingman (CA) or Harlan (IN) were intrathecally pretreated with either vehicle or MK801 (3.0 or 10.0 µg/10 µl) followed by subcutaneous injection of 2.5% formalin (50 µl) in the hindpaw. Two hours later the rats were perfused intracardially with 4% paraformaldehyde and 50 µm sections of lumbar spinal cord were immunostained for Fos protein.

Bantin & Kingman rats had substantially lower background Fos levels than Harlan rats. In Bantin & Kingman rats, MK801 did not have a pronounced effect on behavior elicited by formalin and did not evoke Fos-like immunoreactivity (FLI) above basal levels. Formalin elicited intense ipsilateral FLI in laminae I and II, in the neck of the dorsal horn (laminae V and VI) and in ventral horn laminae VII and VIII. Quantitative analysis revealed that MK801 significantly reduced staining 36% in the superficial dorsal horn ($p < 0.05$).

These data support the hypothesis that NMDA receptors contribute to the induction of Fos by noxious stimulation, particularly in the superficial dorsal horn. [Supported by NIDR - K15 DE00225 (LJK); NIDA - DA01933, DA04274 (GLW) and NINDS - 14627, NIDR - DE08973 (AIB)].

237.9

EFFECTS OF BACLOFEN AND MORPHINE ON THE PERSISTENT EXPRESSION OF C-FOS IN RAT SPINAL CORD FOLLOWING PERIPHERAL NERVE INJURY S.-I. Chi, J.D. Levine* and A.I. Basbaum Depts. of Anatomy, Physiology and Medicine, UCSF, CA 94143.

We previously reported that both baclofen and morphine suppress the short-lived noxious stimulus (formalin)-evoked expression of the Fos product of the *c-fos* proto-oncogene in the spinal cord dorsal horn of the rat. Since nerve injury evokes a persistent expression of Fos (Chi et al, 1989), it was of interest to study the effects of baclofen and morphine in this proposed model of neuropathic pain. To produce a neuroma, the sciatic nerve was cut and ligated in the thigh. The effects of baclofen (7.5mg/kg, i.p) or morphine (5mg/kg, s.c) on Fos expression were studied two weeks later. To maintain drug levels beyond the half life of the Fos-like immunoreactivity (FLI), the drugs were given twice over a four hour period and then the rats were perfused with 4% paraformaldehyde. The lumbar spinal cord was removed and processed for FLI.

Systemic baclofen significantly reduced the number of FLI neurons in laminae 1-7, by 50-60% (n=4, $p<0.05$). The reduction was comparable in all regions of the spinal gray matter. This pattern of inhibition was similar to that produced by the same dose of baclofen on formalin-evoked FLI. In contrast, although morphine produced a profound inhibiton of formalin-evoked Fos (comparable to that seen for baclofen), we found no effect of morphine on the expression of fos in the nerve injury model at two weeks after nerve section.

The effect of baclofen on the persistent Fos expression produced by sciatic nerve section suggests that the GABAergic inhibitory circuitry in the dorsal horn is intact at two weeks after nerve injury. The failure of morphine to reduce nerve injury-evoked FLI, however, suggests that the integrity of the dorsal horn opiate receptor is compromised. Importantly, our results are consistent with the fact that narcotics are often ineffective in the treatment of neuropathic pains in humans. Supported by NS14627 an NS21445.

237.10

MICRODIALYSIS OF SEROTONIN FROM RAT SPINAL CORD: EFFECTS OF OPIOIDS. F. F. Matos, H. Rollema[1] and A. I. Basbaum. Dept. of Anatomy, UCSF and [1]Dept. of Medicinal Chemistry, Univ. of Groningen, The Netherlands.

This study tested the hypothesis that the effects of opioids at supraspinal levels results from activation of serotonin (5-HT) containing neurons of the medullary raphe which project to and inhibit dorsal horn neurons. We used microdialysis coupled to HPLC-ECD to monitor 5-HT release from the spinal cord of the awake rat. A dialysis probe was inserted across the L4 spinal cord segment (Skilling et al., 1988); the next day the probe was perfused with Ringer solution (2µl/min) and sequential samples collected. To study the properties of 5-HT in the dialysate, various drugs were infused through the dialysis probe: tetrodotoxin (TTX, 10µM), K^+ (120mM) or imipramine (1.0mM). Basal levels of 5-HIAA were much higher (400 fold) than 5-HT. Extracellular levels of 5-HT were reduced by TTX (50%) and increased in the presence of K^+ (seven fold) and imipramine (ten fold). Under each of these conditions 5-HIAA decreased, by 20 to 40%. These results indicate that extracellular 5-HIAA levels are a poor indicator of 5-HT release. Although 5-HT release often increased after opioid administration, the results were highly variable. Thus, systemic injection of morphine (0.5mg/kg, i.p.) increased 5-HT by eight fold, in 50% of the animals. 5-HIAA levels, however, decreased, by about 20%, in all animals. Morphine (10µg, i.c.v.) also increased 5-HT, by six fold. The *mu*-selective opioid peptide, DAMGO (0.5 µg, i.c.v.) increased 5-HT release by 13 fold. Our results demonstrate that 5-HT release in the spinal cord of awake rats is primarily derived from neuronal activity. Furthermore, the increased release of 5-HT by morphine and DAMGO is consistent with the hypothesis that narcotics activate descending serotonergic controls that operate at the level of the spinal cord. However, since behavioral analgesia was produced in some rats in which an increase in 5-HT was not observed, our results suggest that release of 5-HT may not be necessary for the analgesic effects of opioids to be manifested. Supported by NS14627 and NS21445.

237.11

THE NORADRENERGIC PROJECTION TO THE RAT DORSAL HORN ARISES FROM SEVERAL BRAINSTEM CATECHOLAMINE CELL GROUPS. G.C. Kwiat and A.I. Basbaum. Dept. of Anatomy, UCSF, CA 94143.

The noradrenergic (NA) innervation of the spinal dorsal horn has recently been reported to arise predominantly, if not exclusively, from the locus coeruleus (Lyons,W.E. et al., *J. Neurosci.*, 9:1481, 1989). Our present study using restricted injections of the retrograde tracer, WGAapoHRP-Au, into the cervical (C5) dorsal horn of rats suggests that the origin of the catecholamine (CA) innervation of the dorsal horn is more widespread.

Approximately 0.5 µl of a WGAapoHRP-Au suspension was injected unilaterally into the dorsal horn gray matter using a glass micropipette. Brainstem sections were silver-enhanced to visualize the retrograde tracer and then reacted for either tyrosine hydroxylase (TH)- or serotonin (5-HT)-immunocytochemical staining using the ABC method. We found that the locus coeruleus, subcoeruleus, A5 and A7 CA cell populations all contribute to the CA innervation of the dorsal horn. In two rats, the tracer injection was concentrated in the superficial laminae of the dorsal horn. In these cases the ipsilateral A5 cell population contained the largest number of TH-immunoreactive projection neurons; up to 40% of the TH-immunoreactive cells in the ipsilateral subcoeruleus projected to the cord. Almost no double labeled cells were found in medullary CA cell groups. In contrast to the widespread origin of the TH projection, the 5-HT-immunoreactive neurons which projected to the dorsal horn were located exclusively in the rostral ventromedial medulla at the level of the nucleus raphe magnus (NRM). About 16% of the retrogradely labeled cells in this region were 5-HT-immunoreactive. It is of interest that the majority of the 5-HT projection neurons were located lateral to the NRM in the n. gigantocellularis pars α and n. paragigantocellularis lateralis.

These results suggest that the NA contribution to antinociceptive descending controls results from activation of several brainstem CA cell groups and that the 5-HT contribution may predominantly arise from activation of 5-HT neurons located lateral to the NRM. Supported by NS14627 and 21445.

237.12

REGULATION OF NOXIOUS STIMULUS-EVOKED FOS EXPRESSION BY INTRATHECAL MORPHINE G. I. Botchkina*, K.R. Gogas, J.D. Levine* and A.I. Basbaum, All-Union Cardiology Center, Moscow, 121552, USSR and Depts. of Anatomy, Physiology and Medicine, UCSF, CA 94143.

Systemic or supraspinal injection of morphine reverses both noxious stimulus-evoked pain behaviors and spinal cord fos-like immunoreactivity (FLI). The present study examines the effects of intrathecal (i.t.) injection of morphine sulfate (MS).

One week after implantation of i.t. catheters, male Sprague-Dawley rats received injections of either saline, MS (0.1-50µg) or MS (5µg) plus naloxone (NX; 10µg). Ten min later, the animals received a formalin injection (100µl, 5%) into the plantar surface of the hindpaw, and behavioral responses were recorded over a 1 hr period. The animals were anesthetized and perfused with 4% paraformaldehyde. The lumbar spinal cord was removed and processed immunocytochemically for fos.

Formalin evoked FLI neurons were most numerous in laminae I, IIo and V of the L4-5 segments of the cord. MS (i.t.) produced a dose-related, NX-reversible inhibition of both the pain behaviors and the FLI evoked by formalin. Although both 5 and 50µg MS produced comparable levels of antinociception in the formalin test, there was approximately 20% greater inhibition of superficial dorsal horn FLI at the 50µg dose. The density of the residual fos in laminae I,II was comparable to that seen after systemic morphine injection (and less than that seen after icv morphine). These data suggest that analgesia produced by systemic or i.t. morphine involves interactions with a dorsal horn opiate receptor that is neither accessed directly nor synaptically via icv opioids. Our preliminary findings show that the delta antagonist ICI174864 partially reduced the analgesic effects of i.t. morphine (5 µg). Although ICI did not reverse the fos suppressive effects of i.t. morphine in the superficial dorsal horn, the density of the residual fos appeared to be increased over that of animals receiving morphine only. These findings suggest that a component of the morphine-induced fos suppression (particularly in superficial dorsal horn) is mediated via interaction with the delta receptor. Supported by NS14627, NS21445 and NIH Training Grant NS07265.

237.13

SPINAL ANTINOCICEPTION BY ADENOSINE ANALOGS AND MORPHINE FOLLOWING INTRATHECAL (i.t.) PRETREATMENT WITH CAPSAICIN, 6-HYDROXYDOPAMINE (6OHDA) AND 5,7-DIHYDROXYTRYPTAMINE (57DHT). J. Sawynok, A. Reid* and D. Nance Depts. Pharmacol. and Anatomy, Dalhousie University, Halifax, NS, Canada B3H 4H7

Spinal antinociception by morphine and Ca^{2+} appears to be due to release of adenosine from capsaicin-sensitive primary afferent neurons. The action of Ca^{2+} also is reduced by i.t. pretreatment with 6OHDA. In this study, the effects of capsaicin, 6OHDA and 57DHT on spinal antinociception by analogs of adenosine (NECA, CHA) and morphine have been examined. All drugs were administered via chronically implanted i.t. cannulas 7-14 days following the toxin or respective vehicle. I.t. pretreatment with capsaicin 50µg (which depletes substance P in the substantia gelatinosa as revealed immunohistochemically) did not alter spinal antinociception by NECA or CHA in the tail flick or hot plate tests, or morphine in the tail flick test, but markedly reduced the action of morphine in the hot plate test. 6OHDA 50µg significantly reduced spinal antinociception by NECA and CHA in both tests, while 57DHT 50µg was without effect. Both 6OHDA and 57DHT were without significant effect on morphine. Depletion of amines with these toxins was confirmed by HPLC. These results suggest (a) i.t. capsaicin may be useful for separating out pre- and postsynaptic components of antinociceptive action within the spinal cord, and (b) the spinal antinociceptive action of purines depends on intact noradrenergic but not serotonergic systems. (Supported by MRC Canada)

237.14

MODULATION OF RECEPTIVE FIELDS OF CAT NOCICEPTIVE THALAMIC NEURONS BY CHOLINERGIC MECHANISMS H. Angus-Leppan, B. Olausson*, G.A. Lambert*, Department of Medicine, Univ. of New South Wales, Australia

Receptive field (RF) modulation represents a change in sensory processing of importance in understanding mechanisms of acute pain, and in particular, allodynia and hyperalgesia. It appears that the phenomenon involves both peripheral and central components. The experiments described here focused on the central component of RF responses relevant to trigeminally-mediated nociceptive pathways, especially those that may be involved in headache and toothache. The effect of cholinergic agonists and antagonists on receptive field properties were examined, as acetylcholine (ACh) is a known mediator of certain ascending pathways to thalamic neurons, including a projection from the parabrachial nucleus, and brainstem pathways considered important in attention and arousal. Cutaneous RFs of thalamic ventrobasal cells receiving nociceptive input from superior sagittal sinus (SSS), tooth pulp (TP) or both were studied. Cats were anesthetized with chloralose. Multi-barreled electrodes with a central tungsten core allowed extra-cellular recording and iontophoresis. Units excited by electrical stimulation of SSS (40-120V, 250µs, 0.2 s^{-1}), TP (train of 3 x 600 Hz pulses,500µA, 0.5ms) or both were identified, and their receptive fields characterised. ACh, atropine (Atr), hexamethonium (Hex), homocysteic acid (HCA) and saline were ejected iontophoretically. Twenty-two units in 9 cats were located, with facial (16) or forelimb (6) RFs. RFs were characterised as wide-dynamic range (11), nociceptor-specific (5) or low-threshold mechanoreceptors(6). In 16 out of 22 units tested, RFs increased in area after iontophoretic ACh. Atr decreased the RF area in 15 units, while Hex had no significant effect on RF size. In addition, changes in RF modality occurred following ACh administration, with increased response to non-noxious (brush) stimulation demonstrated in 2 units, and increased response to noxious (pinch or prick) stimulation in 5 units. These results demonstrate central cholinergic modulation of RF characteristics, of relevance to trigeminally-mediated nociceptive pathways.

237.15

ATROPINE PREVENTS NOXIOUS STIMULUS-INDUCED PERSISTENT DEPRESSION (PD) OF HIPPOCAMPAL CA1 PYRAMIDAL CELL SYNAPTIC EXCITABILITY. S. Khanna* and J. G. Sinclair. Div. of Pharmacol. & Toxicol., Faculty of Pharmaceutical Sciences, Univ. of British Columbia, Vancouver, B.C., Canada, V6T 1W5.

An intense peripheral noxious heat stimulus induces a PD of the dorsal hippocampal CA1 population spike (PS; Khanna and Sinclair, 1989) and activates septo-hippocampal neurones of the medial septal-vertical limb of the diagonal band of Broca (MS-VLDBB; Dutar et al., 1985). Further, MS-VLDBB tetanic stimulation produces a cholinergic mediated depression of the apical dendritic field excitatory postsynaptic potential (dfEPSP; Rovira et al., 1983). Here we report results consistent with the notion that acetylcholine is released in the apical dendrites of CA1 pyramidal cells following a noxious stimulus resulting in depressed CA1 synaptic excitability. Experiments were done in rats (250-350 g) lightly anaesthetized with urethane 1.0 g/kg, i.p. Noxious hot water (55°C, 15 sec) applied to the left hind paw produced a PD of the submaximal CA1 PS evoked upon contralateral CA3 stimulation. Following recovery, preadministration of the cholinergic muscarinic antagonist, atropine sulphate (40 mg/kg, i.p.), prevented the normally produced PD of the PS to noxious heating of the tail. This dose of atropine also antagonized the cholinergic mediated MS-VLDBB tetanus-induced, but not the corresponding paired-pulse, facilitation of the threshold CA1 PS. In different experiments, atropine iontophoresed at the cell body recording site did not prevent the PD of the submaximal CA1 PS following noxious heating of the tail although it antagonized PS facilitation by iontophoretic Ach. However, atropine iontophoresed at the apical dendritic recording site prevented the noxious stimulus-induced PD of the dfEPSP. It also antagonized iontophoretic Ach, but not GABA, induced-depression of the dfEPSP. (Supported by the Medical Research Council of Canada and B.C. Health Care Research Foundation).

237.16

THE ANTINOCICEPTIVE EFFECTS OF INTRATHECAL BACLOFEN IN PATIENTS WITH CHRONIC SPINAL LESIONS. R.M. Herman and S.C. D'Luzansky*, Samaritan Rehabilitation Institute, Phoenix, AZ 85006; Department of Pharmacology, University of Arizona, Tucson, AZ 85724.

Baclofen, the prototypic $GABA_B$ receptor agonist, is often administered to reduce spasticity of spinal origin. When ineffective orally, continuous intrathecal (i.t.) infusion of baclofen by an implanted pump can be clinically beneficial[1]. Prior to implantation of the pump, a double-blind study comprising bolus i.t. injections of baclofen (50 μg) and saline into the L_{1-2} interspace was conducted to adjudge the antispasticity and antinociceptive[2,3] action of baclofen in patients with complete (n=3) and incomplete (n=3) chronic suprasacral spinal lesions. Motor (flexor reflexes) and sensory (discrimination) responses to noxious (pinch, electrocutaneous) stimulation of the foot, and perception (analog scale) of both spasm-related and chronic, radiating pain were analyzed. Our results indicated that i.t. baclofen increases the motor and sensory threshold to a noxious stimulus, and decreases the magnitude of spasm-related and chronic pain (n=3). The antinociceptive action is ascribed to the local, spinal action of i.t. baclofen.

1. Penn D et al. N. Engl. J. Med., 320:1517-21, 1989.
2. Sawynok J, Pharmacol. Biochem. Behav. 26:463-74, 1987.
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237.17

ANTAGONISM OF BACLOFEN-INDUCED ANTINOCICEPTION BY INTRATHECAL PHACLOFEN OR 2-OH-SACLOFEN, BUT NOT δ-AMINOVALERIC ACID. D.L. Hammond and S. Aran. Dept. of Anesthesia and Critical Care, The University of Chicago, Chicago, IL. 60637.

Phaclofen (PHAC), 2-OH-saclofen (SAC) and δ-aminovaleric acid (DAVA) are purported to be $GABA_B$ receptor antagonists. This study examined their ability to antagonize the antinociception produced by intrathecal (i.t.) administration of baclofen (BAC) in the rat.

The dose-response relationships of BAC on the tail flick (TF) and hot plate (HP) tests were first determined in the absence of $GABA_B$ antagonists and then redetermined in the presence of fixed doses of PHAC (10-100 μg i.t.), SAC (10-30 μg i.t.) or DAVA (10-100 μg i.t.). Intrathecal injection of PHAC produced rightward parallel shifts in the dose-response relationship of BAC in both the TF and HP tests. Although SAC (10 μg) produced a parallel shift to the right, no further shifts could be obtained with higher doses of SAC. Although 10-30 μg i.t. DAVA did not antagonize BAC, 100 μg i.t. DAVA augmented the antinociceptive effect of BAC in the TF test.

The results of this study suggest that PHAC is the antagonist of choice to study the role of $GABA_B$ receptors in the spinal cord.

237.18

ADRENAL MEDIATION OF BUSPIRONE-INDUCED HYPOTHERMIA AND ANTALGIA IN RATS. L. Rogers, D. McBride* and J. Giordano. Drake Univ., Des Moines, IA 50311

The 5-HT_{1A} agonist buspirone produces analgesia in rats. Buspirone (1-5 mg/kg,ip) induced dose-related decreases in core temperature (.5-1°C) that were co-terminal with analgesia. Both hypothermic and analgesic effects of buspirone were unaffected by naloxone (1 mg/kg,sc). The lack of opioid involvement and documented role of 5-HT-mediated neuroendocrine substrates in thermoregulation and pain modulation prompted study of adrenal function in these buspirone-induced effects. Administration of the PNMT inhibitor dichloromethylbenzylamine (DCMB:25 mg/kg,ip) did not affect buspirone-induced hypothermia, while significantly reducing analgesia. Pretreatment with the cortisol synthesis inhibitor aminoglutethemide (2x25 mg/kg,ip) did not alter busprirone-elicited hypothermia, but reduced analgesia. This decrease was less than effects produced by DCMB. Sham operated and injected controls did not show changes in buspirone-induced hypothermia or antalgia. These data suggest that buspirone-induced hypothermia and analgesia may be functionally related. Analgesia appears to be reliant upon sympatho-adrenomedullary mechanisms to a greater extent than adrenocortical substrates. It is possible that antalgia due to increased sympathetic-adrenal activity may be a functional response to buspirone-induced hypothermia.

(Supported by Bristol-Myers Scientific Relations Award (JG))

237.19

SUPPRESSION OF MEDIAL THALAMIC RESPONSES TO SPINAL AND NOXIOUS STIMULI BY COCAINE. B.C.Shyu*, J.A.Kiritsy-Roy, T.J.Morrow and K.L.Casey. Depts. of Neurology, Physiology and Inst. of Gerontology, Univ. of Michigan and Neurology Research, V.A. Medical Center, Ann Arbor, MI 48105.

Cocaine is a potent, centrally active non-opioid analgesic in the rat (Brain Res. 479:306-312,1989). We have shown that cocaine inhibits nociceptive responses of units in the medial thalamus and medial reticular formation. The aim of this study was to determine if cocaine suppresses nociceptive responses of thalamic neurons by acting at spinal or supraspinal levels.

Rats were anesthetized with chloral hydrate, paralyzed and ventilated artificially. Stimulating electrodes were positioned at T8 to T10 of the spinal cord and subcutaneously in the foot pad. The dorsal columns were sectioned at about T5 to T6. Recording sites in medial or lateral thalamus were chosen by identification of unit responses evoked by electrical stimulation (0.2 msec duration, 500 μA intensity at spinal cord; 5 - 10 mA intensity at foot pad) or noxious stimulation (pinching of the foot pad).

Cocaine (1mg/kg iv) produced significantly greater suppression of medial thalamic unit responses to noxious somatic stimulation (49.4 ± 8.7% of control) than to spinal cord stimulation (76.2 ± 6.6% of control). The effect of cocaine on unit responses to somatic stimulation was dose related in the range of 0.3 to 3.5 mg/kg and attenuated by eticlopride, a D-2 selective dopamine receptor blocker. Cocaine had no effect on lateral thalamic neuronal responses to either somatic or spinal cord stimulation. Morphine (0.09 to 2.9 mg/kg) also inhibited medial thalamic neuronal responses to somatic stimulation. **These results suggest that the antinociceptive effect of cocaine is mediated by dopaminergic mechanisms and is exerted at thalamic and spinal levels.** (Supported by awards from the Swedish Medical Council, Bristol-Myers Squibb and VA Merit Review).

237.20

SOME DOPAMINE D_2 RECEPTORS MAY NOT BE COUPLED TO ADENYLATE CYCLASE IN THE RAT RETINA. M. Hadjiconstantinou[1,2], X-Z. Qu[*2] and N.H. Neff[2], Departments of Psychiatry[1] and of Pharmacology[2], The Ohio State University College of Medicine, Columbus, Ohio 43210

Dopamine (DA) D_1 and D_2 receptors are present in rat retina. Based on studies with other tissues, D_1 receptors are apparently positively coupled to adenylate cyclase, while some D_2 receptors are negatively coupled and some are not coupled. We have studied DA receptor subtypes in the rat retina following the intraocular injection of 6-hydroxydopamine (6-HODA). Male Sprague-Dawley rats 150 to 200 g were anesthetized and injected intravitreously with 6-HODA or saline. 6-HODA, 30 μg/4 μl, was injected into the right eye, 2 injections 24 hr apart, and an equal volume of sterile saline injected into the left eye. Rats were decapitated 7 days later. After injection of 6-HODA there was depletion of retinal DA by about 90 percent, as well as about a 40 percent decrease of the number of D_1 and D_2 receptor binding sites using [^{3}H]SCH 23390 and [^{3}H]spiperone for D_1 and D_2 sites, respectively. Following the 6-HODA lesion, there was an enhancement of the D_1 receptor-stimulated accumulation of cyclic-AMP, suggesting receptor supersensitivity. Furthermore, there was a loss of responsiveness of D_2 receptors that are negatively coupled to adenylate cyclase. Our results suggest that some retinal D_2 receptors are coupled to adenylate cyclase and some are not. The adenylate cyclase coupled D_2 receptors appear to be associated with 6-HODA sensitive neurons.

238.1

CHRONIC INTRACORTICAL MICROSTIMULATION OF PRIMATE VISUAL CORTEX. I. EVALUATION OF MATERIALS AND METHODS FOR A VISUAL PROSTHESIS. E. M. Schmidt, M. J. Bak, F. T. Hambrecht, C. Kufta* and J. S. McIntosh. Lab. of Neural Control, Neural Prosthesis Program (F.T.H.) and Surgical Neurology (C.K.), NINDS, NIH, Bethesda, MD 20892

Intracortical microstimulation of visual cortex in human subjects elicits the sensation of small points of light called phosphenes (Bak et al., Med. Biol. Eng. & Comput. 28, 1990). Implantation of arrays of intracortical electrodes may provide a means of producing a visual prosthesis for a blind individual. In order to evaluate materials and stimulation parameters that could be used in a visual prosthesis for humans, a primate was implanted with 9 pairs of microelectrodes, under sterile operating conditions, and stimulated over a period of 105 days. The electrodes were fabricated from 37.5 μm iridium wire that was electropolished to a tip diameter of 1-2 μm and coated with Parylene C. The tips were exposed with a high voltage arc and activated to form an iridium oxide on the exposed surface. The spacing between tip pairs ranged from 175 μm to 450 μm. Electrode areas ranged in size from 35 to 300 μm^2. To evaluate stability and tissue reactions, electrodes were stimulated for two hours each day, five days/week, 0.2 ms/phase biphasic pulses at 50Hz, with current densities ranging from zero to 3200 $\mu C/cm^2$ The animal was sacrificed, by intracardiac perfusion with aldehyde fixative, to evaluate the electrodes (this abstract) and tissue reactions (see companion abstract, Azzam, N.A. et al.). Scanning electron micrographs of the electrodes revealed excellent stability of the Parylene C insulation and possible slight erosion of the electrode tips. The insulation was degraded at the tip of one electrode, which was attributed to improper tip exposure. The mechanical and electrical properties of the electrodes look promising for evaluating the feasibility of a visual prosthesis.

238.2

CHRONIC INTRACORTICAL MICROSTIMULATION OF PRIMATE VISUAL CORTEX. II. LIGHT AND ELECTRON MICROSCOPIC EVALUATION FOR A VISUAL PROSTHESIS. N. A. Azzam, A. A. Zalewski, E. M. Schmidt and R. N. Azzam*. Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892

We examined the anatomical response of the visual cortex in a monkey (Macaca mulatta) to chronic implantation and stimulation of nine pairs of microelectrodes. These electrodes were made of iridium and insulated with Parylene C (see companion abstract, Schmidt, E.M. et al.). The design is intended for ultimate use in a visual prosthesis for blind people. When unstimulated and stimulated electrodes were compared, each was associated with minimal neuronal loss that was limited to a few micrometers around and between the electrode tips. The major difference between electrodes was an infiltration of macrophages around the stimulated electrodes, which increased in intensity with higher current densities. Pia mater and astrocytic foot processes ensheathed the shafts and tips of the unstimulated electrodes. However, multinucleated giant cells, with very dense cytoplasm and rich in endoplasmic reticulum, surrounded the tips of the stimulated electrodes. Pial fibroblasts and collagenous fibers surrounded the giant cells and they in turn were isolated from the neuropil by a very dense concentration of reactive astrocytic foot processes. Together they formed a dense basement membrane similar to the glia limitans which suggested the re-establishment of the blood-brain barrier. The larger blood vessels close to the implantation site were similarly ensheathed. Macrophages entered the neuropil around the lesions and were seen in the perivascular compartments and in the pia-arachnoid, indicating movement of these cells, laden with inclusion bodies, to the cerebrospinal fluid. These macrophages had ingested cellular debris as well as electron dense particulate matter, possibly dissoluted iridium. A few homogeneous electron dense intracellular globules were observed and seemed to be fragments of the insulating material Parylene C. The pathology described is not severe enough to contraindicate the use of our prosthetic device in man.

238.3

HUE DISCRIMINATION AFTER FOCAL CEREBRAL EXCISION IN HUMANS. A. Ptito, M. Jones-Gotman and J. Crane. Montreal Neurol. Inst., McGill Univ., Montreal, Canada. H3A 2B4.

The role of inferotemporal cortex (IT) in color discrimination is unclear. In monkeys, IT lesions have resulted in little or no impairment in some studies (e.g. Gross et al, 1971, J. Comp. Physiol. Psychol. 76: 1-7), but severe deficits in others (Heywood et al, 1988, Exp. Brain Res. 437-441). Studies in humans also yielded no definitive results; most reported few cases or ill-defined lesions. In the present study, 61 patients with focal excision from right or left frontal or temporal lobe and 22 normal control (NC) subjects were tested on the Farnsworth-Munsell hue discrimination test. All temporal-lobe lesions included partial removal of IT cortex. Results showed no impairment in either temporal lobectomy group, but both frontal-lobe groups were markedly deficient. Thus it seems that the analysis of color information does not lie in anterior temporal neocortex. However, one patient whose temporal-lobe removal extended posteriorly into anterior occipital lobe performed poorly, supporting the notion that occipitotemporal areas are important in hue discrimination (Pearlman et al, 1979; Ann. Neurol. 5: 263-261). The significance of the deficit in frontal-lobe groups is not clear. It may not reflect a true hue discrimination deficit, but perhaps a difficulty in ordering stimuli. This will be explored in a future study.

238.4

SIGNAL DETECTION IN HUMAN BRAIN LESIONS. M. Rizzo, D. Robin. Department of Neurology, Univ. of Iowa College of Medicine, Iowa City, Iowa 52242.

Signal detection (SD) theory characterizes system performance on perceptual tasks. We evaluated how the SD variables, d' (true sensitivity) and β (response bias), depend on cerebral damage. We tested 35 subjects with focal structural brain lesions on an attention task. They reported on sequential perturbations at unpredictable intervals and locations, of any single element in a random dot display (Starry Night paradigm, Neurology 40:447-455, 1990).

Results showed low d' with occipital and parietal damage mostly from failure to report events in the field opposite the lesions. But low d' could also follow pure frontal lobe lesions in the absence of hemianopia or neglect. β was abnormal in bifrontal cases (n=3) who often reacted to no signal. Parkinson's (n=7), and Alzheimer's (n=8) cases showed combined aberration of d' and β despite normal acuity and fields.

The results suggest that d' and β each depend on multiplex interactions which include bottom-up processes toward the receptor interface, and top-down factors related to knowledge and decision making. Damage in visual cortex and adjoining regions mostly reduce signal strength. Lesions in frontal ("amodal") cortex alter performance in complex ways, explained in SD parlance by an increase of internal noise relative to signal.

238.5

ATTENTIONAL CHANGES AS A FUNCTION OF AGING. D. A. Robin and M. Rizzo. The University of Iowa, Iowa City, IA 52242.

There is relatively little information on the effects of aging on attention. But such changes are important to understanding associated cognitive decline because of attention's broad influence on human behavior. We tested 20 elderly and 20 young adults on five attention paradigms which examined their ability to orient in vision and audition and sustain attention across a visual array. Results showed increased reaction times in elderly subjects on all tasks. They could orient attention similarly to young adults but performed more poorly on the sustaining task. Signal detection analyses showed no age-related differences in response bias (beta), although true sensitivity (d') was reduced in the elderly. These data suggest that aging reduces attentional capacity which may impair performance on perceptual tasks. We hypothesize that aging reduces a pool of attention related neurons similar to those identified in the prestriate region of primates.

238.6

VISUALLY EVOKED MAGNETIC FIELDS: RETINOTOPY AND CORTICAL MAGNIFICATION FACTOR. R.J. Ilmoniemi and S. Ahlfors*. Low Temperature Laboratory, Helsinki University of Technology, Rakentajanaukio 2, 02150 Espoo, Finland.

We have measured the magnetic field over the occipital part of the human brain in response to small visual stimuli with a 24-channel SQUID magnetometer. The black, white, and striped (diameter < 1 degree) stimuli against a gray background were shown on a computer screen (Macintosh™) for 250 ms in different locations of the visual field in order to learn how retinotopic order is reflected in the responses.

Magnetic field measurements make it possible to determine locations of brain activity with an accuracy of 5–10 mm, although shifts in activation sites of 1–2 mm can be detected. To avoid complications from multiple simultaneously active cortical sites, we presented small stimuli and analyzed only the earliest deflections of the evoked response. Locations of cortical activity were obtained as minimum-norm estimates of the source current or as equivalent dipoles. Retinotopic order was evident in the dependence of the source estimates on visual-field locations.

We found that small shifts in the visual-field location of the stimulus normally resulted in regular small shifts in the location of the estimated source current. However, for stimulus shifts in some parts of the visual field, the magnetic field pattern changed dramatically even for relatively small changes in stimulus location. We suspect that these cases correspond to cortical locations where sulci give rise to sharp turns in the surface of the cortex. From the regular shifts, we could estimate the cortical magnification factor, finding agreement with earlier estimates that have been obtained with less direct methods (J. Rovamo and V. Virsu, Exp. Brain Res. 37:495, 1979).

238.7

A MODEL OF SUBLAMINAR AND THALAMOCORTICAL CONTRIBUTIONS TO THE SURFACE VEP: IMPLICATIONS FOR CURRENT SOURCE DENSITY (CSD) ANALYSIS. C.E.Tenke and C.E.Schroeder,Dept. Biopsych./Psychophysiol., NYS. Psych.Inst.,NY and Dept. Neurosci., A.Einstein Coll.Med,Bronx,N.Y.

One dimensional CSD analysis greatly facilitates exploration of both regional neural processes and the physiological basis of surface recorded field potentials. Optimal utilization of a CSD requires a sampling and computational resolution adequate to describe current sources and sinks which are reliable, valid and physiologically relevant. Previous simulation studies have shown that large, open field generators may be reasonably studied using high resolution CSD methods. However, the adequacy of these methods for the study of sharply localized and closed field generators has not been established. High resolution analysis of the CSD profile in macaque striate cortex underlying the surface N40 of the flash visual evoked potential (VEP) reveal one or more prominent current sinks in Lamina 4C, with sources distributed below. However, it has been argued that neither Lamina 4 stellate cells nor their thalamic afferents should behave as open field generators. We therefore simulated these contributions using ensembles of dipoles with variable orientations, dipole lengths and locations. Comparisons with physiological data indicate that: 1) A closed field component of stellate lamina activation produces artifactual sources in suprajacent recording sites, identifiable by changing the differentiation grid; 2) small deviations in symmetry of activation produce an open field generator; 3) the widely distributed sources deep to Lamina 4 are incompatible with a Lamina 4 generator alone, but conform to the open field profile required of current return related to terminal depolarization. **(Supported by MH36295 and MH06723)**

238.8

INTRACORTICAL CONNECTIVITY REVEALED BY EVOKED POTENTIALS IN CAT VISUAL CORTEX. M. Kitano, T. Kasamatsu & A.M. Norcia. Smith-Kettlewell Inst., S.F. 94115

Various functional modules are horizontally connected in visual area V1 and V2. Extensive intracortical and interhemispheric connections are also known anatomically in other visuocortical areas. We studied the extent of the visual field which gives rise to inputs to a small cortical area by recording visual evoked potentials (VEPs) transcortically between a skull electrode and a cannula/electrode placed near the projection of the area centralis. Stimuli consisted of 5 deg patches of phase-reversing gratings (4 or 10 Hz) which were swept in spatial frequency. Test stimuli were presented sequentially on a grid spanning ±30 deg. Response maps were created before, during and after local inactivation of the cortex with a continuous infusion of 10mM muscimol.

We found that: 1) In control, mapping of the visual field revealed a response field larger than that expected on the basis of cortical retinotopy. 2) Stimulation of the nasal and temporal hemi-field evoked VEPs in the contra- and ipsilateral cortex, respectively, beyond the direct retino-geniculo-occipital projection. 3) VEPs diminished gradually after the start of muscimol infusion. 4) By terminating the infusion, VEP responsiveness gradually increased to become larger than control. 5) The muscimol-induced changes were field-specific. They were not confined to visual cortex ipsilateral to the infusion, but were observed concurrently in the contralateral hemisphere as well.

(USPHS grants BRSG 05981, Core Grant EY-06883)

238.9

SPATIOTEMPORAL ANALYSIS OF VISUAL ACTIVITY IN PRIMATES IV: EXTRASTRIATE CONTRIBUTIONS TO THE SURFACE VEP INCLUDING A MANIFESTATION OF DIRECT THALAMOCORTICAL INPUT. S.J.Givre, C.E.Schroeder, & J.C.Arezzo Depts. Neuroscience and Neurology, Albert Einstein Coll. Med. Bronx, NY 10461

The flash-evoked potential (f-VEP) recorded from the occipital surface in the awake macaque includes N40, P55/65, N95 and P120 components. To elucidate their generators, we compared intracortical profiles of f-VEP, current source density (CSD) and multiunit activity (MUA) obtained from Area 17 (V1) with those obtained from extrastriate cortex in the prelunate gyrus of an awake monkey. Profiles were recorded with multicontact electrodes which spanned the cortical laminae. As previously shown, V1 substantially contributes to surface N40 and P55/65 components. In this region, N40 represents thalamocortical afferent activity and the initial postsynaptic activation of Lamina 4C. The associated current sinks have a mean onset of 26.4 msec (±2.9 msec). P55 reflects di/trisynaptic activation of the supragranular laminae; the associated current sinks begin at 34.6 msec (±3.5 msec). Intracortical passes in prelunate cortex (N=6) are characterized by consistent inversions of a small N40, and a robust P120. In medial prelunate gyrus, an inversion of the N95 is also observed. Prelunate N40 appears to represent initial activation of this area. The current sink associated with this component has a mean onset of 28.4 msec (±2.9 msec). It is unlikely that this activity represents input from an earlier cortical stage since it precedes the initial activation of supragranular laminae in V1. Thus, these findings support a direct input from thalamus to prelunate visual areas. CSD analysis shows significant current flow coincident with both N95 and P120 in prelunate cortex supporting a significant contribution of this area to the generation of each of these surface potentials. In contrast, V1 appears to contribute little to these long latency components. The differential generation of surface f-VEP components by striate and extrastriate cortex may increase the clinical utility of this measure in assessing the function and integrity of the cortical visual pathways. (Supported by MH06723).

238.10

SPATIOTEMPORAL ANALYSIS OF VISUAL ACTIVITY IN PRIMATES I: RETINOTOPIC ACTIVATION OF HUMAN VISUAL CORTEX. A.D. Mehta*[1], G.V. Simpson[2], M. Scherg[4], H.G. Vaughan, Jr.[1,2] & K. Weiss*[3]. Depts. Neurosci.[1], Neurol.[2], Radiol.[3], Einstein Coll. Med., Bx., N.Y. 10461 & Max Planck Inst. f. Psychiatr.[4], Munich, FRG.

Application of Brain Electric Source Analysis (BESA) to scalp-recorded VEP yields estimates of the location, orientation and temporal activity pattern of multiple VEP generators (equivalent dipoles). These equivalent dipoles' locations are projected onto the MRI of individual subjects, providing a description of the spatial and temporal brain activation during visual processing. We used this approach to examine the pattern of responses in human visual cortex to stimulation at different locations in the visual field. We presented small, white-on-black square stimuli at different locations in the fovea and at varying degrees of eccentricity in the periphery. Stimuli at different eccentricities were adjusted in size (0.5-2.5 degrees), according to estimates of the human cortical magnification factor, to activate comparably small regions of visual cortex. The results reveal a retinotopic pattern of activation of human visual cortex in which foveal stimuli activate regions at or near the occipital pole, and stimuli at increasing eccentricities in the visual field activate increasingly deeper (anterior) regions along the calcarine fissure. Stimulation at points in the superior vs. inferior and left vs. right fovea activate inferior vs. superior and right vs. left regions of the occipital pole, respectively. Variations in VEP across subjects were correlated with variations in each individual's cortical configuration. The findings correspond with known features of the retinotopic organization of visual cortex based upon prior human studies. This further supports the physiological/anatomical validity of the VEP/BESA/MRI approach. The utility of this method as a means of integrating human and monkey research is addressed in the accompanying poster (Simpson et al.)

238.11

SPATIOTEMPORAL ANALYSIS OF VISUAL ACTIVITY IN PRIMATES II: A METHOD FOR DIRECTLY INTEGRATING HUMAN AND MONKEY DATA. G.V.Simpson, A.Mehta* & C.E.Schroeder Depts. Neurol. & Neurosci., Einstein Coll. Med., Bx, NY 10461.

Brain Electric Source Analysis (BESA) of scalp-recorded VEP yields estimates of the location, orientation and temporal activity pattern of multiple VEP generators (equivalent dipoles). Brain regions represented by BESA equivalent dipoles are approximated by projecting dipole locations onto the subject's MRI. Combined VEP/BESA/MRI describes the spatial and temporal brain activation pattern during visual processing. We applied this approach to comparison of activity patterns engaged by spatial attention vs. pattern discrimination tasks in humans. The results show sequential and temporally overlapping activation of striate, dorsal extrastriate, dorsal parietal and inferior parietal areas in the spatial attention task, versus striate, inferior extrastriate, inferior parietal and inferior temporal areas in the pattern discrimination task. These data are consistent with visual pathway organization as deduced from monkey studies, however, data in monkeys and humans are rarely directly comparable. To integrate the respective strengths of human and monkey research we extended human VEP/BESA/MRI procedures to monkeys. This initial experiment used whole-field flash stimulation, producing sequential temporally overlapping activation of medial and lateral striate and of medial and lateral extrastriate regions in and near the prelunate gyrus. Preliminary indications support an analogous spatiotemporal activation pattern in humans. Our findings demonstrate that VEP/BESA/MRI can provide a view of the temporal and spatial organization of visual processing which directly translates between monkeys and humans. As a consequence, we can improve our understanding of human visual processing by virtue of the anatomic/physiologic specificity achieved in monkeys. An accompanying poster illustrates how VEP/BESA/MRI findings can be validated and extended through intracranial recordings in monkeys.

238.12

SPATIOTEMPORAL ANALYSIS OF VISUAL ACTIVITY IN PRIMATES III: INTEGRATED SURFACE AND INTRACRANIAL INVESTIGATION OF VEP IN AWAKE MONKEYS. C.E.Schroeder, S.J.Givre, G.V.Simpson, N.Nicholson & C.E.Tenke. Depts. Neurosci. & Neurol. Einstein Coll. Med., Bx, NY 10461, Dept. Biopsych. NYS Psych. Inst., NY, NY.

Epidural analysis of flash-VEP in awake macaques outlines a frontolateral N22 component and posterior N40/P55-65 and N95/P120 complexes. Brain Electrical Source Analysis (BESA) of VEP extends the analysis to a hypothetical configuration of intracranial generators, defined by location, orientation and temporal pattern. Mapping the locations defined by BESA to each monkey's MRI supports the origin of: N22 in LGN, N40/P55-65 in striate and (less) in extrastriate cortices and N95/P120 primarily in extrastriate regions in and near the prelunate gyrus (N95 - medial, P125 - medial to lateral). Profiles of concomitant flash-evoked VEP, current source density (CSD), and multiunit activity (MUA) obtained with multicontact electrodes from five awake macaques, evaluated these hypotheses. N22 was traced from its surface maximum to its origin in LGN; it arises primarily from a current sink reflecting depolarization of cells in Lamina 6. As shown previously, a major contribution to N40 arises from current sinks in Lamina 4C of V1, reflecting depolarization of stellate cells and thalamocortical terminals, and P55/65 arises from current sources reflecting depolarization of supragranular pyramidal cells and net hyperpolarization of 4C stellate cells respectively. A similar set of processes, with a slight latency lag (2-5 ms) underlies the smaller contribution of Area V2 to the surface N40/P55-65. The contributions of prelunate extrastriate cortices are detailed in an accompanying poster. Integration of surface and intracranial VEP analyses provides a powerful and unique whole brain view of visual processes, which may directly link human and simian studies. BESA yields detailed hypotheses concerning location and temporal activity pattern in both species. Intracranial recordings in monkeys can test BESA predictions and describe underlying physiology.

238.13

CONTRAST SENSITIVITY FUNCTIONS (CSF) IN PIGMENTED RATS ESTIMATED FROM VISUAL EVOKED POTENTIALS (VEP) ELICITED WITH COUNTERPHASE OR ON/OFF MODULATED GRATINGS. W.K. Boyes[1], E.A. Manring[2] and H.K. Hudnell[1]. [1]US EPA, RTP, NC 27711 and [2]NSI Technology Services.

VEPs recorded using suprathreshold stimuli can be used to extrapolate sensory thresholds. Steady-state VEPs elicited with on/off modulation of sinusoidal gratings have power at both the stimulus rate (1F) and at twice the stimulus rate (2F), but when elicited with counterphase modulation have primary response power only at 2F. On/off VEP amplitude at 1F has a bandpass spatial frequency profile, but at 2F a low pass profile. Amplitude-contrast functions of 2F are more steep than those of 1F. These results prompted us to examine if CSFs derived from VEPs would differ if elicited with counterphase or on/off modulation, and if derived from 1F, 2F or 1F+2F amplitude. Nine male Long-Evans rats were tested in two sessions for either counterphase or on/off modulation. During each session 50 averaged VEPs were recorded in random order; 10 spatial frequency by 5 contrast values. CSFs were based on extrapolations of the amplitude-contrast functions to threshold. Some values were dropped due to unreasonable thresholds estimates. Functions based on 2F gave fewer missing values. CSFs were similar whether based on on/off 1F, 2F, 1F+2F or on counterphase 2F. The results suggest that spatial frequency response profiles differ at threshold and at suprathreshold contrast levels.

238.14

DIAZEPAM INDUCED EFFECTS ON VISUAL EVOKED POTENTIALS AND VISUAL CONTRAST DETECTION THRESHOLDS IN HUMANS. H. Ken Hudnell[1], Will K. Boyes[1] and Wayne B. Counts[2]. [1]Neurotoxicology Div., U.S. EPA, RTP, NC 27711; [2]NSI Environmental Services, RTP, NC 27711.

The benzodiazepine, diazepam (Valium), is an indirect agonist of the inhibitory neurotransmitter, GABA. We previously reported that diazepam altered rat and human steady-state visual evoked potentials (VEPs) elicited by on-off modulation of sine-wave gratings (SFN, 52.3, 1989). Amplitude of the spectral 1F component (1 x the modulation frequency) was uneffected by diazepam, but 2F amplitude was reduced in both species.

We currently investigated diazepam induced effects on the vision of thirty human subjects who received either 10 mg of diazepam or a placebo capsule in a double-blind study. Contrast sensitivity functions for 1F and 2F, and contrast thresholds for detecting pattern and for detecting motion or flicker, were measured before and after treatment. Sine-wave gratings with spatial frequencies of 0.5 and 4.0 cpd, on-off modulated at 5 Hz, were used with both measurement techniques. VEPs were elicited by gratings set to 2,4,8,16 and 32% contrast, whereas, contrast was varied using a modified version of the ascending method of limits to measure detection thresholds. The results indicated that VEP alterations induced by diazepam manifest as alterations in contrast detection thresholds, suggesting that GABA has an important role in processing visual information.

238.15

MODULATION OF VEP N_2 LATENCIES INDUCED BY PATTERN CONVERGENCE AND ORTHOGONALITY. E.Micheli-Tzanakou, N.Cottaris and R.Iezzi.Dept. of Biomedical Engineering,Rutgers Univ.,PO Box 909, Piscataway,NJ 08855-0909

It has recently been shown that the global structure of orthogonally inducing (biasing) patterns appears to be a necessary component in generating certain aftereffects. In this paper patterns that gradually evolved from random to ordered configurations are generated using an optimization technique called ALOPEX. Two types of experiments are then performed using the above sequence of stimuli to record the Visual Evoked Potentials (VEPs) in humans. In the first set of experiments VEPs are recorded using a sequence of fifteen stimuli starting from a random pattern which in time converges to an ordered pattern (a vertical bar). During the OFF period, either a blank pattern or a horizontal bar, which is a biasing pattern orthogonal to the final ON pattern of the series is used. The reverse sequence of ON patterns is also used. In all cases, the N_2 peak of the VEP is examined. Our results show that in the unbiased case, the N_2 peak exhibits a strongly increasing latency, while in the orthogonally biased case there exists a correspondingly strong negative correlation. In the second set of experiments, N_2 latencies demonstrate trends that are exactly opposite to those described earlier.

238.16

WITHIN- AND BETWEEN-SESSION CHANGES IN PEAK N_{160} AMPLITUDE OF FLASH EVOKED POTENTIALS (FEPs). D.W. Herr[1,2], V.T. Griffin[*3], W.P. Watkinson[*1], W.K. Boyes[1], and R.S. Dyer[1]. [1]US EPA, RTP, NC 27711 and [3]NSI Technology Services.

Peak N_{160} amplitude of FEPs may reflect a sensitization and/or habituation process (Dyer, R.S., Physiol. & Behav., 45:355-362, 1989). Manipulation of testing parameters may delineate the influence of these constructs in the development of this peak. In these experiments, we examined changes in the amplitude of peak N_{160} over trials (within-session) and over days (between-sessions). Additionally, we monitored heart rate as an index of behavioral "arousal". Long-Evans hooded rats were implanted with epidural electrodes over the visual cortex. Subcutaneous ECG electrodes were placed over the left and right dorsal chest areas. Blocks of 50 trials and test days were within-subject variables. Relative flash intensity (in dB) was a between-subject factor. On each of 13 consecutive test days, 350 flashes/day (0.3 Hz) were presented. Peak N_{160} amplitude increased over days (between-sessions), but decreased over trials (within-session). These results, combined with decreases in heart rate within the test sessions, suggest there may be processes which resemble between-session sensitization and within-session habituation involved in the development of peak N_{160} amplitude. [2]DWH was supported by a NRC Research Associateship.

238.17

RAT VISUAL SYSTEM 2-DEOXYGLUCOSE UPTAKE IN RESPONSE TO FLASHING-DIFFUSE AND FLASHING-PATTERN STIMULATION AS A FUNCTION OF ILLUMINATION LEVEL. R. M. Cooper, G. A. Thurlow and S. Pepperdine.* Behavioral Neuroscience Group, Psychology Dept., U. Calgary, Calgary, AB, T2N 1N4, Canada.

In our search for CNS structures which mediate intensity discriminations we examined the effects of 5 hz "flashing-diffuse" (eye covered with white diffusing mask) and 5 hz "flashing-pattern" (eye exposed to square-wave gratings) over a 6 log range of luminance on C-14 2-DG uptake in the alert and freely-moving hooded rat. Only flashing pattern increased 2-DG uptake in area 17, an increase equivalent in degree over the 6 log range. In the dorsal and ventral LGN, LPN, and colliculus, however, the increases produced by flashing-diffuse and flashing-pattern were indistinguishable, and declined monotonically as luminance decreased. These 2-DG results, consistent with a sparing of intensity discrimination capacity after cortical ablation, and unit findings which emphasize the importance of contrast rather than luminance level in cortical activation, suggest that in carrying out its major function of allowing visual object perception, area 17 does not process intensity information. This processing is more likely to occur subcortically.

239.1

EFFECT OF AMPHETAMINE ON REGIONAL CEREBRAL METABOLIC RATE IN NORMAL SUBJECTS. J. T. Metz, H. de Wit*, N. Wagner*, and M. Cooper*. University of Chicago, Chicago, IL 60637.

As part of our series of studies on the effects of drugs of abuse on regional cerebral metabolism of glucose (rCMglu), we used positron emission tomography to examine the response of normal subjects to oral administration of amphetamine (AMPH).

PET data were collected with a PETT-VI (5 simultaneous slices, intraslice resolution of 8 mm full width, half max). Subjective effects were evaluated with the Profile of Mood States (POMS). Eleven normal males (aged 21 to 35) were studied. Eight subjects were included in Study 1 in which they received placebo, 0.07 and 0.14 mg/kg AMPH. Drugs were administered double blind in counterbalanced order 90 minutes prior to injection of 8-10 mCi 2FDG. In all three sessions, subjects performed a visual monitoring task (VMT) during the period of FDG equilibration. The other 3 subjects were studied in 4 sessions each during which they received placebo or 0.28 mg/kg AMPH while performing the VMT in 2 sessions and the same doses while resting quietly in the scanner during the other 2 sessions.

Subjects in Study 1 showed a decrease in average rCMglu relative to placebo over the whole brain in response to the higher dose of the drug (means±SD for placebo, 0.07, and 0.14 doses: 8.47 ± 1.78, 8.59 ± 1.62, 7.27 ± 1.38 mg/100g/min). Individual subjects, however, varied in their response to the drug and the decrease was not statistically significant. In Study 2, subjects tended to show increased rCMglu in the AMPH sessions. Sessions in which the VMT was performed generally had higher rCMglu than those with no task; there was no clear drug by task interaction. In both studies, rCMglu changes after AMPH were not limited to particular brain regions although drug-induced mood changes and VMT performance changes were related to localized changes in rCMglu.

These studies demonstrate that the effects of AMPH on rCMglu are complicated and influenced by dose, task demands, and subjective factors.

239.2

TEMPORAL LOBE DYSFUNCTION IN PSYCHIATRIC PATIENTS IDENTIFIED BY ADJUNCTIVE ELECTROENCEPHALOGRAPHY AND REGIONAL CEREBRAL PERFUSION STUDIES WITH SINGLE PHOTON EMISSION COMPUTED TOMOGRAPHY. K.J. Shedlack, B.T. Woods*, J.S. Curtis* and T.C. Hill*. Depts. of Psychiatry & Neurology (McLean Hospital) and Radiology (New England Deaconess Hospital), Harvard Medical School, McLean Hospital, 115 Mill Street, Belmont, MA 02178.

Improved neurodiagnostic specificity in psychiatry is desired as clinical data are often insufficient to support accurate psychiatric diagnosis and rational psychopharmacologic therapy. Among psychiatric patients with abnormal electroencephalogram (EEG) referred for single photon emission computed tomography (SPECT), we have identified cases in which focal regional cerebral blood flow abnormalities were coincident with the electrical abnormality. Psychiatric diagnoses were varied and no case had a history of seizure. EEG, 24-hour EEG, or brain electrical activity mapping (BEAM) revealed focal temporal lobe slowing or sharp activity while 99m Tc-HMPAO (Ceretec) SPECT scan performed on a 12-camera, head-dedicated system revealed unilaterally decreased or increased tracer uptake. Focal areas of SPECT abnormality ranged in size from 7-22% of pixels in the ipsilateral temporal lobe and in magnitude from 8-23% of the mean counts/pixel in the contralateral temporal lobe when measured in a single transverse slice. A subset of cases thus identified have shown remarkable improvement in their clinical symptoms when treated with anticonvulsants suggesting that EEG and SPECT can identify a subpopulation of psychiatric patients who may be best diagnosed as having "temporal lobe dysfunction" and best treated with anticonvulsants rather than customary psychotropic medications.
(Support: Fund for Research in Head Injury/Harvard Medical School)

239.3

RESTING BRAIN GLUCOSE METABOLISM AND EEG FINDINGS IN ALCOHOLIC KORSAKOFF'S SYNDROME.
E.M. Joyce*, J.W. Rohrbaugh*, D.E. Rio* and M.J. Eckardt. LCS/NIAAA, NIH Building 10, Rm 3C102, Bethesda, MD 20892.

Groups of age-matched male normal volunteers (n=10) and abstinent alcoholics with Korsakoff's syndrome (KS, n=11) underwent resting 18FDG PET. EEG recordings were taken at baseline and throughout the FDG uptake period. EEG spectral power analysis showed that alpha was temporarily suppressed immediately after injection in controls but not KS. PET scans were analysed by placing regions of interest over specified brain areas and the rate of glucose metabolism was calculated (rCMRglu). There were no group differences in any absolute rCMRglu comparisons. Subtraction of rCMRglu from the average grey matter CMRglu for each subject revealed , in KS, relative cerebellar hypermetabolism and relative hypometabolism in right sensorimotor cortex, midline parietal cortex and bilateral cingulate cortex. Correlational analysis demonstrated that the groups had the same number of associations within neuroanatomical areas (frontal, temporal, subcortical and cerebellar) but that KS had fewer associations between these regions. In addition, thalamic rCMRglu was positively associated with that of all frontal regions in controls but not that of midline frontal cortex (cingulate and superior frontal gyri) in KS. The data suggest that the groups were in different states of arousal during FDG uptake and that the different metabolic profiles may reflect under-activation of the cerebral cortex in KS. There also appeared to be a functional dissociation between thalamic and cingulate rCMRglu in KS which may contribute to the mnemonic deficits of this group.

239.4

EFFECT OF CHEMICAL STIMULATION OF VENTROLATERAL MEDULLARY DEPRESSOR AREA (VLDA) ON CEREBRAL BLOOD FLOW (CBF) IN THE RAT.
M. Maeda, A.J. Krieger and H.N. Sapru, Departments of Neurosurgery & Pharmacology, UMDNJ-New Jersey Medical School, Newark, NJ 07103.

In anesthetized (chloralose and urethane), paralyzed and artificially ventilated rats, the VLDA was chemically stimulated by microinjections of L-glutamate and the changes in CBF were determined by using microspheres. Unilateral chemical stimulation of VLDA (n=10) decreased CBF on the ipsilateral side significantly ($P<0.05$). The decrease in blood pressure (BP) induced by the chemical stimulation of the VLDA was not responsible for the decrease in CBF because similar decrease in BP induced by controlled hemorrhage did not alter CBF significantly (n=10). In another group of rats (n=6), the BP was raised moderately by transfusion of blood so that it did not decrease below normotensive levels during the chemical stimulation of VLDA. In these rats also, VLDA stimulation decreased CBF ($P<0.05$) and increased cerebrovascular resistance ($P<0.05$) on the ipsilateral side. The reactivity of the cerebral vessels to increase in arterial PCO_2 (n=5) was not altered during the stimulation of the VLDA. These results suggest that VLDA neurons may participate in the regulation of cerebral circulation.
Support: NIH (HL 24347) and Am. Heart Assoc.(NJ).

239.5

TONIC AND PHASIC INCREASE IN CEREBRAL BLOOD FLOW CAUSED BY EXCITATORY AMINO ACID INJECTION K.Nakai, Y.Naka*, K.Kubo*, M.Nakai, T.Itakura*, S.Hayashi*, N.Komai*, T.Shirokawa. Dept. Neurol.Surg., Wakayama Med.College, Wakayama 640 and Dept. Physiol., Akita Univ., School of Medicine, Akita 010, Japan

Lines of evidence have suggested a role of excitatory amino acid in the pathogenesis of lesions due to an ischemic insults to the brain. However, it is still controversial whether an amino acid itself affects cerebral circulation as a result of a metabolic change in the nervous system or through a direct action on the blood vessels. Present study tried to elucidate how excitatory amino acids exert influences on the cerebral blood flow, using the injection technic combined with laser flowmetry (Nakai et al. 1989). Under urethan anesthesia, 1-4 µl of saline containing NMDA or L-glutamate ($1x10^{-5}$, $^{-4}$, $^{-3}$, $^{-2}$M) was injected into the rat parietal cortex over 10 minutes. Doses lower than $1x10^{-4}$M of NMDA and all doses of L-glutamate elicited tonic increase in the blood flow by up to 30% of that before the injection throughout the injection period. Higer doses of NMDA than $1x10^{-3}$M showed a phasic increase in the blood flow for 3-4 minutes by over 100%. Usually two phasic increases were observed during the injection of NMDA, suggesting a different underlying mechanism from that at lower doses. The result together with morphological findings obtained after the injection may imply the existence of both direct and indirect actions of NMDA on cerebral blood vessels.

239.6

PIAL VESSEL REACTIVITY FOLLOWING TOPICAL APPLICATION OF SOMATOSTATIN. D.D. Rigamonti, J.B. Long, A. Martinez-Arizala and C.P. Wingfield. Neuropharmacology Branch, Div. Neuropsychiatry, Walter Reed Army Institute of Research, Washington, DC 20307.

Pial arterioles react to a variety of compounds including adenosine, vasopressin, dynorphin, oxytocin, serotonin, and substance P. The neuropeptide somatostatin (SOM), causes a dose dependent reduction in blood flow following its application to the lumbosacral spinal cord, as well as neuropathological changes and motor deficits including paralysis. To further describe vascular reactions with somatostatin, we examined cortical vessel diameters following its topical application. Adult Sprague-Dawley rats were anesthetized and a craniotomy performed using microsurgical techniques. Animals were divided into three groups: Group I, Saline, Group II, SOM (6.25 nmoles) and Group III, SOM (25 nmoles). Vessel diameters were recorded on film at the beginning of each experiment, following saline exposure and 1, 5, and 10 min after application of SOM. Pial arterioles ranged in size from 0.18 to 1.20 mm and following exposure to saline negligible changes were observed. However, Group II animals showed sustained arteriolar constriction of 47%, 17%, and 31% at 1, 5 and 10 min respectively. Group III animals exhibited changes of 84%, 80% and 83% at the same time intervals. These results suggest that somatostatin causes profound and sustained vasoconstriction of cortical vessels which may be implicated to cause neural injury in the CNS.

239.7

CEREBRAL ISCHAEMIA INDUCED BY ENDOTHELIN-1 IN THE RAT. I.M.Macrae, M.J.Robinson, M.A.McAuley, J.L.Reid and J.McCulloch. Department of Medicine, University of Glasgow, Glasgow G12 8QQ, UK.

The existence of endothelin-1 (ET-1) binding sites within the cerebral circulation and a potent vasoconstrictor activity implicate this peptide in the physiology and pathophysiology of cerebrovascular control.

Intracisternal injection of ET-1 (30pmoles) in conscious rats increased mean arterial pressure by 40±10 mmHg ($p<0.005$,n=5) within 2 mins of administration. Simultaneous measurement of cerebral blood flow (CBF; [^{14}C]-iodoantipyrine autoradiography) revealed profound reductions in flow throughout the caudal medulla and cerebellum (>80% reduction, $p<0.005$) with significant hyperaemia in a number of forebrain structures (eg. 78% increase in sensorimotor cortex, $p<0.005$).

Local application of ET-1 (1nmole) onto the exposed left middle cerebral artery (MCA) of anaesthetised rats produced significant reductions in CBF at 10 mins in those areas supplied by this vessel (eg. 78% reduction in dorsilateral caudate nucleus and 72% reduction in sensorimotor cortex ($p<0.01$,n=5). Hydrogen clearance studies of CBF in the caudate nucleus revealed the prolonged duration of ischaemia induced by ET-1 on the MCA. With 2.5pmoles, CBF in the caudate was 33% of control at 10 mins, 50% at 20 mins, 60% at 1 hr and approaching control values at 2 hrs.

These studies demonstrate the endogenous peptide ET-1 to be capable of reducing CBF to pathologically low levels.

239.8

VASOPRESSIN MODULATES BRAIN BLOOD FLOW AFTER SPREADING CORTICAL DEPRESSION IN AWAKE RATS. R. B. Duckrow. Department of Medicine, Division of Neurology, The Pennsylvania State University, Hershey, PA 17033.

The peptide vasopressin constricts or dilates cerebral arteries *in vitro* but has little effect on cerebral blood flow when studied *in vivo*. However, tone-dependent cerebral vascular responses to vasopressin suggest that vasopressin could mediate tone-dependent cerebral blood flow (CBF) responses measured during spreading cortical depression (SD) in awake and anesthetized rats. To test this a bipolar electrode was placed in the left cerebral hemisphere of pentobarbital-anesthetized rats and secured with dental acrylic. Two days later rats were prepared using halothane-nitrous oxide anesthesia, restrained with a plaster hip-cast, and allowed a one hour recovery period. SD was induced with a 3 mA direct current lasting 5 seconds. [^{14}C]iodoantipyrine was used to measure CBF in regions isolated by gross dissection. Pretreatment with the V_1 receptor blocker MEAVP, 100 μg/kg i.v., prevented hypoperfusion normally measured 2 minutes after the onset of SD in awake rats. MEAVP had no effect on the contralateral hyperperfusion measured in awake rats and had no effect on the pattern of CBF measured during and after SD in rats reanesthetized with pentobarbital. Vasopressin is involved with cerebral vasoconstriction following SD in awake rats but not in anesthetized rats. (Supported by PHS NS24109 and an AHA Established Investigator Award)

239.9

HEMIDECORTICATION IN RAT PUPS: EFFECTS ON LOCAL CEREBRAL METABOLIC RATES FOR GLUCOSE IN ADULTHOOD. J. Kunchandy*, H.T. Chugani, Y. Nassir*. UCLA School of Medicine, Los Angeles, CA 90024.

We previously reported (Soc Neurosci Abstr 15:448, 1989).that measurements of local cerebral metabolic rates for glucose (lCMRGlc) obtained with positron emission tomography (PET) in children following hemidecortication reveal the recovery of crossed cerebellar hypometabolism (diaschisis) and, in one infant, the reappearance of lCMRGlc in the deafferented caudate after a period during which lCMRGlc in this structure was absent on PET. In order to explore further the local cerebral energy requirements of remaining brain tissue in hemidecorticates, we measured lCMRGlc by ^{14}C-2-deoxyglucose autoradiography in a rat model of hemidecortication. Six-day-old rat pups (N=7) underwent gentle suction removal of cerebral cortex of one hemisphere following induction of hypothermia. Striatum and thalamus were spared. Following surgery, the rat pups were returned to their mothers. At maturity (6-9 months), lCMRGlc for 28 neuroanatomical structures were measured and compared to those of sham-operated controls (N=6) using Students's t-test. Most structures contralateral to the side of hemidecortication tended to have lower lCMRGlc than controls. This was highly significant ($p<.001$) for lateral septum, striatum, lateral geniculate, dentate gyrus and visual cortex. lCMRGlc in the surgically spared but largely deafferented striatum was not significantly different from that in controls, or from striatal lCMRGlc in the intact side. Ipsilateral thalamic lCMRGlc (ventral and lateral nuclei), however, were significantly lower than in controls ($p<.001$) and compared to the intact side ($p<.05$). In the cerebellum, there was no lCMRGlc asymmetry in the cortex, white matter or dentate nuclei. Patterns of lCMRGlc for subcortical structures in this model are similar to those obtained with PET scanning in hemidecorticated children and will be further explored in terms of anatomical and biochemical aspects of reorganization.

239.10

SIMULTANEOUS MEASUREMENT OF DOPAMINE AND OXYGEN IN THE RAT CAUDATE NUCLEUS DURING STIMULATED RELEASE OF DOPAMINE. J. Zimmerman and R. M. Wightman. Dept. of Chemistry, Univ. of N. Carolina, Chapel Hill, NC 27599-3290.

Changes in O_2 concentration in the extracellular fluid of the caudate nucleus during electrical stimulation of the medial forebrain bundle (MFB) were studied in an effort to understand some of the rapid metabolic changes that occur during neurotransmission in the striatum. Simultaneous measurement of synaptic dopamine (DA) overflow and oxygen was accomplished with a Nafion-coated carbon-fiber disk microelectrode using fast-scan cyclic voltammetry (300 or 400 V/s). The oxidation current for DA was measured at +0.6 V and reduction current for O_2 at -1.2 V (vs. saturated calomel reference electrode). The MFB was stimulated with a twisted bipolar electrode at frequencies of 5-60 Hz and duration 2 s. The O_2 response showed a marked (40 μM) increase characterized by 2 maxima: one shortly following stimulus initiation (3.5-5 s) and a broader feature much later (15-20 s). The amplitude of the first is frequency-dependent, while that of the second is not. Theophylline (60 mg/kg i.p.) causes rapid disappearance of the second maximum. The effects of α-methyl-p-tyrosine, yohimbine and atropine were also investigated. The increase in O_2 concentration during and following stimulation is apparently caused by local dilation of the cerebral microvasculature mediated by changes in the extracellular fluid composition.

239.11

-3PPP EFFECTS ON LOCAL CEREBRAL GLUCOSE UTILIZATION IN THE RAT J. Pongstaphone*, L. Goodman*, O. Shirakawa, L. Dixon*, J.R. Walters, C.A. Tamminga, Maryland Psychiatric Research Center, University of Maryland at Baltimore, Baltimore, MD 21228, NINDS, NIH, Bethesda, MD 20892

Partial dopamine (DA) agonists, including the prototypical 3-(3-hydroxyphenyl)-N-(1-propyl)piperidine (3PPP), are currently of considerable interest in the treatment of schizophrenia. At low doses, -3PPP selectively stimulates the autoreceptor to reduce DA release and neuronal firing. At higher doses, -3PPP blocks apomorphine's postsynaptic actions. Thus, -3PPP may diminish DA-mediated transmission through two distinct actions. The goal of this study is to: 1) assess -3PPP action on local cerebral glucose utilization (LCGU) in rats to demonstrate its functional localization at low and high doses and, 2) to compare its effects with that of a typical neuroleptic, haloperidol. -3PPP doses were selected from electrophysiologic evidence of DA auto- and postsynaptic-receptor action. Results: Low dose -3PPP produced significantly increased glucose uptake in cortical areas (e.g. medial prefrontal, cingulate, sensorimotor, auditory, $p < .05$) as well as in limbic regions, including hippocampus, septal nucleus, nucleus accumbens, and medial and basolateral amygdala ($p < .05$) compared with saline control. The higher dose of -3PPP produced virtually no significant changes in LCGU. The distribution of metabolic changes induced by either the low (autoreceptor) or high (postsynaptic) dose is distinctive for each dose, as predicted from other preclinical findings. Metabolic data following apomorphine with and without high dose -3PPP will be reported.

239.12

Cultured hippocampal neurons regulate pHi via an amiloride-insensitive Na+/H+ exchanger. K.M. Raley-Susman, R.M. Sapolsky and R.R. Kopito*, Dept. Biol. Sci. Stanford Univ. Stanford, CA 94305.

Regulation of intracellular pH (pHi) is critical for neurons. Excessive acidosis contributes to neuronal damage following ischemia, hypoglycemia and seizure activity. We investigated pHi regulatory mechanisms in cultured hippocampal neurons using the fluorescent pHi indicator dye Bis-carboxyethylcarboxyfluorescein. Mixed neuronal/glial cultures were prepared from day 18 fetal rat hippocampi. Fluorescence of dye-loaded single neurons was continuously monitored and pHi was calculated from a standard curve determined by exposing cells to nigericin-high K+ solutions at different pH's.

The maintenance of resting pH_i was dependent on extracellular Na^+, with or without HCO_3. Resting pH_i maintenance also involved a minor, HCO_3-dependent component, as evidenced by the slightly higher resting pH_i in HCO_3-containing solutions. Recovery from an acute acid load (pulsing with 20 mM NH4Cl for 1-2 min) was also Na^+-dependent, with or without HCO_3. The Na^+-dependent, HCO_3-independent mechanism exhibited many characteristics of the Na^+/H^+ antiporter described in other cells. The recovery from an acid load in nominally HCO_3-free solutions showed a saturable dose-dependence on extracellular Na^+; K_m=26 mM. The recovery rate increased with decreasing intracellular pH; and lithium substituted for sodium as a substrate for the exchanger. The exchanger was electroneutral, as membrane potential collapse did not affect the recovery from an acid load. However, in contrast to reports in other cells, amiloride and its potent 5-amino-substituted analogues did not inhibit the recovery from an acid load. Thus, intracellular pH is regulated in large part by an amiloride-insensitive Na^+/H^+ antiporter.

239.13

MANIPULATION OF HIPPOCAMPAL SLICE ADENYLATE LEVELS BY ADENOSINE AGONISTS AND ANTAGONISTS. T.S. Whittingham, J.J. Klemens*, H. Assaf* and W.D. Lust. Div. of Neurosurgery and School of Dentistry, Case Western Reserve University, Cleveland, OH 44106.

In a previous study, we found that a three hour exposure of hippocampal slices to 1 mM adenosine resulted in a time-dependent 50% increase in total adenylate levels (ATP+ADP+AMP = AXP), and that most of this increase was maintained in the form of ATP. However, the prolonged exposure to high extracellular levels of adenosine appeared to also produce long-lasting changes in orthodromic evoked responses in area CA1 after the adenosine was removed.

We have now tested several additional methods of increasing slice AXP content while trying to avoid electrophysiological deficits. Rats were anesthetized, decapitated and 400 μm transverse hippocampal slices prepared. The slices were allowed to equilibrate in control artificial cerebrospinal fluid (ACSF) for one hour prior to exposure to one of the following ACSF solutions for up to three hours: 1) 1 mM adenosine; 2) 1 mM adenosine + 100 μM IBMX + 100 μM theophylline; 3) 1 mM adenosine + 100 μM dipyridamole; and 4) 1 mM adenine. Appropriate pharmacological control media were tested, and all paradigms also included a one hour washout period.

The results indicate that adenine can also serve as an AXP precursor in brain slices, although it was not as effective as adenosine. The use of adenosine receptor antagonists decreased AXP levels in the absence of exogenous adenosine, and depressed the adenosine-stimulated AXP increase in other slices. Conversely, dipyridamole was used to block adenosine uptake, but was found to increase AXP in both adenosine-treated and untreated slices. These results suggest that brain slice AXP levels could be maximized by preparation in the presence of dipyridamole. A model for the dynamics of brain slice adenylate homeostasis has been constructed, and is currently being tested.

239.14

A SIMPLE AND SENSITIVE COLOROMETRIC ASSAY CAPABLE OF DETECTING PICOMOLE QUANTITIES OF PYRIDINE DINUCLEOTIDES. C.H. Kang and J.W. Kebabian. Neuroscience Research Division, Abbott Laboratories, Abbott Park, IL 60064.

We report a modification of a cycling assay (CA) for pyridine dinucleotide that generates a colored reaction product (in a 96 well microtiter plate format). The CA uses an enzymatic redox cycle with alcohol dehydrogenase, ethanol and iodonitrotetrazolium (INT) to detect either DPN or DPNH. The amount of reduced INT formed in the CA assay is quantified with a microtiter plate reader at 492 nm. Using this assay protocol, less than 1 pmole of NAD or NADH can be detected and a linear standard curve generated between 0.5 and 10 pmole.

We apply this technique to quantify the amount of 2-deoxyglucose-6-phosphate (2DG6P) in the substantia nigra of the rat brain using alkaline phosphatase to convert 2DG6P to 2DG and bacterial glucose dehydrogenase to quantitatively convert the 2DG and NAD to 2-deoxygluconolactone and NADH. Control animals have low levels of glucose and glucose-6-phosphate (2.2 and 1.7 pmole/μg protein). Rats treated with 2-deoxyglucose (1.4 mmole/kg, i.v.) have 10.7 ± 1.3 pmole of deoxyglucose and 17.6 ± 1.26 pmole/μg protein of 2DG6P in the substantia nigra.

239.15

METABOLISM OF HYDROXYEICOSATETRAENOIC ACIDS (HETES) BY MOUSE BRAIN ENDOTHELIUM. M.J. Giordano* and S.A. Moore. Dept. Pathol., Univ. of Iowa, Iowa City, IA 52242.

Various eicosanoids including the HETEs are produced in various types of brain injury. To better understand their effects on brain microvessels, various HETEs and 12(S)-hydroxyeicosapentaenoic acid (12-HEPE) were incubated with cultured mouse cerebromicrovascular endothelium (CME) to ascertain which substances were metabolized by the cells. The eicosanoids listed below were incubated with CME for 1, 2 and 4hr, the media lipids extracted and analyzed by HPLC. As shown below, 5(S)-HETE, 8(RS)-HETE and 9(RS)-HETE are not metabolized by CME. 11(RS)-, 12(R)-, 12(S)-, 15(S)-HETE, and 12(S)-HEPE are metabolized primarily to a single, more polar compound. The data suggests that the enzyme system responsible does not distinguish between enantiomers, however, the enzymatic pathway is sensitive to the position or number of double bonds and is selective based on the position of the hydroxyl group. This selectivity may be one basis for the varied biological effects among the HETEs.

	Metabolite (% of total HETE remaining in medium)		
Substrate	1hr	2hr	4hr
5(S)-HETE	0	0	0
8(RS)-HETE	0	0	0
9(RS)-HETE	0	0	0
11(RS)-HETE	34±6	66±4	88±9
12(S)-HEPE	10±1	25±2	44±1
12(R)-HETE	22±4	49±5	72±12
12(S)-HETE	29±9	49±6	72±10
15(S)-HETE	29±6	74±3	88±7

239.16

LOCALIZED in vivo ^{31}P SPECTRA OF THE RAT BRAIN USING SPECTRAL LOCALIZATION BY IMAGING (SLIM): A BETTER METHOD FOR STUDIES OF BRAIN DEVELOPMENT. E.S. Fletcher, C.D. Gregory*, H. Lee*, W.T. Greenough, M.J. Dawson*, and P.C. Lauterbur, Biomedical Magnetic Resonance Laboratory, Beckman Institute and Neuroscience Program, Univ. Illinois, Urbana, IL 61801

We are investigating the metabolic correlates of neuronal plasticity. The SLIM method[1,2] makes possible the non-invasive, selective chemical analysis of an arbitrarily shaped region of the rat head. SLIM is superior in many ways to other NMR methods of spectral localization.

Anesthetized rats were studied using a 4.7 T, 33 cm bore SISCO imaging spectrometer with a ^{31}P surface coil (2.7 cm) and a ^{1}H imaging saddle coil. 3D image and 4D chemical shift imaging (CSI) data were obtained. The image data were used to define two compartments: brain (2.3 cm^3) and the rest of the head. The SLIM algorithm was then used to obtain compartmental spectra from the CSI data set, compartment information, and the surface coil position. Muscle spectra show the three ATP peaks and a peak for phosphocreatine (PCr). Very little inorganic phosphate (P_i) is seen. Brain spectra also show ATP, PCr, and a small P_i peak, as well as peaks in the phospho- monoester and diester regions.

We are using this new method to study changes in metabolism in the developing brain. Experiments are also underway to assess whether this method detects differences[3] among rats raised in environments varying in complexity. Acknowledgements: MH35321, 5 T32 CA 09067, the Servants United Found. and the Ntl. Center for Supercomputing Appl. Ref.: [1]Hu, X., Levin, D.N., Lauterbur, P.C. & Spraggins, T.A., Mag. Res. Med., 8:314, 1988; [2]Lee, H. & Lauterbur, P.C., Soc. Mag. Res. Med., Bk. of Abs., 1989, p. 651 and p. 1117. [3]Greenough, W. T., & Chang, F.-L. F. In E. G. Jones & A. Peters (Eds.), Cerebral Cortex. Vol. 7. N.Y.: Plenum, 1988, 391-440.

HYPOTHALAMUS

240.1

SUBREGIONAL TOPOGRAPHY OF CAPILLARIES IN THE MEDIAN EMINENCE AND ARCUATE NUCLEUS OF RATS. *S.W. Shaver, J.J. Pang*, K.M. Wall, D.S. Wainman*, and P.M. Gross. Neurosurgical Research Unit, Departments of Surgery & Physiology, Queen's University, Kingston, Canada K7L 3N6*

Cytoarchitectural and neurochemical differentiation of distinct median eminence (ME) zones and adjacent arcuate (ARN) and ventromedial (VMN) nuclei in the hypothalamus prompted our structural and functional studies of their respective capillary networks. From morphometric analysis of 1 μm-thick aldehyde-fixed sections, we determined that capillary densities in the subependymal (SEZ) and internal zones (IZ) of ME were low (range of 147-221 profiles/mm^2) in relation to the rich capillarity of the external zone (EZ, 1079/mm^2). True (3-7.5 μm lumen diameter) and sinusoidal (>7.5 μm) fenestrated capillaries were found throughout ME by electron microscopy. Pericapillary spaces in SEZ and IZ occupied 1-3% of the tissue area and, in EZ, 14%. ARN and VMN had 171-231 capillaries/mm^2 with fine structural characteristics of blood-brain barrier (BBB) endothelium. We noted in ARN a region of transition at the proximal borders with ME where capillary density (323 profiles/mm^2) and pericapillary space area (1%) were higher than in distal ARN. Measurements of capillary blood flow and tissue uptake of the tracer amino acid, [^{14}C]α-aminoisobutyric acid, permitted calculation of permeability x surface area products (PS) which indicated uniformly high PS throughout ME (285 μl g^{-1}min^{-1}), substantial PS in proximal ARN (69 μl g^{-1}min^{-1}), and negligible values (2 μl g^{-1}min^{-1}) in distal ARN and VMN, indicating the presence of a BBB. We speculate that confluent pericapillary and interstitial spaces between ME zones and ARN allow rapid dispersion of blood-borne molecules from permeable EZ capillaries into other ME zones and proximal ARN.

Supported by the Medical Research Council of Canada and the Heart & Stroke Foundation of Ontario

240.2

HISTAMINERGIC NEURONS IN HUMAN TUBEROMAMMILLARY NUCLEUS: NORMAL ANATOMY AND CHANGES IN ALZHEIMER DISEASE. M.S. Airaksinen, A. Paetau*, L. Paljärvi*, K. Reinikainen*, P. Riekkinen and P. Panula. Depts. of Anatomy and Pathology, Univ. Helsinki, and Dept. Neurology, Univ. Kuopio, Finland.

We have reported the presence of histamine-immunoreactive (HA-IR) neurons in the posterior hypothalamus and nerve fibers in the cortex of human adults (Panula, Neurosci., 34:129, 1990). In this study, the anatomy of HA-IR cell bodies in normal human brain and in cases of Alzheimer disease (AD) was examined and compared to the distribution of neurofibrillary tangles (NFT) in the same sections.

As in rodents, HA-IR cell bodies in human brain were found only in the hypothalamus, concentrated in the tuberomammillary (TM) nucleus. Its about 50000 cells formed one continuous cell group that embodied a major part of the hypothalamus. Its major ventral part included the classical TM nucleus, the intercalate nucleus and most of the lateral hypothalamic area. The medial part comprised the supramammillary nucleus and part of the posterior hypothalamic area. Most of the neurons were large and multipolar.

Preliminary results indicated a small reduction of the TM neurons in AD. Although located in similar large neuronal profiles and concentrated in the TM area, hypothalamic NFT were seldom found within the HA-IR neurons. As the nucleus of these profiles did not stain with hematoxylin, they may represent those TM neurons that have died and lost their histamine content.

240.3

HYPOTHALAMIC REGIONAL DIFFERENCES IN NEURONAL RESPONSES TO TEMPERATURE AND CYCLIC AMP. J.D. Griffin, M.L. Kaple and J.A. Boulant. Department of Physiology, College of Medicine, Ohio State University, Columbus, OH 43210.

Our previous experiments suggest that cyclic adenosine monophosphate (cyclic AMP) enhances neuronal thermosensitivity, especially in warm sensitive neurons in the preoptic area and anterior hypothalamus (PO/AH). Using horizontal tissue slices from rats, the present study examined the effect of cyclic AMP on neuronal temperature sensitivity throughout the entire diencephalon. Intracellular cyclic AMP was increased by 10 or 100 uM IBMX (3-isobutyl-1-methylxanthine, a phosphodiesterase inhibitor) or by 0.5-5.0 uM forskolin (an activator of adenylate cyclase). Neurons lacking thermosensitivity were not affected by increasing cyclic AMP, while most PO/AH warm sensitive neurons increased their thermosensitivity. In areas that contain inherently cold sensitive neurons (such as, the posterior hypothalamus), increased cyclic AMP often caused prolonged inhibition during tissue warming. This suggests that cyclic AMP may contribute to the thermosensitivity of both warm and cold sensitive neurons. (Supported by NIH grant NS-14644.)

240.4

EFFECTS OF PARAVENTRICULAR STIMULATION ON ULCER FORMATION: ROLE OF GASTRIC CONTRACTILITY AND GASTRIC ACID. N.S. Morrow, C.V. Grijalva and D. Novin. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

Changes in gastric contractility and gastric acid were measured during electrical stimulation of the paraventricular nucleus (PVN) in urethane anesthetized rats. Gastric contractility was monitored continuously by a pressure transducer connected to an open-ended catheter inserted in the rumen; acid secretions were collected through a gastric cannula positioned in the corpus. To localize PVN sites, blood pressure responses to 5 sec of monopolar stimulation (25 µa, 60 Hz, 100 µs, 200 µa, 100 Hz, .5 ms) were recorded through a femoral artery catheter. After 1 h of stimulation, sites were lesioned. Results indicated that stimulation within the PVN and in surrounding hypothalamic sites (most notably the zona incerta) produced damage in the stomach and somewhat less reliably in the duodenum. Changes in gastric contractility and gastric secretions were correlated with erosion scores. In control rats, stimulation anterior to the PVN was not ulcerogenic and no significant changes in contractility or acid secretion were observed. It is suggested that disturbances in motor and secretory functions mediate the gastrointestinal damage produced by PVN stimulation. [Supported by NIMH Fellowship Grant T32MH17140 and Univ. Research Grant SF86]

240.5

IDENTIFICATION OF HAMSTER PARAVENTRICULAR HYPOTHALAMIC CELLS PROJECTING TO THE SPINAL CORD. J. Blanchard and L.P. Morin. Dept. Psychiatry, HSC, SUNY, Stony Brook, NY 11794.

Hamster brains were processed using the ABC method for immunoreactivity to VP or OXY, or animals received 1 µl 4% FluoroGold (FG) in saline in T1-T2 of the spinal cord followed 10 days later by 100 µg colchicine in the lateral ventricle and perfusion 24 hr later. These brains were processed for immunohistofluorescence using Texas Red.

OXY cells are widely distributed throughout the PVN. The densest concentrations are in the lateral half of mid-rostrocaudal extent of the nucleus. In the lateral "wings," cells tend to be greatly elongated in the mediolateral direction. No obvious subdivisions based on cell size or morphology are found. VP cells are located throughout the same general regions as the OXY cells. Again, there are no obvious anatomical subdivisions. However, VP cells consist of 2 intermingled types: large, darkly staining cells (similar to the magnocellular VP cells of the supraoptic nucleus) or smaller, lightly staining cells.

An average 391 FG cells were counted in the PVN. Rostrally, there is extensive overlap of FG labeled cells with OXY and VP cells, but no double labeling. About 9.1% of all FG cells were also immunoreactive for OXY and 6.2% for VP and they are concentrated in a zone of the caudal PVN along the ventrolateral border extending dorsally and laterally 1-1.5 mm into the "wings."

The data reveal different cell classes according to immunoreactivity, density of immunoreaction, size, general location and co-localization with label retrogradely transported from the spinal cord. However, distinct PVN subdivisions do not appear as obvious as in the rat. NINDS NS22168

240.6

ELECTRICAL AND MORPHOLOGICAL CHARACTERISTICS OF NEURONS IN THE REGION OF THE MALE SEXUALLY DIMORPHIC NUCLEUS (SDN) OF THE RAT MEDIAL PREOPTIC AREA (MPOA). N.W. Hoffman, Y.I. Kim, R.A. Gorski, and F.E. Dudek., Mental Retardation Res. Ctr., UCLA School of Medicine, Los Angeles, CA 90024.

The SDN lies within the MPOA, which has been implicated in several biologically important functions. However, the signalling and morphological properties of SDN and other MPOA neurons have not been well-characterized (though see Curras, M.C., & Boulant, J.A., Soc. Neurosci. Abst., 15: 1090, 1989). We intracellularly recorded (n=19) and labeled these cells with biocytin and determined their positions (n=13) in slices from adult male rats. Neurons displayed a mean resting potential of -61 $\pm$ 2 mV, spike amplitude (from threshold) of 59 $\pm$ 1 mV, spike duration (at half amplitude) of 0.93 $\pm$ 0.1 ms, and post-burst afterhyperpolarizations. In response to positive pulses superimposed on steady hyperpolarizing current, all cells displayed low-threshold potentials that generated Na^* spikes. Mean input resistance and membrane time constant was 192 $\pm$ 24 MΩ and 14.9 $\pm$ 1.9 ms, respectively. Current-voltage plots were linear in the hyperpolarizing direction. Dorsal stimulation evoked an EPSP-IPSP complex in 93% of recorded neurons; IPSPs were reversed at -73 $\pm$ 2 mV (n=5) and blocked with bicuculline (n=1). Local axon collaterals may mediate the IPSPs, since labeled collaterals appeared to terminate near the stained cell. Labeled neurons generally had horizontal or vertical orientations, somata diameters (longest axis) of 10-20 µm, and 2-4 sparsely spiny to aspiny primary dendrites. They resided in SDN (n=6) or elsewhere in MPOA (n=7). Preliminary findings suggest similarity between SDN and other MPOA neurons. Future studies will address whether sex steroids, in addition to increasing cell number, developmentally alter electrical and morphological properties of male SDN neurons.

240.7

SUPRACHIASMATIC NUCLEUS (SCN) NEURONS RECEIVING RETINAL INPUTS ARE UNDER THE CONTROL OF γ-AMINOBUTYRIC ACID (GABA). Y.I. Kim and F.E. Dudek. Mental Retardation Res. Ctr., UCLA Sch. of Med., Los Angeles, CA 90024.

The neurotransmitter GABA has been implicated in the light-induced phase shifts of free-running locomotor rhythms of hamsters (Ralph, M.R. and Menaker, M., J. Neurosci., 9:2858, 1989). Light-sensitive neurons in the SCN are considered as a possible site of the GABAergic action.

To provide neurophysiological evidence that the light-sensitive neurons receive GABAergic inputs, intracellular recordings were obtained from SCN neurons in parasagittal brain slices (500-650 µm) of adult male rats (n=11) and guinea pigs (n=5). Twenty-six neurons (16 from rats and 10 from guinea pigs) that responded with excitatory postsynaptic potentials (EPSPs) to optic nerve stimulation were selected for investigation. Since no apparent difference was present between data from the two different species, they were combined. In normal perfusion medium, virtually all neurons studied (15 of 16) showed spontaneous, fast inhibitory postsynaptic potentials (IPSPs). Stimulation of a site dorsocaudal to the SCN usually evoked an IPSP (13 of 16), which was often preceded by a small EPSP. Bicuculline (50 µM), a $GABA_A$ receptor anatgonist, completely blocked both the spontaneous (n=2) and evoked (n=6) IPSPs. The neurons encountered in 50-µM bicuculline (n=10) exhibited neither spontaneous nor evoked IPSPs.

The results indicate that SCN neurons receiving retinal inputs are under GABAergic control and $GABA_A$ receptors mediate the neurotransmission. Supported by U.S. Air Force Grants (87-0361 and 90-0056) to F.E.D.

240.8

RESPONSES OF PARAVENTRICULAR NUCLEUS NEURONS TO AN OPIOID PEPTIDE SELECTIVE FOR MU-RECEPTORS IN GUINEA PIG. M. Kasai*, J.P. Wuarin and F.E. Dudek Mental Retardation Res. Ctr., UCLA Sch. of Med., Los Angeles, CA 90024.

Recent intracellular studies concerning the effects of a mu-receptor agonist, [D-Ala^2, NMe-Phe^4, Gly^5-ol] enkephalin (DAGO, 10^{-6} M), on neurons in the supraoptic and paraventricular nuclei have suggested a direct action through an increase in potassium conductance in about half of the cells (Wuarin, J.P. and Dudek, F.E. Neuroscience, in press). Experiments combining intracellular recording and staining with neurophysin immunocytochemistry in the paraventricular nucleus have defined three cell types: (1) magnocellular neuroendocrine cells that lack low-threshold calcium spikes (LTS), (2) parvocellular neurons with small LTS responses that do not generate bursts, and (3) neurons near the paraventricular nucleus which have LTS potentials that evoke bursts (Hoffman, N.W., Tasker, J.G. and Dudek, F.E. Soc. Neurosci. Abstr. 14:1178, 1988; ibid, 15:1088, 1989). Bath application of DAGO (10^{-6}M) inhibited 40% of the non-LTS cells, 38% of the non-bursting LTS neurons, and 80% of the bursting LTS cells. In preliminary experiments quinine (1 mM), which blocks calcium-activated potassium channels, reduced the inhibitory effect of DAGO (n=3). These results suggest that DAGO inhibits approximately half of the magnocellular and parvocellular neurons in the paraventricular nucleus, and nearly all of the bursting LTS cells in the perinuclear region. These data are consistent with an action of mu-agonists on calcium-activated potassium channels, similar to previous studies in the locus coeruleus (North, R.A. and Williams, J.T. J. Physiol. 364:265, 1985).

240.9

CURRENT-CLAMP PROPERTIES OF MAGNOCELLULAR NEUROSECRETORY CELLS (MNCs) ACUTELY DISSOCIATED FROM THE SUPRAOPTIC NUCLEUS OF THE ADULT RAT. Oliet S.H.R.* and Bourque C.W. *Centre for Research in Neuroscience. Montreal General Hospital and McGill University, Montreal, Canada. H3G 1A4.*

MNCs were acutely dissociated from the supraoptic nuclei of adult rats using the procedure of Kay & Wong (J. Neurosci. Meth. 16, 1986). The cells obtained were plated onto petri dishes and superfused (1-2 ml/min) with artificial CSF. All recordings were obtained from phase bright somata exceeding 17 X 17 μm in size and corresponding to the morphology of neurons immunoreactive for vasopressin and oxytocin. Intracellular impalements lasting up to 34 min were obtained from 22 cells showing a mean resting potential of -57.9 ± 1.4 mV, input resistance of 290.7 ± 50.8 MΩ and spike amplitude of 74.2 ± 2.2 mV. When evoked at 0.5 Hz, spike duration averaged 2.1 ± 0.5 ms at 29°C, or 3.1 ± 0.4 ms at 23°C. In response to current injection, repetitive firing was associated with progressive spike broadening and was followed by a protracted afterhyperpolarization. When measured from holding potentials (V_H) near rest, voltage-current (V-I) relationships were linear below spike threshold. Each of 18 cells tested from V_H <-80 mV displayed a prominent transient outward rectification during depolarizing pulses. The characteristics described above are typical of those of adult rat MNCs recorded in vivo in anesthetized rats, or in vitro in slices or explants of hypothalamus. Dissociated MNCs, therefore, represent a useful preparation for the study of MNCs. Supported by FCAR, FRSQ and MRC.

240.10

AN IN VITRO NEUROPHYSIOLOGICAL APPROACH TO THE IDENTIFICATION CONNECTIONS AND ELECTROPHYSIOLOGICAL PROPERTIES OF HYPOTHALAMIC ARCUATE NEURONES. S.J.A. MacMillan and C.W.Bourque, *Centre for Research in Neuroscience, Montreal General Hospital & McGill University, Montreal, Canada. H3G 1A4*

Although the hypothalamic slice has enabled us to classify neurones into sub-populations on the basis of extracellullar discharge patterns and permits the combined application of intracellular recording and immunocytochemical identification, input-output relationships are limited by the plane of section. We have therefore evaluated the use of an intact superfused hypothalamic explant preparation, in which extensive pertinent afferent and efferent circuitry is preserved, for physiological studies.

Stable intracellular recordings were obtained from 78 neurones in the ventro-medial aspect of the arcuate nucleus. These cells which were recorded for between 20 mins and 6 hrs exhibited stable membrane potential (Em) of -55 to -73mV, action potential amplitudes of 66 to 95mV and input resistances of 174 to 422MΩ. When held at hyperpolarized potentials (beyond -80mV), depolarizing current pulses activated either 'low threshold responses' and bursts of action potentials in 35% of the cells, or transient outward rectification and delayed firing characteristics of 'A'-current activation in another 20% cells. Electrical stimulation of the median eminence served to identify antidromically 14 tuberoinfundibular neurones (onset latencies, 3 to 10ms). Stimulation of the NIL evoked antidromic responses from 2 cells, excitatory PSP's from 9 cells, and mixed excitatory and inhibitory PSP's in 2 others. Selective stimulation of the SON activated antidromically 8 putative ß-endorphin neurones (onset latencies, 8 to 16ms). Stimulation of the medial preoptic area evoked mixed compound synaptic responses in 10 arcuate cells tested. This preparation, therefore, is suitable to characterize the physiology and pharmacology of arcuate neurones with identified anatomical connections. (SJAM is a Beit Fellow. Supported by The Royal Society, The Wellcome Trust & MRC(Canada).

240.11

SERIAL SECTION ANALYSIS OF SYNAPSE FORMATION ASSOCIATED WITH MOTHERHOOD IN THE RAT SUPRAOPTIC NUCLEUS (SON). J. L. Smithson* and G. I. Hatton. Neuroscience & Biological Sciences Programs, Michigan State University, E. Lansing, MI 48824.

The SON dendritic zone has been shown to exhibit an increased occurrence of double synapses (i.e. one axon terminal simultaneously contacting two post-synaptic dendrites, each possessing a post-synaptic density, PSD) in response to stimuli associated with motherhood (Perlmutter, L. S. et al., *Neurosci.*, 13:769, 1984). Recently, it has been shown that axon terminals in the SON somatic region which only appose a cell body will eventually form a conventional synapse possessing a PSD (Modney, B. K. & Hatton, G. I., *J. Neuroendo.*, 1:21, 1989). The present study sought to determine whether a similar situation exists within the SON dendritic zone during conditions associated with motherhood. Using tissue from a previous study (Taubitz, I. L. et al., *Soc. Neurosci. Abst.*, 13:1594, 1987), a representative animal from each of the following groups was serial sectioned: virgin female, prepartum (day 21 of gestation), 2-24 h postpartum, 14 day lactating and 30 day postweaning rats. In all conditions, 90% of dendrites eventually formed a conventional synapse in serial sections. We estimated PSD length and apposition length between a terminal and a dendrite based on the number of sections which displayed these features. The lengths of the PSD and terminal apposition in single synapses were decreased in the prepartum animal, suggesting the occurrence of a synaptic reorganization. In the postpartum animal, the length of terminal apposition in multiple synapses increased compared to the prepartum animal. A given multiple synapse with ≥ 2 PSDs appeared in more sections in postpartum animals compared to other groups. In conclusion, multiple synapses while not uncommon, are uniquely sensitive to the stimulation of parturition. Supported by NS 09140.

240.12

MORPHOLOGICAL CHANGES IN THE POSTERIOR PITUITARY IN RESPONSE TO ACUTE DEHYDRATION. G.H. Beagley, B.K. Modney & G.I.Hatton. Psych. Dept.& Neurosci. Program, Michigan State Univ., E. Lansing, MI 48824.

In the posterior pituitary, nerve terminals contact the basal lamina (BL) of the neurohypophysial vascular zone where they release the hormones vasopressin and oxytocin. Glial cells (pituicytes) also contact the BL and alter their morphology in response to physiological challenges. Nerve terminal contact with the BL was compared in male rats given intraperitoneal injections of hypertonic (1.5 M) or normal (0.15 M) NaCl solution. Rats were sacrificed five hours after the injections. Morphometric analysis of electron micrographs revealed that the three animals in the 1.5 M NaCl condition showed 66%, 72% and 77% (mean = 73%) nerve terminal contact with the BL. Animals in the normal NaCl condition both showed 42% neural contact. These data suggest that the relationship of pituicytes to the BL can change rapidly in accordance with the hypothesis that pituicyte processes withdraw from the BL to allow increased neural contact to facilitate hormone release under appropriate physiological conditions. Supported by NS 09140

240.13

INVOLVEMENT OF INWARD SODIUM CURRENTS IN PHASIC FIRING OF RAT HYPOTHALAMIC SUPRAOPTIC (SON) NEURONS. K. INENAGA*, S. YAMAMOTO*, N. AKAMATSU*, H. KANNAN and H. YAMASHITA, Dept. Physiol., Univ. Occupational & Environmental Health, Sch. of Med., Kitakyushu 807, Japan.

A phasic firing pattern consisting of a plateau potential and bursting discharges was observed in the SON of rat hypothalamus. It has been reported that Ca currents and Ca-activated K currents are involved in the generation mechanisms of phasic firing. To investigate whether Na currents are involved in the generation, intracellular recordings were made from phasically firing neurons in the SON of rat slice preparations. Application of low Na solution suppressed phasic firing. In a Ca free medium or a Ca free/EGTA medium containing veratridine, the phasic firing remained and a plateau potential evoked by depolarizing pulses through the recording electrode was also observed. Amplitude and duration of the plateau potential increased with the number of pulses. TTX blocked the veratridine-induced phasic firing and the pulse-evoked plateau potential in the Ca free medium. Application of extracellular TEA or intracellular Cs ions failed to block the phasic firing but prolonged duration of the plateau potentials. From these results, it is suggested that inward currents by Na ions as well as Ca currents and Ca-activated K currents may play an important role in generation of the phasic firing of rat SON neurosecretory cells. This work was entrusted to UOEH by the Science and Technology Agency.

240.14

CHANGES IN RENAL SYMPATHETIC NERVE ACTIVITY,HEART RATE AND ARTERIAL BLOOD PRESSURE ASSOCIATED WITH DRINKING IN RATS. H.KANNAN[1], T.NAKAMURA[2*], Y.HAYASHIDA[2*] and H.YAMASHITA[1]. Dept.Physiol[1]. and Dept. Systems Physiol[2]., University of Occupational and Environmental Health, Kitakyushu 807, Japan

Renal sympathetic nerve activity(RSNA), heart rate(HR) and arterial blood pressure(AP) were simultaneously recorded in conscious rats during drinking which was induced or occured spontaneously. In rats not allowed to drink, intraventricularly(ivt) administered angiotensin II(AII)(10ng) elicited an increase in AP and decreases in HR and RSNA. When rats were allowed to drink, an additional increase in AP occured concomitantly with onset of drinking induced by ivt AII, while the decreases in HR and RSNA were significantly attenuated, compared with those in non-drinking rats. Similar drinking-dependent responses were observed with drinking induced by ivt carbachol(200ng) and hypertonic sodium solution(5%,2ul). Spontaneous drinking elicited increases in AP, HR and RSNA. Cardiac-related grouped discharges of RSNA, which were observed at rest, increased during drinking. We conclude that RSNA increases in association with drinking behavior and that activation of the sympathetic efferent activity to the kidney is involved in the cardiovascular adjustment associated with drinking behavior.(This work was entrusted to University of Occupational and Environmental Health, using the Special Coordination Funds for Promoting Science and Technology)

240.15

SEX DIFFERENCES IN THE VASOPRESSIN INNERVATION OF THE GERBIL FOREBRAIN UNDER VARIOUS HORMONAL CONDITIONS. B. Crenshaw, G.J. De Vries, and P. Yahr, Dept. Psychology and Prog. of Neurosci. and Behav., Univ. Mass., Amherst, and Dept. Psychobiol. Univ. Cal., Irvine.

The medial preoptic-anterior hypothalamic region of gerbils contains a sexually dimorphic area (SDA) which has a subgroup--the SDA pars compacta (SDApc)--that is absent in females. Many vasopressin-immunoreactive (AVP-IR) fibers are present in the medial SDA and SDApc. Previously, we found that the bed nucleus of the stria terminalis (BST) and medial amygdaloid nucleus (MA) project to the SDA. Since the SDA in gerbils and AVP projections of the BST and MA in rats respond to steroids in adulthood, we studied how gonadectomy and testosterone treatment affected AVP-IR fibers in the SDA and lateral septum in both sexes.

In both sexes, AVP-IR fibers were clustered in the region that in males houses the SDApc. In every treatment group, this cluster was larger in males than in females (ANOVA; $P<0.0001$). Males had also more AVP-IR fibers in the medial SDA than females ($P<0.005$). There were no treatment effects for either the SDApc or the medial SDA, but sex and treatment interacted to affect the innervation of the SDApc. Gonadectomy decreased the cluster size in males and increased it in females. Testosterone reversed these effects ($P<0.05$). This differs from hormonal effects on the AVP innervation of the lateral septum, which comes from the BST. In this area AVP-IR fiber density was much higher in males than in females ($P<0.0002$), and gonadectomy decreased--and subsequent testosterone treatment increased--VP fiber staining in both sexes ($P<0.0001$).

The fact that AVP-IR fibers in the SDA and lateral septum differ, suggests that they come from a different source. The SDA-fibers might come from AVP-IR cells that we have found in this study at the dorsal aspect of the suprachiasmatic nucleus.

240.16

INVOLVEMENT OF DOPAMINERGIC MECHANISMS IN GASTRIC EROSIONS INDUCED BY LATERAL HYPOTHALAMIC (LH) LESIONS. B. Roland and C.V. Grijalva. Dept. Psychology, UCLA, Los Angeles, CA 90024.

Dopamine (DA) agonists provide protection against gastric erosions induced by cold restraint or certain types of brain damage, whereas DA antagonists facilitate gastric erosion formation. LH damage alters brain dopamine levels and results in gastric pathology. The present study examined whether DA agonists or antagonists would alter the incidence of LH lesion-induced gastric erosions.

Six groups of male rats were given bilateral electrolytic LH lesions preceded by a subcutaneous injection of either apomorphine (DA agonist) or domperidone (peripheral DA antagonist) in the following doses: 1, 5, or 10 mg/kg. An additional LH group was injected with an equivolume of vehicle (2 ml/kg). A second injection was given 6-8 hr. postoperatively. Stomachs were analyzed 24 hr. following surgery. Only the highest dose of apomorphine significantly reduced the incidence of gastric erosion ($p<.02$). No significant facilitation of gastric damage was seen with domperidone. The findings suggest alterations in dopamine levels induced by LH damage may contribute to gastrointestinal pathophysiology. (Supported by UCLA University Research Grant).

240.17

ROLE OF THE PARAVENTRICULAR NUCLEUS IN THE PROJECTION FROM NUCLEUS OF THE SOLITARY TRACT TO THE OLFACTORY BULB. R. Guevara-Guzman, D.E. Garcia-Diaz, L.P. Solano-Flores and M.J. Wayner. Depto. de Fisiologia, Fac. de Medicina, U.N.A.M. and Div. of Life Sciences, Univ. of Texas at San Antonio, San Antonio, Texas, USA.

The paraventricular nucleus (PVN) is clearly involved in feeding behavior. Morphological and electrophysiological evidence demonstrate a bidirectional connection between the nucleus of the solitary tract (NTS) and the PVN. In a previous report we established the existence of a functional connection between visceral vagal afferents and cells of the periglomerular layer of the olfactory bulb (OB). The purpose of the present study was to determine the connections between the NTS, PVN, and the OB. The experiments were conducted in anesthetized rats. Evoked potentials were recorded by means of concentric electrodes in the PVN and the OB in response to electrical stimulation of the NTS, before and after electrolytic lesions (2-10 mA DC for 30-60 s) were produced in the PVN. We found that the lesions of the PVN enhance the amplitude of the positive and negative components of the evoked potential in the OB. Activation of the pathway from the PVN to OB apparently exerts a suppressive influence on the effects of the NTS on the OB.

240.18

ULTRASTRUCTURAL EVIDENCE FOR SYNAPTIC INPUT TO THE RAT SUPRAOPTIC NUCLEUS FROM THE OLFACTORY BULB AND THE SUBFORNICAL ORGAN. M.L. Weiss, A. Tackman*, L.E. Koran*, F. Marzban* & G.I. Hatton. Neuroscience Prog., Psychology and Anatomy Depts., Michigan State University, E. Lansing, MI 48824-1117.

We have previously described inputs to the supraoptic nucleus from both the main olfactory bulb (MOB; Smithson et al., Neurosci., 31:277, 1989) and the subfornical organ (SFO; Weiss et al., Soc. Neurosci. abstr., 10:609, 1984) using light microscopic techniques. Here the input from SFO or MOB to supraoptic nucleus (SON) was examined ultrastructurally using anterograde transport of either Phaseolus vulgaris leucoagglutinin or a horseradish peroxidase-based tracer. The input from the MOB terminates on small dendrites, most likely from SON neurons. On the other hand, the SFO axons terminate upon dendrites closer to SON somata. Our data corroborate the results of previous tract-tracing and electrophysiological studies and demonstrate monosynaptic inputs to SON from these two telencephalic structures. Supported by NSF BNS-8919898 and NIH 09140.

240.19

THE EFFERENT CONNECTIONS OF THE VENTRAL PREMAMMILLARY NUCLEUS: A *PHASEOLUS VULGARIS* LEUCOAGGLUTININ TRACT-TRACING STUDY IN THE RAT. N.S. Canteras*, R.B. Simerly and L.W. Swanson, Dept. of Biology, University of Southern California, Los Angeles, CA 90089 & Oregon Regional Primate Research Center, Beaverton, OR 97006.

The ventral premammillary nucleus (PMv) is a compact nucleus in the tuberal region of the hypothalamus immediately caudal to the ventromedial nucleus and has been implicated in the control of gonadotropin secretion. Cells in this nucleus appear to express receptors for sex-steroid hormones and also receive strong inputs from several nuclei in the forebrain linked to the sexual dimorphic circuit, including the amygdalo-hippocampal area, medial nucleus of the amygdala, encapsulated nucleus of the bed nuclei of the stria terminalis, and medial preoptic nucleus. Nevertheless the projections of the PMv remain largely unknown. In order to clarify the organization of the efferent connections of the PMv we applied the *Phaseolus vulgaris* leucoagglutinin (PHA-L) anterograde tract-tracing method. Iontophoretic injections of PHA-L were made into the region of the PMv in 25 male rats and the brains processed as described elsewhere (Gerfen & Sawchenko, '84).

From injection sites centered in the PMv labeled fibers ascend through the periventricular zone of the hypothalamus and appear to provide massive inputs to all parts of the periventricular nucleus, the arcuate nucleus, and anteroventral periventricular nucleus of the preoptic region. In addition, a dense plexus of labeled fibers was found in the medial preoptic nucleus, and was largely localized to its medial subdivision. Labeled fibers also pass dorsolaterally from the periventricular zone toward the bed nuclei of the stria terminalis and provide inputs to the encapsulated nucleus, as well as to the ventral part of lateral septal nucleus. Fibers from this pathway continue through the stria terminalis and end as a dense terminal field in the posterodorsal part of the medial nucleus of the amygdala and the amygdalo-hippocampal area.

These results suggest that the PMv may convey hormone-sensitive information to regions of the forebrain primarily involved in the neuroendocrine regulation of the anterior pituitary.

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241.1

LYAPUNOV CHARACTERISTIC EXPONENT AND VARIATION IN THE FIRST DERIVATIVE QUANTIFY THE DYNAMICAL COMPLEXITY OF EVENT-RELATED BRAIN POTENTIALS. Gene V. Wallenstein and Allan J. Nash, Center for Complex Systems, Florida Atlantic University, Boca Raton, Fl 33431.

The first order Lyapunov Characteristic Exponent was used to assess the relative stability (i.e. convergence or divergence) of the event-related potentials produced during an oddball paradigm. Five subjects were asked to keep a running mental count of one of two clearly discriminable tones, and report this at designated time intervals. The target tones (1500 Hz) occurred with a probability of 20% and evoked a large positive-going wave, the P300, which was dramatically reduced or absent to non-target (500 Hz) tones. The brain's electrical response to these stimuli were recorded from the central (Cz) and parietal (Pz) midline locations. Twenty trials were averaged under each of the target and non-target conditions, producing a time series that was initially embedded in an 8-dimensional state space. Using the Grassberger-Procaccia algorithm, the correlation integral was obtained to derive some estimation of the signal's structure, and subsequently give information as to whether a larger embedding space would be necessary. In all cases, this procedure yielded dimension estimates of between 2 and 5, although there was considerable overlap both within and between subjects in terms of target and non-target stimuli. The Lyapunov Characteristic Exponent and variation in the first derivative of the time series data were computed using a method proposed by Wolf et al (1985). The averaged target stimuli consistently produced positive Lyapunov exponents indicating exponential divergence of nearby initial conditions, while the nontarget signals typically resulted in a marked reduction in this value, and in some instances yielded negative estimations suggesting convergence to a regime of lower periodicity. Variation in the first derivative also proved to be an effective measure for differentiating between the two stimulus conditions, with the targets having a substantial increase in this value relative to non-targets.

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241.2

MIDLATENCY AUDITORY EVOKED RESPONSES IN A PATIENT WITH A BILATERAL THALAMIC INFARCTION. R.J. Erwin, B. Malamut*, and M. Mawhinney-Hee*. Dept. of Psychiatry, Univ. of Penn., Philadelphia, PA 19104.

Evidence from both the human and cat model suggests that the P1 (50-65 msec latency) component of the midlatency auditory evoked response (MLR) is generated in midline thalamic regions (Erwin and Buchwald, EEG suppl., 40:461, 1987). To further investigate this hypothesis, the topographic distribution of P1 was examined in a previously healthy 31 year old male with bilateral and symmetrical midline thalamic infarctions. An MRI localized the lesion to include the anterior nuclei and portions of the medial dorsal nucleus. There was no evidence of damage to other subcortical or cortical regions. Recording procedures were identical to those previously described (Erwin et al. Neurosci. Abst., 15:746, 1989) using a 1/sec stimulus rate.

The distribution of P1 in this individual was characterized by distinct frontal maxima and temporal minima. In contrast, central maxima for P1 were observed in controls at the 1/sec stimulus rate (Erwin et al., 1989). This distribution resembled that of controls at a faster stimulus rate (10/sec) where the central maxima was diminished. These findings provide further evidence that multiple MLR components exist at the P1 latency and that the P1 component observed centrally has a thalamic generator source.

241.3

ELECTROPHYSIOLOGICAL INDICANTS OF VISUAL SPATIAL ATTENTION AND READING ABILITY: A LONGITUDINAL STUDY. L. Anllo-Vento, S.L. Miller* and M.R Harter#. Psychology Dept., UNC-Greensboro, NC 27412-5001.

Event-related brain potentials (ERPs) were obtained to assess developmental changes in neural indicants of spatial attention in normal and reading disabled young males. Two spatial tasks were employed. Each required a reaction time response to a small square, flashed peripherally in the visual field cued by an antecedent, foveally presented arrow. ERPs were recorded from the same subjects when they were approximately 10.8 years old, and again at age 16.

Changes in P1-N1 and P3 amplitude due to stimulus relevance were comparable across groups, despite significant differences in verbal intelligence and reading ability. Thus, it appears that dyslexic subjects have normal visual-spatial abilities.

There was, however, a significant difference in the posterior-anterior gradient of P1-N1 amplitude. Dyslexics showed smaller differences in activation between posterior and anterior brain regions than did normal subjects. This difference was developmentally stable and consistent across tasks.

Thus, it appears that neural activation is less focal in disabled than in normal readers, even when attentional and verbal task demands are controlled.

Supported by NINCDS Grant RO1 NS19413-07

#Deceased 3/24/90

241.4

EVIDENCE FOR AN ERP CORRELATE OF ATTENTIONAL FILTERING. S.J. Luck* & S.A. Hillyard. Deptartment of Neurosciences, M-008, University of California, San Diego, La Jolla, CA 92093.

In visual search tasks, subjects must determine whether or not a target item is present within an array of distractor items. In order to accomplish this task, subjects may have to filter out the distractor items, allowing only the target item to reach object identification processes. Here we report two experiments suggesting that an ERP component -- an N2 wave that appears over contralateral posterior sites -- may be an electrophysiological index of this filtering process.

There were 8 items in each visual display, except as noted; the items were either identical or else one item differed from the others and appeared to "pop out." In Experiment 1, the target item was defined by its color or its orientation. In one condition, the target was presented within an array of distractor items; in a second condition, the same target item was presented in isolation, without any distractors. Attentional filtering was presumably unnecessary in the latter condition. As predicted, the N2 component was greatly attenuated in this condition compared to the condition in which the target was embedded in an array of distractors. Experiment 2 tested the effects of increasing the amount of processing required to identify the target item. Each pop-out item was composed of four colors, and a pop-out item was classified as a target if these colors were presented in one spatial arrangement and as a non-target if any other spatial arrangement of the same colors was presented. The number of non-target arrangements was varied across conditions; as the number of arrangements increased, the difficulty of target identification increased. The amplitude of the posterior N2 component increased in parallel with the difficulty of the identification process. From these results, we conclude that the posterior N2 component indexes a spatial filtering process that is used to assist object identification processes. This component reaches its maximal amplitude near the occipital-temporal border, and may reflect the attentional processing that has been demonstrated in areas V4 and IT.

241.5

SELECTIVE ATTENTION MODULATES PROCESSING IN NON-TONOTOPIC AUDITORY CORTEX IN HUMANS. D. L. Woods, A. Algazi, and K. Alho#, Dept of Neurology, U.C. Davis, VAMC, Martinez, CA, 94553, and #Dept of Psychology, University of Helsinki, Finland.

Attention alters auditory event-related brain potentials (ERPs). These attention effects can be isolated as attentional difference waves (ADWs) by subtracting ERPs evoked by ignored sounds from ERPs elicited by the same sounds when attended. We investigated the tonotopic organization of the generators of ERPs and ADWs by determining if their scalp distributions changed when evoked by tonebursts of widely different frequencies (250-4000 Hz).

In experiment 1 subjects attended either to auditory or visual stimuli, and ignored stimuli in the competing modality. In experiment 2 subjects attended to monaural tones of one pitch and ignored competing tones. In both experiments, the N1 component (latency 110 ms) evoked by nonattended tones changed progressively in scalp distribution as a function of frequency. In contrast, no frequency-related changes occurred in the distributions of ADWs. The results suggest that the cortical processing of nonattended tones occurs primarily in tonotopically organized fields, whereas auditory areas modulated by attention are largely non-tonotopic. (Supported by the VA Research Service, the NIDCD, and a Fogarty International Fellowship to KA).

241.6

AUDITORY STREAMING INFLUENCES ONSET OF SELECTIVE ATTENTION EFFECT ON AUDITORY ERPs. C. Alain, A. Achim & F. Richer. Laboratoire de Neuroscience de la Cognition, Université du Québec à Montréal, Montréal, Canada.

We tested whether the attention effect on ERPs could be manipulated by auditory context, while keeping constant physical separation of alternate attended channels. ERPs were recorded at Fz, Cz and Pz from 10 subjects paying attention to one pitch (1000 or 1682 Hz) and ignoring tones of three other pitches, all lasting 60 ms (p=.85) or 130 ms, presented at 200-500 ms intervals. In the baseline condition, the four channels were equally spaced by 3 half-tones (1000; 1189; 1414; 1682 Hz). In the grouped-channels condition, the middle pitches were replaced by two others, separated from the extreme tones by one half-tone (1000; 1059; 1587; 1682 Hz) creating two pairs of closely spaced channels. Subjects detected the longer tones in the attended channel. Reaction times were not different between conditions; subjects made more false alarms and misses in the grouped-channels condition. The mean amplitude of the difference potentials (Nds) for attended and unattended tones were measured over consecutive 40-ms periods. Significant Nds began at the 45-80 ms interval for the grouped-channels condition and at 125-160 ms for the baseline condition. Nds were different between the two conditions during the 45-120 ms interval. The data show that the onset of the Nd wave can be manipulated by the perceptual context created by auditory inputs and is not exclusively dependent on a stimulus matching process.

241.7

EFFECTS OF ASSOCIATION CORTEX LESIONS ON THE SOMATOSENSORY P300

S.Yamaguchi and R.T.Knight Dept. of Neurology, Univ. of California, Davis, VAMC, Martinez, CA 94553

Novel stimuli requiring no overt response generate a frontal-central P3a whereas correctly detected target stimuli generate a parietal P3b. Lesion studies employing auditory and visual stimuli support prefrontal and temporal-parietal junction cortex involvement in P3a and P3b generation. We investigated the contribution of anterior and posterior association cortex to somatosensory P3 generation by recording ERPs in controls (n=10) and patients with unilateral lesions in lateral parietal cortex (n=8), temporal-parietal junction (n=8) and dorsolateral prefrontal cortex (n=10). Subjects pressed a button to mechanical taps of the fifth finger (targets, p=.12), randomly interposed in sequences of taps to the second (standards, p=.76) and third or fourth finger (tactile novels, p=.06). Occasional shock stimuli were delivered to the wrist (shock novels, p=.06). Temporal-parietal lesions abolished or reduced P3a and P3b at posterior sites to contralateral and ipsilateral stimuli. Frontal lesions reduced the P3a and P3b at frontal scalp sites. Parietal patients produced no focal reduction of the P3b although the P3a to contralateral shock novel stimuli was reduced. The data indicate that multi-modal cortex in the temporal-parietal junction and dorsolateral prefrontal lobe contributes to the scalp P3a and P3b.

241.8

MONKEY P3 IN A VISUAL AND A MULTIMODAL ODDBALL PARADIGM.

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It has generally been assumed that human P3 reflects modality-independent mechanisms because of its "cognitive" nature, its scalp distribution, and the similarities in task and probability effects following auditory, visual, and somatosensory stimulation. These similarities suggest two models of P3 genesis: 1) that each modality engages comparable, but independent mechanisms; or 2) that aspects common to all modalities arise from the same neural source.

To test these hypotheses, ERPs were recorded from chronically implanted squirrel monkeys (Saimiri sciureus) in a visual (VOP) or a multimodal (MOP) oddball paradigm. VOP consisted of blue rectangles (2.3^{o} x 1.15^{o}, 90%, centered, 1 sec ISI), as background, and yellow rectangles (7.6^{o} x 1.15^{o}, 10%), as "oddballs", presented pseudo-randomly in different locations on a screen. ERPs were recorded before and after administration of the adrenergic agonist, clonidine (0.1 mg/kg, IM), and the antagonist, L753,643 (0.05 mg/kg, IM). MOP consisted of tones (3 KHz, 300 msec, 70 dB nHL, 80%, 1 sec ISI), as background, a different pitch tone (1 KHz, 300 msec, 70 dB nHL, 10%), as "auditory oddball" (AO), and a red rectangle (7.6^{o} x 1.15^{o}, centered, 10%) as "visual oddball" (VO). P3 amplitudes increased in five subjects following clonidine administration and decreased following L753,643. P3s to VO were larger in amplitude, longer in latency, and exhibited a broader scalp distribution than AO responses. These data suggest that monkey P3 is the result of both modality-independent and modality-dependent mechanisms, and that one likely substrate for the modality-independent aspects is the noradrenergic system.

241.9

VISUAL SELECTIVE ATTENTION TO SPATIAL LOCATION: EVENT-RELATED BRAIN POTENTIAL AND CURRENT DENSITY ANALYSES.

G.R. Mangun(1), J.C. Hansen(2) and S.A. Hillyard(2). (1) Dept. of Psychiatry and Program in Cognitive Neuroscience, Dartmouth Medical School, Hanover, N.H., and (2) Dept. of Neurosciences, UCSD, La Jolla, CA.

Past studies have reported polarity inversions in certain of the early occipital event-related potentials (ERPs) with upper versus lower field stimuli. Such changes can be related to cortical anatomy. In one model, components that inverted in polarity were localized to striate cortex, while those that did not, were localized to extrastriate cortical regions. The present study examined the behavior of the early, attention-sensitive P1 and N1 components with upper and lower field stimuli in order to investigate their neural generators. ERPs were recorded from 30 scalp sites in response to stimuli flashed in rapid, randomized sequences to the four quadrants of the visual field. Subjects attended the stimuli in one quadrant while ignoring stimuli in the other three quadrants: each quadrant was attended in a different run. Spherical spline interpolation was used to obtain topographic maps and current density maps and waveforms. Stimuli in upper versus lower field locations elicited polarity inversions in a midline, parieto-occipital N80 component that was not influenced by attention. In contrast, the occipital P120 (P1) and parietal N180 (N1) peaks did not invert in polarity, but were significantly amplitude modulated with attention. These data are interpreted in terms of the levels of visual processing affected by sensory gating during selective attention. Conducted at UCSD with grants to SAH.

241.10

EVENT-RELATED BRAIN POTENTIALS AND SCALP CURRENT DENSITY MAPS DURING COLOR SELECTIVE ATTENTION IN HUMANS

K.S. Mangun(1)*, G.R. Mangun(2) and S.A. Hillyard(3). (1) Division of Neurosurgery and (2) Dept. of Psychiatry, Dartmouth Medical School, Hanover, N.H., and (3) Dept. of Neurosciences, UCSD, La Jolla, CA.

Selective attention to stimulus color is usually associated with a broad negative event-related potential (ERP) deflection between 150-300 msec latency (selection negativity). The present study investigated the scalp distribution of ERP color attention effects using multichannel voltage and scalp current density analyses. Subjects (N=12) were presented with purple and blue squares flashed in rapid, random sequence to a single location on the vertical meridian above fixation. In separate runs, subjects were required to attend to one color while ignoring the other. Color selection effects were first observed over occipital scalp as a positivity between 120-260 msec latency that peaked at about 170 msec. This positivity tended to be larger over the right hemisphere and was associated with a current source-sink configuration over occipital scalp. A left occipito-temporal negativity elicited by the attended color was observed between 200-300 msec and was associated with a current sink-source configuration distinct from that associated with the earlier posterior, right hemisphere positivity. Over anterior scalp regions the principal effects of color selection were a fronto-central P200-N290 sequence. These data are interpreted in terms of the activation of cortical areas specialized for color processing during attention. (Conducted at UCSD and supported by grants to SAH).

241.11

SPATIOTEMPORAL DYNAMICS OF HUMAN WORKING MEMORY.

Brian Cutillo*, Steve Bressler and Alan Gevins. EEG Systems Lab., San Francisco, CA 94107. Five USAF test pilots performed several hundred trials of a task requiring precise finger pressures proportional to the stimulus number seen 2 trials (about 12 seconds) previously. They had to maintain a constantly changing sequence of two numbers in working memory, and deal with numerical feedback about response accuracy on each trial. The control task eliminated the working memory component by having subjects respond immediately to the current stimulus number. 20% of the trials were no-response catch trials, which consisted of the number "0" in the control condition, and a match between the 2-back number and the current stimulus number in the memory condition. Cortical functional network activity patterns were computed from spatially and temporally enhanced evoked potentials during 4 split-second intervals. In the prestimulus interval, evidence was found for verbal codings for working memory in patterns focused on sites over left-hemisphere visual association and anteroparietal areas, while similar patterns were found in the control condition over the right hemisphere. Evidence was also found for very early (50 msec poststimulus) pattern matching over left-hemisphere anteroparietal sites in the memory condition only. During the interval spanning the P300 wave in catch trials, anterocentral, central, and left anteroparietal patterns dominated the memory condition, while the control condtion had a bilateral pattern over visual association and right anteroparietal areas. This implies that inhibition of response involved anterior premotor areas in the memory condtion, and visual association areas in the control condition. During response execution, both conditions had the same left central and anteroparietal pattern we have seen previously with right index finger flexions. These results may be the first temporally and spatially detailed measures of the dynamics of working memory in the human brain.

241.12

SLOWING WITH AGE: MODERATE CORRELATIONS AMONG BEHAVIORAL AND ELECTROPHYSIOLOGICAL INDICATORS OF SPEED OF PROCESSING

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Slowing is the change most characteristic of human aging. A central timing mechanism which slows with age has been advanced as a possible explanation. On this view all those processes which depend on timing slow as well. To evaluate this hypothesis 48 women (ages 20, 40, 60, 75±2 yrs) were tested on several perceptual (figural synthesis, CFF, backward and forward masking), reaction time (simple, choice RT; digit symbol substitution) and short-term memory (Sternberg) tasks, all of which measure various aspects of speed of processing. The EEG was recorded during testing. ERPs were extracted for the RT and memory tasks. For the perceptual data, power spectrum analysis of the EEG was performed. Mood, health, and intellectual status of subjects were also evaluated since these factors are thought by some to be related to speed of processing. Examination of covariation among timed behavioral and electrophysiological measures, mood, health and intellectual status and their association with age showed moderate support for the hypothesis that generalized slowing of a timing mechanism or mechanisms is associated with age-related slowing. While not all behavioral and electrophysiological measures were significantly correlated, a large number were. Clustering by type of measure was not observed, as would be expected if domain-specific timing mechanisms were operating.

241.13

A STATISTICAL MODEL BASED ON POOLED ACTIVITY PREDICTS VIBROTACTILE ATTENTIONAL ASYMMETRY. K. C. Whang, H. Burton, and G. L. Shulman*, Depts. of Anatomy & Neurobiology, Neurology & Neurol. Surg., Washington Univ. Sch. of Med., St. Louis, MO 63110. (Supported by NIDCD 00096.)

Human performance studies showed that detecting the presence of a vibrotactile amplitude change does not demand selective spatial attention, but detecting the absence of such a change does.

The present model proposes a single mechanism to account for these asymmetrical attentional demands. Populations of detectors sensitive to amplitude change are postulated to feed into a pooled activity unit, which takes a weighted sum of inputs from multiple fingertips. The probability of a correct response in two-interval forced-choice trials is computed in terms of the means and variances of detector firing rates, given an assumed distribution of detector thresholds. The weights of the inputs from each fingertip are chosen to maximize predicted overall task performance.

The model predicts probabilities that are close to the human performance data when detector thresholds are distributed log-normally and the firing rates across fingertips are highly correlated. The relative weighting of the fingertips affects the probability of detecting the absence, but not the presence, of an amplitude change. A similar model based on Treisman and Souther's (1985) Weber fraction model did not predict attentional asymmetry in all of the experimental conditions in which it was observed.

241.14

PREATTENTIVE versus ATTENTIVE TEXTURE PERCEPTION BY THE TACTILE SYSTEM K.Sathian and H.Burton, Department of Anatomy & Neurobiology and McDonnell Center for Higher Brain Function, Washington University School of Medicine, St. Louis, MO 63110. (Supported by NIDCD 00096.)

Human subjects stroked four gratings simultaneously with the 2d and 3d fingerpads of each hand, to detect the presence of a target grating. A tap to one of the four digits cued the target location with a probability of 0.8. Values of grating spatial parameters were chosen to keep overall performance around threshold (75% correct).

When the task was to detect (1) the *presence of a change in roughness* along the grating, performance did not differ significantly on validly and invalidly cued trials, suggesting that this target "pops out" **preattentively**. A significant cueing effect, suggesting that **attention** (spatially directed by the cue) is required for target detection, was found when the task was to detect (2) the *absence of a change in roughness* along the grating, (3) the *direction* of such a change or (4) a grating that *differed in roughness* from the others. In tasks (1) & (2), the results were similar whether groove width or ridge width was varied.

Thus, *texture change* is a feature that appears to be processed preferentially by the preattentive tactile system. Such processing is not restricted to surface spatial parameters (e.g. groove width) that are encoded in peripheral afferent firing rate.

241.15

VISUAL ATTENTION DISORDERS:NEUROPATHOLOGY AND DISSOCIATION Dr.D.Hodgkin* (Spon: Brain Research Association) Physiology Department, Cambridge Univ. Cambridge,CB2 3EG

Disorders of visual attention are a frequent,transient or permanent consequence of damage to several brain areas, principally right parietal,occipital and frontal cortex. Information processing techniques (Triesman,A. and Gormican S.Psychological Revue,95:15,1988)were used to examine five patients suffering from right parietal/occipital damage. They demonstrate that pre-attentive and focal attentional processes can break down independently and in parallel, rather than sequentially and dependently, as much current theory predicts. These separable sub processes also recover at different rates. A long term study of one visual agnosic demonstrates quantitative and qualitative differences in the rate and degree of recovery of pre-attentive and focal processes. A complementary study examined the physical basis for such variations in neural plasticity. Early lesions, associated with improved behavioral recovery, reduced astro-cytic scarring and dopamine production, also expressed increased levels of Heat Shock Protein which has a generally protective, developmental function in many biological systems. The combined information processing and physiological studies suggest that different attentional subsystems may vary not only in their function,anatomy and neurochemistry but also in their resistance to damage. Ongoing experiments are examining differences in their developmental profiles.

241.16

INHIBITORY SPATIAL TAGGING AND VISUAL ATTENTION: CONTRIBUTION OF PREFRONTAL CORTEX AND BASAL GANGLIA. A. Henik*, Ben-Gurnion University, Israel. R.D. Rafal and R.T. Knight, UC Davis, Martinez VAMC, CA 94553.

A peripheral visual signal has a biphasic effect on attention: the initial orienting is superseded by an inhibition of return which inhibits detection at that location. This mechanism favors novelty in visual scanning and has been shown to be generated through extrageniculate pathways. We measured inhibition of return in patients with unilateral lesions of prefrontal cortex or prefrontal cortex plus basal ganglia. Subjects made reaction time (RT) responses to targets appearing in the left or right field. Each trial began with the presentation of a flashing box (precue) in the periphery followed by a target which appeared with equal probability either at the cued location or in the opposite field. Inhibition of return was measured as a relative slowing of RT to targets at precued locations. Patients with lesions of prefrontal cortex showed deficient inhibition of return. Lesions involving both prefrontal cortex and striatum restored inhibition of return in the field contralateral to the lesion. We conclude that the inhibitory visual tagging system involves a neural circuit including superior colliculus, prefrontal cortex and basal ganglia.

241.17

SELECTIVE CORTICAL ENHANCEMENT EFFECTS PRODUCED BY A THALAMIC CIRCUIT MODEL BASED ON CURRENT NEUROANATOMICAL FINDINGS. David LaBerge, Vincent Brown, and Marc Carter, Department of Cognitive Science, University of California, Irvine, CA 92717.

PET and single-cell studies have suggested that a principal nucleus of the dorsal thalamus (pulvinar) may be involved in selectively attending to locations in visual space. We ask how the neural circuitry of a typical thalamic nucleus might operate to control selection in a cortical area to which it projects. A model of a thalamic circuit was constructed based on current neuroanatomical findings, and afferent inputs were compared with thalamocortical outputs. The neurons of the circuit model were afferent cells, thalamocortical (relay) cells, inhibitory interneurons, inhibitory reticular nucleus cells, and reciprocally connected corticothalamic cells. The net input to each cell was transformed by a function with a minimum value of zero and an asymptotic value based on current estimates of the maximum firing rate of the particular type of cell.

An afferent input (e.g., from V1) to the circuit was introduced in a target cell (in the center) and flanker cells (in the surround) such that the output of the center cell was initially slightly greater than the output of a surround cell. Following afferent onset, thalamocortical and corticothalamic center and surround cells quickly increased their firing rates, but the center cells increased their rates considerably faster than the surround cell. This thalamic center-surround enhancement effect was also produced reciprocally when the corticothalamic cells provided the sole input to the thalamic circuit. It appears that an algorithm exhibited by this thalamic circuit model can augment center-surround differences originating in cells at both the input and output ends of the circuit.

241.18

ATTENTIONAL DEFICITS IN INDIVIDUALS WITH CANCER.* B. Cimprich. The University of Michigan, Ann Arbor, MI 48109

Attentional fatigue typically follows intense exertion of mental effort and is manifested as a decline in the capacity to direct attention, i.e., inhibit competing stimuli. Despite intense mental demands associated with diagnosis of cancer, little is known about the problem of attentional fatigue. Sixteen women with localized breast cancer and no history of cognitive disorders were studied to determine whether there was a discernible pattern of decline in the capacity to direct attention over the initial phase of treatment. Repeated measures were obtained at 3, 18, 60, and 90 days following breast surgery. Measures included Digit Span Forward (DSF) and Backward (DSB) and Necker Cube Pattern Control (NCPC), a newly developed test requiring active inhibition of a competing pattern stimulus. At 3 days post-surgery, prior to receiving chemotherapy or radiation therapy, 44% of the subjects showed marginal or impaired performance on DSF ($\leq$6) and DSB ($\leq$4). Mean scores in DSF did not change significantly over time. Also, mean scores in DSB did not improve significantly until 60 days following surgery (X $\pm$ SD: 4.5 $\pm$ 1 vs 5.4 $\pm$ 1.5; 18 and 60 days, respectively; p=.02). Finally, there was a significant loss in NCPC at 90 days (X% Reduction in NCPC $\pm$ SD: 17% $\pm$ 35) compared to 3 days (34% $\pm$ 26; p=.03) and 18 days (37% $\pm$ 25; p=.02) post-surgery. This study provides initial evidence of attentional deficits in individuals with cancer. The sustained decline in attentional capacity observed following surgery for breast cancer most likely reflects fatigue of directed attention.

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241.19

NEUROPSYCHOLOGICAL AND PSYCHOACOUSTIC EFFECTS OF BILATERAL LESIONS IN HUMAN AUDITORY CORTEX. S.W. Anderson, H. Damasio, D.A. Robin, & L. Krain*. Div. of Behav. Neurology & Cognitive Neuroscience, U. Iowa College of Medicine, Iowa City, IA 52242.

There has been limited study of the effects of bilateral damage to auditory cortices in humans. Here we report on four patients in whom the primary and association auditory cortices were damaged bilaterally by stroke (demonstrated by analysis of magnetic resonance, computerized tomography, and emission tomography). The striking feature in all cases was a severe impairment in the recognition of speech and environmental sounds, despite normal audiometric thresholds for sound detection. More detailed psychoacoustic analysis of one case revealed impaired reaction time to auditory stimuli, abnormal gap detection, and impairments of frequency discrimination and pitch matching. Sound localization, brain stem auditory evoked potentials, and recognition in visual and tactile modalities were normal. Repeated presentation of over 100 sounds revealed that 56% were never recognized, 14% were reliably recognized, and 30% were recognized inconsistently. These findings indicate that bilateral damage to human auditory cortex may not disrupt localization of sound or perception of volume, but severely distorts auditory signals at an early perceptual level.

241.20

IMPAIRED PERCEPTION OF RELATIVE PURE TONE PITCH FOLLOWING BILATERAL LESIONS OF AUDITORY CORTEX IN MAN M.J. Tramo, Program in Cognitive Neuroscience, Dartmouth-Hitchcock Med Ctr, Hanover, NH 03756

While neurons within the primary auditory fields of the cat (Woolsey & Walzl 1942; Merzenich et al. 1975) and monkey (Merzenich & Brugge 1973; Imig et al. 1977) demonstrate frequency-specific responses to pure tones and topographical organization of frequency receptive fields, the results of lesion studies in animals have been interpreted as evidence that the discrimination of simple pitch differences is subserved by subcortical components of the central auditory system (Neff et al. 1975). However, recent data obtained following bilateral ablations of auditory cortex in monkeys have challenged the notion that "elementary" psychoacoustic functions do not rely upon the integrity of auditory cortex (Heffner & Heffner 1986). The examination of simple pitch perception following bilateral lesions of auditory cortex in man has been limited by the rarity of their natural occurrence, particularly in young, alert patients in whom peripheral hearing loss and/or dementia do not cloud experimental observations.

Data from a case with bilateral lesions involving all of Heschl's gyri and parts of the superior temporal gyri are presented. The patient is a thirty year old man who seven years ago presented with deafness following his second middle cerebral artery distribution stroke secondary to cardiogenic emboli (Mendez & Geehan 1988). Pure tone sensation thresholds at 250-8000cps (duration=500msec) were normal at the time of the present observations (Tramo et al. 1990). However, on a standardized test requiring relative pitch judgments of two pure tones differing by 2-17cps (frequency centered at 500cps, loudness=35-40dB above threshold, duration=600msec, ISI<600msec), he scored in the 15th percentile (Seashore et al. 1960). When the two tones differed by 2-4cps, his performance fell to below chance (7/18); when the tones differed by 5-17cps, he scored well above chance (26/32). These results suggest that fine-grained discriminations of relative pure tone pitch do rely upon the integrity of auditory cortex, and that the size of the frequency difference between the standard and comparison tones used in an experiment is likely to influence the intepretation of structure-function relationships governing simple pitch perception. Previous studies in the animal and clinical literature are reviewed in this light. (Supported by NIH 5PO1 NS17778-08)

DRUGS OF ABUSE: COCAINE AND OTHERS

242.1

NEUROANATOMICAL STUDIES OF COCAINE TREATMENT ON DOPAMINERGIC NEURONS: TH AND GFAP IMMUNOCYTOCHEMICAL STUDIES. X.L. Chen and M. Gupta, Dept. of Anatomical Sci. & Neurobiology, Univ. Louisville Sch. Med., Louisville, KY 40292.

Cocaine is a psychomotor stimulant that appears to exert its action by blockade of the reuptake of catecholamines resulting in increased dopamine and norepinephrine at synaptic levels. Vast majority of studies on cocaine research have focused on its behavioral effects and very little attention has been paid to its neuroanatomical actions. The present studies were undertaken to investigate the long-term effects of cocaine on the dopaminergic systems in mice and on gliosis in the striatum and nucleus accumbens following neuronal injury in the midbrain nuclei. Young adult male C57BL/6 mice at 2-3 months of age were given daily injections of cocaine HCl (10 or 20mg/kg) or saline vehicle for 9 consecutive days. The animals were sacrificed three weeks later and brains were processed immunocytochemically for tyrosine hydroxylase (TH) and Glial Acidic Fibrillary Protein (GFAP). The results of these studies show that cocaine treatment produced a siginificant decrease in the number of TH-positive neurons in the substantia nigra. The dopaminergic neurons of the ventral tegmental area appeared to be reduced in a dose-dependent manner but were not significantly different from controls. Quantitative analysis of the astrocytic response using GFAP immunocytochemistry in the striatum and nucleus accumbens is in progress. These data demonstrate that chronic coacine treatment produces loss of dopaminergic neurons in the substantia nigra. Supported by USPHS grant R29 NS24291 to MG.

242.2

CAFFEINE PREEXPOSURE SENSITIZES RATS TO THE MOTOR ACTIVATING AND REWARD PROPERTIES OF COCAINE B.A. Horger and S. Schenk, Dept. Psychol., Texas A&M Univ., College Station, TX., 77843.

Rats were pretreated with 10 daily injections of either caffeine (20 mg/kg, IP) or the saline vehicle. Thereafter, the motor activating effects of cocaine (10 mg/kg, IP) or the acquisition of intravenous cocaine self-administration (0.25 mg/kg/infusion) were assessed. The caffeine pretreated rats were more sensitive to the motor activating effects of cocaine. They exhibited higher cocaine-induced activity at 10 and 20 min following the drug injection when compared to the saline pretreated rats. The caffeine preexposed rats also responded at higher rates for infusions of cocaine. Non-reinforced responding was not similarly increased thus ruling out the possibility of non-specific increases in activity as the basis for the enhanced responding for cocaine infusions. These data suggested a shift to the left in the dose/response curve (i.e. sensitization) for both behavioral actions of cocaine following caffeine pretreatment.

242.3

CAFFEINE FACILITATES RECALL OF NARRATIVE AND EXPOSITORY PROSE IN ORAL-CONTRACEPTIVE-FREE YOUNG WOMEN. B. E. Beckwith, T. V. Petros, and J. Brouse*. Psychol. Dept., Univ. of North Dakota, Box 7187, Grand Forks, ND 58202.

Although several studies have demonstrated that caffeine has a complex effect on memory for simple stimulus materials as a function of gender, time of day, and impulsivity, no published study has explored the effect of caffeine on recall of complex stimulus materials. In the present study, female subjects during the first 5 days of their menstrual cycle were administered 0, 2, or 4 mg/kg of caffeine and, 30 mins. later, asked to read and to immediately recall three expository and three narrative passages. Subjects receiving either 2 or 4 mg/kg of caffeine recalled a greater number of idea units of high importance than subjects given the placebo. Furthermore, there was a facilitation in recall of idea units of low importance as a function of dose of caffeine and no effect of caffeine on recall of idea units of medium importance. It appears that caffeine improves memory for essential and trivial details of narrative and expository prose when administered to healthy young women during the menstrual phase of their cycle.

242.4

ANXIOGENIC STIMULI PRODUCED BY COCAINE ARE POTENTIATED DURING PROTRACTED WITHDRAWAL FROM CONCURRENTLY ADMINISTERED COCAINE AND ETHANOL IN RATS. P.L. Prather and H. Lal, Dept. of Pharmacology, Texas College of Osteopathic Medicine, Ft. Worth, TX 76107.

Cocaine abusers often experience persistent anxiety and panic attacks even after discontinuation of cocaine abuse. These experiences are likely to promote abuse of drugs with anxiolytic properties. Thus, ethanol, a mild anxiolytic, is often co-abused with cocaine. At present, the neurobiological consequences of the coabuse of ethanol with cocaine are not known. Therefore, this study was undertaken to examine the anxiogenic effects of cocaine in subjects chronically treated with otherwise non-addicting doses of both ethanol and cocaine. Rats were trained to respond on one lever following an injection of saline and the alternate lever after the anxiogenic drug, pentylenetetrazol (PTZ) (20 mg/kg), according to a FR10 schedule of food reinforcement. Animals were then treated for 5 days with either cocaine (20 mg/kg, ip) three times daily, ethanol (daily 100 ml of liquid diet containing ethanol, 2.25% w/v), or a combination of both. The ability of cocaine to produce a PTZ-like stimulus was determined 60 h after the last drug treatment. Cocaine produced a PTZ-like stimulus only in a small number of animals (30% at 10mg/kg) treated chronically with either cocaine or ethanol. In contrast, concurrent pretreatment with cocaine and ethanol resulted in dose-dependent and full elicitation of a PTZ-like stimulus by cocaine, with over 85% of the rats selecting the PTZ lever at the 10 mg/kg dose. These results indicate that during protracted withdrawal from co-administration of cocaine and ethanol, the anxiogenic stimuli produced by cocaine are markedly enhanced. (Supported by NIAAA grant AA06890)

242.5

CLINICAL NEUROBIOLOGY OF COCAINE WITHDRAWAL. L.H. Price, M.D., C.J. McDougle, M.D., J. Palumbo, M.D., T.R. Kosten, M.D., H.D. Kleber, M.D., G.R. Heninger, M.D., Yale Univ. Dept. of Psychiatry, 34 Park St., New Haven, CT 06519.

Three phases of withdrawal have been described in chronic cocaine abusers (Phase 1: Crash, Phase 2: Withdrawal, Phase 3: Extinction). Pharmacological challenge with L-DOPA 250 mg/carbidopa 25 mg (Sinemet) was used to investigate DA function during phases 1 and 2 of cocaine withdrawal in humans. Methods: Six male inpatients with DSM-III-R Cocaine Dependence received 1.5 mg/kg of cocaine p.o. 3X/day for three consecutive days (maintenance treatment period 1 (MTP1)). Subjects then received placebo cocaine 3X/day for nine consecutive days (maintenance treatment period 2 (MTP2)). Subjects and ward staff were blind to the content of the cocaine capsules. Each subject received randomized challenge tests of active or placebo Sinemet on the two days following MTP1 (Phase 1 withdrawal), and one week following MTP2 (Phase 2 withdrawal). Plasma prolactin (PRL), growth hormone (GH), MHPG, and HVA (all reported as ng/ml), were obtained following each challenge. Results: PRL (N=6) decreased following Sinemet during challenge sets 1 (mean±SD, -3.6±1.5 vs -1.1±1.4, $p<0.04$) and 2 (-4.2±1.7 vs -1.0±1.1, $p<0.001$) compared to placebo. Change in PRL was not different between challenge sets 1 and 2. GH (N=6) increased following Sinemet during challenge set 1 (26.2±15.4 vs 3.1±4.4, $p<0.02$) but not during set 2, compared to placebo. There was a trend toward increased GH response to Sinemet compared to placebo between challenge sets 1 and 2 (23.2±15.7 vs 6.3±11.2, $p<0.08$). MHPG (N=6) increased following Sinemet during challenge sets 1 (2.7±1.0 vs 1.1±1.0, $p<0.0001$) and 2 (2.3±1.4 vs 1.0±1.0, $p<0.005$) compared to placebo. Change in MHPG was not different between challenge sets 1 and 2. HVA (N=6) increased following Sinemet during challenge sets 1 (367.2±99.0 vs -0.2±1.2, $p<0.0001$) and 2 (330.2±120.4 vs 0.2±3.4, $p<0.001$) compared to placebo. There was also an increased HVA response to Sinemet compared to placebo between challenge sets 1 and 2 (367.4±99.5 vs 330.0±119.8, $p<0.05$). Conclusion: These data suggest that Phase 1 (Crash) of cocaine withdrawal may be associated with altered DA metabolism compared to Phase 2 (Withdrawal). Behavioral and neurobiological data will be presented.

242.6

ANXIETY OR STRESS DUE TO COCAINE. N.E. Goeders, G.F. Guerin, X.M. Yang* and A.J. Dunn. Dept. of Pharmacology & Therapeutics, LSU Medical Center, Shreveport, LA 71130.

Initial cocaine use usually produces profound subjective feelings of well-being and a decrease in anxiety in humans. However, some of the major symptoms reported during cocaine withdrawal include severe agitation, anxiety, restlessness and depression. In rats, cocaine increases plasma ACTH, β-endorphin and corticosterone, probably through a corticotropin-releasing factor (CRF)-related mechanism. Recent data from our laboratory demonstrated that chronic cocaine administration results in decreases in CRF binding sites primarily in brain areas associated with the mesolimbic-mesocortical dopaminergic system in rats. The experiments described below were designed to investigate the behavioral and physiological correlates of cocaine administration and the potential effects of exogenous CRF on cocaine reinforcement in rats. In the first experiment, the anxiogenic effects of cocaine were investigated using the defensive withdrawal paradigm in rats. Chronic (7 or 14 days) exposure to cocaine (20 mg/kg, ip) resulted in significant increases in the latency to emerge from a small enclosed chamber in an open field and in the total time spent in the chamber, a stress- or anxiety-related response. Plasma corticosterone concentrations were also increased 81% in the cocaine-treated animals compared to saline controls. In the second experiment, CRF pretreatment (20 to 100 ng, icv) produced dose-related changes in intravenous cocaine self-administration in rats suggesting that drug-intake can be influenced by the pharmacological introduction of a "stress-like" state in rats. Anxiety mediated via CRF-related mechanisms may therefore be involved in some of the behavioral and pharmacological effects of cocaine.

[Supported by grants NS27283 from NINCDS (AJD) and DA04293 from NIDA (NEG) and a LSUMC fellowship (XMY)]

242.7

TREATMENT OF COCAINE WITHDRAWAL WITH BUSPIRONE. A. J. Giannini, R.H. Loiselle, and D. J. Folts*. Department of Psychiatry, Ohio State University, Northeastern Ohio Universities College of Medicine, P. O. Box 2169, Youngstown, Ohio 44504

Chronic abuse of cocaine leads to dopamine and norepinephrine and possible serotonin depletion. Buspirone, an anxiolytic, enhances dopaminergic and noradrenergic firing but suppresses serotonergic firing. Twenty cocaine abusers volunteered to be detoxified in this study. Ten received buspirone 10 mg. t.i.d., p.o. and 10 received placebo t.i.d., p.o. Both groups were studied for 30 days. After the fifth day, the buspirone group reported significantly less symptoms ($p < .05$). Measurements on the 10th ($p < .05$), 15th ($p < .02$), 20th ($p < .01$), 25th ($p < .001$) and 30th day ($p < .001$) showed that this rate of improvement continued. All symptoms were evaluated by the BPRS scale.

242.8

COCAINE TOXICITY: DISTINCT NEUROTRANSMITTER SYSTEMS ARE ASSOCIATED WITH SEIZURES AND DEATH. M.C. Ritz* and F.R. George. NIDA-ARC, Baltimore, MD 21224

Cocaine use is increasingly associated with serious toxic effects, including seizures and lethality. This study identifies brain receptors which appear to mediate these effects of cocaine and pharmacologically related compounds. The seizurgenic ED_{50} values and LD_{50} values for death within 15 minutes postinjection were determined concurrently for each drug in C57Bl/6J mice. Binding potencies of all test compounds were assessed at dopamine, norepinephrine and serotonin transporter sites and at M_1 and M_2 cholinergic and sigma opiate receptor sites. Multiple regression analyses determined the proportion of influence of each of these receptors on seizurgenesis or lethality. The serotonin transporter is the primary binding site associated with the potency of cocaine and related drugs in seizurgenesis. However, drug binding at either sigma, M_1 or M_2 receptors appears to attenuate this effect. In contrast, drug binding to the dopamine transporter appears to be associated with the lethal potency of these drugs, while muscarinic receptors appear to play a secondary role in lethality. These biochemical findings are supported by pharmacological potentiation or antagonism of seizures by serotonin uptake inhibition or blockade of $5HT_2$ receptors, respectively. In contrast, cocaine lethality is antagonized specifically by D_1 and M_1 blockers.

242.9

ELECTROPHYSIOLOGICAL ACTIONS OF COCAINE ON RAT MEDIAL PREFRONTAL CORTICAL NEURONS. S.S. Jahromi and P.L. Carlen. Playfair Neuroscience Unit, Addiction Research Foundation, Departments of Physiology and Medicine (Neurology), The Toronto Western Hospital, University of Toronto, Toronto, Ontario, M5T 2S8.

It is well known that animals self-administer cocaine into the medial prefrontal cortex (Goeders, N.E. and Smith, J.E., NIDA Res. Monogr., 55, 132-7). We perfused brain slices from the medial prefrontal cortex with cocaine hydrochloride at concentrations of 1-20 uM and recorded intracellularly from the deeper layers of the cortex with 3 M potassium methylsulphate electrodes in over 20 neurons.

In the majority of cells, cocaine reduced both evoked excitatory and inhibitory postsynaptic potentials. This effect was reversible with washout. In some cells, there was a delayed depolarization on the falling phase of the EPSP with exposure to cocaine. At 10 and 20 uM, cocaine often increased the neuronal input resistance and increased the membrane threshold for spike generation. In most cells, spike frequency adaptation was either unaffected or increased, and the resting membrane potential was unchanged. The spike train afterhyperpolarization was usually reduced (10-20%) by cocaine. These preliminary results indicate both excitatory and inhibitory effects of cocaine on rat medial prefrontal cortical neurons. Supported by the MRC and The Hospital for Sick Children Foundation.

242.10

CHRONIC COCAINE AND EEG POWER SPECTRA: EFFECTS OF DRUG HISTORY AND PREDICTORS OF LETHALITY G.T. Livezey and S.B. Sparber, Dept. of Pharmacology, University of Minnesota, Minneapolis, MN 55455

The effects of cocaine, at doses used to treat rats during their last trimester of pregnancy in a perinatal drug exposure study, were studied in nonpregnant females for its residual, acute and chronic actions upon the EEG. The effects of the high dose readministered 2 months after initial treatment were studied in order to determine if cocaine challenge was necessary to unmask otherwise undetectable residual effects (e.g. kindling). Twelve SD rats were given either water, 15 mg or 45 mg cocaine/kg, p.o. 3 times a day (9AM, 1PM, 5PM) for 7 days (History). Two months later all 12 were implanted with radiotransmitters for sampling EEG. Subsequent treatment for all 12 consisted of water for 19 days and cocaine (45 mg/kgX3) for 19 days. Six of the 12 animals died between days 6 and 13 of cocaine gavage (2 from each History group). EEG was analyzed from one baseline day (prior to water Rx), one water day, one early cocaine day (day2) and the last day of cocaine upon which all animals were alive (day5). Five, 30sec EEG samples from 1 hr before morning gavage and 1 hr after morning gavage were used to derive averaged power spectra (1-100Hz). Power was analyzed as total (1-100Hz) or in 20Hz bins. The immediate response was measured between EEG samples after water and after cocaine (days 2,5).Animals originally treated with 45 or 135mg cocaine/kg/day, in divided doses, did not show evidence of a residual effect upon total power (1-100Hz) or upon distributed power (any 20Hz bin). However, treatment with cocaine after implantation of radiotransmitters caused a significant decrease in total power (1-100Hz) between the post-water gavage value and the post-cocaine value on the 5th treatment day. Cocaine treatment for 2 days failed to cause a significant change in total power. Bin 21-40Hz showed a significant reduction and bins 61-80Hz and 81-100Hz showed signnificant increases in power after both 2 and 5 days of cocaine. Bin 1-20Hz showed decreased power only after 5 days cocaine. Total power (1-100Hz) prior to starting water gavage, was increased and 61-80Hz activity was decreased for rats that did not survive chronic cocaine treatment, compared to survivors.Thus, predictors of chronic cocaine lethality may be evident prior to chronic treatment. Supported by USPHS DA 04979 , T32DA07097 and NS 23289.

242.11

AMYGDALA CENTRAL NUCLEUS NEURONAL DISCHARGE FOLLOWING LOCALIZED MICROINJECTION OF COCAINE. H. Ni, J.X. Zhang, R.K. Harper and R.M. Harper. Brain Research Institute and Department of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024.

The central nucleus of the amygdala (ACE) projects heavily to cardiac and respiratory regions of the parabrachial pons, periaqueductal gray, and nucleus of the solitary tract in the cat; electrical stimulation and cold blockade of the ACE exert profound respiratory and cardiac changes. Intravenous and intraventricular cocaine administration lead to pronounced tachypnea and tachycardia. Using extracellular single-cell recording and microinjection techniques, we examined the effects of cocaine (100μg in 0.2μL) on activity of ACE neurons in chronically instrumented freely moving cats and used artificial CSF as a control. Thirteen single neurons were recorded during a baseline sleep-waking period. Six neurons slowed profoundly in response to cocaine administration relative to the slowest rates in any baseline condition; two neurons were depressed for 2 hours postadministration. One neuron increased its discharge rate by a factor of 10; discharge of the remaining cells was unchanged. A portion of the cocaine effect on cardiac and respiratory patterning may be mediated by ACE neurons.

Supported by R01-DA04913.

242.12

EFFECTS OF IV COCAINE ON REGIONAL CEREBRAL BLOOD FLOW IN THE RAT. E.A.Stein and S.A.Fuller*. Department of Psychiatry, Medical College of Wisconsin, Milwaukee, WI 53226.

Cocaine is a potent CNS stimulant whose primary mechanism of action is reuptake inhibition of the monoamines. Cocaine's reinforcing and motor activating properties are thought to be mediated primarily by dopamine and binding to components of the dopamine transporter has been observed. While metabolic mapping studies have recently been performed, due to cocaine's short duration of action and complex behavioral symptoms, we wanted to employ a short time-window marker to ultimately identify neuronal structures involved in different aspects of its pharmacologic profile. We now report the effects of cocaine on regional cerebral blood flow in the rat. Conscious rats were injected IV with either 0, 0.1, 0.5, 1 or 5 mg/kg cocaine, 1 min prior to radiolabel. [^{14}C]Iodoantipyrene was used to determine rCBF according to the method of Sakurada et al. Analyses of variance indicated that all 58 measured structures were altered by cocaine. Biphasic responses were seen at the lowest dose (0.1mg/kg), with such limbic structures as the anterior, lateral, ventomedial and dorsomedial hypothalamus and MD thalamus depressed by this dose but activated, in a dose response fashion, by the two higher doses of cocaine. A second group of structures responded with a dose related activation including the nucleus accumbens, olfactory tubercle and LD thalamus at 0.5 mg/kg threshold. Higher threshold (1.0 mg/kg) effects were observed in such structures as the claustrum, frontal cortex and substantia nigra. Thus, not only does rCBF appear to be a sensitive measure of cocaine's effects, but as a brief temporal marker, may have significant advantages over other metabolic measures of early neuronal activity. (Supported by NIDA grant DA 05012).

242.13

RELATIONSHIP BETWEEN VENTRICLE/BRAIN RATIO, A MEASURE OF CEREBRAL ATROPHY, AND SUBJECTIVE RESPONSES TO INTRAVENOUS COCAINE IN HUMAN SUBSTANCE ABUSERS. M.J. Morgan, N.G. Cascella*, J.M. Stapleton, E.K. Shaya*, D.F. Wong and E.D. London, NIDA Addiction Research Center, Baltimore, MD 21224 and Johns Hopkins Medical Institutions, Baltimore, MD 21205.

The relationship between subjective responses to cocaine and the ventricle/brain ratio (VBR) (Synek, V. et al., Neurology, 26:231, 1976), measured by X-ray computed tomography, was examined in 8 human volunteers with histories of polydrug abuse. A negative rank correlation was found between a measure of the subjective effect of cocaine (40 mg, i.v.) and individual VBRs, ($p < 0.05$). This measure was the response to the question "How much do you feel the drug?", which was rated on a scale of 0 to 4 and was elicited by a "beep" prompt 1, 3, 4, 5, 7, 8, 9, and 10 min after cocaine injection. Negative rank correlations were also found between individual VBRs and subjective ratings of various drug effects on a visual analog scale. Subjects rated drug effects on this scale 30 min before and 30 min after i.v. administration of cocaine. The post-injection data were subtracted from pre-injection baselines to provide difference scores which were then examined for correlations with VBRs. The responses to the following questions yielded significant negative rank correlations: "How energetic did the drug make you feel?" ($p < 0.05$); "How much did you like the drug?" ($p < 0.05$); and "How much did you want to take the drug again?" ($p < 0.05$). These data suggest that the relative integrity of the brain may be a significant determinant of the stimulant and rewarding effects of cocaine in humans.

242.14

BEHAVIORAL CHARACTERIZATION OF PCP-INDUCED CHANGES IN RAT SOCIAL BEHAVIOR: A POSSIBLE MODEL OF SCHIZOPHRENIC SYMPTOMS. R.E. Steinpreis, C. Longyhore* and J.D. Salamone. Department of Psychology, University of Connecticut, Storrs, CT 06269-1020.

PCP-induced psychosis in humans is virtually indistinguishable from an acute episode of schizophrenia. While the motor effects of PCP in rats have been well described, the effects on social interaction could provide a more useful tool in the understanding of psychotic behavior. An "intruder" paradigm was employed in which rats were injected with either saline, 1.0, 2.0, or 4.0 mg/kg PCP, placed in a stable colony of three other rats, and observed for 40 minutes to quantify the PCP-induced changes in social behavior. PCP produced a syndrome of abnormal behaviors characterized mainly by social withdrawal. There was no effect at lower doses, but at 4.0 mg/kg there was a reduction in aggressive and approach behaviors, and an avoidance of social contact. A second study employed microdialysis methods to measure PCP-induced increases in dopamine (DA) activity. The neurochemical and behavioral effects of PCP in rats provide additional evidence that PCP may serve as a useful drug model of schizophrenic symptoms.

242.15

APOMORPHINE INDUCED HYPERLOCOMOTION: ELEMENTS OF PLASTICITY AND STEREOTYPY. Ernest N. Damianopoulos and Robert J. Carey VA Medical Center, Syracuse, NY 13210

Hyperlocomotion induced by repeated apomorphine stimulation in the intact rat was investigated using a Pavlovian conditioning protocol. Animals were administered apomorphine (2.0 mg/kg SC) daily for 7 days either paired or unpaired with a 10 min test environment placement. Direct recording of distance traversed (m) showed a gradual development of hyperlocomotion in response to repeated apomorphine stimulation but only in the animals of the paired treatment group. Locomotion rotation patterns, grouped into four categories of diameter size, were also analyzed. On Day 1, animals of both the paired and unpaired treatment groups exhibited equal distribution of rotation frequency across all four categories of diameter size. This pattern remained stable in the unpaired group. In the paired animals, however, a new pattern of rotation emerged by Day 7 which was a shift to a highly skewed distribution of rotations toward the highest possible diameter size. This unique pattern of rotation was retained after a 7-day drug withdrawal period but was exhibited only under drug test conditions. The results were interpreted as showing a learned, environment induced effect on the unconditioned drug response to apomorphine.

242.16

GENETIC INFLUENCES ON NICOTINE TOLERANCE EVALUATED BY CONTINUOUS MONITORING OF RESPONSIVENESS. M.J. Marks, S.F. Robinson, J.R. Pauly and A.C. Collins. Institute for Behavioral Genetics, University of Colorado, Boulder, CO.

The ability of mice to develop tolerance to the effects of nicotine is influenced by the genotype of the animal. In general, those mice initially more sensitive to the effects of nicotine require chronic treatment with lower doses to induce tolerance. To test the hypothesis that nicotine must produce a physiological effect before tolerance develops, mice from several inbred strains were implanted with transmitters (Mini-Mitter) to measure body temperature and activity in their home cages before and during nicotine treatment. Nicotine was administered by intravenous infusion. Tolerance was assessed by administration of a pulse of drug once each day. The relative sensitivity of the mouse strains to the effects of nicotine were similar to that measured after acute injection of drug (C57BL/6>DBA/2>BUB/Bn). Those strains that were affected by the drug were more likely to develop tolerance to the effects of nicotine. The extent of tolerance development was also influenced by the dosage and timing of drug administration. The results indicate that the magnitude of tolerance development is closely related to the magnitude of initial effect both within and among strains.

(Supported by Grant DA03194 from NIDA).

242.17

NICOTINE MODULATES PROENKEPHALIN GENE EXPRESSION IN BRAIN AND PITUITARY. S.R. George, M. Kertesz* and J. Tsatsos*. Pharmacology Dept., University of Toronto, Toronto, Ontario, Canada M5S 1A8.

Nicotine effects in brain have been postulated to be mediated through opioid mechanisms, at least in part. In the present study, the effect of nicotine administration on proenkephalin (PE) gene expression in brain and pituitary were examined. Male Sprague-Dawley rats were housed in environmental rooms and injected s.c. with nicotine 1 mg/kg/day or saline vehicle for varying lengths of time. Animals were sacrificed and brain regions analyzed for PE mRNA, free Met-enkephalin and cryptic Met-enkephalin as an index of PE peptide. Met-enkephalin was measured by radioimmunoassay; cryptic levels by sequential enzymatic digestion with trypsin and carboxypeptidase B, followed by RIA. PE mRNA was detected by Northern blotting with hybridization of a 32P-labelled 935 bp cDNA probe. Nicotine treatment resulted in a decrease of free Met-enkephalin peptide in striatum with no change in the levels of cryptic Met-enkephalin and an increase in PE mRNA. Met-enkephalin levels in medulla-pons and anterior pituitary were increased, with no apparent change in PE mRNA. In neurointermediate pituitary there was a decrease in the ratio of basal to cryptic Met-enkephalin and a decrease in PE mRNA. These results suggest that nicotine alters proenkephalin gene transcription and proenkephalin peptide processing in various brain regions.

242.18

CONDITIONED PLACE PREFERENCE (CPP) INDUCED BY VENTRAL TEGMENTAL AREA (VTA) INJECTIONS OF THE NICOTINIC AGONIST CYTISINE. E. Museo* and R. A. Wise. Center for Studies in Behavioral Neurobiology, Department of Psychology, Concordia University, Montreal, Canada, H3G 1M8.

Male Long-Evans rats were given intracerebral injections of the nicotinic agonist cytisine (10 nmol/0.5 μL/side) when placed in one side of a balanced, 3-compartment, CPP apparatus. These injections alternated with saline injections that were paired with the opposite compartment. Four pairings of each were given. Animals given VTA injections (n=7) subsequently spent significantly more time in the cytisine-paired compartment than in the saline-paired compartment. Animals receiving cytisine injections dorsal to the VTA showed no such CPP. Nicotinic receptors are reported to be on dopaminergic cells in the VTA, and these cells are implicated in the CPP induced by morphine and amphetamine. The present data raise the possibility that these cells also play a role in the CPP established by systemic nicotine.

242.19

PHENCYCLIDINE, BUT NOT MK-801, PRODUCES INCREASES IN EXTRACELLULAR DOPAMINE LEVELS AS ASSESSED BY *IN VIVO* MICRODIALYSIS. C.B. Hubner and A. Pert. BPB/NIMH, Bethesda, MD 20892.

Both phencyclidine (PCP) and MK-801 produce behavioral effects such as locomotor stimulation which suggest involvement of the dopamine system. The purpose of this study was to evaluate the effects of these two compounds on extracelluar DA using *in vivo* microdialysis. Using a cumulative dosing regimen, the effect of systemically administered PCP (3.0-30.0 mg/kg) on extracellular DA levels in the striatum, nucleus accumbens, and frontal cortex of the rat were determined. When compared with vehicle, PCP produced a significant dose-dependent increase in extracellular DA levels in all three brain regions tested. The removal of Ca^{2+} from, and the addition of Mg^{++} to, the dialysate significantly attenuated the increases in dopamine overflow induced by PCP, suggesting that the dopaminergic effects of PCP are due to an exocytotic process. These effects of PCP could be mediated either through its actions at the NMDA receptor or through its effects at the DA uptake site. MK-801 is a potent and selective non-competitive NMDA antagonist which has little affinity for the DA uptake site and a behavioral profile similar to PCP. Systemic injections of MK-801 (0.1-3.0 mg/kg) did not produce any increases in extracellular DA in the nucleus accumbens. These findings suggest that blockade of NMDA function is not sufficient to account for increases in DA produced by PCP. In addition, it does not appear that the locomotor stimulation produced by MK-801 is determined through DA activation.

242.20

PERSEVERATIVE BEHAVIOR IN THE RADIAL ARM MAZE IS THE AUGMENTED BY DRUGS OF ABUSE. E.A. Loh, A. Smith* and D.C.S. Roberts Department of Psychology, Carleton University, Ottawa, Canada K1S 5B6.

The hypothesis that drugs of abuse induce perseverative patterns of foraging behavior was investigated. We have previously shown that the pattern of exploration for food becomes perseverative following injection of nicotine, amphetamine, cocaine, heroin, alcohol or diazepam. In the present experiment we tested two centrally acting drugs known to be without abuse potential - haloperidol and scopolamine. Food deprived rats were trained to obtain food positioned at the ends of an eight arm radial maze. The food cup was rebaited each time the animal left the arm; thus every arm entry was reinforced. In contrast to drugs of abuse, we found that haloperidol and scopolamine decrease the perseverative nature of the foraging pattern. We conclude that an examination of the effects of drugs on foraging behavior may be a useful method of evaluating drug-induced reinforcement. (Supported by M.R.C.).

DRUGS OF ABUSE: AMPHETAMINE

243.1

THE CHARACTERIZATION OF BRAIN PROTEINS IN AMPHETAMINE TREATED MICE BY GEL ELECTROPHORESIS. L.O. Chambers* and M.A. Blackshear. Dept. of Biological Sciences, Tennessee State University, Nashville, TN 37209-1561.

The toxic effects of amphetamine on crowded mouse behavior and brain polyribosomes have been previously reported (Blackshear et al., 1979). To further examine the effects of crowding on amphetamine-induced changes in protein synthesis, we studied the effects of amphetamine on brain proteins found in the pH 5 enzyme fraction and the polyribosomal fraction, the cellular components of protein synthesis. Swiss ICR male mice (20-26g) received a single injection of 20 mg/kg of dl-amphetamine and were crowded for thirty minutes. Control animals crowded in a similar manner received 0.01 ml/gm physiological saline. The whole brains (minus cerebellum) were dissected and the proteins in the pH 5 enzymes and polyribosomes were analyzed by polyacrylamide gel electrophoresis. The administration of amphetamine caused a decrease in the proteins contained in the pH 5 enzyme fraction and in the proteins contained in the polysome fraction in comparison to saline controls. These findings suggest that amphetamine acts post-translationally to inhibit protein synthesis. In addition, these findings suggest that amphetamine may act at more than one site to inhibit protein synthesis.

(Supported by NIH-RCMI Grant G12RR03033).

243.2

GENDER DIFFERENCES IN THE EFFECTS OF PRENATAL COCAINE EXPOSURE ON LOCOMOTOR ACTIVITY IN 21-22 DAY OLD RATS. H.E. Hughes, L. A. Freed, L. M. Donohue* and D. L. Dow-Edwards. Laboratory of Cerebral Metabolism, Department of Neurosurgery, SUNY Health Science Center, Brooklyn, N.Y., 11203.

Our lab has demonstrated that early postnatal cocaine exposure in female rats alters the adult behavioral response to amphetamine. The present study investigated locomotor activity in male and female weanling rats treated prenatally with cocaine. Pregnant rats were gastrically intubated with 30 or 60 mg/kg/day cocaine HCl or vehicle only during gestational days 8-22. Vehicle treated rats were pair-fed/watered to rats receiving 60 mg/kg cocaine. A non-treated control group was also maintained. At parturition, litters from all four groups were surrogate fostered. At 21 or 22 days of age, two offspring per litter were sequentially placed in a Digiscan Activity Monitor. Activity counts were collected in one minute intervals over a 15 min. baseline period. Subjects then received one of two doses of d-amphetamine sulfate sc followed by a 95 min. period of activity monitoring. The data suggest a gender difference in the baseline activity of control rats. Females traveled more distance than males. Baseline activity for both sexes in the 60 mg/kg group was similar to that of control males. Prenatal cocaine exposure appears to nullify the gender difference observed in control rats, with females displaying a "male-like" amount of locomotor activity. Whether prenatal cocaine exposure alters developing brain systems that directly underlie motor activity or whether it results in a masculinizing effect in females is unknown. Data indicating the effects of prenatal cocaine exposure on activity in response to amphetamine are forthcoming. Supported by ADAMHA grant #DA04118.

243.3

THE EFFECT OF COCAINE AND AMPHETAMINE ON THE MICRO-EVENTS OF UNCONDITIONED LOCOMOTOR BEHAVIOR. M.P. Paulus and M.A. Geyer. Dept Psychiatry, Lab of Biol Dynamics and Theoret Med, Univ California at San Diego, La Jolla, CA 92093.

Substances influencing central dopaminergic systems have been suggested to change motor behavior either towards varied locomotion or increased perseveration. To test the hypothesis that substances having similar neurochemical effects will yield similar changes in behavior, measures derived from the thermodynamic description of dynamical systems and from the description of point processes in physics were applied to locomotor paths recorded for 60 min in the Behavioral Pattern Monitor. Briefly, amphetamine (0.25 - 4 mg/kg, s.c.) and cocaine (2.5 - 40 mg/kg, i.p.) were injected 10 min prior testing. Both drugs increased locomotion dose-dependently. A global geometric descriptor, the spatial scaling exponent *d*, a global dynamic descriptor, the metric entropy of the rat locomotor sequences *h*, the distribution of geometric singularities *f(d)*, and the dynamic singularities *s(h)* were obtained from the rat locomotor paths. A wavelet transform was applied to the temporal sequence of measures to characterize the chronological order of drug-induced changes in the different local descriptors. The two substances produced different and dose-dependent changes in *d* and *h*. While amphetamine increased *h* without affecting *d*, cocaine, especially at high doses, decreased both *h* and *d*. The functions *f(d)* and *s(h)* specified particular changes induced by each drug in the distribution of local geometrical and dynamical scaling exponents. Specifically, amphetamine increases the frequencies of both probable and unpredictable sequences, cocaine increases the unpredictable and the highly predictable (perseverative) but not the probable sequences of micro-events. In conclusion, psychomotor stimulant drugs acting via dopaminergic systems lead to comparable increases in the amount of activity but have specific signatures in their effects on the structural and sequential characteristics of unconditioned locomotor behavior. Supported by DA06325.

243.4

THE EFFECTS OF REPEATED AMPHETAMINE AND VARIED OPEN-FIELD CONFIGURATION ON $\hat{\gamma}$, A PUTATIVE INDEX OF LOCOMOTOR STEREOTYPY. P.M. Kunko and K. Mueller. Dept. of Psych., Texas Christian Univ., Fort Worth, TX 76129.

Locomotor stereotypy, the tendency to repeat patterns of locomotion in a limited area of the environment, is an amphetamine (AM)-induced behavior which has recently drawn considerable attention. Attempts to quantify this behavior have resulted in a variety of procedures, including the gamma ($\hat{\gamma}$) statistic. Acute AM reliably produces a constellation of dose-dependent behaviors in rats. Changes in some of these behaviors also vary reliably following chronic AM administration. Two experiments were done to determine if changes in AM-induced locomotor stereotypy ($\hat{\gamma}$) following chronic AM administration are functionally related to changes in other AM-induced behaviors, or if $\hat{\gamma}$ is a procedural artifact. In experiment one, 12 daily injections of AM (1 or 2mg/kg) were given and behaviors were assessed on the first and last days. In experiment two, rats were injected with saline, caffeine, or AM prior to assessment in two different configurations of an open field. Suprisingly, locomotor stereotypy changed little over the time course of repeated AM, suggesting that AM-induced behaviors are mediated by different neurochemical mechanisms. Additionally, locomotor stereotypy does not appear to be an artifact of open field configuration, as $\hat{\gamma}$ scores from different configurations were highly correlated.

243.5

DUAL EFFECT OF AMPHETAMINE ON LOCOMOTION OF PONTINE-DAMAGED RATS. Rebecca M. Chesire. Univ. Hawaii-Manoa, Honolulu, HI 96822.

Damage of the nucleus reticularis tegmenti pontis (NRTP) can produce rapid forward locomotion that is not abolished by morphine, haloperidol or ethanol (1-6), indicating that the NRTP mediates some inhibitory locomotor effects of these drugs. A suggestion has been made that amphetamine (AMPH) might abolish NRTP damage-induced locomotion (7). This report describes both excitatory and inhibitory effects of AMPH on NRTP-damaged rats. 16 male Long-Evans hooded rats were given bilateral electrolytic lesions of the NRTP or used as controls (n=4). Between postop. days 1 & 57 they were pretested for amount and form of locomotion, injected once with 5 mg/kg d-AMPH sulfate, and retested at 10-80 min. Control rats showed rapid locomotion and eventual "trapping" in stereotyped movement (8,9). NRTP-damaged rats showed: (1) an initial increase in amount and rapidity of locomotion followed by a decrease or (2) a repetitive alternation between more rapid locomotion and loss of support. The results suggest that NRTP damage can partly block the increase in locomotion and stereotypy produced by AMPH, but that such effects are not unitary, and appear to be biphasic.

243.6

NEUROPHARMACOLOGICAL ASSESSMENT OF COCAINE-INDUCED CONDITIONED PLACE PREFERENCE USING INTRA-CRANIAL MICROINJECTIONS. **S.E. Hemby, G.H. Jones, J.B. Justice, Jr. & D.B. Neill; Departments of Psychology and Chemistry, Emory University, Atlanta, GA 30322**

Cocaine and amphetamine (AMPH) have both been suggested to exert their reinforcing and locomotor effects via nucleus accumbens (NACC) dopamine. Rats will self-administer AMPH directly into the NACC and intra-NACC infusions of AMPH produce locomotor activation as well as a conditioned place preference (CPP). However, although intra-NACC cocaine increases locomotor activity, it has been shown that rats will not self-administer cocaine into the NACC. Furthermore, 6-OHDA lesions of the NACC completely abolish IV AMPH self-administration whereas similar lesions merely attenuate IV self-administration of cocaine. This evidence suggests different mechanisms for AMPH and cocaine-induced reinforcement. The present studies were conducted to further delineate the neural substrates of cocaine-induced reinforcement using the CPP paradigm.

Male Wistar rats were implanted with bilateral guide cannula aimed at the NACC. Following pre-exposure, animals were assigned to either compartment of the CPP apparatus for drug pairing in a totally counterbalanced design. Rats received bilateral infusions of cocaine (12.5, 25, 50, or 100 μg/μl; n=10 per group) into the NACC and were immediately placed in the drug-paired compartment. These doses were chosen as they produce pronounced locomotor activation). Two additional groups of animals received either intra-accumbens d-AMPH (10 μg/μl; n=9) or IP cocaine (5 mg/kg; n=12). On alternate days rats received vehicle infusions and were placed in the opposite compartment. All animals were given two drug and two vehicle pairings.

Both the AMPH and IP cocaine groups exhibited a CPP for the drug-paired environment. However, no dose of intra-accumbens cocaine produced a place preference. These results are further evidence that the NACC may not be an essential substrate for cocaine reinforcement as measured by CPP.

243.7

THE ASSESSMENT OF THE ORGANIZING INFLUENCE OF AMPHETAMINE ON BEHAVIOR: INTERACTION BETWEEN PLACE PREFERENCE AND TASTE AVERSION. *A.L. Errico, D.V. Gauvin, & F.A. Holloway. Univ. of Oklahoma Health Sciences Center, Oklahoma City, OK 73190-3000.*

Some psychoactive drugs such as amphetamine have been found to induce conditioned taste aversions (CTAs) within a dose range similar to that which is self-administered by laboratory animals. Such findings have been aptly described as presenting an "apparent paradox" (Hunt & Amit, *Neurosci. & Biobeh. Rev., 11, 107-130, 1987*). The present study assessed the interaction between the associative "valences" between a CTA and conditioned place preference (CPP) using d-amphetamine as the conditioning stimulus (UCS). Male S-D rats were randomly assigned to two groups (N=12/group). One group received CTA with a 1% saccharin solution prior to CPP pairings (Grp. CTA/CPP) and the other group received CPP pairings prior to development of CTA (Grp. CPP/CTA). Each of these groups were further subdivided into saline control *vs* amphetamine treatment groups. Prior learning of either the aversive (Grp.CTA/CPP) or rewarding (Grp. CPP/CTA) properties of amphetamine did not interfere with the later development of "associative learning" of either CPP (approach, Grp. CTA/CPP) or CTA (avoidance, Grp. CPP/CTA). Both amphetamine tx. groups developed CPP's ($p<.01$) and CTA's ($p<.001$) which did *not* differ in magnitude between subgroups (i.e. Amphetamine subjects in CPP/CTA did not differ from their cohorts in CTA/CPP and *vice versa*). Saline treated animals developed neither CPP or CTA. At the conclusion of the dual associative tasks, animals were tested in the CPP chambers with calibrated drinking tubes installed inside the alley-ways to test the interaction between associative approach (CPP) and avoidance (CTA). Saccharin solutions were placed on the CS(-) side and water on the CS(+) side. Data supports the view that associative learning of the "aversive" and "rewarding" properties of amphetamine may operate in parallel with no *a priori* dominant influence of one over the other.

243.8

POTENTIATION OF THE DISCRIMINATIVE STIMULUS PROPERTIES OF COCAINE AND AMPHETAMINE BY THE DIHYDROERGOTAMINE DERIVATIVE, PPI-389. B. Geter, D. Dick* and A.L. Riley. The American University, Washington, D.C. 20016.

Cocaine users often complain of migraine headaches following binges. Cocaine alleviates these headaches. The ergot alkaloid, ergotamine, does so as well (Satel, S.L. and Gawin, F.H., JAMA, 261:2995-2996, 1989). That ergotamine substitutes for cocaine in relieving such headaches suggests that they have similar properties. The following studies assessed these similarities through the drug discrimination procedure. In experiment 1, following the establishment of drug discrimination learning with cocaine (10 mg/kg), rats were tested for the substitution potential of a range of doses of the dihydroergotamine derivative, PPI-389 (5.6 to 32 mg/kg). For all doses, subjects displayed vehicle-appropriate responding. However, when various doses of PPI-389 (5.6 and 18 mg/kg) were given in combination with the training dose of cocaine and when PPI-389 (5.6 mg/kg) was given in combination with various doses of cocaine (5.6 to 8.9 mg/kg), the dose-response function for cocaine was shifted to the left. In a second experiment, PPI-389 (5.6 mg/kg) also shifted to the left the dose-response function for amphetamine in cocaine-trained rats. The fact that PPI-389 was able to affect the dose-response function for cocaine (and amphetamine) suggests a potentiating effect of PPI-389. The mechanism remains unknown.

243.9

AMPHETAMINE-INDUCED BEHAVIORAL SENSITIZATION OF THE MESOLIMBIC NORADRENERGIC SYSTEM IN RATS: ROLE OF CORTICOSTEROIDS. A.R. Cools, Dept. Pharmacology University of Nijmegen, 6500 HB Nijmegen, The Netherlands.

The involvement of corticosteroids in the amphetamine-induced sensitization of the mesolimbic noradrenergic system was investigated. Locomotor activity induced by injections of the alpha-adrenergic agonist phenylephrine into the accumbens of rats primed 24 hr earlier with an intra-accumbens injection of 10 µg dexamphetamine (Cools et al., Beh. Pharmacol., 1, Suppl. 1: 6, 1990) was used to assess the effect of adrenalectomy and of corticosteroid replacement treatments (500 mg/kg, s.c., daily for 8 days). Sham adrenalectomy had no effect at all. Adrenalectomy attenuated the sensitization to phenylephrine without changing the response to amphetamine. Dexoycortisone and corticosterone re-instated fully and partially respectively the phenylephrine response without changing the amphetamine response. Dexamethasone increased the amphetamine response and re-instated the response to phenylephrine. It is concluded that mineralocorticoids are necessary for the sensitization of the mesolimbic alpha-noradrenergic system, and that glucocorticoids are necessary for the mesolimbic dopaminergic and, possibly, noradrenergic system.

243.10

GENETIC AND ENVIRONMENTAL FACTORS MODULATE SENSITIVITY TO THE BEHAVIORAL EFFECTS OF AMPHETAMINE. S. Puglisi-Allegra, S. Cabib*, A. Badiani*, Istituto di Psicobiologia e Psicofarmacologia (CNR), via Reno 1, I-00198 Roma, Italy

It has been shown that mice of the DBA/2 and C57BL/6 are characterized by different of adaptations of the brain dopamine (DA) systems to repeated stress (Cabib et al., 1985; Puglisi-Allegra et al., 1990). In the present study, the behavioral effects of amphetamine were examined in mice of these two strains following repeated restraint stress (120 min daily x 10 days).

Twenty four hours after the last stressful experience, mice of the DBA/2 strain exhibited a significant increase of responsivity to amphetamine in a locomotor test. By contrast, no changes in this behavioral effect of amphetamine was observed in chronically stressed C57BL/6 mice which exhibited a significant increase of spontaneous (drug-free) locomotion in comparison with unstressed controls. These genotype-dependent differences were observed either in mice habituated or naive to the test situation. Finally, pretreatment with DA antagonists selective for the D1 and D2 receptor types indicated that the two types of DA receptors are differently implicated in the hyperactivity responses induced by amphetamine and chronic stress in C57BL/6 mice.

These results are discussed in terms of the interaction between genetic and environmental factors modulating behavioral sensitivity to drugs of abuse acting on brain DA systems.

Cabib S., Puglisi-Allegra S., Oliverio A. (1985) Behav. Neural. Biol., 44:239-248.
Puglisi-Allegra S., Kempf E., Cabib S. (1990) Neurosci. Biobehav. Rev. in press.

243.11

INDIVIDUAL DIFFERENCES IN AMPHETAMINE-INDUCED LOCOMOTION COULD BE PREDICTED FROM AMOUNT OF SUGAR INGESTED. T. L. Sills and F. J. Vaccarino, Department of Psychology, University of Toronto, Toronto, Ont., M5S 1A1

Recent evidence suggests that there are individual differences in locomotor responsivity to amphetamine (AMP) and that these differences can be predicted from baseline activity (Piazza et al., 1989). Our lab has also demonstrated individual differences in feeding responsivity to AMP, which could be predicted from baseline feeding. The present study extended these findings by examining whether baseline feeding would be predictive of locomotor responsivity to AMP.

Sixteen male Wistar rats (Charles River, Quebec) were presented with powdered chow and sugar in their home cages and baseline intake was measured for one hour each day for seven days. Animals were divided into two subgroups on the basis of sugar intake level (above and below the median value); half (n = 7) were classified as low feeders and half (n = 7) as high feeders. Subsequently each group was tested for AMP-induced locomotion. Rats were administered vehicle (saline) and three doses of AMP (0.125, 0.25, 0.50 mg/kg) on separate days. To measure locomotion, rats were placed in cages equipped with photocell beams and counts of beam interruptions were recorded for 1.5 hours.

Results indicate that low baseline feeders exhibited more baseline locomotion than high baseline feeders. Importantly, low baseline feeders were more responsive to AMP(at the highest dose) than high baseline feeders. These findings demonstrate that individual differences in locomotor responsivity to AMP can be predicted from how much sugar rats ingest under baseline conditions.

This research supported by a NSERC grant to FJV.

243.12

RESPONSE TO NOVELTY AS A PREDICTOR OF THE EFFECTS OF ACUTE AND REPEATED DRUG ADMINISTRATION. M.S. Hooks, A.D. Smith, G.H. Jones, J.B.Justice, Jr. Dept. of Chem., Emory Univ., Atlanta, GA, 30322.

Some behavioral effects of amphetamine (AMPH) in rats have been shown to be largely predictable from the locomotor response to a novel environment (Piazza, *Science*, 245,1989). This experiment was designed to examine whether this relationship is consistent for both acute and repeated exposure to other classes of drug.

On day 1, subjects (male rats; n=64) were placed in individual photocell cages for a 3 h period. Animals were divided into high (HR) and low responders (LR) based on whether their locomotor activity (LA) scores for the first hour were above or below the median. On days 3, 5, and 7, rats were placed in the test cages for a 90 min habituation period prior to intraperitoneal administration of either AMPH (0.5 mg/kg), cocaine (COC; 10 mg/kg), scopolamine (SCOP; 0.5 mg/kg), or saline (n=16 for each drug; HR=8 and LR=8) and LA was measured for a further 2 hrs. On days 4 and 6 animals received the relevent drug in the home cage. Doses of drug were chosen to produce similar levels of locomotor activation.

HR rats had significantly greater LA than LR rats for all three drugs ($p<0.005$). There were no differences between HR and LR groups for the 30 min period before drug administration. Saline treated HR and LR rats did not differ. AMPH treated animals showed sensitization to locomotor stimulating properties of the drug ($p<0.01$). However, this effect was strikingly different in the HR and LR rats as only the HR rats showed increasing activity levels across days 3-7 ($p<0.02$). The COC treated rats showed a similar effect. SCOP treated HR and LR rats both showed tolerance to the drug with activity scores decreasing with repeated administration ($p<0.01$). LA for the habituation period on day 3 did not differ between HR and LR for any drug group. However, treatment with COC apparently reinstated the differences between HR and LR rats. Thus, on days 5 and 7 the HR rats in the COC group showed significantly greater LA than LR rats for the habituation period. The possible dopaminergic basis for these individual differences in LA was studied with microdialysis.

243.13

AMPHETAMINE-INDUCED CORTICOSTERONE DECREASE PREDICTS INDIVIDUAL VULNERABILITY TO DEVELOP AMPHETAMINE SELF-ADMINISTRATION IN THE RAT. S. Maccari*, P.V. Piazza*, J.M. Deminière*, P.M. Mormède*, M. Le Moal and H. Simon*, Lab. des Comportements Adaptatifs INSERM. U259-Univ. Bordeaux II. Domaine de Carreire 33077 Bordeaux Cedex-France.

An individual vulnerability to the reinforcing effect of addictive drugs is considered as an important factor influencing the development of addiction in human. Thus, the possibility to develop biological indices allowing an identification of individuals at risk is of great clinical interest. In a recent work we have shown that individual behavioral response to amphetamine (amph) provided a fairly good prediction of the reinforcing effect of the drug (Science 1989, 245: 1511-1513). In addition to this behavioral index we thought it was important to correlate a biological marker with the reinforcing value of the drug. It has been shown that amph modifies corticosterone levels and this hormone is related to the individual vulnerability to develop amph self-administration (SA) (Soc. Neurosci. Abst. 1989, 15: 1186). In the present, we have correlated the effects of amph (0.3 mg/kg, i.v.) on corticosterone release experiments with animal's propensity to develop SA. Four different groups of rats have been used for this study. Three groups were submitted to life events which increase amphetamine-taking behavior: repeated tail pinch, prenatal stress or social stress. The thourth group was raised in basal standard laboratory condition. Amphetamine injection induced an increase of corticosterone levels in control animals that showed later the lower levels of SA, while a significant decrease of the hormone levels was observed in stressed subjects which showed the higher levels of drug SA. These results suggest that an amphetamine-induced corticosterone decrease may be proposed as a biological predictive factor of the vulnerability to develop psychostimulant drug-seeking behavior.

243.14

INDIVIDUAL VULNERABILITY TO AMPHETAMINE SELF-ADMINISTRATION IS CORRELATED WITH DOPAMINERGIC ACTIVITY IN FRONTAL CORTEX AND NUCLEUS ACCUMBENS. P.V. Piazza*, F. Rougé-Pont*, J.M. Deminière*, M. Kharouby*, M. Le Moal and H. Simon*, Lab. des Comportements Adaptatifs INSERM. U259-Univ. Bordeaux II. Domaine de Carreire 33077 Bordeaux Cedex-France.

Humans and animals show wide individual differences for the vulnerabilty to develop drug-taking behavior. In the rat, individuals at risk for psychostimulant self-administration (SA) may be identified on the basis of their locomotor reactivity to a novel environment (Science 1989, 245: 1511-1513). Animals with the higher locomotor responses to novelty (HR group) will acquire amphetamine (amph) SA, while animals with the lower responses (LR group) will not. Since DA neurons seem to be involved in behavioral response to novelty and to psychostimulants, we hypothesized that differential activity of the DA neurons was one of the biological substrates sustaining individual vulnerability to amph SA. Thus, we have compared DA activity in different brain regions of rats with an high (HR) and low risk (LR) to acquire amphetamine SA. Animals from both groups have been sacrificed in basal condition and after 2hrs of exposition to a novel environment. Various brain regions were dissected. The DOPAC/DA ratio has been computed as an index of DA utilization. The HR rats, with the higher risk to acquire amph SA displayed a specific neurochemical pattern: a higher ratio in the nucleus accumbens plus a lower one in the frontal cortex. These data reveal a selective imbalance between these two regions for these rats and suggest that individual vulnerability to amph SA may depend on differences in the activity of DA neurons. Post-mortem studies were duplicated by *in vivo* microdialysis. In conclusion, animals at risk for drug SA present in basal conditions higher (accumbens) and lower (frontal cortex) dopamine utilization.

243.15

METHAMPHETAMINE LOWERS BRAIN STIMULATION REWARD THRESHOLD. M. Sarkar*, G.T. Bain* and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston Univ. Sch. of Med., Boston, MA 02118.

Most abused drugs increase the sensitivity of animals to brain-stimulation reward (BSR) a model of drug-induced euphoria. Because of the recent increase in the abuse of methamphetamine (MAMP) (street name "Ice") we determined if (+) MAMP would lower the threshold for BSR in a similar manner to other psychomotor stimulants. Four F-344 (Charles River Laboratories) male albino rats were stereotaxically implanted with bipolar stainless steel electrodes aimed at the medial forebrain bundle at the level of lateral hypothalamus. A rate independent method was used to determine the BSR thresholds. Acute effects of MAMP (0.0325-2.0 mg/kg, ip) were studied in 4 animals. Three out of the 4 showed significant threshold lowering at 0.25 mg/kg with marked stereotypic head moving and sniffing behavior at doses greater than 1.0 mg/kg. The 4th animal showed significant threshold lowering only at 2.0 mg/kg with stereotypy at higher doses. The results of this study suggest that the reinforcing property of MAMP is mediated, at least partially, via the same reward pathway as the other abused psychomotor stimulants. (Supported by NIDA grant DA02326 and Research Scientist Award DA00099 to CK).

243.16

THE EFFECTS OF 4-METHYLAMINOREX AND METHAMPHETAMINE ON CATECHOLAMINE RELEASE IN PC-12 CELLS. J.M. Beaton, A.M. Freeman, III*, F. Benington*, J.A. Monti, and R.D. Morin*, Dept. of Psychiatry and Behavioral Neurobiology, University of Alabama at Birmingham, Birmingham, AL 35294.

4-Methylaminorex (2-amino-4-methyl-5-phenyl-2-oxazoline, 4-MAX) and methamphetamine (MET-AMP) have been reported to be possible candidates for the street drug, "ICE". The effects of 1,10,50,100 and 500 μM of each drug on catecholamine release from PC-12 cells were determined with quantification of the released norepinephrine (NE) and dopamine (DA) being by HPLC-EC. The amount of NE and DA released, expressed as a percentage of the release in samples with no drug added (control) is shown below.

	4-MAX		MET-AMP	
Dose (μM)	NE	DA	NE	DA
Control	100	100	100	100
1	86	97	94	90
10	102	103	101	102
50	98	111	98	108
100	121	127	94	112
500	114	137	108	132

The data indicate: (1) 4-MAX and MET-AMP are more potent in stimulating DA release than NE release, (2) both significantly increased the DA release at the higher doses. This increase in DA release is in accord with the finding of Misenheimer and Glennon, (Soc. Neurosci. Abst., 15, Part 2, 1187, 1989), showing that 4-MAX produced amphetamine-like stimulating effects. (Supported in part by the Alabama Consumer Fund).

243.17

Effects of In-Utero Methamphetamine on Rat Offspring. S. Caldecott-Hazard, R. Clark, K. Derrig*, K. McCunniff*. USC, LA, CA 90089

Adult, female rats were injected with saline (SAL), low doses of methamphetamine (MAMP, maximum 2 mg/kg), or high doses of MAMP (max. 10 mg/kg). These drugs were given daily until drug-induced changes in temperature and food consumption returned to pre-drug levels. Females were then bred with naive male rats, and MAMP injections continued to the females throughout pregnancy. All pups born to MAMP or SAL mothers were fostered to SAL mothers, and all injections were discontinued. Beginning at 30 days of age, SAL and MAMP pups were tested in an open field, physically restrained for 2 hrs then re-tested in the open field, and tested in a Morris water maze. High dose MAMP pups crossed significantly fewer squares in the open field, under no-stress and stress conditions, as compared to controls. These pups did not differ from controls in latencies to find a platform in the water maze. Low dose MAMP pups did not differ from controls on any of the tests. Studies using 14C 2-deoxy-glucose are in progress to compare brain glucose utilization in the 3 groups of pups (NINDS #K07NS00979; USC FRIF and BRSG grants).

243.18

PHARMACOKINETICS OF SYSTEMICALLY ADMINISTERED 3,4-METHYLENEDIOXYMETHAMPHETAMINE (MDMA) AND MONOAMINE RELEASE IN BRAIN DIALYSATE. M. Hiramatsu*[1,2], E.W. DiStefano*[2], T. Kameyama*[1] and A.K. Cho[2]. [1]Dept. of Chemical Pharmacology, Meijo Univ., Nagoya 468, Japan and [2]Dept. of Pharmacology, UCLA School of Medicine, Los Angeles, CA 90024

MDMA (3,4-methylenedioxymethamphetamine) and its metabolite, MDA (3,4-methylenedioxyamphetamine), were measured in the plasma of rats injected s.c. with enantiomers of MDMA. Microdialysis techniques were used to monitor monoamines and their metabolites in the striatum after (+)MDMA. MDMA concentrations in plasma reached peak values 70 min after administration of both isomers of MDMA. Plasma levels of MDA after (+)MDMA were about 3 times higher than those after (-)MDMA and reached peak values at 160-190 min, although the plasma concentrations of the parent drugs were comparable. (+)MDMA caused a rapid increase in dialysate levels of dopamine and a decrease in DOPAC and HVA immediately after dosage. Dopamine release in striatal dialysate correlated with plasma MDMA, but not with plasma MDA. These results indicate that MDMA interacts with dopamine neurons stereoselectively to release dopamine. The higher plasma MDA levels after (+)MDMA and/or high levels of extracellular dopamine may contribute to enantiomeric differences in the behavioral and neurotoxicological effects of (+)MDMA. Supported by DA04206.

243.19

THE DISCRIMINATIVE STIMULUS PROPERTIES OF OVER-THE-COUNTER BINARY STIMULANT MIXTURES. *D.V. Gauvin, K.R. Moore*, B.D. Youngblood,*, & F.A. Holloway. University of Oklahoma Health Sciences Center, Oklahoma City, OK 73190-3000.*

It is becoming increasingly rare for drug users to encounter or use a single drug in isolation without co-administering a second drug concomitantly or in temporal sequence. Even over-the-counter (OTC) diet and cold remedies are usually manufactured as binary drug compounds containing caffeine (CAF), ephedrine (EPH), and/or phenylpropanolamine (PPA). Very little is known about the factors or "rules" that govern the perception of drugs in mixtures. The present study sought to examine the discriminative stimulus properties of binary drug mixtures of legal OTC stimulants. Three groups of male Sprague-Dawley rats (n=12/group) were trained to discriminate between saline and either (1) CAF+PPA (**CP**, 10+10 mg/kg), (2) CAF+EPH (**CE**, 10+5 mg/kg), or (3) EPH+PPA (**EP**, 6+12 mg/kg) in a two-choice, food-motivated lever-press operant task. Whereas both EPH and PPA were equally salient stimuli (i.e., each training dose tested singly resulted in approximately 50% drug-lever responding) in the **EP** group, EPH and PPA were perceptually dominant over the CAF stimulus in both the **CP** and **CE** groups. CAF alone failed to generalize across a wide range of doses to either the **CP** or to the **CE** binary compound stimulus. The **EP** and **CE** groups generalized both cocaine and amphetamine to the training stimuli. However, the **CP** group only generalized to amphetamine. These data suggest that (1) some legal OTC stimulants produce internal subjective states similar to both cocaine and amphetamine, and (2) the perceptual processing analyses of binary drug mixtures parallel those of similar chemoreceptive systems (i.e., olfaction).

Supported by NIDA DA04444 and Oklahoma Center for Science and Technology Grant #1686 to F.A. Holloway.

243.20

SILICONE PELLET FOR CONTINUOUS COCAINE ADMINISTRATION: COMPARISON WITH CONTINUOUS AMPHETAMINE. Jack Lipton, Steve Zeigler, Jeffrey Wilkins*, and Gaylord Ellison. Depts of Psychology and Psychiatry, UCLA, Los Angeles, CA, 90024.

An inexpensive silicone pellet is described for the continuous adminstration of cocaine for up to 5 days. Rats implanted with this pellet show with minimal skin irritation and go through distinct behavioral stages, with an initial period of hyperactivity followed by motor stereotypies. Then, at 3-4 days after implantation, a variety of "hallucinogen-like" behaviors appear; these include limb flicks, sudden startle responses, and repetitive mid-air grasping movements. Compared to continuous d-amphetamine, continuous cocaine induces decreased motor stereotypies but heightened "late-stage" behaviors. HPLC analysis of both the subcutaneous pellet and serum levels suggest a relatively stable rate of cocaine release for the first 4 days. Rats in automated activity cages developed tolerance to cocaine in a nine day administration (during which pellets were replaced on day 4). Their activity peaked by day 3 and gradually declined through day 9.

244.1

IDAZOXAN, AN ALPHA-2 ADRENOCEPTOR ANTAGONIST, REVERSES THE EFFECTS OF B-HT 920, A DOPAMINE AUTORECEPTOR AGONIST, ON CONDITIONED AVOIDANCE RESPONDING IN RATS. A.T. Shropshire and K.L. Marquis. Wyeth-Ayerst Research, CN8000, Princeton, NJ 08543.

Antagonism of conditioned avoidance responding (CAR) in rats is a sensitive test for dopamine receptor antagonists; however, alpha 1 adrenoceptor antagonists such as prazosin and alpha 2 adrenoceptor agonists such as clonidine block CAR and appear as false positives in this test and others used to predict preclinical antipsychotic activity (Arnt, J., Acta Pharmacol. Toxicol. 51:321, 1982; Hawkins and Monti, Eur. J. Pharmacol. 58:53, 1979). Meltzer, *et al.*, (Eur. J. Pharmacol. 170:105, 1989) have shown that alpha 2 adrenoceptor antagonists can unmask the postsynaptic dopaminergic agonist activity of the dopamine autoreceptor agonist B-HT 920 by blocking its alpha 2 agonist effects. Moreover, we have demonstrated that moderate to high doses of B-HT 920 block CAR in rats (Marquis et al., at this meeting). To define a possible mechanism for the CAR effect we treated rats, previously trained to avoid a shock by making a shelf jump or lever press response in a discrete trial procedure, with various doses of clonidine, prazosin and B-HT 920. All drugs reduced avoidance responding and increased escape responding at doses which did not increase the number of no-response trials. Combined ad-ministration of the alpha 2 antagonist idazoxan and clonidine, or idazoxan and B-HT 920, reversed the avoidance deficits and the increased escape responding produced by each drug alone. These data suggest that some dopamine autoreceptor agonists which have activities at alpha adrenoceptors could inhibit CAR by an adrenergic mechanism as opposed to a dopaminergic mechanism.

244.2

EFFECTS OF DOPAMINE AUTORECEPTOR AGONISTS ON CONDITIONED AVOIDANCE RESPONDING (CAR) IN RATS. K.L. Marquis, A.T. Shropshire, T.G. Demetriou*, T.H. Andree, and J.A. Moyer. Wyeth-Ayerst Research, CN8000,Princeton, NJ 08543.

Typical and atypical antipsychotics, which may exert their therapeutic action by antagonizing the D_2 receptor, block CAR in rats. However, many of these agents also produce extrapyramidal side effects (EPS). It has been proposed that a dopamine (DA) partial agonist or selective autoreceptor agonist will be antipsychotic without producing EPS (Tamminga and Schaffer, Science 200:567, 1978); yet, there is very little information available regarding the ability of some of the more recently developed DA autoreceptor agonists to block CAR. Male Sprague-Dawley rats, previously trained to avoid a shock by making a shelf jump response, were treated with various doses of apomorphine, (-)3PPP, B-HT 920, EMD 23448, n-propyl-norapomorphine, PD 116795, OPC 4392 or CGS 15855A. All drugs were autoreceptor agonists as demonstrated by a reversal of GBL-induced accumulation of DOPA in rat limbic tissue and by a reduction in mouse locomotor activity. Only B-HT 920, PD 116795 and OPC 4392 significantly reduced CAR at doses which did not produce overt behaviors in mice (stereotypy and climbing) indicative of postsynaptic D_2 agonism. EMD 23448 and (-)3PPP produced only modest decreases in CAR at high doses. Since each drug affecting CAR did so at doses well above those which produce hypolocomotion, it can be concluded that CAR effects may occur as a result of postsynaptic D_2 antagonism rather than autoreceptor agonism. In addition, other mechanisms within the noradrenergic sytem could also play a role in CAR (Shropshire and Marquis, this meeting).

244.3

EFFECT OF TYPICAL AND ATYPICAL ANTIPSYCHOTIC DRUGS (APD) ON FRONTAL CORTICAL (FC) SEROTONIN$_2$ (5-HT$_2$) AND STRIATAL (STR) DOPAMINE$_2$ (D_2) BINDING IN VIVO Herbert Y. Meltzer, Ying Zhang* and Craig Stockmeier, Dept. Psychiatry, Pharmacology and Neuroscience, Case Western Reserve University, Cleveland, Ohio 44106

It has been suggested that atypical APD can be distinguished from typical APD on the basis of higher ratios of pKi values for FC 5-HT$_2$ to STR D_2 binding sites for the atypical APD (JPET 254, 238, 1989). To determine if this distinction is also obtained in vivo, FC and STR binding of ^{3}H-N-methyl-spiperone was validated as measures of 5-HT$_2$ and D_2 binding by preliminary studies with ritanserin and raclopride, respectively. The typical APD haloperidol (0.15, 0.50 mg/kg), chlorpromazine (10 mg/kg), thioridazine (2.5 mg/kg), loxapine (1, 2.5 mg/kg) and risperidone (5 mg/kg) occupied STR D_2 receptors $\geq$ FC 5-HT$_2$ receptors. The atypical APD clozapine (5,10,20,40,60 mg/kg), melperone (2.5, 5.0, 10 mg/kg) and amperozide (10, 20 mg/kg) occupied FC 5-HT$_2$ > STR D_2 receptors. Even at the highest doses of clozapine studied, D_2 occupancy did not exceed 50%. Amperozide did not decrease D_2 sites occupied by ^{3}H-N-methyl-spiperone in vivo. The results support the conclusion that the 5-HT$_2$/D_2 ratio may be important for atypical APD. Amperozide may achieve its antischizophrenic action without blocking D_2 receptors in vivo. Supported by MH 41684 and Laureate Foundation/NARSAD grants.

244.4

EFFECTS OF HALOPERIDOL ON THE KINETICS OF ENDOGENOUS DOPAMINE RELEASE *IN VITRO* USING ROTATING DISK ELECTRODE VOLTAMMETRY. J.S. McElvain and J.O. Schenk. Dept. of Chemistry and Program in Biochemistry, Washington State University, Pullman, WA 99164.

The rotating disk electrode (RDE) system used to montior the kinetics of endogenous dopamine (DA) release and reuptake from isolated rat striatal homogenates has been described previously (McElvain and Schenk, Neurosci. Abst., 48.20, 1989). Striatal homogenates were incubated at 37°C in 500 µL of physiological buffer in the presence or absence of haloperidol (HAL) and depolarized with 15 and 30 mM KCl. The resulting DA released was monitored by the RDE and was analyzed kinetically by treating the resulting DA concentration-time profile as intermediate between two consecutive, irreversible first order reactions (release and reuptake, respectively). In the presence of low concentrations of HAL (5-100 nM), it was found that the maximum of the 30 mM KCl stimulated DA release signal (S_{max}) and the first order rate constant for release (k_{rel}) increased over control stimulations in a dose dependent manner. At higher concentrations of HAL (10-50 µM) it was found that the time to the maximum of the DA release signal increased over control values while the initial DA release rates, the S_{max}, and the k_{rel} decreased. HAL had little effect on the first order rate constant for the reuptake of DA (k_{up}) at low concentrations (1-1000 nM) but decreased the k_{up} significantly at higher doses (10-50 µM). Thus HAL has only an amplitude modulating influence on DA release at HAL concentrations relevent to autoreceptor functioning. At higher concentrations of HAL, frequency as well as amplitude modulation of DA release is observed. Supported by MH 42759 and the state of Washington.

244.5

CHRONIC HALOPERIDOL AND CLOZAPINE DIFFERENTIALLY ALTER THE DENSITY OF MET-ENKEPHALIN-LIKE IMMUNO-REACTIVE PERIKARYA IN RAT CAUDATE VERSUS ACCUMBENS NUCLEI. A.P. Auchus and V.M. Pickel. Dept. of Neurology and Neuroscience, Div. of Neurobiol., Cornell U. Med. College, NY, NY 10021

We examined the capacity of the classic neuroleptic haloperidol (HAL) and the atypical neuroleptic clozapine (CLOZ) to alter met-enkephalin-like immunoreactivity (MELI) in caudate vs accumbens nuclei. Rats received 1 mg/kg/d HAL or 20 mg/kg/d CLOZ via their drinking water. After 21 days, the animals (and controls) were sacrificed and their brains were processed using a 1:20,000 dilution of ME antibody and immunoperoxidase. In caudate, HAL, but not CLOZ, resulted in a significant ($p<.05$) increase in ME cell density (immunoreactive perikarya per unit area) cf controls. In accumbens, CLOZ, but not HAL, resulted in a significant ($p<.05$) decrease in ME cell density cf controls. These results demonstrate an effective method for comparing intraperikarya MELI between different drug treatment groups and suggest the relevance of differential changes in caudate vs accumbens enkephalin systems to the pathogenesis of neuroleptic-induced movement disorders. (Supported by grants HL18974, DA04600 MH40342, training grant NS07141 and a fellowship from the Norman and Rosita Winston Foundation)

244.6

MCPP EFFECTS IN SCHIZOPHRENIC PATIENTS: EFFECTS OF PHARMACOTHERAPY. J.H. Krystal, J.P. Seibyl, L.H. Price, S.W. Woods, G.R. Heninger, D.S. Charney. Schiz. Biol. Research Cntr., Psychiatry Svc., West Haven VA Med. Cntr., West Haven, CT 06516

M-chlorophenylpiperazine (MCPP) is a non-selective serotonin (5-HT) partial agonist. In order to evaluate the contribution of 5-HT systems to the symptoms of schizophrenia, MCPP effects were studied in schizophrenic patients (PTS) and healthy subjects (HS). **Methods:** In an ongoing study, PTS meeting DSM-III-R criteria for schizophrenia and HS (N=10) participated in 2 test days in a randomized order: MCPP (0.1 mg/kg, i.v. over 20 min.) or placebo. PTS were tested under 3 treatment conditions: 1) 2 wks. neuroleptic-free (N=11), 2) 4 wks. haloperidol treatment .3 mg/kg/d, p.o. (N=5), and 3) after clozapine treatment (800 mg/d) (N=3). **Results:** 9/11 unmedicated PTS and 0/10 HS experienced increases in the positive symptoms of schizophrenia (visual and auditory hallucinations, conceptual disorganization, and suspiciousness). MCPP-induced anxiety was comparable in unmedicated PTS and in HS and showed a time course distinct from positive symptoms. Neuroleptic-free PTS did not differ in the extent to which MCPP increased plasma levels of growth hormone, cortisol, or prolactin. Preliminary analyses suggest that clozapine produced greater reduction in MCPP behavioral effects than did haloperidol. **Implications:** MCPP appears to exacerbate psychosis in a high percentage of PTS, suggesting a role for 5-HT in the symptoms of schizophrenia. The high affinity of MCPP for 5-HT$_{1c}$, 5-HT$_2$, and 5-HT$_3$ receptors supports the impression antagonists of these receptors may be useful in the treatment of schizophrenia symptoms that are refractory to typical neuroleptic (i.e., haloperidol) treatment.

244.7

AUTORADIOGRAPHIC EVIDENCE FOR CLOZAPINE SITE-SELECTIVITY AT THE DOPAMINE RECEPTOR. T. F. Seeger, A.W. Schmidt and P. Suzdak. Neuroscience Dept., Pfizer Central Research, Groton, CT 06340

Using the technique of receptor binding autoradiography in rat brain sections, the atypical antipsychotic clozapine was found to displace the dopamine agonist ligand 3H-NPA from mesolimbic dopamine D-2 receptors with a greater affinity than it showed for D-2 receptors in the striatum. However, when this comparison was made using tissue homogenate binding, clozapine displaced 3H-NPA equally well in the two regions.

In order to investigate this discrepancy, a functional in vivo measure of dopamine interaction was devised using the quantitative 2-deoxyglucose method in rats. A fixed dose of the dopamine agonist D-amphetamine (1 mg/kg s.c.) was used to activate cerebral metabolism in both mesolimbic and striatal areas. Pretreatment with clozapine (5 mg/kg s.c.) was found to partially antagonize the amphetamine-induced increases in glucose utilization while not causing significant changes when given alone. In agreement with the previous autoradiography studies, clozapine caused a preferential antagonism in limbic areas (nucleus accumbens and olfactory tubercle) relative to the striatum. These results support the concept of clozapine having a functional antagonist selectivity for the mesolimbic dopamine system.

244.8

BEHAVIORAL COMPARISONS IN RATS OF CHRONIC CLOZAPINE WITH HALOPERIDOL. M.F. Egan, L.L.Wing, T.L.Bryant, D.G. Kirch and R.J. Wyatt. Neuropsychiatry Branch, NIMH @ St. Elizabeths, Washington, D.C. 20032.

The antipsychotic clozapine, unlike haloperidol, has been demonstrated to possess serotonergic and cholinergic antagonist properties in addition to D1 and D2 receptor blockade. A comparison of the behavioral effects of chronic administration of both was made.

Thirty-six male Sprague-Dawley rats (350g +15g) were given daily i.p. injections of either clozapine (10 mg/kg), haloperidol (4 mg/kg) or the vehicle (distilled water, NaOH and glacial acetic acid). Animals were tested for 1 hr in Omnitech activity monitors after 4 wks of treatment. Both clozapine- and haloperidol-treated animals displayed less locomotor activity than vehicle-treated controls. Nevertheless, while the overall activity of the drug-treated groups were not significantly different from one another, clozapine-injected rats spent significantly more time in the corners and along the walls of the test chamber. In contrast, the novel environmental stimulus cues of the test chamber were less aversive to the haloperidol-injected animals. Although less active than controls, the haloperidol-injected animals spent the most time exploring the center of the chamber, and the least time in the corners or along the walls of the test chamber than did either the vehicle- or clozapine-treated rats. Thus, both antipsychotics produced vastly different behavioral profiles.

244.9

SELECTIVE EFFECT OF CLOZAPINE IN DA TURNOVER IN THE PREFRONTAL CORTEX IN RATS. L. Hernandez[1,2] and B. G. Hoebel[1]. [1]Dept. Psychology, Princeton Univ., Princeton, NJ 08544-1010; [2]Laboratorio de Fisiologia de la Conducta, Escuela de Medicina, Universidad de Los Andes, Merida, 5101-A, Venezuela

Triple microdialysis studies showed that acute injections of the typical neuroleptic haloperidol increase dopamine (DA) turnover in the prefrontal cortex (PFC), nucleus accumbens (NA) and striatum (STR) in rats. Chronic administration of haloperidol lowered the basal level of DA in the PFC; it also decreased DA turnover in the PFC and the STR but not in the NAC. No tolerance was observed. These effects may explain both psychotic symptom suppression (decreased DA turnover in the PFC) and extrapyramidal side effects (decreased DA turnover in the STR) caused by haloperidol[1]. Clozapine (CLOZ), an atypical neuroleptic that is devoid of extrapyramidal side effects should not affect DA turnover in the striatum, but should do so in the PFC. To test this prediction, triple microdialysis was performed in 24 rats. Acute clozapine increased DA turnover in the PFC. A dose-response relationship was found. Only the highest dose of acute clozapine (40 mg/kg) significantly increased DA turnover in the NAC. No effect was observed in the STR. Chronic administration of clozapine (20 mg/kg) decreased DA levels and turnover in the PFC but did not affect DA turnover in the NAC or STR. These results suggest that CLOZ preferentially affects the mesocortical system to ameliorate psychotic symptoms.

[1]Hernandez, L. & Hoebel, B. G. *Brain Res. Bull.*, 1989, 22, 763-769.

244.10

DOPAMINE AUTORECEPTOR SUPERSENSITIVITY AND POSTSYNAPTIC SUBSENSITIVITY WITH CHRONIC NEUROLEPTIC TREATMENT. M.R.Lynch, C.Haskins* and J.Woo*, Res. Serv., VAMC and Depts. Psychiat. and Pathol., SUNY Health Sci. Ctr., Syracuse, N.Y. 13210

Delayed onset antipsychotic effects of neuroleptics have been explained by depolarization block and autoreceptor supersensitivity hypotheses. We have previously reported a progressively enhanced locomotor suppression with chronic haloperidol(HAL)(Biol.Psychiat.,24:941,1988), mimicking this delayed onset. To probe for neurochemical mechanisms of this enhancement, rats received 20 days of 0.1 mg/kg HAL or vehicle (VEH), were withdrawn for 48 hr and then tested with 0.07 mg/kg apomorphine (APO). (Prolactin assay results confirmed that HAL was no longer bound to central DA receptors at the 48 hr test.) HPLC-EC, to assess APO-inhibition of DA metabolism, revealed autoreceptor supersensitivity in mesolimbic (not nigrostriatal) tissue from HAL animals (significant decrease in HVA/DA ratio). Conversely, HAL rats failed to show the APO-induced hypomotility and yawning observed in VEH animals. Therefore, it appears that autoreceptor supersensitivity was induced concomitant with subsensitivity in an agonist-sensitive population of postsynaptic DA receptors mediating these APO behavioral effects. However, there was no evidence of depolarization block: At 48 hr withdrawal HAL activity returned to baseline; DA turnover was normal and was not increased by APO.

244.11

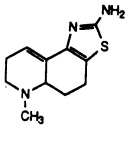
INTERACTION OF THE POTENTIAL ANTIPSYCHOTIC BMY 14802 AND STRUCTURALLY-RELATED SIGMA LIGANDS WITH M_1 MUSCARINIC SITES. Duncan P. Taylor, Jennifer Defnet, Susan H. Behling and Diana Marrero. CNS Biology, Bristol-Myers Squibb Company, Wallingford, CT 06492-7660.

A distinct class of sigma binding sites has been identified which can be differentiated from NMDA receptor-associated phencyclidine binding sites as well as dopamine and opioid receptors. Efforts to investigate the receptor role of the sigma binding site have been hampered by the lack of a functional assay. Bowen et al. (1988) reported that sigma ligands attenuated carbachol-stimulated phosphoinositide turnover in rat brain. They have recently shown that the rank orders of potency for ten unrelated compounds were similar for inhibition of in vitro binding to sigma sites or [^{3}H]oxotremorine-M binding to M_1 muscarinic cholinergic sites (Spearman $\rho = 0.88$, $p < 0.01$). We have investigated the correlation of the rank orders of potency for 32 structurally-related analogs of the putative sigma antipsychotic BMY 14802 (Taylor et al., 1990) in both sigma and M_1 binding assays. For these agents we find no statistically significant correlation between rank orders of potency in the two assays (Spearman $\rho = 0.11$). These data suggest that in the guinea pig brain the M_1 site does not have the same structural requirement for binding as the sigma site, or, alternatively, that these two sites are not allosterically coupled.

244.12

THE IDENTIFICATION OF PD 128483 AS A DOPAMINE AUTORECEPTOR AGONIST WITH ANTIPSYCHOTIC-LIKE PROPERTIES. **L.D. Wise, J.C. Jaen*, B.W. Caprathe*, T.G. Heffner, L.T. Meltzer, T.A. Pugsley. Parke-Davis Pharmaceutical Research Division Warner-Lambert Company, Ann Arbor, MI 48105.**

Although PD 118440, a compound previously proposed as a bioisosteric replacement for 3-PPP, is a potent, orally active dopamine (DA) agonist, it lacks sufficient pre- vs postsynaptic receptor selectivity for development as an antipsychotic agent (Soc. Neurosci. Absts., 13:461, 1987). To identify a more selective compound, a series of related 6-substituted-4,5,5a,6,7,8-hexahydrothiazolo[4,5-f]quinolin-2-amines was explored. The 6-methyl, ethyl, propyl, and allyl compounds are potent DA autoreceptor agonists; they bind to DA receptors in vitro, and in rats reverse GBL-stimulated increases in DOPA synthesis, inhibit DA neuronal firing activity, and inhibit spontaneous locomotion. PD 128483, the maleate salt of the methyl analog, was selected for development based on its overall profile. While both of its enantiomers are partial DA agonists, optimal selectivity is observed with the racemate.



PD 128483

244.13

BIOCHEMICAL CHARACTERIZATION OF PD 128483, A NOVEL DOPAMINE AUTORECEPTOR AGONIST, IN RAT BRAIN. T.A. Pugsley, Y.H. Shih*, S.Z. Whetzel* and R.D. Schwarz. Department of Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, MI 48105.

PD 128483 ((±) - 4,5,5a,6,7,8-hexahydro-6-methyl-thiazolo[4,5-f]quinolin-2-amine) and its enantiomers were evaluated biochemically for their dopamine (DA) agonist and other activities. In the (gamma)-butyrolactone (GBL) model of DA autoreceptor function, PD 128483 had an ED_{50} value of 0.7 mg/kg i.p., in striatum and the effect was haloperidol-reversible; the values for the (+) - and (-)-enantiomers were 0.5 and >10 mg/kg i.p. In the GBL model DA autoreceptor activity was retained for 6 hours after a single p.o. dose and no tolerance was seen after 10 days of treatment. PD 128483 exhibited affinity for DA D-2 receptors with negligible affinity for other receptors except for a_2-adrenoceptor binding. PD 128483 exerted little activity on stimulated acetylcholine release from striatal slices, a test for postsynaptic D-2 receptors, indicating a relatively selective action on presynaptic DA receptors. It decreased DA synthesis and turnover without altering extensively that of norepinephrine and serotonin. These data suggest that PD 128483 is a selective DA autoreceptor agonist.

244.14

EFFECTS OF THE NOVEL DOPAMINE AUTORECEPTOR AGONIST PD 128483 ON THE ACTIVITY OF SUBSTANTIA NIGRA DOPAMINE NEURONS. C.L. Christoffersen, K.A. Serpa, T.G. Heffner and L.T. Meltzer. Dept. of Pharmacology, Parke-Davis Pharm. Res. Div., Warner-Lambert Co., Ann Arbor, MI 48105.

PD 128483, (±)-4,5,5a,6,7,8-hexahydro-6-methylthiazolo-[4,5-f]quinolin-2-amine, binds to dopamine (DA) D-2 receptors and is active in biochemical tests of DA autoreceptor agonist activity (Pugsley et al. this meeting) and in behavioral studies shows selectivity for the DA autoreceptors versus postsynaptic DA receptors (Corbin et al., this meeting). In the present studies we compared the effects of PD 128483 and its enantiomers with those of apomorphine and EMD 23448 on the firing activity of A9 DA neurons recorded extracellularly in chloral hydrate anesthetized male S-D rats. PD 128483 produced a dose-related inhibition of A9 DA neuron firing. The maximal inhibition at 1.28 mg/kg IV was approximately 75% of the baseline firing rate. The (+)- and (-)-isomers of PD 128483 produced maximal inhibitions of 90% and 63%, respectively. Apomorphine (128 ug/kg IV) and EMD 23448 (1 mg/kg IV) produced maximal inhibitions of 100% and 63%, respectively. PD 128483 inhibited DA neuron activity in rats depleted of DA by treatment with reserpine plus alpha-methyl-paratyrosine, indicating direct DA agonist actions. These data indicate that PD 128483 is a partial DA agonist, with intrinsic activity similar to EMD 23448, but less intrinsic activity than apomorphine.

244.15

EFFECTS OF THE DOPAMINE AUTORECEPTOR AGONIST PD 128483 ON NEUROTRANSMITTER RELEASE AS ESTIMATED BY INTRACEREBRAL MICRODIALYSIS IN RATS. L.M. Ball, M.D. Davis and T.G. Heffner. Dept. of Pharmacology, Parke-Davis Pharmaceutical Research Divison, Warner-Lambert Co., Ann Arbor, MI 48105.

PD 128483,(±)-4,5,5a,6,7,8-hexahydro-6-methylthiazolo-[4,5-f]quinolin-2-amine, is a dopamine (DA) autoreceptor agonist as shown in neurophysiological, neurochemical and behavioral tests. DA D2 agonists are known to depress DA neurotransmission by presynaptic actions. The present studies sought to determine if PD 128483 inhibits the release of DA from central neurons in vivo. Intracerebral microdialysis (ICMD) probes were placed in the striatum of rats and amines in striatal dialysates were measured by HPLC-EC. PD 128483 i.p. caused a substantial decrease in the levels of DA and its metabolites DOPAC and HVA in dialysates from anesthetized animals. Similar effects were produced by the DA autoreceptor agonist B-HT-920, while the DA antagonist haloperidol increased these levels. Striatal dialysate levels of the serotonin metabolite 5-HIAA were not altered by PD 128483. ICMD was also used in conscious freely-moving rats by inserting dialysis probes in chronically-implanted guide cannula. Behaviorally-active doses of PD 128483 given orally were found to produce effects in the conscious rat similar to those observed in anesthetized animals. These results suggest that PD 128483 decreases the release of DA from central neurons and is orally active in rats.

244.16

EFFECTS OF THE NOVEL DOPAMINE AUTORECEPTOR AGONIST PD 128483 IN RODENT BEHAVIORAL TESTS. A.E. Corbin, J.N. Wiley* and T.G. Heffner. Department of Pharmacology, Parke-Davis Pharmaceutical Research Division, Warner-Lambert Co., Ann Arbor, MI 48105.

PD 128483,(±)-4,5,5a,6,7,8-hexahydro-6-methylthiazolo-[4,5-f]quinolin-2-amine, is a dopamine (DA) autoreceptor agonist as shown in neurophysiological and neurochemical tests. PD 128483 inhibits spontaneous and amphetamine-stimulated locomotion in mice and rats, consistent with activation of presynaptic brain DA receptors. Selectivity for pre- vs postsynaptic DA receptors is evidenced by lack of stimulation and stereotypy in normal rats as well as in rats pretreated with reserpine or a D1 agonist. Absence of post-synaptic DA antagonist effects is indicated by lack of antagonism of the climbing induced by the direct-acting DA agonist apomorphine. Like other DA agonists, PD 128483 produces stimulation of locomotion in rats depleted of brain DA by central injections of 6-hydroxydopamine. PD 128483 has oral activity that persists for up to 6 hours and does not show diminished behavioral efficacy after daily dosing for 10 days. Postsynapatic DA agonist effects seen with the (+)-enantiomer of PD 128483 and the weak presynaptic effects of the (-)-enantiomer indicate that the racemate is the optimal form for development. These results indicate that PD 128483 is an orally active brain DA autoreceptor agonist.

244.17

EFFECTS OF THE NOVEL DOPAMINE AUTORECEPTOR AGONIST PD 128483 IN PRIMATES. T.G. Heffner, C.L. Christoffersen, L.W. Cooke, M.D. Davis, L.T. Meltzer and F.W. Ninteman*. Dept. of Pharmacology, Parke-Davis Pharmaceutical Res. Division, Warner-Lambert Co., Ann Arbor, MI 48105.

PD 128483,(±)-4,5,5a,6,7,8-hexahydro-6-methylthiazolo-[4,5-f]quinolin-2-amine, is a dopamine (DA) autoreceptor agonist as shown in neurophysiological, neurochemical and behavioral tests in rodents. Like known antipsychotics, PD 128483 inhibited Sidman avoidance in squirrel monkeys, a test commonly used to predict antipsychotic efficacy. In contrast, the nonselective DA agonists apomorphine and bromocriptine failed to inhibit avoidance responding in monkeys and caused only stimulation of responding. PD 128483 displayed oral activity in monkeys (ED_{50}: 4.8 mg/kg po) and its effects persisted for 6 hours. Intracerebral microdialysis in the squirrel monkey caudate putamen indicated that behaviorally-active doses of PD 128483 decreased the overflow of DA from central neurons. In squirrel monkeys sensitized to the acute extrapyramidal side effects (EPS) of haloperidol, PD 128483 did not cause the dystonias produced with a variety of antipsychotics that produce EPS in man. These preclinical results indicate that PD 128483 is a DA autoreceptor agonist with effects in primates that suggest antipsychotic efficacy, a DA autoreceptor mechanism of action and no liability for the acute neurological side effects of available DA antagonist antipsychotics.

244.18

Chromate Alters Haloperidol Brain/Plasma Concentration Ratio. T.M. Smith*, D. Allen*, H.L. Evans and G.C. Stone. N.S. Kline Inst., Orangeburg, N.Y. 10962 and Inst. Environ. Med., NYU Medical Ctr., Tuxedo, N.Y. 10987.

To equate blood levels of psychotherapeutic agents with clinical response is of value in psychiatry. Since the target tissue of these agents is the brain, it is of interest to determine how much of the drug would be in the brain compared to what is found in blood. This brain/plasma concentration ratio has been determined for a number of drugs by others. Exogenous substances which change this ratio may effect treatment or lead to toxicity. We have investigated whether chromate ($K_2C_rO_4$), a persistent environmental contaminant which has the potential for human exposure via drinking water, may effect the bioavailability of haloperidol (HAL) in brain. Steady state plasma levels of HAL in rats was obtained by Alzet pumps. At a dose of 1 mg/day the plasma levels of HAL in the rat mimicked the human therapeutic range. While receiving HAL, experimental rats also received chromate via drinking water at a level of 500 ppm. Control rats received only HAL. At the end of 12 days, blood and 5 brain regions were analyzed for HAL by GC. Except for the cerebellum, all tested brain regions of chromate exposed rats had higher ratios with the frontal cortex showing the greatest increase ($p = < 0.02$).

244.19

CHRONIC HALOPERIDOL ALTERATION OF LICKING PATTERNS IN RATS AND MICE: A BEHAVIORAL ANALYSIS FOR NIGROSTRIATAL FUNCTION. K.M. Kantak and J.J. Vogel*. Lab. of Behav. Neurosci., Dept. Psychol., Boston Univ., Boston, MA 02215.

Analysis of licking patterns in mice has previously revealed a progressive increase in the number of licks per bout over 3 months. When mice received daily haloperidol, alterations in the pattern of licking occurred upon haloperidol withdrawal and spontaneously after 2 and 3 months of injections. The pattern resulted from increases in the number of bouts of licking and decreases in the number of licks per bout, indicating a disrupted pattern of licking. In the present studies these effects in mice were replicated for a fourth time. In rats, there also was a progressive increase in the number of licks per bout over 3 months. Rats reacted differently to chronic haloperidol. Upon withdrawal of haloperidol after month 3, there was an increase in the number of licks per bout and decreases in the number of bouts of licking, indicating a more stereotypic pattern of licking than controls. Thus, the presumed dopamine supersensitivity which occurs following chronic haloperidol produces different types of alterations in licking patterns in rats and mice.

244.20

ANTIPSYCHOTIC ACTION OF SM-9018, A CENTRALLY ACTING DOPAMINE D_2 AND SEROTONIN $(5\text{-HT})_2$ RECEPTOR ANTAGONIST. M. Nakamura*, Y. Ohno, A. Hirose*, H. Shimizu*, H. Tanaka* and T. Kato*. Res. Labs., Sumitomo Pharmaceuticals Co., Ltd., Konohana-ku, Osaka 554, Japan.

Studies were performed in rats and mice to clarify the pharmacological profile of SM-9018 (cis-2-{4-[4-(1,2-benzisothiazol-3-yl)-1-piperazinyl] butyl} hexahydro- 1H-isoindole-1,3-[2H]-dione HCl), a potential antipsychotic agent. In a receptor binding study, SM-9018 exhibited very high affinities both for 5-HT_2 and D_2 receptors and moderate affinities for α_1, 5-HT_1 and D_1 receptors. Orally administered SM-9018 markedly inhibited 5-HT_2 receptor-mediated behaviors (i.e., head twitches, wet dog shakes and clonic seizures) induced by 5-HT stimulants and blocked dopamine agonist-induced behaviors (i.e., stereotypy, hyperactivity and climbing behavior), with ED_{50} values of 1.4-5.8 mg/kg. Low-dose SM-9018 selectively inhibited the conditioned avoidance response in rats (ED_{50}=3.3 mg/kg, p.o.) with a potency greater than that of chlorpromazine while only weakly reducing the escape response. In addition, SM-9018 was much weaker than chlorpromazine and haloperidol in producing catalepsy, bradykinesia in the pole-test, disturbance of motor coordination and suppression of spontaneous locomotion. These results suggest that SM-9018 is a novel antipsychotic agent with less extrapyramidal and CNS depressive side-effects and it produces a combined blockade of dopaminergic and serotonergic transmission.

244.21

EFFECTS OF HALOPERIDOL ON NMDA AND $GABA_A$ RECEPTORS IN MEDIAL PREFRONTAL CORTEX. S.L. Vincent, R. Allen*, J.P. SanGiovanni* and F.M. Benes. Department of Psychiatry and Program in Neuroscience, Harvard Medical School and Mailman Research Center, McLean Hospital, Belmont, MA 02178.

Previous studies have shown that treatment with haloperidol (HAL) induces a change in the relative number of asymmetric and symmetric synapses in layer VI of rat medial prefrontal cortex (MPF) where the mesocortical projection terminates. Such neuroleptic induced shifts of excitatory and inhibitory cortical synapses raises the possibility that these drugs may affect the distribution of certain populations of receptors in MPF. To explore this question further, the present study has examined the relative distribution of NMDA and $GABA_A$ receptor sites using L-[^{3}H]glutamic acid and [^{3}H]muscimol, respectively, in rat MPF following treatment with haloperidol decanoate (dose equivalent of 1 mg/Kg/day) for 2 weeks. Quantitative high-resolution analysis of autoradiograms was carried out in neuropil regions and neuronal cell bodies in layers II/III, V and VI of MPF. In neuropil, specific NMDA-sensitive glutamate binding was similar between control and HAL-treated groups, while on neurons, it was decreased by 40% in layer V only. Specific $GABA_A$-sensitive muscimol binding (bicuculline-inhibited) in neuropil was decreased by 19% for layers Ia and II in the HAL-treated group, but on cell bodies in layers II/III, V and VI, it was reduced by 76%, 61% and 71%, respectively. These data suggest that HAL administration is associated with a generalized down-regulation of $GABA_A$ receptors in the neuropil and on neuronal cell bodies of MPF; while it resulted in a highly focalized reduction of NMDA receptors on neuronal somata of layer V only. These data are consistent with the idea that neuroleptic drugs may alter the activity of intrinsic transmitter systems in rat MPF. Supported by NIMH grant MH-00423.

MOTIVATION AND SELF-STIMULATION

245.1

REFRACTORY PERIODS OF DORSOMEDIAL HYPOTHALAMIC UNITS DIRECTLY DRIVEN BY REWARDING STIMULATION OF THE VENTRAL TEGMENTAL AREA MATCH PSYCHOPHYSICALLY-DERIVED ESTIMATES FOR REWARD-RELATED NEURONS. K.L. Conover and P. Shizgal, CSBN, Department of Psychology, Concordia University, Montreal, Quebec, H3G 1M8.

Neuroanatomical and behavioral data suggest that cell bodies in the Dorsomedial Hypothalamic Nucleus (DMH) contribute to the rewarding effect of electrically stimulating of the Ventral Tegmental Area (VTA). The present study reinforces this view by demonstrating that neurons with somata in or near the DMH can be antidromically activated by rewarding VTA stimulation in rats, and that the refractory periods of most of these directly-driven cells resemble those of neurons responsible for the rewarding effect.

Responses were obtained from 186 units, 38 of which were antidromically-driven by VTA stimulation. Eighteen of the sites at which the antidromic responses were seen lay in the DMH or nearby (within 200 μm). Ten DMH units were driven using stimulation sites, currents and pulse durations that were previously shown to support self-stimulation and were used to psychophysically estimate recovery from refractoriness in reward neurons. The refractory periods of 8 of these 10 cells fell within the range of the psychophysically-derived estimates, as did the refractory periods of an additional 8 neurons driven by stimulation of VTA sites that had not been behaviorally screened.

245.2

CAUDAL MEDIAL FOREBRAIN BUNDLE TRANSECTIONS DISRUPT THE REWARDING EFFECT OF LATERAL HYPOTHALAMIC SELF-STIMULATION. Meg A. Waraczynski, Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA 19104

This study investigates changes in self-stimulation of the lateral hypothalamic (LH) medial forebrain bundle (MFB) in the rat following transection of the caudal MFB and ventral tegmental area (VTA). Rate-frequency functions were collected daily at a single current intensity from 10 rats. Following baseline behavioral stabilization, a coronal plane knife cut was made behind the stimulating electrode. Three rats showed a long-term 0.4 to 0.6 log unit increase in the frequency required to sustain half-maximal responding; 3 rats showed a 0.1 to 0.2 log unit increase, and 4 rats showed no change. Cuts were most effective when the electrode was in the posterior LH/MFB and the cut was not more than 1 mm caudal to the electrode, and when damage was to the MFB just rostral to the anterior border of the VTA. Cuts were least effective when placed ventrally in the MFB, or in the VTA and substantia nigra (SN). One rat with considerable damage to the VTA and SN showed no change in frequency required to sustain half-maximal responding, suggesting that cut effectiveness did not result solely from damage to dopaminergic neurons.

245.3

CONDITIONED PREFERENCE PRODUCED BY INJECTION OF GLUTAMATE INTO THE ANTERO-LATERAL HYPOTHALAMUS. B. Murray and P. Shizgal. CSBN, Concordia University, Montreal, Quebec H3G 1M8.

A preference task was used to determine whether chemical stimulation of cells in the anterior lateral hypothalamus (LH) is rewarding. For 15 min per day, male rats were placed in an open field with a floor consisting of 4 removable panels made from either rods or wire mesh. On Days 1 and 3, the entire floor was composed of panels of one material (the CS^+), and l-glutamatic acid (.5 µl, .5 M, pH 7.6) was injected into the anterior LH while the subjects were in the open field. On Days 2 and 4, the panels were of the alternate material (the CS^-), and artificial CSF (.5 µl, pH 7.8) was administered. On the test day, the floor was composed of two panels of each material, arranged in a checkerboard pattern. CSF was administered, and the position of the subject was recorded on videotape.

The group (n = 11) with histologically verified injection sites in the anterior LH spent significantly more time on the flooring paired with the glutamate injections than on the flooring paired with CSF. Two subjects with cannulae aimed 1 mm dorsal to this site failed to show a conditioned preference for the glutamate-paired flooring. These data suggest that activation of somata in the anterior LH is rewarding, a result complementary to the attenuation of medial forebrain bundle self-stimulation by lesions of the anterior LH.

245.4

FAILURE OF ESTROGEN TO ALTER LATERAL HYPOTHALAMIC BRAIN STIMULATION REWARD. C.M. Pudiak & M.A. Bozarth, *Department of Psychology, University at Buffalo, Buffalo, NY 14260.*

Several measures were used to determine the effects of ovariectomy and subsequent estrogen replacement on brain stimulation reward (BSR). Female, Long-Evans rats were implanted with monopolar electrodes aimed at the lateral hypothalamic level of the medial forebrain bundle. After 5 to 10 days recovery from surgery, they were trained to press for monophasic cathodal stimulation pulses using one of three different BSR techniques. The frequency-rate method determined 2-min response rates for a descending series of stimulation frequencies. The autotitration procedure determined the self-selected minimum current intensity necessary to maintain responding. And, the threshold tracking procedure determined the minimum stimulation frequency necessary to maintain 30 responses/min (see Bozarth et al., *Soc. Neurosci. Abstr.*, 1990).

Ovariectomy had no effect on BSR thresholds obtained with any of the three measures. 17-β-Estradiol injections (0.2 to 20 µg) also failed to alter BSR thresholds. These data indicate that estrogen does not influence the rewarding impact of lateral hypothalamic BSR and that earlier studies reporting estrogen modulation of BSR are probably attributable to differences in electrode placements or to nonspecific enhancement of lever-press rates.

245.5

A THRESHOLD TRACKING PROCEDURE FOR STUDYING BRAIN STIMULATION REWARD. M.A. Bozarth, C.M. Pudiak, J. Norcera*, & C.M. Specht*, *Department of Psychology, University at Buffalo, Buffalo, NY 14260.*

An experimental procedure is described that permits continuous tracking of brain stimulation reward (BSR) thresholds. This method determines the minimum stimulation frequency that maintains a predetermined response rate (e.g., 30 presses/min). Subjects are initially presented with a series of descending stimulation frequencies (0.1 log units/step). One-minute response rates are determined for each frequency, and the minimum frequency that maintains the criterion response rate is defined as threshold. When the response rate is less than the criterion, the stimulation frequency increases (0.1 log units/min) until responding is reinstated (≥ criterion).

Threshold tracking yields threshold values that are similar to those obtained using the frequency-rate method. The latter method, however, is laborious and requires 15 to 20 min for a single threshold determination, while threshold tracking is fully automated and can determine BSR thresholds in as little as 1 to 3 min. Thresholds obtained with this method are stable (±5%) over several months of continuous or intermittent testing. d-Amphetamine (0.1 to 1.0 mg/kg, i.p.) produces dose-dependent threshold lowering (14 to 60% ↓), with a detection limit of ≤ 0.1 mg/kg. Threshold tracking provides a fast, reliable, and sensitive measure of BSR thresholds and may offer several important advantages over traditional BSR measures.

245.6

EFFECTS OF MEDIAL AND POSTERIOR HYPOTHALAMIC KNIFE-CUTS ON LATERAL HYPOTHALAMIC SELF-STIMULATION REWARD J.R. Stellar, F. Scott Hall, and Hugh Albert: Psychol. Dept., Northeastern Univ. Boston MA 02115.

Rats with a lateral hypothalamic (LH) electrode were given a sagittal knife-cut between the LH and the dorsalmedial nucleus of the hypothalamus (DMH). A second group with posterior LH electrodes were given a coronal knife-cut in the mid-LH. Both groups were tested on a self-stimulation (SS) task using a rate-frequency curve-shift method at up to five stimulation currents daily before and for three weeks after the knife-cut. In the first group, sagittal knife-cuts had little or no effect on SS reward. In the second group, coronal knife-cuts produced a substantial SS reward decrease (LOR increase) which was larger at lower currents. We conclude that DMH fibers do not directly mediate LH SS reward as may have been inferred from other work (cf. Glimcher et al., Neurosci Abst,1989), and that previous lesion-SS studies with only one stimulating current per subject may have missed some SS reward effects.

(Supported by the Whitehall Foundation)

245.7

HALOPERIDOL PREVENTS THE ESTABLISHMENT OF BRAIN STIMULATION INDUCED CONDITIONED PLACE PREFERENCES BUT NOT AVERSIONS IN RATS. C.L. Duvauchelle and A. Ettenberg. Dept. Psychology, Univ. Calif., Santa Barbara, CA 93106

Male rats were implanted with indwelling intracranial electrodes aimed at either the lateral hypothalamus (LH), prefrontal cortex (PFC), ventral tegmental area (VTA) or the dorsomedial tegmentum (DMT). The pairing of brain-stimulation with one of two distinct conditioning environments led to subsequent conditioned place preferences (CPPs) for the stimulation-paired environment in LH, PFC and VTA subjects and a conditioned place aversion in DMT-stimulated rats. Pretreatment on conditioning trials with the dopamine antagonist haloperidol (0.15-0.3 mg/kg), prevented the establishment of all CPPs but had no effect on the learned aversions of the DMT group. These results suggest that a) memory encoding processing remain intact under neuroleptic challenge, and b) haloperidol attenuated the rewarding properties of LH, PFC and VTA stimulation.

245.8

EFFECTS OF ACCUMBENS OPIATE MICROINJECTION ON INTRACRANIAL SELF-STIMULATION FOLLOWING NEONATAL DOPAMINE LESION. P. Johnson1, K.S. Sidhu1, J.R. Stellar1, and J. Bruno2. 1-Psychol. Dept., Northeastern Univ., Boston, Ma., 02115; 2-Psychol. Dept. Ohio State University, Columbus Ohio 43210.

Neonatal day 3 rats given intraventricular injections of 6-OHDA yield adults with severe permanent dopamine (DA) lesions, but relatively spared lateral hypothalamic self-stimulation (LHSS) reward function (Stellar et al. Pharm Biochem & Behav 30:365,1988). We now report that neonatal DA-lesioned rats are hypersensitive to accumbens microinjections of d-ala-met-enkephalinamide (DALA) in a manner that parallels our previous report of DALA hyper-sensitivity in rats given accumbens DA lesions as adults (Johnson et al. Neurosci. Abst. 1989). However, unlike our adult lesion results, the DALA results with neonatal DA lesions are not temporary.

(Supported by the Whitehall Foundation)

245.9

NUCLEUS ACCUMBENS SINGLE UNIT ACTIVITY IN THE RAT DURING VTA SELF-STIMULATION. M. Wolske, P-P. Rompre, R.A. Wise, and M.O. West Dept. of Psychology, Rutgers Univ., New Brunswick, NJ 08903 and Dept. of Psychology, Concordia Univ., Montreal, Quebec, Canada.

The present study used extracellular single unit recordings in the freely moving rat to study the effects of ventral tegmental area (VTA) self-stimulation on nucleus accumbens neurons. Male Long-Evans rats were implanted with a detachable microdrive for recording in the accumbens and with stimulating electrodes in the ipsilateral VTA and fimbria. A 500 msec duration train of VTA pulses (0.1-0.5 mA, 0.1 msec, 20-30 Hz) was used as a reinforcer for lever pressing (FI 1.5 sec). Unit responses (N=74 units) to trains of stimulation showed 4 different patterns in peri-event histograms: 1) Loosely time-locked neurons (LTL; N=15/74) showed excitatory time-locked responses of long duration (3-25 msec). 2) Tightly time-locked neurons (TTL; N=18/74) showed time-locked single spike responses with invariant latency (range 11-16 msec across units). 3) Excitatory responses (EXC; N=16/74) consisted of overall increases during the train, not time-locked to individual pulses. 4) Inhibitory responses (INH; N=25/74) consisted of overall decreases during the train, not time-locked to individual pulses. Statistical analysis showed that LTL exhibited significantly higher between-train firing rates (range 0.40 to 23.81 Hz) than neurons in all other categories (range 0.00 to 3.99 Hz). TTL tested for high frequency following (n=5) followed 200-1200 Hz stimulation. Two of these units were tested for collision and both collided. These results suggest that TTL responses reflect antidromic activation whereas LTL, EXC and INH represent orthodromic responses (mono- or polysynaptic) of accumbens neurons to VTA self-stimulation. Supported by BNS-8708523, DA 04551 and RR 07058-21.

245.10

ENHANCED RESPONSIVENESS OF NUCLEUS ACCUMBENS UNITS TO ODORS ASSOCIATED WITH AMPHETAMINE ADMINISTRATION IN RATS. Charles H.K. West and Richard P. Michael. Department of Psychiatry, Emory University School of Medicine, and Georgia Mental Health Institute, 1256 Briarcliff Road, Atlanta, GA 30306.

It has been proposed that the mesolimbic dopamine (DA) system is involved in sensory filtering and reward processes. We previously reported that the responsiveness of neurons in mesolimbic terminal regions to sensory stimuli was increased by conditioning animals to associate sensory cues with rewarding brain stimulation. Here we report that the effectiveness of odors in evoking responses in mesolimbic units is increased by pairing the odors with the rewarding effects of a psychostimulant drug. Adult male rats were trained to associate administration of d-amphetamine (0.5 mg/kg, sc) with one odorant and administration of saline with another (4 sessions/drug, 40 min each). Additional rats were exposed in their home cages (4 sessions) to a previously unfamiliar odor. Subsequently, all rats (n = 16) were anesthetized with urethane (1.4 g/kg, ip), and single units were recorded in caudate-putamen, nucleus accumbens (NAC) and olfactory tubercle and were tested with 10 odorants. Pairing odors with amphetamine increased their effectiveness at evoking unit responses. This effect was most marked in NAC where the proportion of units responsive to paired and non-paired odors was 72% and 24%, respectively. In contrast, pairing odors with saline did not alter the proportion of responsive NAC units. However, familiarizing an animal with an odorant increased the proportion of units responsive to that odorant from 17% (unfamiliar) to 43% (familiar). These results support a role for neurons in mesolimbic DA terminal regions in modulating sensory stimuli relevant to reward processes.

(Supported by the Georgia Department of Human Resources.)

245.11

EFFECT OF RAPHE STIMULATION ON SINGLE UNIT ACTIVITY IN THE NUCLEUS ACCUMBENS SEPTI (NAS) OF THE RAT. C.W. Callaway. and S.J. Henriksen. Research Institute of Scripps Clinic, La Jolla, CA 92037.

The NAS is thought to be involved with the action of psychostimulant drugs and with the maintenance of rewarded behavior. Because behavioral and biochemical evidence suggests that the neurotransmitter serotonin (5-HT) can affect these NAS-mediated behaviors and because most forebrain 5-HT arises from the midbrain raphe nuclei, we examined the effects of raphe stimulation on firing of single NAS units. Stimulating electrodes were placed in the fimbria and the dorsal raphe nuclei of halothane-anesthetized male rats, and unit activity was recorded in the NAS using single-barrel glass micropipettes. Single pulse stimulation of the raphe was followed by 50-100 ms inhibition of spontaneous firing of NAS units, although a few cells responded with brief (50 ms) increases in firing rate. The inhibitory response was antagonized by cyproheptadine (2 mg/kg, s.c.). Stimulation of hippocampal afferents in the fimbria produced monosynaptically evoked discharges in NAS units with latencies of 4-9 ms and polysynaptically evoked discharges with latencies of 15-25 ms. Conditioning stimulation of the raphe (10-20 hz, 0.5-1.0 sec train) increased the current required to evoke the longer latency fimbria-evoked discharges, but did not affect units with monosynaptic latencies. Preliminary tests indicate that the 15-25 ms latency fimbria-evoked discharges result from stimulation of the hippocampal formation, suggesting that raphe stimulation may modify firing of NAS units in part by altering input from at least one major afferent to the NAS.

245.12

CHARACTERISTICS OF REWARD NEURONS IN THE CAUDATE-PUTAMEN : REFRACTORY PERIOD ESTIMATES. M. M. Trzcińska and C. H. Bielajew. University of Ottawa, School of Psychology, 275 Nicholas, Ottawa, Ontario, K1N 6N5.

The excitability characteristics of the substrate underlying brain-stimulation reward in the caudate-putamen were investigated. Male Hooded rats were implanted with a moveable electrode aimed at the rostral caudate nucleus (AP: +1.7 mm ML: 1.3-1.4 mm V: 1.8-3.3 mm). Stimulation consisted of 500 msec trains of 0.1 msec rectangular, cathodal pulses delivered on a continuous reinforcement schedule. Stable bar pressing behavior was observed at currents of 600-800 uA, with acquisition of self-stimulation occurring within the first shaping session. Maximum rates ranged from 10 to 40 responses per minute. Seven refractory period estimates were obtained from seven different sites in two animals. The time course of recovery from refractoriness had the following profile: Recovery was observed at 0.5 and 1.0 msec; the end of recovery increased from roughly 2.0 to 10.0 msec as the electrode was moved ventrally. In the first subject, asymptote effectiveness values did not exceed 50%, while in the second subject, almost 100% effectiveness was obtained. These results are consistent with the observation that the refractory periods underlying forebrain self-stimulation tend to be longer than the ones reported for midbrain regions.

This research was supported by NSERC grant #UO514 to C.B.

245.13

SELF-STIMULATION THRESHOLDS ALTERED BY DOPAMINE AGONISTS AND ANTAGONISTS. S. Nakajima and N.B. O'Regan, Dalhousie University, Halifax, Nova Scotia, Canada

Dopamine antagonists generally attenuate brain self-stimulation, and some dopamine agonists facilitate it. Whether the behavioral changes are caused by alterations in the reinforcing effect of stimulation or by interference with motor performance has been controversial. One way to dissociate motor effects from reinforcement itself in such a situation is to examine the response rates across a range of stimulation frequencies and plot a frequency-response curve.

Rats were trained to press a bar for lateral hypothalamic stimulation, and tested 30 min after injection of a selective dopamine D1 or D2 agonist or antagonist. Either SKF 38393 HCl (D1 agonist) 0.4 mg/kg or quinpirole (D2 agonist) 0.1 mg/kg shifted the frequency-response curve to a lower range, and either SCH 23390 maleate (D1 antagonist) 0.04 mg/kg or raclopride (D2 antagonist) 0.04 mg/kg shifted it to a higher range. The results suggest that both D1 and D2 receptors are critically involved in producing the rein-forcing effect of brain stimulation.

245.14

EFFECTS OF D1 AND D2 ANTAGONISTS ON LATERAL HYPOTHALAMIC SELF-STIMULATION REWARD IN ADULT RATS DOPAMINE-LESIONED ON NEONATAL DAY 3. K.S. Sidhu[1], J.R. Stellar[1], J. Bruno[2]. 1-Psychology Department, Northeastern Univ., Boston, MA 02115, 2-Psychology Department, Ohio State Univ., Columbus, Ohio 43210.

Rats were given bilateral ICV 6-OHDA injections (75ug each side) on Day 3 of neonatal life to produce severe adult dopamine (DA) depletions. As adults, these rats were tested on a rate-frequency paradigm for lateral hypothalamic self-stimulation (LHSS) reward under 4 systemic doses of D1 (SCH 23390, 0.01- 0.15mg/kg) and D2 (Raclopride 0.02-0.6 mg/kg) DA receptor blockers. Results indicate that both drugs were effective in reducing LHSS reward (increasing LOR), but compared to sham-lesioned controls, the neonatal DA-lesioned rats appeared somewhat subsensitive to Raclopride in agreement with our earlier work (Stellar et al. PB&B 30:365,1988). Combining the 3rd highest dose from each drug produced a LOR increase that was similar to the arithmetic sum of the separate LOR effects of the two doses alone, indicating no strong advantage of the combination drug treatment.

(Supported by the Whitehall Foundation)

245.15

DOPAMINE BLOCKADE IN NUCLEUS ACCUMBENS REDUCES BAR-PRESS BUT NOT PLACE PREFERENCE MEASURES OF LH STIMULATION REWARD. J. M. Goodman, D. B. Neill, and J. B. Justice, Jr. Depts. of Psychology and Chemistry, Emory University, Atlanta, GA 30322.

We used the conditioned place preference procedure as a rate-free measure of lateral hypothalamic stimulation reward. Rats received noncontingent hypothalamic stimulation while exposed to one of two environments. Conditioning took place on two consecutive days and consisted of one twenty minute exposure to one of the environments per day. When given free access to both environments, rats showed a preference for the one that was paired with the brain stimulation. This preference was impaired by systemic (0.8 but not 0.25 mg/kg), but not intra-accumbens (50 ug/ul bilaterally) administration of the dopamine receptor blocker *cis*-flupenthixol. The systemic and intra-accumbens doses which did not reduce place preference severely impaired bar-pressing for brain stimulation. We conclude that the reward of hypothalamic stimulation is not reduced by blockade of accumbens dopamine receptors and that a major portion of reduced bar-pressing for the stimulation is motoric in nature.

245.16

AUTOTITRATION ANALYSIS OF CHOLINERGIC INVOLVEMENT IN REWARDING HYPOTHALAMIC STIMULATION. D. B. Neill and K. Krebs*. Dept. of Psychology, Emory University, Atlanta, GA 30322.

Enhanced transmission of the neurotransmitter acetylcholine (ACh) has been linked with depression in humans. We observed the effect of elevated ACh on an hypothesized animal indicator of depression, decreased responding to intracranial self-stimulation. The anticholinesterase physostigmine was injected *i.p.* (0.05-0.10 mg/kg) into rats trained in autotitration self-stimulation of the lateral hypothalamus. Physostigmine reliably produced suppression of responses, but had no significant effect on reset intensity. To explain this unusual pattern of results we hypothesize the existence of two separate cholinergic systems. Activation of one system decreases reward (higher reset intensity; anhedonia); activation of the other decreases behavioral activation (lower reset intensity; anergy). Simultaneous activation of these systems, such as by systemic administration of physostigmine, could result in a cancelling out and no change in reset intensity, the pattern observed in this study. Decreased responding, produced by activation of either system, remains as the most consistent result of physostigmine administration.

245.17

EFFECT OF REPEATED NICOTINE ADMINISTRATION ON DORSAL RAPHE BRAIN STIMULATION REWARD. P. Bauco and R.A. Wise. Ctr. Stud. Behav. Neurobiol., Dept. Psychol., Concordia U., Montreal, Canada.

The effects of nicotine (0.05, 0.1, 0.2, or 0.4 mg/kg, s.c.) on dorsal raphe self-stimulation were assessed daily for 10 days in independent groups of rats. Nicotine caused a leftward shift of the function relating response rate to stimulation frequency; thus nicotine reduced the "dose" of rewarding stimulation necessary to produce responding at normal levels. The effects of nicotine were consistent from day to day; there was no evidence of either tolerance or sensitization. The reward-facilitating effects were strongest with the 0.1 mg/kg dose. Duration of facilitation increased with nicotine dose. Treatment with the nicotine antagonist mecamylamine (1 mg/kg, s.c.) had no significant effect on baseline responding, but blocked the effect of nicotine at each of the doses tested. Thus nicotine, like other habit-forming drugs, increases the impact of brain stimulation reward.

245.18

THE EFFECT OF CHRONIC INTRACRANIAL SELF-STIMULATION ON CYTOCHROME OXIDASE LEVELS IN CNS.
C. Gow, C. Kateb, K. Harman, and C. Bielajew
School of Psychology, University of Ottawa, K1N 6N5.
The relationship between cytochrome oxidase levels and the amount of activity in developing and mature CNS neurons has been previously demonstrated (Wong-Riley, TINS, 1989, 12, 94). We have reported slight increases in cytochrome oxidase levels in several CNS structures following long-term administration (20 min/day) of rewarding brain stimulation in the lateral hypothalamus. In order to enhance the metabolic demands of the critical substrate, we conducted the present study in which stimulation trials were significantly lengthened (4 hr/day) and cytochrome oxidase activity across the entire brain was quantified using optical densitometric methods. Immediately following the final stimulation session, animals were perfused and the brains prepared for cytochrome oxidase histochemistry using the procedure described by Wong-Riley (Brain Res, 1979, 171, 11-28). Generally, the stimulated hemisphere showed greater increases in cytochrome oxidase activity than did the unstimulated hemisphere, measured in relative optical density units. Additionally, darker reaction products were noted in central brain regions, as defined anteriorly by the caudate nucleus and posteriorly by the substantia nigra. Outside this region, enzymatic changes were less noticeable.
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245.19

SUPPRESSION OF SEIZURES WITHOUT ALTERING EXCITABILITY PROPERTIES OF REWARD-RELATED NEURONS BY THE NOVEL BENZODIAZEPINE - BROTIZOLAM
T. Harris and C. Bielajew. School of Psychology, University of Ottawa, Ottawa, Ontario, Canada, K1N 6N5.
Brotizolam, a novel benzodiazepine, with longer-acting and stronger anti-convulsant properties than the traditional diazepine derivatives, was investigated for its ability to control stimulation-induced seizures without altering the time course of the behaviourally derived refractory period profiles. Three rats were implanted with LPO electrodes and one rat with an LH electrode. Frequency thresholds were interpolated at a fixed criterion of 35 responses/minute from ascending rate/frequency functions. Brotizolam (7.5mg/kg i.p.) and its vehicle were administered on alternate days with at least two days between drug sessions. Three hours post-injection, refractory periods were determined at currents which maximized the probability of inducing seizures (800-1000μA). Seizure activity was rated using Racine's five-point classification system. Brotizolam suppressed overt seizures during the average 105 minute testing sessions without altering the recovery from refractoriness, estimated to be roughly 0.4 to 2.0 msec across animals. These results suggest that brotizolam could be a useful agent for investigating the properties of the directly stimulated substrate mediating brain-stimulation reward in seizure-prone sites.
Supported by NSERC Grant #U0514 to C.B.

245.20

EFFECTS OF HOUSING ON RESPONSE TO HANDLING, LOCOMOTOR ACTIVITY AND THE ACQUISITION OF INTRACRANIAL SELF-STIMULATION IN RATS. G.J.Schaefer and R.P. Michael, Department of Psychiatry, Emory University, School of Medicine, Georgia Mental Health Institute, 1256 Briarcliff Rd., Atlanta, GA 30306.

Rats (N=24) were implanted with electrodes in the lateral hypothalamus. Following recovery from surgery, they were divided into two groups of equal mean body weights. Animals in one group were housed individually, while animals in the other group were housed four to a cage. These housing conditions were maintained throughout the experiment. After four weeks in these housing conditions, animals were tested individually for changes in locomotor activity (15 min/day for 5 days) and in their response to handling during the fifth week. During the sixth and seventh weeks, animals were tested for the acquisition (self-shaping) of intracranial self-stimulation (ICSS) (15 min/day, 5 days/week). Compared with animals housed individually, animals housed in groups were less re-active to handling and had higher locomotor activity scores. Animals housed in groups also made more responses during the acquisition of the ICSS response than did animals housed individually. These results demonstrated that changes in housing conditions for a period of four weeks can influence both unconditioned behavior (locomotor activity and response to handling) and the acquisition of ICSS. (Supported by the Georgia Department of Human Resources.)

245.21

EVIDENCE OF A NON-UNITARY SCALE OF REINFORCEMENT AS MEASURED BY PSYCHOPHYSICAL PREFERENCE TESTS. R. Raymond and E. Miliaressis. Behavioral Neurophysiology Lab. University of Ottawa, Ottawa, Ont., K1N 6N5.

Since 1964, brain stimulation reward researchers have considered the reinforcement system to be unitary. Malette and Miliaressis (in press) have shown that pulse frequencies that elicit identical proportions of the maximum self-stimulation (SS) rate within the same structure are equipreferred. The purpose of this study then, was to determine if upon stimulation of two different stuctures, such pulse frequencies would also be equipreferred. Therefore, male Sprague-Dawley rats implanted with monopolar moveable electrodes were given a choice between stimulation of the ventral tegmental area (VTA) and the lateral hypothalamus (LH). It was hypothesized that in the event that the rats fail to equate behaviorally two stimuli that elicit identical proportions of the maximum SS rate, two separate scales of reinforcement may be in play. Results of this study show that at pulse frequencies that elicit identical proportions of the maximum SS rate, rats did indeed choose the VTA over the LH at a mean preference ratio of .9. These results clearly support a non-unitary system of reinforcement.
Supported by a Natural Sciences and Engineering Research Council grant, A8625, to E.M.

245.22

A STUDY OF THE AXONAL LINKS BETWEEN REWARD-RELEVANT NEURONS IN THE BILATERAL HYPOTHALAMIC NUCLEI. J. Malette and E. Miliaressis, School of Psychology, University of Ottawa, Ottawa, On., Canada, K1N 6N5.

Unilateral self-stimulation (SS) has been reported to result in a bilateral activation of the dopaminergic terminals (Porrino et al., Science, 1984, 224, 4646). Forebrain ablations performed by Colle and Wise (Brain Res., 1987, 407) on self-stimulating animals suggest that the SS system may not entirely be unilateralized. The aim of our study was to investigate the reward-relevant bilateral linkage within the medial forebrain bundle. Male Sprague-Dawley rats were implanted with moveable electrodes aimed bilaterally at the lateral hypothalamic nuclei (LH). The behavioral version of the collision test was used and consisted of the delivery of trains of pairs of pulses, with the conditioning pulse (C) delivered to a first electrode and the test pulse (T) delivered to the contralateral electrode. C-T intervals varied from 0.2 to 5.0 ms. Collision was inferred from an increase in SS thresholds at short C-T intervals. High SS thresholds were obtained at C-T intervals inferior or equal to the refractory period of the LH. Consequently, direct reward-relevant axons do not seem to link the bilateral LH. A heterosynaptic summation, rather, seems to take place between these bilateral nuclei.
Supported by a Natural Sciences and Engineering Research grant, A8625, to E.M.

INVERTEBRATE LEARNING AND BEHAVIOR II

246.1

CHANGES IN LEARNING AND MEMORY THROUGHOUT THE LIFESPAN OF THE NEMATODE *C. ELEGANS*. C. D. O. Beck, S. R. Cerniuk* & C. H. Rankin, Dept. of Psychology, Univ. British Columbia, Vancouver, BC, V6T 1Y7.

Adult *C. elegans* are capable of simple forms of non-associative learning such as habituation, dishabituation and sensitization as well as both short and long-term memory (Rankin, Beck & Chiba, *Behav. Br. Res.*, 37: 89, 1990). We have examined the changes in these forms of learning and memory over both early (prereproductive) and late (postreproductive) development. Based on anatomical evidence, there are three distinct patterns of neural connectivity: an embryonic pattern, a transitional larval pattern, and an adult pattern. Developmental studies of non-associative learning indicate that these different patterns of neural connectivity are reflected in differential learning abilities. *C. elegans* shows habituation in all stages of development; however larval animals require shorter ISI's than adults (adult=4 days old). In addition larval animals appear to recover from habituation more rapidly than adults. Dishabituation is expressed in all developmental stages except during the second larval stage. In postreproductive development, changes in the degree of habituation and the lack of apparent dishabituation in animals 7, 10, and 12 days of age suggest functional changes in the aging nervous system.

These studies of learning and memory throughout the lifespan of *C. elegans* will allow a characterization of some of the properties of the mechanisms underlying changes in learning and memory early in development when postembryonic neurons are arising and forming connections and during aging when the nervous system may be degenerating functionally.

246.2

INCREASE IN BRANCHING AND NUMBER OF VARICOSITIES IN MOTOR AXON TERMINALS OF *DROSOPHILA* MEMORY MUTANTS WITH ALTERED cAMP LEVELS. Y. Zhong, V. Budnik & C.-F. Wu. Dept. of Biology, Univ. of Iowa, Iowa city, IA 52242

Modulation of synaptic efficacy may result from changes in morphology or membrane properties at pre- or postsynaptic sites. Such cellular modifications have been demonstrated to accompany long-term behavioral acquisition. In these processes, modulation of membrane currents has been shown to be mediated by second messengers such as cAMP, Ca^{2+}, and phospholipids. *Drosophila* memory mutants at the *dunce* (*dnc*) locus reduce or eliminate activity of phosphodiesterase (PDE) II so that cAMP level is increased. We have shown that the synaptic plasticity, such as facilitation and potentiation, at neuromuscular junctions of these mutants is altered (Zhong & Wu, Soc. Neurosci. Abstr. 15:1141). Therefore, it is of interest to determine whether morphological changes can also be induced by disrupted cAMP metabolism. We now report that the number of terminal branches and varicosities are significantly increased at the neuromuscular junction in *dnc* mutants.

Anti-HRP immunocytochemistry was employed to visualize nerve terminals innervating body-wall muscles of the third instar larva. Only the terminals on muscle fibers 13 and 14 in abdominal segment 3 were scored to examine the mutational effect. The general innervation pattern specific to each identified muscle fiber was preserved in different *dnc* alleles. However, the results indicated that both dnc^{1} and dnc^{M14} have a greater number of varicosities (338±55 and 313±36, respectively) than normal larvae (243±44, mean±SD). The increase in terminal branching was due mainly to a larger number of higher-order branches. The heterozygote of dnc^{1}/dnc^{M11} showed an even stronger increase in number of varicosities (381±99) and terminal branches. Our results suggest a possibility that elevating cAMP levels can lead to an increase of synaptic contacts between nerve terminals and their targets. However, it remains to be determined how the increment in synaptic contact contributes to changes in synaptic efficacy.

246.3

ISOLATION OF NEW SINGLE-GENE MUTANTS AFFECTING MEMORY IN *DROSOPHILA MELANOGASTER*.
S. Boynton*, A. Villella*, and T. Tully. Department of Biology, Brandeis University, Waltham, MA. 02254.

Classical conditioning of an olfactory avoidance response produces strong learning and retention in wild-type *Drosophila*. Previous genetic dissection of conditioned behavior in *Drosophila* has isolated six mutant strains which exhibit reduced learning or memory of this task. In agreement with one current model of memory formation, memory in *Drosophila* appears to require normal cAMP regulation, as two of the learning/memory mutants affect enzymatic components of the cAMP pathway.

We have begun a screen for new mutations affecting conditioned olfactory avoidance behavior. Mutant strains, generated by a single p-element transposition, were tested for memory loss three hours after training. Those strains consistently scoring less than 70% of wild-type then were studied further. One strain, *latheo*, shows abnormally low learning and retention, with a more rapid decay of memory in the first 30 minutes after training. The mutant *latheo* appears to be normal in its ability to smell the odorants, to locomote, and to sense electric shock. Genetic analyses confirm that the retention deficit in *latheo* indeed is a single-gene effect caused by a p-element insertion. Significantly, the p-element insertion in the *latheo* gene constitutes a "molecular tag," which can be used to obtain the relevant DNA sequences. In this manner, we have begun to clone this new gene involved with memory formation in *Drosophila*.
Supported by grants from the McKnight Foundation, NIH GM33205, and NIMH MH09946.

246.4

APIS MELLIFERA AS A NEUROPHYSIOLOGICAL MODEL FOR UNDERSTANDING THE ROLE OF NEUROTRANSMITTERS IN BEHAVIOR. J. Last*, R. Rosse*, I. Mefford*, S. Sheppard and W. Goldberg. Dept. Psychiatry, Georgetown Univ. Hosp., Washington DC 20009 and VA Med. Center, Washington DC, 20422.

Different strains of the honeybee, Apis mellifera, were studied to explore neurophysiological differences between more docile European strains and a highly aggressive Africanized strain. Africanized and one European bee strain specimens were collected from S. America by the USDA and preserved in frozen storage. Another European bee strain was collected in N. America (Maryland). Chromatographic analysis of brain neurotransmitters (dopamine, DOPAC, 5HT, 5HIAA), appear to demonstrate a significant lower brain level of dopamine in Africanized bees. Behavioral studies were performed in the field on haloperidol-fed Africanized bees (10mg/hive). Haloperidol treated hives appear to demonstrate a significant decrease in stinging response compared to placebo (glucose solution) treated Africanized hives as determined by number of stings/sq. inch on a mechanical agitator. Immunohistochemical studies of dopamine in brain tissue of Africanized bee brain appear to demonstrate increased density of dopamine reactivity when compared to the European strain.

246.5

MOTONEURONS INNERVATING THE MUSCULATURE OF THE FLY PROBOSCIS HAVE PROJECTIONS IN FUNCTIONALLY DISTINCT REGIONS OF THE SUBESOPHAGEAL GANGLION OF THE CNS.

L. C. Sudlow and L.L. Murdock*. Purdue Univ., W.Lafayette, IN 47907.

We sought to better understand the cytoarchitecture of the subesophageal ganglion (SEG), the site of the control of feeding in the fly. We mapped CNS projections of the motoneurons involved in ingestion and proboscis extension and retraction. Experiments were replicated in *Phormia regina* and *Calliphora vacina*. Cobalt, introduced via modified suction electrodes, followed by Timm's intensification was used to label specific cut nerves. Additionally, DiI was used to label the Retractor furca motoneurons in both species. The muscles involved in extension (Protractor fulcrum, Extensor of the haustellum and Adductor of the apodemes) and retraction (Retractor rostrum, Flexor of the hausetellum and Accessory Retractor rostrum) have projections found predominantly in the ventrolateral and ventral neuropil of the SEG. The Retractor furca, involved in labellar lobe spreading, has projections in the dorsomedial neuropil, slightly ventral to the labrofrontal nerve root. The Gracilis motoneuron and the motoneurons of the common medial cibarial dialator nerve have projections in an anterior region of the anterodorsal neuropil of the SEG, centered around the labrofrontal nerve roots. This region of the SEG receives sensory projections from the labellar and tarsal chemosensory hairs (Edgecomb, R.S. & L.L.Murdock, J. comp. Neurol., Ms submitted). The lateral cibarial nerves appear to contain only sensory neurons that arise within the rostrum. Many of these sensory neurons are cobalt-coupled to neurons in the anterodorsal neuropil. These secondary fills of SEG neurons were eliminated by labelling with Texas Red. These results suggest that the motoneurons of the proboscis musculature project to functionally distinct regions of the SEG neuropil.

246.6

Serotonin as a Postural Control "Gain-setter" in the Lobster.

P.M.Ma and E.A.Kravitz, Dept. Neurobiology, Harvard Medical School, Boston, MA 02115. Serotonin (5-HT) injection triggers the appearance of a flexed posture in lobsters which resembles the stance assumed by dominant animals. This suggests a role for the amine in the postural component of this behavior. In isolated ventral nerve cords, the application of 5-HT promotes the read-out of flexion motor programs. We have identified 5-HT-containing neurons in the lobster 1st abdominal ganglion which are likely to be involved in postural regulation and have attempted to examine the mechanism by which 5-HT might promote postural flexion. Direct stimulation of the 5-HT-containing cells does not affect motor output programs. Instead, 5-HT acts by facilitating command inputs to postural motoneurons. Flexion commands usually excite, while extension commands inhibit the 5-HT-containing cells. In further exploring the relationship between command neurons and the 5-HT-containing cells, we found that simultaneous activation of the 5-HT-containing neurons and flexion command fibers significantly amplified the efficacy of the command input, whether excitatory or inhibitory, to about 25% of the postural motoneurons we monitored. Activation of 5-HT-containing cells along with postural extension commands, which usually inhibit the 5-HT-containing cells, also results in amplification of extension command effects. These results suggest that 5-HT can function as a "gain-setting" element enhancing excitatory and inhibitory inputs to cells in various settings, and may be recuited to do so via multiple circuitries under different physiological or behavioral conditions. (Supported by NIH and MDA).

246.7

IN SITU INTRACELLULAR RECORDINGS FROM PROCEREBRAL NEURONS DURING COHERENT NETWORK OSCILLATIONS IN THE OLFACTORY PROCESSING SYSTEM OF *LIMAX MAXIMUS*.

A. Gelperin, J. Flores* and D. W. Tank. Biophysics Research Dept., AT&T Bell Laboratories, Murray Hill, N.J. 07974

Computational analysis of odor learning in *Limax* led us to examine the procerebrum (PC) of the cerebral ganglion, which receives direct olfactory input and contains the majority of the neurons in the CNS. The PC displays an endogenous oscillation in its local field potential (LFP) (1 Hz) which is modulated by odor input (**Nature**, in press). We have obtained stable long-term intracellular recordings from the small (8 μm diameter) PC neurons in the intact PC using nystatin-filled cell-attached patch electrodes. PC cells receive prominent IPSPs which appear unitary but which may represent input from a tightly coupled set of synchronously-active presynaptic cells. These hyperpolarizing IPSPs are periodic and occur phase-locked to the LFP oscillation. The onset of the IPSP is coincident with the onset of local current flow during the LFP oscillation. Between these hyperpolarizing events PC neurons show 0 - 5 action potentials . Depolarizing current applied to the somatic recording site greatly increases spike frequency within a burst but does not change the rate of burst occurrence, which is determined by the periodic hyperpolarizing event. Hyperpolarizing current eliminates spiking without altering the frequency of the periodic hyperpolarizing event and can reverse the polarity of the hyperpolarizing event. Computer models of the neural circuit underlying LFP oscillation, which are consistent with known anatomy and electrophysiology, are being developed and studied to aid in the circuit analysis of the PC and the functional analysis of the oscillating LFP.

246.8

VOLTAGE- AND CURRENT-CLAMP SIMULATION OF HERMISSENDA TYPE B CELL RESPONSES.

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Repeated pairings of light and rotation result in an enhanced light-generated response (LGR) of H.c. Type B photoreceptors, which has been attributed to reductions in two K currents: I(A) and I(K-Ca). We have constructed Hodgkin-Huxley style models of the major voltage-, light-, and calcium dependent ionic currents in B cells from voltage-clamp data, with the aim of reconstructing the LGRs measured from trained and control B cells under current-clamp. To simulate the previously documented 30-40% reductions in I(A) and 50-60% reductions in I(K-Ca) for conditioned cells, the maximal conductances for each current were correspondingly reduced, first alone and then in combination. Because it is difficult to completely separate I(Ca) and I(K-Ca) experimentally, they were modeled as a single current. The conductances for the light-induced current and I(leak) were unchanged. Reducing g max A by 30% resulted in a 2.2 mV enhancement of the transient component of the LGR and a negligible change in the steady-state component. The 30% reduction of g max A resulted in only an 8% reduction in peak I(A) during the LGR. Reducing I(K-Ca) by 50% had negligible effect upon the peak of the LGR, but enhanced the steady-state component by 2.3 mV. Reduction of both conductances enhanced all components of the LGR. These increases were greater than the additive effects of reducing each alone.

246.9

THE LARVAL AND JUVENILE CENTRAL NERVOUS SYSTEM OF THE NUDIBRANCH BERGHIA VERRUCICORNIS.

D.J.Carroll* and S.C.Kempf. Dept. of Zoology and Wildlife, Auburn University, Al. 36849.

In order to cultivate Berghia verrucicornis as an animal model for future neurodevelopmental and behavioral studies, the larval and post-metamorphic central nervous system was studied using 1 um serial sections. We found that ganglia characteristic of the adult nervous system are present when the lecithotrophic larvae hatch from the egg mass. The arrangement of the neural constituents is similar to that found for larvae of other opisthobranch species (Kempf et al., J. Neurobiology, 18:217-236, 1987; Bickell and Kempf, Biol. Bull., 165:119-138, 1983); however, the cerebral and pleural ganglia appear to be fused in newly hatched larvae. In serial sections of newly metamorphosed juveniles, the cerebral and pleural ganglia are discrete, connected to each other via a cerebral-pleural connective. In both larvae and juveniles, the paired pedal ganglia are joined by a commissure, and linked to their respective cerebral ganglia via a connective. Also linked to the cerebral ganglia are the buccal ganglia, situated posterio-dorsal to the developing buccal mass. In the juvenile, a visceral ganglion is present posterior to the buccal commissure. Analysis of serial sections suggest its presence as part of the pleurovisceral loop running from one pleural ganglion to the other.

246.10

BAG-CELL PEPTIDE(S) INHIBITS TAIL-SIPHON WITHDRAWAL REFLEX AND ATTENUATES SENSORY TO MOTOR NEURON SYNAPSE UNDERLYING TAIL-WITHDRAWAL IN *APLYSIA*.

J.R. Goldsmith and J.H. Byrne. Department of Neurobiology & Anatomy, University of Texas Medical School, Houston, TX 77225.

The peptides released prior to and during egg-laying in *Aplysia californica* are responsible for eliciting or modulating a repertoire of behaviors preceding and accompanying egg-laying. Our interest was in determining the role these peptides may play in modulating non-reproductive behaviors. Specifically, we examined the effects of the bag-cell peptides (BCPs) on a simple behavior, the tail-siphon withdrawal reflex, which has been well characterized and whose underlying circuitry is well defined. We recorded the total duration of siphon withdrawal in response to test stimuli, delivered once every 5 min, to the tail. Pretest and posttest periods consisted of 5 trials each. Immediately following the last stimulus in the pretest, one of four solutions was injected: bag-cell extract (BCE, n=23), egg-laying hormone (ELH, n=22), artificial seawater (ASW, n=20) or abdominal ganglion extract (minus the bag-cell clusters; ABG, n=19). The posttest was begun 20 min after the injection. Whereas both experimental solutions (BCE and ELH) inhibited the baseline tail-siphon withdrawal reflex (median values: 64.0% and 64.5% of pretest, respectively), only the BCE group differed significantly ($p<0.05$) from the control groups (ASW and ABG; median values: 104.5% and 93.0% of pretest, respectively).

We are presently using an *in vitro* analogue of the tail-component of the reflex to determine which peptide or peptides may mediate this effect as well as a possible site(s) of action. In these experiments single spikes are elicited in tail sensory neurons (SN) and the monosynaptic EPSPs elicited in motor neurons (MN) are recorded. As in the behavioral experiments, there are 5 trials (ISI= 5 min) in each the pretest and the posttest. Peptides are bath applied 2.5 min after the last stimulus in the pretest. The posttest is begun 2.5 min after the bath application. Peptides being studied include α-, β- and γ-BCP and ELH (1 μm). In addition, we are also examining arginine vasotocin (1 μm) to test the possibility of non-specific peptide effects, and to determine whether this peptide has a similar effect to that demonstrated on the SN to gill and siphon MN synapses (Lukowiak, 1987). Preliminary data indicate that at least one peptide, β-BCP, attenuates the SN-to-motor connection. These results indicate that the bag-cell peptides are involved in the modulation of at least one non-reproductive behavior. The significance of this modulation may be to permit the animal to ignore extraneous non-reproductive stimuli, thereby allowing it to continue laying eggs.

246.11

THE MOBILIZATION PROCESS OF FACILITATION PRODUCED BY SEROTONIN IS INHIBITED BY FMRFAMIDE IN SENSORY NEURONS OF *APLYSIA*. J.P. Pieroni and J.H. Byrne. Dept. of Neurobiology and Anatomy, University of Texas Medical School, Houston, TX 77225

Previous studies of sensory neurons (SNs) mediating defensive reflex responses in *Aplysia* indicated that at least two processes, spike broadening and mobilization, contribute to presynaptic facilitation of transmitter release (Gingrich et al., 1985, 1988; Hochner et al., 1986). Recently, we demonstrated that while both serotonin (5-HT) and small cardioactive peptide (SCP) generate spike broadening, only 5-HT induces mobilization (Pieroni and Byrne, 1989). This mobilization effect was most pronounced 3-4 min after the application of 5-HT. In contrast to the facilitatory effects of 5-HT and SCP, FMRFa has inhibitory effects. Although FMRFa produces spike narrowing and decreases synaptic transmission (Abrams et al., 1984), it is not known whether FMRFa affects mobilization. To examine this issue, we have compared the effects of 5-HT on processes contributing to facilitation of EPSPs in depressed SN-follower neuron synapses in the presence and absence of FMRFa.

Abdominal ganglia were pretreated with TEA (100 mM) in order to broaden the SN spikes into a range of durations for which additional broadening alone has little or no effect on release (Gingrich et al., 1985, 1988; Hochner et al., 1986). Under this condition, changes in release are believed to be due primarily to mobilization. Single spikes were evoked in LE SNs at 60 s intervals, and the resultant monosynaptic EPSPs were monitored in follower neurons.

Following the induction of moderate homosynaptic depression (4-6 spikes), FMRFa (15-30 μM) or vehicle was added to the bath. FMRFa decreased both the amplitude of the EPSP and the duration of the SN spike (although the spike duration was still within the aforementioned range) compared to vehicle during the subsequent 5 spikes (n=8). The effects of 5-HT (20-25 μM) were then assessed with FMRFa or vehicle still present in the bath. The facilitatory effect of 5-HT on the amplitude of the EPSP was significantly attenuated in the presence of FMRFa compared to vehicle. Moreover, this inhibitory effect manifested itself 3-4 min after addition of 5-HT, a period consistent with the latency for mobilization suggested previously. Interestingly, although FMRFa reduced the amplitude of the facilitated EPSP, it did not significantly reduce the effect of 5-HT on spike broadening. These data suggest that FMRFa has an inhibitory effect on the mobilization process that may be involved in replenishing transmitter levels in the presynaptic active zone.

246.12

SHORT-TERM AND LONG-TERM CHANGES OF PROTEINS IN SENSORY NEURONS OF *APLYSIA* PRODUCED BY AN *IN VITRO* ANALOGUE OF SENSITIZATION TRAINING. F. Noel, A. Eskin, R. Homayoni and J.H. Byrne. Dept. of Neurobiol. & Anat., Univ. of Tex. Med. Sch., Houston, TX 77225, and Dept of Biochem., Univ. of Houston, TX 77204.

A critical difference between short- and long-term changes in *Aplysia* sensory neurons (SNs) induced by sensitization training or procedures that mimic sensitization training is that long-term, but not short-term changes require synthesis of proteins (Castellucci et al., 1989; Montarolo et al., 1989). To study the proteins involved in long-term sensitization, we developed an *in vitro* analogue that mimics behavioral training and consists of stimulating peripheral nerves of pleural-pedal ganglia with 4 trains of shocks over a 1.5 hr period. Pleural SNs were affected in a similar way by behavioral training and electrical stimulation of peripheral nerves. Nerve stimulation led to reduction of net outward current, a long-term enhancement of the SN-motor neuron connection, and produced changes of specific proteins of SNs (Goldsmith and Byrne, 1989; Noel et al., 1989). Most of these protein changes were similar to those produced by 5-HT or cAMP. To extend these studies we investigated changes of proteins that occured 24 hr after stimulation and compared these changes with those observed just after stimulation.

For each experiment three experimental and matched control ganglia were placed into two separate chambers. The ganglia were exposed to ^{35}S-methionine for a 2 hr period starting ½ hr before stimulation for the short-term experiments (n=4) or 24 hr after stimulation for the long-term experiments (n=13). At the end of the labeling period, samples from sensory clusters were run on 2D PAGE.

The incorporation of label into 2 proteins (#120 and #130) was increased both at the end of stimulation and 24 hr after stimulation. These proteins may be involved in events common to short- and long-term sensitization. Two proteins (#85 and P9) were affected just after the stimulation but not 24 hr later. These two proteins may be involved in the induction of sensitization. On the other hand, 2 proteins (#17 and #84) were increased 24 hr after the stimulation but were unchanged just after stimulation. These 2 proteins may be involved in the mediation or maintenance of sensitization. In addition, the phosphorylation of protein #17 was increased by application of 5-HT (1.5 hr) or of cAMP (25 min). Thus, training appears to produce a temporal spectrum of effects on proteins; some proteins are affected only early, some only late, and others are affected both early and late. Amino acid sequencing of these proteins will help determine their specific roles in the induction, mediation, and maintenance of sensitization.

246.13

SEROTONIN REGULATES THE EXPRESSION OF mRNA IN THE PLEURAL-PEDAL GANGLIA OF *APLYSIA*, AS DETERMINED BY *IN VITRO* TRANSLATION. R.E. Zwartjes, M.T. Crow*$^{+}$, J.H. Byrne^{+}, and A. Eskin. Dept. of Biochemistry, Univ. of Houston, 77204, and U. T. Med. School^{+}, Houston, TX 77225.

The formation of long-term memories in *Aplysia* and other animals appears to be dependent upon translation of proteins and perhaps gene transcription. For example, in *Aplysia*, inhibitors of protein synthesis disrupt the induction of long-term sensitization, and the turnover of a number of proteins is modified by treatments that produce or mimic long-term sensitization (Castelucci, *et al.*, 1988, 1989; Montarolo, *et al.*, 1986; Eskin, *et al.*, 1989; Barzilai, *et al.*, 1989; Noel, *et al.*, 1989). Moreover, transcription also appears to be required for the induction of long-term presynaptic facilitation (Montarolo, *et al.*, 1986). To further test the hypothesis that long-term sensitization is induced via alterations in gene expression, we have begun to investigate whether mRNA expression is altered by training procedures. *In vitro* translation of mRNA is being used to determine which messages are transcribed during the induction of learning.

PolyA^{+} RNA was extracted from control and matched experimental pleural-pedal ganglia just after treatment with serotonin (5-HT) for 1.5 hr. ^{35}S-methionine-labeled proteins were produced by *in vitro* translation of the RNA using a rabbit reticulocyte lysate. The proteins were analyzed by 2D-PAGE. Several hundred proteins were visible on the autoradiograms and the pattern of these proteins corresponded quite well with the pattern obtained from gels of proteins labeled in intact ganglia. The 5-HT treatment increased incorporation of methionine into 4 proteins. Therefore, 5-HT affected the expression of mRNA and it probably did so by increasing the levels of mRNA for these 4 proteins. This preparation and these techniques appear well suited for studying the role of gene expression in learning and memory.

246.14

NOVEL EFFECTS ON PROTEINS PRODUCED BY PAIRING SEROTONIN WITH DEPOLARIZATION. A. Eskin, F. Noel, U. Raju, R.G. Cook, M. Núñez-Regueiro, J.H. Byrne. Dept. of Biochem., Univ. of Houston, TX, 77204, and Dept of Neurobiol. & Anat., Univ. of Tex. Med. Sch., Houston, TX, 77225.

A mechanism for classical conditioning in *Aplysia* is believed to involve the convergence of activity in a neuron with the diffuse modulatory effects of a transmitter on that neuron. One way to mimic associative learning at the cellular level is to treat cells with a modulatory transmitter while the activity of the cell is increased with a depolarizing agent such as elevated potassium (high K^{+}) (Ocorr et al., 1985). We have begun to investigate the effects of pairing high K^{+} and 5-HT to determine potential sites of interactions of second messenger systems upon proteins and to set the stage for studying proteins involved in associative learning.

Abdominal ganglia were subjected to 4 different conditions: 1) 1 hr of high K^{+} (80 mM) alone; 2) 2 hr of 5-HT (5 μM) alone; 3) high K^{+} paired with 5-HT with the high K^{+} overlapping by ½ hr the 2 hr of 5-HT; 4) no treatment. The ganglia were incubated in ^{3}H-leucine for 2 hr beginning at a time corresponding to 5 hr after the end of the 5-HT treatment. After incubation, ganglia were processed for 2D PAGE. We focused on proteins in which pairing high K^{+} and 5-HT produced different effects than those produced by a summation of the individual treatments. At least 3 proteins seemed to be affected in a non-additive way by the pairing procedure. The incorporation of label into 2 proteins (#3 and #45) was decreased by 5-HT alone, was not affected by high K^{+} alone, but was increased in response to the paired procedure. The other protein (#2) was decreased by both high K^{+} and 5-HT alone and was not affected by the paired procedure.

To study the functional significance of these results, we have begun to identify the proteins by obtaining their amino acid sequences. Thus far, we obtained the following partial sequences from two peptides derived from protein #3:
A.)ValAsnLeuGlyAspValPheProAsnPheThrAlaAsnThrSerLysGlyGluIleLysPhe,
B.)LeuGlyMetIlePheProAspGluArgThrLysGluGlyLeuProLeuThrAlaArgAlaVal.

We are now in the process of using these sequences to identify the cellular function of protein #3.

These results indicate that an analogue of a classical conditioning procedure produces what appears to be pairing specific changes in incorporation of label into proteins. Furthermore, the results indicate that associative learning may produce changes in specific proteins that differ qualitatively from the changes produced by nonassociative learning (e.g. application of the modulatory transmitter alone). We are currently examining this phenomenon by using explicitly unpaired controls.

246.15

EXTRA POST-TRAINING UNCONDITIONED STIMULI DO NOT DECREASE ASSOCIATIVE PLASTICITY IN A CELLULAR ANALOGUE OF CONTINGENCY IN *APLYSIA*. D.V. Buonomano and J.H. Byrne. Dept. of Neurobiology and Anatomy, Univ. of Texas Med. Sch., Houston, TX 77225.

Mechanisms for simple forms of both nonassociative and associative learning have been analyzed in *Aplysia*. As an initial effort in extending these analyses to higher-order forms of associative learning we have used a neural analogue of classical conditioning to study contingency. Contingency influences associative learning in that it reflects the probability that the unconditioned stimulus (US) will be predicted by the conditioned stimulus (CS). In vertebrates, extra USs before, after or intermixed with training can decrease conditioning. Extra USs interspersed in a classical conditioning protocol also decrease associative learning in *Aplysia* (Hawkins et al., 1986). As an initial approach to a cellular analysis of contingency we used a paradigm with extra post-training USs. The analogue is similar to that used previously for classical conditioning (Buonomano and Byrne, 1989).

In the control group, two pleural sensory neurons (SN), which synapsed onto a common motor neuron (MN), were fired to establish baseline EPSPs. Training consisted of activating one SN (SN+, analogous to a CS+) with a 1 s train of pulses 400 ms before the onset of a 500 ms electric shock to nerve P9 or P8 (analogous to the US). The second SN (SN-, analogous to a CS-) was activated with the same parameters either 2.5 min before or after the shock. There were a total of 3 training trials with an interval of 5 min. The effect of training was examined by testing the connections 5 and 20 min after the last train of action potentials in each SN. The experimental group (extra USs) was similar to the control group except that 3 extra nerve shocks were administered at 5 min intervals between the 5 and 20 min posttest. EPSPs were expressed as a percent of pretest. Associative plasticity was defined as the difference score between the values of the SN+ and SN- EPSPs within an experiment.

Average associative plasticity for the control (n=6) and experimental (n=16) group was 59±29% and 40±15% at the 5 min posttest, and 24±16% and 45±10% (mean±SEM) at the 20 min posttest. There was no significant difference between the experimental and control groups at either posttest. Thus, extra USs in the experimental group did not have a detrimental effect on associative plasticity. The extra USs appeared to decrease the decay of associative plasticity within the 15 min between the 5 and 20 min posttest. These results argue against a contingency effect when extra USs are applied after training. It remains to be determined whether extra USs during or before training produce a contingency effect.

246.16

NEURAL MECHANISMS OF RESPONSE SPECIFICITY. I. TAIL AND MANTLE NERVE SHOCK PRODUCE DIFFERENTIAL EFFECTS ON THE SIPHON-WITHDRAWAL NEURONAL CIRCUIT IN *APLYSIA*. X. Fang and G. A. Clark. Program in Neuroscience, Princeton University, Princeton, NJ 08544.

Both sensitization and classical conditioning of the siphon-withdrawal response in *Aplysia* exhibit response specificity. For example, the conditioned siphon-withdrawal response to a tactile siphon stimulus resembles the response to tail shock (tailward bending) when tail shock has been used as the unconditioned stimulus (US) during training, but resembles the response to mantle shock (bending toward the mantle) when mantle shock has been used as the US. Various neural mechanisms have been proposed to explain this effect, including synapse-specific facilitation in siphon sensory cells, changes in "labeled-line" interneurons, and changes in specific motor neurons. As an initial step toward investigating these possibilities, we examined the responses to tail and mantle nerve shock in various neural elements of the siphon-withdrawal circuit, including the LFS (Type B) and LBS siphon motor neurons, as well as the interneuron L25 of the Interneuron II (respiratory pumping) network. We found that LFS-B motor neurons (which move the siphon tailward when stimulated intracellularly) fired significantly more during tail nerve shock than during mantle nerve shock ($p < .001$), and were in fact often inhibited by the latter. In contrast, LBS motor neurons (which move the siphon toward the mantle when stimulated intracellularly) and interneuron L25 both showed strong excitation during mantle nerve shock, but only weak excitation and/or inhibition during tail nerve shock ($p < .005$ and $p < .01$, respectively). Moreover, mantle nerve shock produced a differential increase in the number of Interneuron II events occurring during the five minute post-shock period ($p < .001$). These results suggest that LFS-B motor neurons are preferentially involved in producing siphon-withdrawal responses elicited by tail shock, while LBS motor neurons are preferentially involved in responses elicited by mantle shock. In addition, the greater increase in Interneuron II events following mantle nerve shock may contribute in part to mantle-like response specificity.

246.17

Crowding Affects Sensitization in Juvenile Aplysia. J. Flinn, S. Kurtz, L. Alexander*, S. West*. Dept. of Psychology, George Mason University, Fairfax, VA 22030.

Juvenile Aplysia were raised from 30 to 124 days post-metamorphosis in crowded or uncrowded conditions. The survival rate was 82% in the uncrowded and 60% in the crowded group. The weight range ~.5-24 gms was similar, with fewer mid-range animals in the crowded group. Sensitization, using number of steps moved following tail shock, was examined for the 2 groups matched by weight over the range of greatest weight overlap, .5-12 gms. Uncrowded animals showed a significant increase in escape behavior following sensitizing shock (t=4.04,df=15,p<.01) while crowded animals did not (t=1.13,df=15,ns). Both groups in this weight range showed a sig nificant correlation between weight and sensitization level, r=.52, r=.67,p<.05 respectively. 5HT levels were examined in a subset of animals using HPLC, at Am. Med. Labs. There was a significant correlation between sensitization and 5HT levels for the uncrowded r=.71,p<.05. but not the crowded group, r=.17, ns. These results show that animals of the same age showed different levels of learning depending on both weight and rearing conditions.

246.18

INDUCTION OF AN IMMUNE REACTION IN APLYSIA IS ACCOMPANIED BY LONG-TERM ENHANCEMENT OF SENSORY NEURON EXCITABILITY. H.Alizadeh, A.L.Clatworthy, G.A.Castro, and E.T. Walters. Dept. of Physiology & Cell Biology, Univ. Texas Medical School, Houston, TX 77225.

Stress elicits integrated responses of the nervous and immune systems in many species. Long-term sensitization in Aplysia is produced by cutaneous shock, which mimics afferent input from peripheral injury. Because injury activates the molluscan immune system, factors released from immunocytes might contribute to the sensory changes involved in long-term sensitization. We induced an immune response by implanting a cotton string in the hemocoel under $MgCl_2$ anesthesia. The string was looped around either the left or right pedal nerves. One week later, when string and nerves were thickly encapsulated by amoebocytes, 5-12 VC sensory neurons per pleural ganglion (with axons in pedal nerves) were stimulated intracellularly with 1 sec depolarizing pulses to test excitability of the soma. Mean responses of somata on the encapsulated side were significantly greater (p<.05) than responses on the contralateral side (14.9 vs. 8.8 spikes; n=12 animals), or in somata of sham-operated (7.8 spikes, n=11) or naive animals (11.2 spikes, n=15). This suggests that defensive encapsulation causes a long-term enhancement of excitability of nearby sensory neurons, while surgical trauma decreases excitability of sensory neurons with receptive fields distant from the incision.

246.19

AGE AND CHRONIC STIMULATION AFFECT HEMOLYMPH PROTEINS IN APLYSIA. M.Srivatsan*, B. Hallahan*, B.Peretz, and R.Talwalker*. Dept. of Physiology, Univ. of Kentucky, Lexington KY 40536.

With increased age the gill withdrawal reflex (GWR) weakens in Aplysia. Paralleling the age effects on the GWR we now have found that with SDS-PAGE the band patterns of hemolymph (Hml) proteins changed with age. Of the many bands present, 6 were prominent in Hml from young animals (shell diam. - 11 mm, 80 days old), with MWs from 14,000 to 300,000. Hml from mature (s.d. - 26 mm, 160 days old) and old (s.d. - 59 mm, 240 days old) animals had fewer prominent bands, only 4 and 3, respectively; MWs were from 45,000 to 300,000. Two bands common to all three ages differed quantitatively. The topmost band of 300,000 was hemocyanin; the other prominent bands were not breakdown products of hemocyanin. Possibly changes of some Hml proteins are related to those of the GWR; factors in Hml can alter the responsiveness of the GWR (e.g. Lukowiak, Neurosci. Lett., 77:205,1987). In old Aplysia chronic stimulation (ChSt) of the siphon over a period of 4 weeks improved the GWR and its substrates (Zolman & Peretz, Behav. Neurosci.,101:1,1987). Taken together these results prompted us to examine the effects of ChSt on Hml proteins. Freely behaving mature and old adults underwent ChSt (1 sec., 8g/cm^2 water jet; applied 1/20 min., 10/day) for 4 weeks. Hml was drawn weekly before, during, and after the ChSt period. Mature and old animals showed a significant change in the GWR after the ChSt period. Results from SDS-PAGE showed that one prominent band of ca 97,000 MW, which was well defined in unstimulated mature animals and poorly defined in old animals, increased in Hml from ChSt animals but not from a control group. Thus far no significant increase in the concentration of Hml proteins was measured. Yet, enhancement of the 97,000 MW band in both age groups suggests that ChSt may have triggered its increased synthesis, resulting in a redistribution of protein constituents in the Hml.

246.20

***Aplysia's* feeding apparatus modulates its force output in response to changes in mechanical load.** H. J. Chiel, N. Weiner, M. Bamberger and D. W. Morton, Depts. of Biology, Neuroscience, and Mechanical and Aerospace Engineering, Case Western Reserve University, Cleveland, OH 44106.

Sensory inputs can have a profound impact on the rhythmic behaviors of animals. We have studied the ability of *Aplysia californica* to rapidly adapt its feeding behavior in response to changes in mechanical loads similar to those that it encounters in its natural habitat. We initially studied the responses of animals to two different seaweeds on which it feeds, *Laurencia* and *Ulva*. The seaweeds were attached to a force transducer, and the forces generated by animals were recorded while their behavior was videotaped. We observed significant variations in the force output by the animals in response to natural seaweed. In order to quantify the response to a defined mechanical load, we have devised two techniques to measure forces generated during feeding: an "artificial seaweed" consisting of a piece of canvas dipped in seaweed extract and attached to a force transducer, and a pair of forceps to which strain gauges are attached. Animals were induced to swallow these stimuli, and their force output was measured while they were videotaped. Even though these were fixed mechanical loads, animals' responses were far more irregular than when their feeding apparatus was unloaded, which occurred when they attempted to bite, but were not allowed to consume food. Extracellular recordings from buccal nerves 1, 2, and 3 in intact animals provided evidence for rapid changes in the output of the neural controller for feeding in response to loads. Lesions of the cartilage of the jaws significantly reduced the ability of animals to "clamp down" on large mechanical loads, and to exert pulling forces. In contrast, lesions of buccal nerve 2, which innervates the jaws and provides sensory feedback to the buccal ganglion, reduced the animal's ability to clamp down, but animals showed increased pulling forces. These studies provide a basis for determining the neural correlates of adaptation to changes in mechanical load. [Supported by NSF grant BNS-8810757 to H.J.C.]

246.21

THE EFFECTS OF THE FOOD AROUSAL NEURON CPR ON ANTERIOR POSTURAL MUSCLES INVOLVED IN FEEDING IN *APLYSIA*.
T. Nagahama*, T. Teyke*, K.R. Weiss and I. Kupfermann.
Center Neurobiology and Behavior, Columbia University and NYS Psychiat. Inst., New York, N.Y. 10032.

The cerebral neuron CPR may function as a command element for the complex food-induced arousal state in *Aplysia*. We previously reported that firing the CPR evokes bilateral contractions of the neck muscles, which could serve to lift the head of the animal into the feeding posture, the most apparent manifestation of food arousal. We now report that CPR activity increases activity in pedal nerves, particularly those innervating the anterior part of the body. Firing the CPR produced weak movements of the anterior foot region (via anterior foot nerve P1), which appear to relax the tissue. We found different types of neurons with axons in nerve P1. One type received monosynaptic EPSPs from the CPR. Firing these cells, however, did not cause contractions of the muscles. A second group of neurons, which contracted the anterior foot, received polysynaptic inhibitory input, which decreased their spontaneous activity, and may relax the muscles. Furthermore, contractions of the anterior foot, which were evoked by firing one of these motoneurons, were altered after the CPR was fired, indicating that the CPR also has indirect modulatory effects. In contrast, motoneurons which innervate the neck muscles (via nerve P4) received polysynaptic EPSPs from the CPR, and in some cases, brief stimulation of the CPR produced prolonged excitation of these neurons. These results indicate that the CPR can promote different types of motor effects by virtue of its connections to pedal motoneurons. First, CPR activity may promote relaxation of the anterior portion of the foot, which could serve to detach the foot from the substrate. Second, it may also cause contractions of the neck muscles which lift the head. Both motor effects are consistent with the notion that the CPR is involved in mediating the appetitive head-up posture of the animal.

246.22

A MODEL OF DECISION-MAKING IN *APLYSIA FASCIATA*. I. Ziv*, C. Lustig* & A.J. Susswein, Dept Life Sci, Bar-Ilan Univ., Ramat Gan 52 900, Israel

To begin studying the neural basis of decision-making in *Aplysia*, we examined rules governing transitions between bouts of behaviors. Over 85% of transitions were via a single intermediate behavior, moving in place (MIP; consisting of head-waving, and similar activities), which tended to precede and follow crawling, swimming, immobile and feeding. After MIP, animals generally return to the behavior performed before MIP. Additional preferred sequences were courtship to mating, and crawling to swimming.

The large number of transitions via MIP suggest that MIP is a behavioral correlate of decision-making *per se*. If this is so, since animals must return to MIP to decide after most bouts of other behaviors, MIP should always represent a fixed proportion of all bouts. When observation conditions were modified by adding or removing food or mates, the number of bouts of other behaviors varied markedly, while MIP always represented ~40% of all bouts.

Previous data suggested that feeding and mating are regulated by a common arousal (CA) mechanism that plays a major role in governing the time budget. If MIP is a behavioral monitor of decision-making, MIP and CA level should be significantly correlated. Formulas were developed to estimate the CA level, based on overall time spent feeding and/or mating. We then found a significant correlation (r=0.84) between CA and a normalized measure of MIP. Thus, MIP may be a behavioral monitor of CA.

A 1/day oscillator affects various behaviors. CA and MIP do not show a daily oscillation, suggesting that the daily oscillator affects other levels of behavioral control, perhaps transition probabilty from MIP to affected behaviors.

A simulation can test whether integration over a full day of variability in CA, and change in transition probability, account for time devoted to each behavior, and specific times that behaviors are seen.

Muscles, nerves and neural circuits responsible for generating head-waving, a form of MIP, have been identified. This circuitry may be partially responsible for decision-making, raising the exciting possibility that the neural basis of higher-order decision-making in *Aplysia* will be accessible to analysis.

247.1

BEHAVIORAL CHANGES AFTER RIGHT AND LEFT CAUDATE LESIONS IN RHESUS MONKEYS. J.B.Bryer*, S.E. Starkstein, R. Richardson, J.P. Fedoroff*, J. Higley*, T.H. Moran, M. DeLong, R.G. Robinson. Dept. Psychiatry, Johns Hopkins Univ. Sch. Med., Balto., MD 21205.

Recent studies have demonstrated that patients with lesions in the head of the left caudate show a high frequency of depression. Right caudate lesions, however, produce indifference, euphoria, and even manic states. The aim of our study was to examine whether similar lesions in non-human primates produce similar behavioral changes. The behavior of two male adult rhesus monkeys was recorded over a 6 month baseline period. Behaviors included locomotion, exploration, play, autoeroticism, stereotypy, passivity, and huddling. Both monkeys were given sham lesions followed, four weeks later, by either left (ibotenic acid) or right (radiofrequency) lesion of the head of the caudate. Neither of the monkeys showed significant behavioral changes after the sham surgery. The left head of the caudate lesion produced huddling behavior (a behavioral equivalent of depression in primates), that lasted for 6 weeks post-surgery. This was accompanied by weight loss, and a decrease in exploration and autoeroticism (less than 50% of the baseline values). The right head of the caudate lesion did not result in huddling, but produced repetitive locomotor activity (up 100% from baseline values), that lasted more than 6 weeks. This preliminary study suggests that a significant association between behavioral changes and side of lesion exists in both human and non-human primates.

247.2

ENHANCEMENT OF GABA NEUROTRANSMISSION IN THE CARDIOSTIMULATORY REGION OF THE POSTERIOR HYPOTHALAMUS HAS ANXIOLYTIC EFFECTS IN THE ELEVATED PLUS-MAZE. A. Shekhar and L. L. Sims*, Dept. of Psychiatry, Indiana Univ. Sch. of Med., Indianapolis, IN 46202.

Blockade of GABA in the region of the posterior hypothalamus (PH) of rats elicits a constellation of responses characterized by increases in heart rate (HR), blood pressure (BP), respiratory rate (RR), locomotor activity and the level of "anxiety" in the conflict test. The aim of the present study was to test the effect of enhancing GABA in the PH on the elevated plus-maze model of "anxiety." Male Sprague-dawley rats with arterial and venous catheters in place for physiological measurements were implanted bilaterally with chronic microinjection cannulae in the region of the PH where bicuculline methiodide (25 ng/250 nl) elicited significant increases in HR and RR. After recovery, the effects of microinjecting saline and muscimol (1, 5 & 10 ng/250 nl) bilaterally into the PH in the elevated plus-maze were as follows:

Treatment	n	Time-open arm (sec)	Time-closed arm (sec)
Saline	5	42 ± 12	218 ± 19
Musci.10ng	5	98 ± 17*	126 ± 32*

(*Significantly different from saline by ANOVA, P 0.05) In addition, microinjection of muscimol attenuated the increases in HR and BP observed in the elevated plus-maze. These results suggest that enhancing GABA in the PH decreases anxiety. (Supported by R29 MH 45362-01).

247.3

RAT 22 KHZ ULTRASOUNDS TO PREDATORS: ALARM CRIES? R.J. Blanchard, D.C. Blanchard, S. Weiss* and R. Agullana* Dept. of Psychology and Bekesy Lab. of Neurobiology, Univ. of Hawaii, Honolulu, HI 96822

Laboratory rats maintained in mixed-sex groups in a visible burrow situation (VBS) emit 22 kHz ultrasonic vocalizations when a nonattacking cat is presented in the open area of this habitat. One or more colony animals vocalized about 50% of the time while the cat was present and during the first 30 min. after the cat was removed, reliably more than when a plush toy cat was presented. During the high vocalization period virtually all group animals remained in the tunnel/chamber system of the VBS.

When individual rats were confronted by the cat in an open area of similar size, they averaged only 10 sec. of ultrasonic vocalization in the 45-min cat and post-cat period, suggesting that concealment in a burrow system facilitates anti-predator ultrasonic vocalization, and/or that it may function primarily as a warning cry directed at conspecifics. The latter possibility is strengthened by preliminary studies suggesting that concealed group members not in visual contact with the cat, including pups as young as 4 weeks, may show recruitment of ultrasonic vocalization and subsequent changes in defensive behaviors. Ultrasonic vocalization may thus be an important mechanism for communicating the presence of a threat stimulus within social rat groups. The effects of ethanol and diazepam on these behaviors will be presented.

247.4

SUBORDINATION AS AN ANIMAL MODEL OF DEPRESSION. D. C. Blanchard, R. J. Blanchard, R. Hammer, D. Clow, M. Bardo, and J. K. Rowlett. Békésy Lab. of Neurobiology, and Dept. of Psychology, Univ. of Hawaii, Honolulu, HI 96822.

Good animal models of human psychopathology make it possible to isolate behavior patterns and analyze their origins, pathophysiology and responsiveness to treatment techniques. Ethoexperimental analysis of social and agonistic interactions in rat groups in semi-natural burrowing situations has revealed complex patterns of behavior change for subordinate males, including increased alcohol intake, reduced social, sexual and aggressive activity, restriction of locomotor and exploratory activity, changes in sleep cycles, weight loss associated with lowered food intake, and impaired information processing, which appear to be isomorphic to the behavioral symptomatology of clinical depression. The magnitude of these changes predicts early subordinate mortality as much as 200 days before death. This analysis provides a subordination model enabling experimental evaluation of the view that downward mobility in dominance hierarchies is a major experiential determinant of depression, also enabling detailed analysis of associated changes in brain regional neurochemical systems to elucidate the neurobiology of defensive patterns related to depression. We report increased regional 5-HT metabolism as indexed by levels of its major metabolite, 5-HIAA, in a number of brain areas from cortex to spinal cord, in subordinates relative to dominants or controls.

247.5

SEPTAL LESIONS INHIBIT FEAR REACTIONS IN THE ELEVATED PLUS-MAZE. C. Pesold* and D. Treit. University of Alberta, Canada T6G 2E9.

Recent research in our laboratory has shown that electrolytic lesions of the posterior (but not anterior) septum reduce fear reactions in animal tests of anxiolytic drug action (e.g., significant increases in rats' open arm activity in the elevated plus-maze). The purpose of the present experiment was to further characterize the neuroanatomical specificity of these anxiolytic-like effects by comparing kainic acid septal lesions to electrolytic septal lesions in the plus-maze test. Following sodium pentobarbital anaesthesia, rats were given either kainic acid, electrolytic, or sham lesions of the posterior septum and then tested 15 days later in the elevated plus maze. Both kainic acid and electrolytic lesioned animals showed significant increases in open arm activity compared to sham-lesioned controls. These results provide further evidence that posterior regions of the septum play an important role in the control of anxiety in the rat.

247.6

PRE- AND SUBTENTORIAL PERIAQUEDUCTAL GREY OF THE RAT MEDIATES DIFFERENT DEFENSE RESPONSES ASSOCIATED WITH HYPERTENSION K.A. Keay A. Depaulis* M.J. Breakspear and R. Bandler. Dept. of Anatomy University of Sydney NSW Australia 2006. *DNBC Centre de Neurochimie du CNRS 67084 Strasbourg, France.

The midbrain periaqueductal grey (PAG) of the cat mediates two distinct behavioral and cardiovascular patterns of defense; threat display and flight, which are evoked from the pre- and subtentorial regions respectively. These responses resemble those evoked by natural stimuli in the freely moving cat. This raised the question, does a similar functional organization exist within the PAG of the rat ? Two types of experiment were performed. A) Rats were prepared with chronic intracranial implants and, in the presence of another rat, the behavioural effects of microinjections of small doses (40 pmol) of kainic acid (KA) into the PAG were observed. B) Rats were decerebrated at a precollicular level and the effect of microinjections of DL-homocysteic acid (DLH 10nmols) on blood pressure and heart rate were observed. The decerebrate preparation allowed the observation of both behavioural and cardiovascular variables without the interference of anesthesia. The data showed the following: 1) KA microinjections into the lateral pretentorial PAG of the freely moving rat evoked, in response to the approach of another rat, defensive upright postures and backing away from the partner; whereas, injections into the lateral subtentorial region evoked vigorous forward locomotion away from the partner. 2) DLH microinjections in the lateral pretentorial and subtentorial PAG of the decerebrate rat evoked large pressor responses (+20% to +200%) accompanied by increases in heart rate. In addition, pretentorial PAG pressor responses were accompanied by "hunching" of the back and limb movements which were directed to push the animal backwards, whereas subtentorially, vigorous running movements were associated with the pressor responses. At many sites in the pretentorial region sonic vocalizations were also observed; these were rarely encountered in the subtentorial region. These data indicate that the PAG of the rat controls two different types of defensive movements which are accompanied by pressor responses and tachycardia and suggests that the rat PAG may be organized functionally in a similar manner to that of the cat. (Supported by: NHMRC (Aust) and H.F.Guggenheim Fdn.)

247.7

ALARM RESPONSES IN ADULT SQUIRREL MONKEYS: GENDER DIFFERENCES AND STIMULUS SPECIFICITY. L. J. Crepeau and J. D. Newman
Laboratory of Comparative Ethology, NICHD, NIH, Poolesville, MD 20837-0289.

Captive squirrel monkeys (*Saimiri*) respond to alarming stimuli by modulating locomotor activity and vocalization rates. Alarm reactions of adult squirrel monkeys are mediated by cholinergic mechanisms (Glowa and Newman, *Psychopharm 90*: 457-460, 1986). We evaluated the relative potency of various stimuli to elicit alarm in the presence and absence of the central muscarinic antagonist benactyzine hydrochloride (BNZ) using socially-housed adult animals (6 males, 5 females). Animals received BNZ (2 mg/kg) or vehicle s.c. 5 min prior to a 15 min isolation test, followed by alarm testing. Animals were presented with an alarm stimulus series twice per test session. The alarm stimulus series included: mirror-reflected self image (MIR); a large hand monkey puppet covered with synthetic fur (PUP); a leather glove used for capture (GLO); a net used for capture (NET); experimenter 1 (EXP1); experimenter 2 (EXP2); and both experimenters (BOTH), facing the subject with a raised gloved hand. Inanimate stimuli were presented for 30 sec, separated by 30 sec ISIs (I1-I4); experimenters sequentially presented themselves for 30 sec without ISIs.

In isolation, undrugged males exhibited higher levels of isolation peeps (IPs) and locomotion (LOC), indicated by test cage quadrant changes. Benactyzine eliminated this difference, and the two sexes were essentially identical following BNZ administration.

During stimulus presentation, undrugged males exhibited higher LOC levels, in a stimulus-specific manner. Male LOC levels were highest during PUP, GLO, & EXP1, moderate during MIR, NET, & EXP2, and low during ISI & BOTH trials. Female LOC levels remained low across all stimulus trials. Benactyzine eliminated this difference, and the two sexes were essentially identical following BNZ administration. However, undrugged females exhibited higher levels of alarm calling during PUP, EXP2, & BOTH. BNZ induced reliably higher alarm calling rates in a stimulus-specific manner, as well as reliably lower levels of locomotor behavior in both sexes. BNZ condition calling rates were highest for both sexes during EXP2 & BOTH, moderate during PUP & EXP1, and low during all other trials. Female calling rates were higher than male rates during EXP2 & BOTH trials.

The selective responses by males and females to isolation and various alarm stimuli suggests that the two sexes employ separate defensive strategies when encountering environmental challenges in the wild, which has been documented in other mammals. Males' increased locomotor behavior may serve to distract intruders so that others in the group can escape, while females' higher vocal reactivity may serve to alert their young, as well as other adults in the social group.

247.8

FUNCTIONAL MAPPING OF THE RAT BRAIN DURING VOCALIZATIONS: A 2-DEOXYGLUCOSE STUDY.
R.J. Frysztak & F. Gonzalez-Lima, Department of Anatomy, College of Medicine, Texas A&M Univ, College Station, TX 77843

Autoradiographic [^{14}C] 2-deoxyglucose (2-DG) procedures were used to map the functional activity in the CNS during vocalizations elicited by electrical stimulation of the midbrain reticular formation (MRF) in behaving rats. Six male albino rats weighing 180-200g were used. Following injection of 2-DG, three rats received MRF stimulation through two stainless steel electrodes (0.5s duration, 40Hz, 0.2ms pulses) using irregular intervals (range 5-10s) over 90 min. Yoked controls received 2-DG injection followed by playback of the recorded vocalizations. Relative differences in peak isotope uptake (gray/white matter ratios) in 23 structures related to vocalization were compared between the two groups.

The major findings in this study were localized to hypothalamus, midbrain and brainstem structures. Significant increases in 2-DG uptake were noted in the following structures in MRF stimulated rats: dorsolateral central gray (PAG), MRF, lateral hypothalamus (LH), ventromedial hypothalamus (VmH), paraventricular nucleus (PVN) and nucleus ambiguus (NA). Cortical structures were not activated during MRF stimulation. The PAG and NA are known to be important relays in the production of vocalizations. The PAG is known to receive input from "higher" vocalization centers such as the hypothalamus, amygdala and anterior limbic cortex, as well as the MRF. Additionally, LH, VmH and PVN have reciprocal connections with the PAG and have been previously associated with emotional states which induce vocalization. Stimulation of the MRF, therefore, may have resulted in the excitation of these areas. The laryngeal, pharyngeal and soft palate muscles are innervated by motoneurons in the NA, with the laryngeal motoneurons being located in the caudal two-thirds of the nucleus. MRF stimulation, therefore, activates the motor output pathways for vocalization, but does not appear to activate cortical and limbic motivational centers. (Supported by NIMH grant RO1-MH43353)

247.9

5HT1A RECEPTOR MODULATION AND SEPARATION DISTRESS IN DOMESTIC CHICKS. L. Normansell and J. Panksepp.
Muskingum College, New Concord, OH and Bowling Green State University, Bowling Green, OH.

A series of experiments was conducted to evaluate the role of the 5HT1A receptor in the control of separation induced distress vocalization (DV) in chicks. Following i.p. injection of buspirone (1 & 5mg/kg), isolated chicks vocalized 3 times more often than control animals when tested in boxes containing mirrored inserts which normally act to suppress DVs. In plain boxes, BUS-treated birds did not differ from controls. This effect was dose dependent and was apparent over the first 30 min of testing. Administration of 1mg/kg 8-OH-DPAT produced an effect essentially identical to the high dose of BUS.

Central injection of BUS (5 or 25µg administered free-hand into the vicinity of the 4th ventricle) also led to dose-dependent increases in DVs in the mirrored boxes, whereas central DPAT (1,5, or 10µg) reduced DVs in both the mirrored and plain testing boxes.

Chicks injected 3 times per day with 1mg/kg BUS and tested for 4 days showed no indication of the development of tolerance. Chicks repeatedly injected with 5ug DPAT and tested (4 injections, once every 45 min) also showed no tolerance.

Both BUS and DPAT showed anxiolytic activity in a novel test of fear. The latency to jump from an elevated platform to rejoin the flock was reduced with either drug.

247.10

A POSSIBLE NEURAL SUBSTRATE FOR GENDER DIFFERENCES IN VOCAL BEHAVIOR BY RHESUS MACAQUE INFANTS DURING BRIEF PERIODS OF SOCIAL SEPARATION. J.D. Newman, J. Bachevalier*, M. Michieda and S.J. Suomi. Lab. of Comparative Ethology, NICHD, NIH and *Lab. of Neuropsychology, NIMH, Bethesda, MD 20892.

During infancy, rhesus macaques (Macaca mulatta) exhibit a pronounced vocal response to separation from familiar conspecifics, frequently used as a behavioral measure in previous studies of the physiological correlates of separation distress. While there is little information regarding the neural substrate mediating production of isolation calls in infant macaques, a previous study (Newman and Bachevalier, Neurosci.Abstr., 14, 1988) indicated that the amygdala might be involved in regulating the affective quality of separation-induced 'coos.' To investigate further the neural substrate of this behavior, we recorded vocalizations during brief periods of social separation in three groups of infant monkeys. Subjects, all less than one year of age, consisted of a group of 8 males and 5 females that had received neonatal bilateral ablations of the anterior inferotemporal cortical area TE, a group of 2 males and 4 females subjected to fetal hydrocephalus induced by injections of 10 mg/kg triamcinolone acetonide to pregnant females at 20-24 days gestation followed by corrective shunt surgery prior to delivery, and a control group of 10 males and 6 females which received only sham operations. All vocalizations during the 5 minutes following the first vocal utterance were viewed with a real-time sound spectrograph and classified as a 'coo', 'leap,' or 'scream' according to their detailed acoustic characteristics. Statistically significant differences between males and females occurred in the control group, with males making fewer noisy vocalizations (leaps and screams). This gender difference did not exist in either experimental group, where males and females both made more noisy vocalizations than control males. We conclude that the inferotemporal cortex may be part of a neural system important in determining gender differences in the vocal response to social separation by infant monkeys.

247.11

SOCIAL-ISOLATION INDUCED DESPAIR IN CHICKS AND MICE: SIMPLE ANIMAL MODELS OF REACTIVE DEPRESSION. J. Panksepp, G. Yates*, E. Nelson*, S. Ikemoto* and R. Conner*, Department of Psychology, Bowling Green State University, Bowling Green, OH 43403

Despair following social isolation promotes depression. Simplified animal models of isolation-induced despair useful psychobiological research remain to be developed. In this work, we eavaluted effects of social isolation on swimming performance of young mice and distress vocalizations (DVs) of young chicks.

Previous work has failed to observe potentiation of despair-like immobility in the Porsolt swimming test following social isolation in adult Swiss mice (Hilakivi, et al. (1989) *Pharmacol Biochem Behav, 33*: 371). In the present work, a single day of social isolation was sufficient to increase immobility by > 500% in 17 - 18 day old Swiss-Webster mice but the effect dissapeared by 26 days of age. The effect was amplified by prolonged 15 min tests as compared to standard 5 min tests, and required a larger test chamber than traditionally used in this test. In short, the mouse exhibits only short periods of robust sensitivity to social loss.

Individual housing of chicks at 1 (but not 5) days of age yielded a failure to thrive syndrome resembling "anaclitic depression" in one third of our animals. Protest gradually diminishes following chronic social isolation, and this index of despair is potentiated more by low doses of reserpine (0.25-0.5 mg/kg) in isolated as compared to socially housed birds. During this despair phase, imipramine (5-10 mg/kg) was selectively effective in elevating DVs which had been reduced by reserpine. In socially housed animals, imipramine could also counteract the decline in DVs that normally occurs during extended periods of separation testing (e.g., 2-5 hrs). These findings suggest that one can obtain social-isolation induced despair effects in various lower animals. Such simplified models of protest, despair and depression may be useful in pursuing basic neuroscience issues that cannot be readily pursued in available primate models.

247.12

OLFACTORY BULBECTOMY AND ZONA INCERTA LESIONS ELIMINATE MATING IN MALE RATS: PARTNER-PREFERENCE TESTS DISCRIMINATE EFFECTS ON SEXUAL MOTIVATION AND SEXUAL PERFORMANCE. C. Tardivel, K. T. Griffis*, S. D. Isaacs* and D. A. Edwards, Dept. of Psychology, Emory University, Atlanta, GA 30322.

Brain damage may affect the ability to perform the movements of copulation without necessarily affecting sexual motivation. Sexually active male rats prefer sexually receptive females to nonreceptive females, and partner-preference tests provide one measure of sexual motivation. Control and brain-damaged male rats were tested in an arena where the male could choose to spend time with (and mate with) a sexually receptive female, a nonreceptive female, or be in a neutral compartment.

After surgery, olfactory bulbectomized males did not mate. They showed no preference for a receptive female, spending their time equally between the receptive female and the nonreceptive female. Bulbectomized males almost never mounted, and episodes of anogenital investigation were rare.

After surgery, males with zona incerta lesions did not mate. Lesioned males continued to show a strong preference for a receptive female over a nonreceptive female. Although lesioned males did not mount females in the usual sense, mounts (invariably directed at the receptive female) without pelvic thrusting or palpation were common. Lesioned males showed frequent anogenital investigation of the receptive female.

Taking partner preference as a measure of sexual interest, olfactory bulbectomy is associated with a dramatic decrease in sexual motivation. In contrast, males with zona incerta lesions show a strong preference for receptive females, and the failure of lesioned males to mate presumably refects an inability to engage the locomotor responses required for copulation. Supported by NSF grant BNS-8718797.

247.13

VENTRAL SOMATOSENSORY DETERMINANTS OF NURSING BEHAVIOR IN RATS: EFFECTS OF NIPPLE LOSS OR ANESTHESIA. J.M. Stern, L. Dix*, C. Pointek* and C.T. Thramann*. Department of Psychology, Rutgers University, New Brunswick, NJ 08903.

By manipulating the quality and quantity of pups, Stern & Johnson showed that ventral stimulation from rooting and suckling pups causes the dam to become immobile and to flex her ventrum, resulting in a high crouch (Physiol. Behav., 14(5), 1990). We now show that a full litter of capable pups cannot effectively stimulate nursing behavior in dams without nipples or with their ventrum anesthetized.

In Expt. 1, nipples were removed (thelectomy) on day 7 of gestation; postpartum, litters were rotated daily with sham-operated controls and with a donor ♀. In Expt. 2, all 12 nipples were anesthetized with 0.2 ml sc 0.375% bupivicaine (Sensorcaine, Astra) (which blocks milk-ejection) and in Expt. 3, all nipples + 4 mid-ventrum sites were anesthestized (0.28%); controls received Sensorcaine ip or saline injections of ventrum. Maternal behavior was observed for 30 min continuously following a dam-litter separation of 2 hr (on day 4, Expt. 1) or 4 hr (on day 7, Expts. 2 and 3) and at intervals up to 2 hr. In experimental dams, the high crouch posture was not observed and only a small portion of them became immobile, after a much longer latency than in controls, while retrieval and licking were normal. Thus, immobility and ventroflexion are dependent upon ventral somatosensory afferents, particularly from nipples.

(Supported by MH-40459).

247.14

ANTINOCICEPTION PRODUCED BY MATERNAL CONTACT IN INFANT RATS IS NOT MEDIATED BY MU RECEPTORS. D.J. Shide and E.M. Blass. Dept. of Psychology, Johns Hopkins Univ., Baltimore, MD 21218.

The following experiments examined analgesia produced through maternal contact, and interactions between contact and gustatory stimulation. In Exp. 1, 10-day-old Sprague-Dawley rat pups were tested for responsivity to a focal thermal stimulus (45°, 48°, or 51°C; 0.3 cm diam.) across four contact conditions: individual isolation; housed with siblings; non-nutritive suckling; and suckling while receiving an infusion of milk through a tongue cannula. Withdrawal latency (WL) was defined as the latency with which a rat removed its head from the heat source. The first two WLs were averaged to represent the baseline (BL). After BL was determined for the last pup all rats were injected with naltrexone (1.0 mg/kg), and WLs were sampled 20 and 40 min after injection. Initial contact BLs differed significantly across the treatment groups (48°C). Isolated pups averaged 7 sec; contact with siblings essentially doubled this to 16 sec. WL increased to 33 sec in suckling pups, and to 85 sec in pups tested during infusion, a tenfold increase compared to isolates. Naltrexone had no significant effect on WLs at either 20 or 40 min postinjection. In Exp. 2, Day 10 pups were placed in one of four contact conditions: individual isolation; group housed; contact with the mother (no suckling); and non-nutritive suckling. After baseline WLs were obtained pups received an intraoral infusion (0.06 ml/min for 3 min) of either distilled water, 7.5% sucrose, or no substance. WLs were determined 0, 3, 6, 9 and 12 min after infusion termination. As in Exp. 1, initial BL WLs differed according to the type of contact; isolates averaged about 8 sec, contact with siblings or the dam increased this to 16 sec, while suckling pups averaged around 30 sec. WLs also differed according to infusion; pups receiving infusions of sucrose were significantly more analgesic than water-infused or no infusion controls. Sucrose was most effective in isolated rats, least in suckling rats.

247.15

PARADOXICAL EFFECTS OF MORPHINE AND NALOXONE ON PREFERENCE FOR SACCHARIN AS A FUNCTION OF SACCHARIN CONCENTRATION.
K.Akarid*, K. Touzani* and L.Velley, Lab. Psychophysiologie, URA CNRS 339, Univ. Bordeaux I, Av. Facultés 33405 Talence France.

The aim of this study was to further analyse our recent observation that, in rats, a moderate dose of morphine may induce either an increase or a decrease in preference for saccharin, the direction of change depending on the concentration of the sweetener.

In a series of experiments, with drug administered subcutaneously, we studied the effects of increasing doses of morphine (0.1, 0.3 and 1 mg/kg) and naloxone (0.01, 0.1 and 1 mg/kg) on rats placed in a two-bottle choice task between water and one of three (0.3, 1 and 1.7 mM) saccharin concentrations. The results showed that, when the saccharin concentration was around the threshold value for preference (0.3 mM), both morphine (1 mg/kg) and naloxone (0.01 mg/kg) acted in a direction opposite to their classically observed effects i.e. as an apparent antagonist and agonist respectively.

In an attempt to define the brain sites where these paradoxical effects are mediated we tested the effects of increasing doses of morphine (50-800 ng) and methylnaloxonium (50-250 ng) directly injected into parabrachial area, the second gustatory relay-station. The results obtained using intracerebral injections were analogous to those obtained using subcutaneous administration. Considered together, these data suggest that the paradoxical effect could result either from stimulation of autoreceptors or from a differential influence on opioid receptor sub-types.

247.16

PIMOZIDE DOES NOT BLOCK THE CONDITIONED INCENTIVE MOTIVATIONAL EFFECTS OF FOOD-RELATED STIMULI. J.C. Horvitz and A. Ettenberg. Dept. of Psych., U. of Calif., Santa Barbara, CA 93106.

Hungry rats were exposed to a LIGHT stimulus just prior to the delivery of their daily food. After approximately one month of these daily LIGHT-FOOD pairings, animals were treated with either 0, 0.5, 0.75 or 1.0 mg/kg of dopamine antagonist pimozide, and the LIGHT (CS) was presented alone. Locomotor activity was measured during 10 minute intervals both before and after CS presentation. While pimozide produced a dose-dependent suppression of overall activity levels, the incentive motivational effects of the food-related stimulus appeared unaffected. Both vehicle and pimozide-treated animals showed dramatic increases in locomotor activity following CS presentation; for all groups, activity during CS presentation was approximately 300% of pre-CS activity levels. For control animals, LIGHT OFFSET was established as the conditioned stimulus for food, and similar results were observed.

247.17

REWARDING AND REINFORCING EFFECTS OF DRINKING SUCROSE: ROLE OF OPIOIDS AND DOPAMINE. A. Galván*, B. L. Talamantes* and A. Agmo. Dept. of Psychology, Universidad Anáhuac, México City.

It has been proposed that place preference conditioning is a 3 stage process. First, the reinforcing event must produce an affective arousal. Second, this affective arousal must become associated with environmental stimuli. Third, this association must be remembered. The first stage corresponds to reward, and the third to reinforcement. The purpose of the present studies was to evaluate the neurochemical basis of these stages.

Male rats were trained to drink 18% sucrose in water in a lickometer apparatus. They were allowed to make 2000 licks or 15 min exposure to the sucrose solution, whichever occurred first. Immediatly after they were transferred to place preference cages for 30 min. Conditioning consisted of 3 reinforced and 3 nonreinforced sessions.

Sucrose produced a clear place preference. This was completely blocked by cis(z)-flupentixol, 0.5 mg/kg. The drug did not affect sucrose consumption. Naloxone, 16 mg/kg, not only blocked the place preference produced by sucrose drinking, but also induced place aversion. Sucrose consumption was drastically reduced. Indeed, drinking was similar to that observed in animals given plain water instead of sucrose.

These data suggest that opioid mechanisms are involved in reward and dopamine is critical for reinforcement.

247.18

REDUCTIONS IN FOOD REINFORCEMENT QUALITY PRODUCE ELEVATIONS IN THE FORCE EXERTED DURING OPERANT RESPONDING IN RATS.
E.O'S.Hammond, P.Baskin*, & A.Ettenberg
Dept of Psyc, Univ of CA, Santa Barbara, CA 93106

Hungry rats were trained to press a force-sensing operandum for 2.5 sec access to sweetened condensed milk. In previous work, animals responding for food reinforcement have been shown to exert increased force both during conditions of extinction and during reinforced trials conducted in the presence of neuroleptic drugs. Is the increase in peak force seen with neuroleptics a result of a decrease in reward or, as others have suggested, a motoric-postural phenomenon? In the present study we examined the effects of manipulating reinforcer quantity (0.02 to 0.1 ml) or quality (25% to 50% dilutions) on the peak force emitted during 5 min operant test sessions. Increases in peak force were observed only after downward shifts in the quality of reinforcement. No such changes were produced by increases in reinforcer quality nor shifts in the quantity of reinforcement. These preliminary results suggest that neuroleptic-induced increases in peak force may reflect reductions in the rewarding quality of food reinforcement.

247.19

NEUROLEPTICS BLOCK THE DRIVE AND DRIVE REDUCTION,BUT NOT THE INCENTIVE, MOTIVATIONAL EFFECTS OF OPIATES AND FOOD. A.Bechara, F.Harrington, T.Stefurak, and D. van der Kooy, Neurobiology Research Group, Dept. of Anatomy, Univ. of Toronto, Toronto,Ont.,Canada,M5S 1A8.

The motivational properties of opiates and food are derived from both their incentive and their drive and drive reduction effects. Drug naive or food sated rats express conditioned preferences for morphine or food paired places, respectively, over unfamiliar neutral ones. In contrast, opiate dependent or food deprived rats avoid places previously paired with the absence of morphine (i.e.withdrawal) or the absence of food (i.e.hunger), respectively. The incentive effects produced by morphine in opiate naive , or food in food sated, rats are abolished by ibotenic acid lesions of the tegmental pedunculopontine nucleus (TPP). These lesions do not affect the drive and drive reduction effects associated with withdrawal in opiate dependent rats or with hunger in food deprived rats.

We now report that interfering with dopamine systems blocks the motivational effects associated with the drive and drive reduction effects of both opiates and food. The place preferences seen in opiate dependent rats conditioned with morphine (2 mg/kg) or heroin (50 ug/kg) were blocked by pretreatment with α-flupenthixal or pimozide. The same place preferences seen in opiate naive rats were not affected by neuroleptics. Similarly, when using food rather than opiates, we found that α-flupenthixal blocked the conditioned aversions for places previously paired with the absence of food in food deprived (23 hours) rats (i.e. drive effects), but not the conditioned place preferences produced by food itself in food sated rats(i.e. incentive effects). These results double dissociate two idependent neural substrates subserving the incentive versus drive and drive reduction effects of motivation. The TPP serves as a nodal brainstem substrate mediating the impact of incentive stimuli, whereas a dopamine substrate appears to underly the drive and drive reduction effects of motivational stimuli.

247.20

ILLUMINATION LEVEL AND OPEN-FIELD ACTIVITY IN GENETICALLY DEFINED MICE. D. F. Peeler, Neurosurgery Dept., Univ. Miss. Med. Ctr, Jackson, MS 39216

A previous study comparing locomotor activity and investigatory activity in a runway (Peeler & Nowakowski, Behav. Neur. Biol.,48:90, 1987) did not assess the effects of illumination level. In the present study, 72 male mice from the progenitor strains C57BL/6ByJ and BALB/cByJ and the seven RI strains derived from them were tested for 5 min in an open field under bright and dim ambient light. The area was marked with a grid describing 36 squares, which were considered as periphery, interior, or center sections. Five objects, 4 in the interior and 1 in the center sections, were placed in the field. Activity was recorded as locomotor (line crossings) or investigatory (object contacts) by an observer. There were significant interactions ($p<.05$) of strain with level of illumination in all 3 open-field areas. The interaction was not significant for investigatory activity. Strain CXBD had greater locomotor and investigatory activity than all other strains under most conditions, and increased locomotor activity under bright light. BALB/c, CXBE, CXBG and CXBK had a decrease. The response to conditions of illumination is a function in part of genetic background.

247.21

STIMULUS CONTROL MODULATES EFFECTS OF IONIZING RADIATION ON OPERANT PERFORMANCE. P.C. Mele and J.H. McDonough. Behavioral Sciences Dept., Armed Forces Radiobiology Res. Inst., Bethesda, MD 20814-5145.

Rats responded under a fixed-consecutive-number (FCN) schedule of reinforcement. Milk was presented when eight or more responses on one lever were followed by a single response on a second lever. Under alternating conditions (a multiple schedule), the completion of eight responses on the first lever was either cued with external discriminative stimuli (lights and a tone) or was not cued. Separate groups of rats (n=7/group) received a single whole-body exposure to 6.0 or 7.5 Gray (Gy) of gamma radiation, or two exposures to 4.5 Gy delivered 2 wk apart. Response rates under cued and noncued conditions were reduced by each dose of radiation during the 1-3 wk post-exposure period. Dose-related reductions were evident in terms of the magnitude of effect and in the proportion of animals affected. Response rates were frequently reduced more in the noncued condition, especially at the 6.0 and 7.5 Gy doses. Rate reductions after the second 4.5 Gy exposure were not consistently greater than after the first. Intermittent decreases in response rates in the noncued condition occurred during wks 3-7 after exposure in a number of rats across dose groups. Accuracies of switching between the two levers were reduced in 6/14 rats by 6.0 and 7.5 Gy; accuracies were generally reduced more under the noncued condition. Strong stimulus control can attenuate radiation-induced disruptions in operant performance.

247.22

FEAR-POTENTIATED STARTLE IN HUMANS DURING ANTICIPATORY ANXIETY. C. Grillon, R. Ameli*, S. W. Woods, K. Merikangas*, and M. Davis. Dept. of Psychiatry, Yale Univ. Sch. of Med., New haven, CT 06510.

Fear-potentiated startle was investigated in humans using a paradigm involving anticipation of shocks to produce anxiety. The eyeblink component of the startle reflex was measured during either the anticipation of electric shocks (anticipatory anxiety) or periods in which no shocks were anticipated (safe period). The eyeblink was consistently earlier and larger during periods when the subjects (N=9) anticipated shocks, compared to the safe periods. This effect was statistically significant before the subjects actually received any shock. These results indicate that anticipatory anxiety can be measured objectively in humans using the fear-potentiated startle reflex in a paradigm not actually requiring any shock. Because a great deal is known about the neuroanatomical and pharmacological mechanisms of fear-potentiated startle in animals, this test procedure may be especially useful in humans to investigate the neurobiologic substrate of anxiety disorders and their pharmacological treatments.

BIOLOGICAL RHYTHMS AND SLEEP I

248.1

DEVELOPMENT OF THE RETINO-HYPOTHALAMIC TRACT (RHT) IN FETAL SHEEP.. K.L. Fletcher*, M. Terman, A.J. Silverman and R.I. Stark* Depts. Pediatrics & Anat. & Cell Biol., Columbia Univ. NY and NYS Psychiatric Inst. NY.

Circadian rhythms have been described in behavioral and endocrine functions of the fetal sheep in the last third of gestation (term = 147 d). Although fetal rhythms may be entrained by maternal signals, it is of interest to determine when the suprachiasmatic nucleus (SCN) becomes mature in this species. One aspect of maturity is the arrival of the RHT. Intraoccular injections of various tracers were made at 68, 98, 128 and 137d of gestation, after birth and in adulthood (n=2 per age). The most successful tracer was a 30% DiI solution containing in 0.9%NaCl, 0.1% TX 100 and 5% (v/v) DMSO. After hysterectomy with direct visualization, fetal injections were made with volumes based on the size of the eye (0.1 to 0.5 ml). Fetuses were delivered 7-10d after injection, perfused transcerebrally with 4% paraformaldehyde and vibratome sections cut. Selected sections were reserved for immunocytochemical localization of SCN peptides. In vivo application of DiI resulted in brilliant labeling of the optic nerve, chiasm and tract. Labeled axons were traced as far caudalward as the lateral geniculate nucleus and superior colliculus. At all ages studied the region of the SCN received a robust retinal innervation on both ipsilateral and contralateral sides. In cresyl violet stained sections, the cell-dense clusters just dorsal to the chiasm, the putative SCN, were small while the labeled terminals occupied larger oval areas similar in morphology to the SCN of the hamster. These data indicate that the RHT is well established as early as half-way through gestation. Ongoing immunocytochemical studies will assess the phenotypic maturation of the SCN neurons. HD 10665 (AJS), HD 13062 (RIS)

248.2

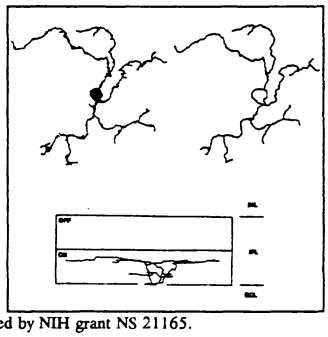
LOCALIZATION OF CHOLINERGIC AND NERVE GROWTH FACTOR-RECEPTOR NEURONS PROJECTING TO THE SUPRACHIASMATIC NUCLEUS OF THE RAT K.G. Bina, K. Semba and B. Rusak, Departments of Psychology and Anatomy, Dalhousie University, Halifax, Canada. B3H 4J1

Circadian rhythms in mammals are regulated by neural mechanisms centered in the suprachiasmatic nucleus (SCN) of the anterior hypothalamus Recent immunohistochemical findings indicate that both choline acetyltransferase (ChAT), the synthesizing enzyme for acetylcholine, and nerve growth factor-receptor (NGF-R) are present in the SCN. In order to identify the source of this immunoreactivity, a combination of retrograde tracing and immunohistochemistry for ChAT and NGF-R was performed. Following iontophoretic injections of fluorogold into the SCN, in addition to previously described areas, retrogradely labelled neurons were seen in the substantia innominata, the part of the magnocellular basal nuclear complex near the SCN. Some of these retrogradely labelled cells were also single- or double-labelled with ChAT and NGF-R. Unilateral knife cuts directed lateral to the SCN decreased NGF-R immunoreactivity in the ipsilateral SCN. Bilateral blinding had no effect on NGF-R labelling in the SCN. This work was supported by NSERC and MRC of Canada.

248.3

MORPHOLOGICAL FEATURES OF LUCIFER YELLOW (LY) FILLED RETINAL GANGLION CELLS INNERVATING THE SUPRACHIASMATIC NUCLEUS (SCN). **G.E. Pickard and E. Friauf. Depts. of Psychiatry and Anatomy, University of Pennsylvania, Philadelphia, PA 19104 and Dept. of Neurobiology, Stanford University, Stanford, CA 94305.**

The sub-type of retinal ganglion cell projecting to the hypothalamic SCN is unknown. We have begun to describe these cells in the golden hamster retina. Ganglion cells were identified by the retrograde transport of rhodamine-labeled microspheres injected into the SCN in vivo. LY was injected intracellularly into microsphere-labeled cells in living retinas maintained in vitro to reveal their complete structure. LY filled cells were photoconverted (Fig. 1 left), logged into a computer (Fig. 1 right) and rotated 90° about the X-axis to reveal dendritic stratification in the inner plexiform layer (IPL) (Fig. 1 bottom). Ganglion cells innervating the SCN have a large soma (dia=13.5 μm), 3-4 primary dendrites, which branch sparingly and stratify in the inner half of the IPL and a large dendritic field (225-295μm). The morphology of ganglion cells afferent to the SCN implies a high degree of convergence with a subsequent loss of spatial resolution. Supported by NIH grant NS 21165.



248.4

LOCALIZATION OF CHOLINE ACETYLTRANSFERASE IMMUNOREACTIVE (CAT-I) CELLS AFFERENT TO THE SUPRACHIASMATIC NUCLEI (SCN) IN THE GOLDEN HAMSTER. M.R. Dwyer, M.E. Harrington and T. Rahmani*, Dept. Psych., Smith College, Northampton, MA 01063 and Neurosci. and Behav. Program, Univ. Mass., Amherst, MA 01030.

Acetylcholine may play a role in mediation of photic responses of circadian rhythms (Physiol. Rev. 69:671-707, 1989). In this study, sources of cholinergic input to the putative circadian pacemaker in the SCN were localized.

Injections of rhodamine-conjugated latex microspheres (0.05ul) were aimed at one SCN of golden hamsters. After 48 h or longer, animals were sacrificed with a sodium pentobarbital overdose. Tissue was processed for CAT immunohistochemisty using a polyclonal human placental CAT antisera (Chemicon). The avidin-biotin method was used with either diaminobenzidine or flourescein as the chomagen.

Distribution of CAT-I cells was similar to that reported for the rat brain (Brian Res. 415:49-62, 1987; 495: 271-297, 1989) with a few exceptions. Notably, CAT-I diagonal band cells were not continuous with CAT-I cells in the medial septum. Cells afferent to the SCN were found in areas previously described (J.Comp.Neurol., 211:65-83, 1982) as well as in several other areas.

Overlap in these markers was seen in the basal forebrain, arcuate, paradorsal raphe, dorsal tegmental nucleus/parabrachial area and the parabigeminal nuclei. Double-labeled CAT-I cells afferent to the SCN were observed in the dorsal tegmental/parabrachial area, extending laterally into the parabigeminal nucleus. A few double-labeled cells were also observed in the vertical limb of the diagonal band. Double-labeled cells which were only lightly CAT-I were located in the area immediately around the caudal SCN.

These results indicate that cholinergic input to the SCN may arise from several distinct groups of cells. Visually responsive parabigeminal or peri-SCN neurons may be involved in mediating light-induced phase shifts of circadian rhythms.

(Supported by NIH.)

248.5

SYNAPTOGENESIS AND RETINOHYPOTHALAMIC TRACT (RHT) DEVELOPMENT IN THE HAMSTER SUPRACHIASMATIC NUCLEUS (SCN) J.C. Speh and R.Y. Moore, Depts. of Neurology and Neurobiology, SUNY, Stony Brook, NY 11794

Synaptogenesis in the rat SCN is a largely postnatal event with the largest production of synapses occurring in postnatal days 4-8 (P4-P8, Moore & Bernstein, 1989). This corresponds to the period of maximal development of RHT (Speh & Moore, 1988) but is significantly later than the onset of functional development, as demonstrated by the 2-deoxyglucose method (Reppert & Schwartz, 1984).

We studied synaptogenesis and RHT development in the hamster using Synapsin I immunohistochemistry and anterograde transport of cholera toxin-HRP, respectively. In contrast to the rat, the hamster SCN contains numerous synapsin-like immunoreactive structures at P1 and is essentially comparable to the adult appearance by P4. However, there are only very sparse, scattered RHT axons in the SCN, lateral hyothalamus (LH) and anterior hypothalamic area (AHA) at P4, and no projections to the preoptic area (POA), retrochiasmatic area (RC) and basal forebrain. By P6-8 projections to the SCN, LH and AHA are more dense and projections to the POA, RCA and basal forebrain are present. On P15 all projections are present and appear comparable to the adult (Johnson et al, 1988). These observations indicate that there are striking differences between the rat and hamster in the timing of development of synapses and the RHT in the SCN. (Supported by NIH grant NS-15304).

248.6

THE HUMAN SUPRACHIASMATIC NUCLEUS (SCN) **R.Y. Moore and J.C. Speh, Depts. of Neurology and Neurobiology, SUNY, Stony Brook, NY 11794.**

The human SCN has been characterized in Nissl material (Brockhaus, 1942; Braak and Braak, 1987) and using immunohistochemistry (Swaab et al, 1984; Stopa et al, 1984; Moore, 1989). The present study was undertaken to provide a more detailed analysis of the human SCN.

Human hypothalami were obtained from routine postmortem material fixed in buffered formaldehyde. Coronal sections were prepared with antisera to vasoactive intestinal polypeptide (VIP), neuropeptide Y (NPY), vasopressin (VP) and neurotensin (NT). The SCN is the most rostral definable hypothalamic nucleus. It appears as a cluster of cells within the dorsal chiasm which extend into the rostral chiasmatic hypothalamus immediately caudal to the lamina terminalis. More caudally the SCN is an ovoid nucleus adjacent to the third ventricle between the paraventricular nucleus dorsally and the supraoptic nucleus laterally. Caudally the SCN becomes smaller to disappear at the level of the caudal optic chiasm. VIP neurons form the central region of the SCN. NPY neurons are present in the VIP zone and extend beyond it. VP neurons extend beyond the NPY zone and there is an extensive group of NT neurons extending over the entire area. The human SCN differs from that of other mammals in that it contains NPY neurons and in the large number of NT neurons. Supported by a grant from the United States Air Force.

248.7

VIP NEURONS IN THE HUMAN SCN FORM A DISCREET PROJECTION TO THE "SUBPARAVENTRICULAR ZONE"

Stopa, E.G., Chorsky,R.*, King, J.C. and Albers, H.E., Dept. of Path., SUNY Health Sci. Ctr., Syracuse, NY. Dept. of Anat. and Cell Biol., Tufts U. Sch. of Med., Boston, MA and Depts. of Biol. and Psychol., Georgia State U., Atlanta, GA.

The suprachiasmatic nucleus (SCN) in the anterior hypothalamus is essential for the neural control of circadian rhythms. In the rat, the majority of SCN efferent fibers project to immediately adjacent regions, including an area dorsal to the SCN designated the "subparaventricular zone" (Watts, A.G. & Swanson, L.W., J. Comp. Neurol. 258:230-252, 1987). In this study, we examined the efferent projection of the vasoactive intestinal peptide (VIP)-containing neurons within the human SCN. In the rat, these neurons receive the majority of SCN afferent fibers and are thought to be involved in the first order processing of light information.

Human hypothalami (n=15) were fixed by immersion in either 5% acrolein or Zamboni's fixative and sectioned in the coronal, sagittal, and horizontal planes. The immunocytochemical procedures were performed on serially obtained sections using either the ABC or PAP method after incubation in primary antibody for 72 hrs. VIP neurons were evident throughout the SCN, concentrated ventrally near the optic chiasm. Immunoreactive fibers were seen arborizing within the nucleus, and formed a discreet projection which could be traced to the "subparaventricular zone." Our data suggest that the "subparaventricular zone" may act as an integrator for photic circadian information in humans, as in other mammals, and that this information is relayed primarily by the VIP-containing neuronal subpopulation. (Supported by AG00295).

248.8

LABELING OF THE HUMAN RETINOHYPOTHALAMIC TRACT WITH THE CARBOCYANINE DYE, DiI. [1]D.I. Friedman, E.G. Stopa, and [2]J.K. Johnson. [1]Depts. of Neurol., Ophthalmol., and Path., SUNY Health Science Center, Syracuse, NY, 13210, and [2]Inst. for Sensory Res., Syracuse Univ.,Syracuse, NY 13244.

A retinohypothalamic tract (RHT) has been demonstrated in human post-mortem brains with documented optic nerve damage prior to death (Brain Res 1985;340:243) and in rats (J Comp Neurol 1972;146:1). It has been suggested that a RHT exists in animals to mediate endogenous rhythms. We used the lipophilic fluorescent probe, DiI, to attempt localization of the RHT in normal human post-mortem brain.

Human brains (n=4) were obtained within 18 hours of death. Hypothalami were removed and placed in 4% paraformaldehyde in 0.1M phosphate buffer. After 24 hours in fixative, crystals of DiI (1,1'-diocladecyl-3,3,3',3-tetramethyl indocarbocyanine perchlorate, Molecular Probes) were imbedded into either optic nerve. Tissue was maintained at 36°C in darkness for two to three months. Serial coronal vibratome sections (60 um) were cut, collected on gelatin-coated slides and mounted in an aqueous mounting medium (Gelmount, Biomeda Corp., Foster City, CA).

In all brains studied, labeled fibers were seen extending from the optic chiasm to the ventral surface of the SCN. Our data indicate that the membrane probe, DiI, provides a reliable method for demonstrating a RHT in normal human post-mortem brain tissue.

(Supported by grant AG00295 and NIH grant EY06064.)

248.9

ENDOCRINE RHYTHMS IN SEASONAL AFFECTIVE DISORDER DURING A CONSTANT ROUTINE. K.Dahl*, D.Avery*, M. Savage*, G.Brengelmann*, M.Kenny*, A.Lewy*, L.Larsen*, M.Vitiello*, and P.Prinz. University of Washington, Seattle, WA 98195.

The phase of circadian rhythms in 9 Seasonal Affective Disorder (SAD) subjects and 7 controls was assessed using a constant routine (CR). Eight SAD subjects were restudied following a minimum of one month early morning bright light therapy (2500 lux, 6AM to 8AM). During the CR subjects were sleep deprived at bed rest with light held at 60 lux for 27 hours. Blood samples for TSH and cortisol were drawn 1/hour. Samples to determine the Dim Light Melatonin Onset (DLMO) were drawn 2/hour from 6PM to 12AM. Rectal temperature was monitored 1/min. The cosinor acrophases of cortisol, TSH and temperature of SAD subjects were significantly ($p<.05$) phase-delayed relative to controls, while there was a nonsignificant trend for DLMO of SAD subjects to be phase-delayed. AM light therapy significantly phase-advanced the cortisol acrophase, and produced a nonsignificant trend toward phase-advance of melatonin, TSH and temperature rhythms in the SAD subjects. The endocrine rhythms correlated with the temperature rhythm as well as with each other (combined groups):

	Cortisol	TSH	DLMO
TSH	r=.80, p<.001		
DLMO	r=.85, p<.001	r=.47, p=.014	
Temp	r=.61, p<.01	r=.67, p<.001	r=.45, p=.023

248.10

BIOLOGICAL RHYTHM DISTURBANCES IN SEVERELY AND PROFOUNDLY RETARDED INDIVIDUALS: PREVALENCE AND CHARACTERISTICS. Ch. E. Olmstead, Robert H. Chaney* and Carolyne Givens.* Olmstead Assocs, Playa del Rey, CA 90296 and UCLA/MRRC Group at Lanterman Developmental Ctr, Pomona, CA 91769.

The records of all residents of a state residential facility for the developmentally disabled were surveyed for disorders of body rhythms and regulated behaviors. Of the 1082 residents, 22.5% showed disturbances of the sleep-waking cycle (N=132), defective thermoregulation (N=45), polydipsia (N=38) or dysphagia (N=82). Twenty-four hour observations of either behavioral activity (N=21) or body temperature (N=37) were carried out to further characterize the rhythms.

Sleep-waking cycle disturbances ranged from mild to severe with total sleep times that ranged from 3.0 to 10.25 hours distributed in from 2 to 18 bouts. Only 7 of the 21 subjects showed normal periods of uninterrupted sleep during the night. The basic rest activity cycle (BRAC) estimated by a combination of activity and sleep measures ranged from 74 to 113 minutes. A variety of disturbances of core body temperature were seen ranging from no circadian variation to inverted or phase shifted rhythms. In general, those individuals whose brain damage was attributable to post-natal causes such as trauma or infection were the most likely to show near normal circadian variations, while those with prenatal etiologies were the most severely disrupted.

248.11

HUMAN INTERLEUKIN-1 RECEPTOR ANTAGONIST (IL1ra) BLOCKS IL1-INDUCED FEVER AND NON-RAPID-EYE-MOVEMENT SLEEP (NREMS) IN RABBITS. M. R. Opp and J. M. Krueger. Univ. of TN, Memphis TN 38163

IL1 is a cytokine that plays a fundamental role in regulating the host defense response. The interactions of IL1 with other cytokines are complex and poorly understood. Recently, a human IL1 receptor antagonist has been characterized and cloned (1), thus providing a means to further elucidate IL1 effects. IL1ra is a polypeptide of 18-22 kD that can block several IL1 effects, e.g. leukopenia (2). We report here that hu-r-IL1ra (Synergen) attenuates IL1-induced febrile responses and blocks IL1 somnogenic actions in rabbits for periods of 6-h or more. Sleep-wake activity and brain temperature (Tbr) were recorded for 6-h immediately after injection 100 ug IL1ra and/or 10 ng IL1-beta (R&D, Inc.). Injection of IL1 alone resulted in a characteristic fever that reached 1.8°C by the end of the 6-h period, while NREMS was increased by 13.3% across this same time period. Pre-treatment with IL1ra attenuated the IL1-induced febrile response; Tbr at the end of the recording period was only 0.5 C higher than SALINE control. IL1-induced NREMS was completely abolished by pre-treatment with IL1ra; NREMS across the recording period was -1.0 % relative to SALINE control. We conclude that hu-r-IL1ra is capable of inhibiting at least two of the CNS actions of IL1, sleep and fever.

(1) Eisenberg, S. et al. Nature 343:341, 1990.
(2) Ohlsson, K. et al. Cytokine 1:131, 1989.
Supported in part by: NS25378

248.12

A PIECEWISE-LINEAR DIFFERENCE EQUATION MODEL OF THE HUMAN SLEEP-WAKE CYCLE Kevin A. O'Connor*, Arnold J. Mandell, Suzanne Knapp. Department of Psychiatry, School of Medicine, Univ. of California San Diego, San Diego, California 92093

Human temporal isolation studies often show desynchrony, in which sleep onsets occur at a variety of phases of the temperature cycle. Despite the seeming disorder of the sleep onsets, desynchrony shows a number of regular features: (1) sleep length as a ramp-like function of sleep onset; (2) longer average wake length than in synchrony; (3) a relation between wake length and next sleep onset; (4) wake-maintenance zones; (5) sleep-maintenance zones; (6) longer average sleeps than in synchrony; (7) longer or shorter sleep fraction in desynchrony (Strogatz, S., *The Mathematical Structure of the Human Sleep-Wake Cycle* (Berlin: Springer-Verlag, 1986)). A variety of models can generate feature 1 (Strogatz, *ibid.*), but not all of the other features. Using features 1 and 2 we derive a difference equation model that produces features 3 to 7. The model also explains the appearance and disappearance of desynchrony as tangent bifurcations of the system, and phase-trapping as a noised system with a pair of near-tangent fixed points. The model assumes one oscillator, associated with temperature cycle (normalized to 1), and a 3-piece ramp function, SF(n), yielding sleep length given sleep onset: $S(n+1) = S(n) + SF(n) + W \pmod 1$, where S(n) is the temperature phase of the nth sleep onset, and W is the wake length. Parameters (wake length, maximum and minimum sleep duration) control transitions among phase-locked behavior, quasiperiodicity, period-doubling, and chaotic dynamics. Many of the findings are generalizable to piecewise sinusoidal or polynomial approximations of the sleep duration function. This model yields testable predictions of changes in sleep-wake pattern with certain manipulations of wake or sleep lengths.

248.13

THE APPLICATION OF AN ARTIFICIAL NEURAL NETWORK TO THE DISCRIMINATION OF SLEEP DEPRIVED FROM RESTED EEG. G. Belenky, H. Sing*, Y. Shaham*, M. Thomas*, N. Shepanek*, D. Thorne*, T. Balkin*, U. McCann, J. Fertig, D. Redmond*. Dept. of Behavioral Biology, Walter Reed Army Institute of Research, Washington, DC 20307-5100.

We applied an artificial neural network (ANN) to the discrimination of EEG obtained from the same normal volunteer when sleep deprived and when well rested. In the sleep deprived condition, the subject spent 64 hours in the laboratory with no scheduled sleep. In the rested condition, the subject spent 64 hours in the laboratory but with the opportunity for 7 hours sleep each night. In both conditions, at 50 hours into the study, the subject was required to do a computer-based serial addition and subtraction task for 30 min. EEG, EOG, and EMG were measured continuously. By standard polysomnographic criteria, the subject was awake during both runs, having less than 10 sec of scorable stage 1 sleep in either condition during the 30 min. The 30 min records were divided into 32 sec segments. Each segment was run through a fast fourier transformation (FFT) and expressed as relative EEG amplitude for each 0.25 Hz segments from 0.75-31 Hz. Alternate 32 sec FFT transforms for both sleep deprived and rested conditions (59 each) were assigned to either the training set or test set for the ANN. The ANN paradigm was a feed forward network with training conducted using back propagation and the generalized delta rule. The ANN consisted of 122 input units, 61 hidden units, and 2 output units. The ANN was run through the training set 6,000 times. Training took approximately 18 hrs on a 25 MHz 386 PC with math co-processor. Once trained, the ANN was run through the test set once. The ANN correctly classified 53/59 (91%) of the test set as rested or sleep deprived. Each classification took a fraction of a second. Trained ANNs have utility in the on-line, real-time assessment of alertness.

248.14

AUTOMATED STAGING OF SLEEP USING NEURAL NETWORKS. A.Mamelak*, J.Quattrochi and J.A.Hobson. Laboratory of Neurophysiology, Harvard Medical School, Boston, MA 02115.

Manual staging of sleep based on visual EEG criteria is a laborious and time-consuming task. In an effort to automate sleep staging, we have developed a neural network that "learns" to stage sleep on the basis of waveband count data alone in the cat. Waveband count data are collected on a microcomputer, using period-amplitude analysis. Delta waves, spindle bursts, PGO waves, EOG, EMG, and movement artifact amplitudes are collected, and then used to "train" the network to score sleep by testing these data against the corresponding manually staged record which serves as a "teacher". We demonstrate that, when used to score the states of wake, slow wave sleep, desynchronized sleep, and transition periods, these neural networks agree with manual scoring a mean of 93.3% for all epochs scored. Neural network programs can learn both rules and exceptions, and since the nets teach themselves these rules automatically, a minimum of human effort is required. Because programming requirements are small, this approach is readily adaptable to microcomputer based systems, and is widely applicable to both animal and human EEG analyses. The utility of this approach for the evaluation of clinical disorders is discussed. Supported by NIH grant MH13923.

248.15

AN ANALYSIS OF THE BRAIN'S AMPLITUDE-FREQUENCY-CHARACTERISTICS DURING DIFFERENT SLEEP STAGES

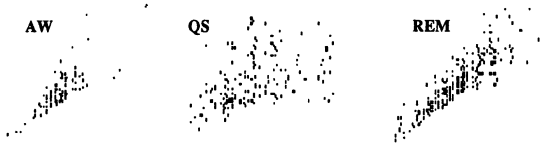
J. RÖSCHKE*, K. MANN*, J.B. ALDENHOFF, Dept. of Psychiatry, University of Mainz, F.R.G.

We studied late components of auditory and visually evoked potentials during sleep. Ten healthy male subjects were randomly stimulated by tone bursts and light flashes. According to Rechtschaffen and Kales (1968) we performed an off-line scoring procedure of sleep-EEG and averaged the AEPs and VEPs of five different periods, corresponding to sleep stages I, II, III, IV and REM. From the averaged evoked potentials we computed the amplitude-frequency-characteristic (AFC) of the brain (Basar, 1980) during different sleep stages. In general AFCs characterize transfer properties of an oscillating system. The comparison of the AFCs at the different sleep stages has shown that the excitability of the brain depicts an clear alpha resonance during stage I, a pronounced delta resonance during stages II, III and IV, and a theta resonance during REM sleep. Depending on the depth of sleep also other resonant frequencies were detectable. A comparison of these results from linear system theory with the nonlinear dynamical behavior of the CNS, such as dimensionality and the degrees of freedom of the sleep-EEG, was performed.

248.16

MODES OF R-R INTERVAL VARIATION DURING SLEEP AND WAKING STATES. S.L. Raetz, C.A. Richard, A. Garfinkel, R.M. Harper, Brain Research Institute and Department of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024.

Assessment of cardiac R-R intervals during different sleep states by procedures that evaluate total variation indicates that cardiac R-R interval variation is much higher in rapid eye movement sleep (REM) than in quiet sleep (QS). Measures such as total variance, however, suffer from their summary nature, and are unable to provide information on instantaneous or dynamic aspects of beat-to-beat control. A simple procedure for assessing beat-to-beat control is to plot each interval against the next; this procedure provides an indication of the probability of occurrence of one interval from its predecessor. Cardiac R-R intervals were determined over 3-12 minute periods of waking, QS, or REM from 4 cats; each cardiac interval was plotted on the x-axis against the next value on the y-axis. The resulting plots provide evidence that the *correlation* between successive R-R intervals was greater in REM than in QS, even though



the overall *variance* is greater in REM. The resulting plots scattered in a bimodal fashion across all states; short R-R intervals were under tighter control than longer intervals. These results suggest that cardiac control during REM may be influenced by much more closely regulated mechanisms than suggested by classic concepts. Supported by R01-HL-22418-13.

248.17

EEG PATTERNS DURING TM PRACTICE AND HYPNAGOGIC SLEEP. E.T. Travis, Maharishi International University, Fairfield, IA 52556

EEG patterns during TM practice, ie. alpha activity spreading anteriorly and slowing 1-2 c/sec, was first reported by Wallace (1). Later, Fenwick (2) and others (3,4) noted a marked similarity of this EEG pattern with EEG during the hypnagogic state experienced between waking and sleeping. They concluded that TM balances the awareness between waking and sleeping. The present study compared EEG patterns during TM practice (10 TM Ss), recorded from F_3,F_4,C_3,C_4,P_3,P_4, to that during an eyes-closed rest period (10 matched non-meditating Ss). Power and coherence were not significantly different between TM practice and the period between eyes-closed and Stage 1 in the comparison group; both peaked between 7-9 Hz (theta/alpha). However, during TM practice this EEG pattern persisted for 10 minutes (the entire session), while the hynagogic state in the control subjects lasted only three minutes on the average. While this supports the idea that awareness is balanced between waking and sleeping during TM practice, Maharishi (5) has also proposed a "junction point model": that waking, dreaming and sleeping are active modes of a continuous field of pure consciousness that can be experienced in the transition between active states of consciousness or, at will ,during TM practice.

REFERENCES: (1) Wallace RK. (1970). *Physiological effects of Trasncendental Meditation* , MIU Press, 1973. (2) Fenwick PBC et al. (1977). *Bio Psych*. 51: 101-118. (3) Stigsby B. (1981). *Electroenceph Clin Neurophysio*. 81:431-112. (4) Pagano & Warrenburg (1983). Consciousness and self-regulation: Advances in research and theory. Plenum press. (5) Maharishi. (1972). *The Science of Creative Intelligence*. MIU Press.

LEARNING AND MEMORY: ANATOMY I

249.1

PERFORANT PATH DEAFFERENTATION ALTERS EXPLORATION AND EXTINCTION BUT NOT SPONTANEOUS ALTERNATION IN RATS. R.L. Port, K.S. Curtis*, P.W. Parsons* and K.S. Seybold. Depts of Psychology, Slippery Rock University, Slippery Rock, PA, 16057 and Grove City College, Grove City, PA 16127.

The hippocampal formation is a highly structured brain system that is known to participate in a variety of learned behaviors. Among the effects of gross hippocampal damage are deficits in spatial learning and extinction. The present study investigates the specific networks responsible for these behaviors. Adult hooded rats were randomly assigned to control (n=8), lateral (n=8) or medial (n=8) perforant path (PP) deafferentation groups. Surgical procedures, adapted from Myhrer (Physiol Behav, 42:1988), involved mechanical severation of pathways using stereotaxic coordinates. Animals were tested in spontaneous alternation, acquisition and extinction of shuttlebox avoidance. Damage to the medial or lateral PP had no effect on spontaneous alternation. However, during adaptation, lateral PP animals were less active than controls. During extinction, medial PP animals were found to respond less frequently than other groups.

249.2

THE AUDITORY CORTICOPONTINE PROJECTION IN THE RABBIT STUDIED WITH HRP-WGA. B.J. Knowlton, C. Weiss, J.K. Thompson & R.F. Thompson, Neurosciences Program, University of Southern California, Los Angeles, CA. 90089-2520.

The pontine nuclei may be a major site for cerebral cortical modulation of the brainstem and cerebellar circuits involved in classical conditioning. In order to study the auditory corticopontine projections, HRP-WGA was injected into the auditory cortex in 11 rabbits. Recordings were made from the injection site to confirm placement in the auditory cortex. The injection volume was 30-100 nl of 1% HRP-WGA. Survival time after injection was 2 days. HRP was visualized with TMB processing. In all cases, retrogradely labeled cells in the thalamus were confined to the medial geniculate nucleus, demonstrating that the injection was confined to the auditory cortex. Anterograde labeling in the pontine nuclei occurred in 2 ipsilateral regions; a dorsolateral region in mid-rostral sections, and a region in the caudal pontine nuclei, that extended from the lateral pontine nuclei to the ventral edge of the cerebral peduncle. There was also some labeling in the caudal medial pontine nuclei in 5 rabbits. Anterograde labeling was also seen bilaterally in the inferior colliculus, most prominently in the ipsilateral pericentral and external regions.

Supported by NSF (BNS-8718300) & the McKnight Foundation grants to RFT.

249.3

A COMPARISON OF THE EFFECTS OF EXCITOTOXIC LESIONS TO SEPTAL NUCLEI AND THE HIPPOCAMPAL FORMATION ON THE ACQUISITION OF DECISION RULES. T.W. Robbins, H.M. Marston*, L.S. Wilkinson* and B.J. Everitt (Spon. EBBS) Depts. of Experimental Psychology and Anatomy, University of Cambridge, Downing Street, Cambridge, CB2 3EB, U.K.

The effects of (i) lesions aimed at the cholinergic septo-cortical projections and (ii) lesions of the hippocampal formation, were compared on the rate of acquisition of (i) a conditional visual discrimination (CVD) and (ii) the Morris swim maze task.

Septal lesions were made by infusing either 0.5 ul quisqualic acid (0.12 M) or vehicle at four sites bilaterally. Hippocampal lesions were made by infusing ibotenic acid (0.06 M) at 11 sites bilaterally. *Post mortem* biochemical analysis of choline acetyltransferase activity (ChAT) revealed that the septal group's lesions were of three types according to the primary region of ChAT reduction; (a) cingulate cortex (S-C group), (b) hippocampus (S-H) and (c) both structures (S-CH). Additional analysis confirmed that there were no significant reductions in the levels of cortical monoamines. Histological examination of the hippocampal ibotenate group revealed extensive damage in the intended regions.

The CVD was between fast and slow flashing lights, responses having to be made either to the left or right, depending upon stimulus frequency. Hippocampal lesions produced only slight impairments in acquiring the task, but the S-C and S-CH groups were profoundly impaired. The S-H group learned normally. In the water maze task, the hippocampal group were impaired both in acquisition and on the memory probe trial. The septal group only showed minimal acquisition.

These results suggest that an intact cingulate cortex is a prerequisite for the efficient learning of conditional rules. The hippocampus, while not essential for rule learning, does appear to be important for the precise integration of spatial information.

249.4

INVOLVEMENT OF THE LATERAL NUCLEUS OF THE AMYGDALA IN AMPHETAMINE AND FOOD CONDITIONED PLACE PREFERENCES (CPP) N. Hiroi, R.J. McDonald* and N.M. White, Department of Psychology, McGill University, 1205 Dr. Penfield Ave, Montreal, Quebec, Canada H3A 1B1.

The CPP paradigm utilized an apparatus with two distinct, equally preferred environments. Rats experienced pairings of a primary reward with one of the environments and an equal amount of non-paired experience with the other environment, in a counterbalanced manner. When the primary reward was a subcutaneous injection of 2 mg/Kg d-amphetamine sulphate, electrolytic lesions localized to the lateral nucleus of the amygdala, made before or after training significantly attenuated the CPP. In contrast, electrolytic lesions localized to the central or basolateral amygdaloid nuclei, the endopyriform nucleus, ventral hippocampus or fornix/fimbria were without effect. Neurotoxic (NMDA) lesions including the lateral amygdaloid nucleus had the same effect as electrolytic lesions. Similar neurotoxic lesions also significantly attenuated the CPP when consumption of food was the primary reward. The results suggest that the lateral amygdaloid nucleus, but not other amygdaloid nuclei or the hippocampal system, is involved in mediating certain conditioned rewarding effects. Taken together with other findings (eg, Hiroi & White, Br Res, 510:33,1990; Cador, Robbins & Everitt, Neurosci, 30:77-86,1989), these results may imply that the lateral nucleus of the amygdala interacts with the nucleus accumbens in acquiring and expressing memory for stimulus-reward associations.

249.5

A TASK DESIGNED TO DEMONSTRATE A DECLARATIVE MEMORY REPRESENTATION OF ODOR CUES IN RATS. C.G. Wible, H. Eichenbaum, and T. Otto. Biology Department, Wellesley College, Wellesley, MA 02181.

A task was designed for rats that used nonspatial declarative memory similar to the memory requirements used in tasks with human amnesics. Correct performance on probe trials after training on the task required the establishment of a representation of odors that was both flexible and could be accessed in a novel situation that did not simply reproduce the original training conditions. The serial order task consisted of initial training on 4 odor discriminations with shared elements (A+B-, B+C-, C+D-, D+E-). The task was designed to encourage a transitive representation between the odor representations such that A>B>C>D>E. Probe trials were given with the pairs A-E and B-D. The correct choice of odor A in pair A-E did not require the establishment of a transitive representation, because odor A was always rewarded and E was never rewarded in training. The correct choice of odor B in pair B-D did require access to a transitive representation of the odors, because B and D were rewarded equally often during training. Rats with fimbria/fornix (FFX) lesions and SHAM rats learned the task to a criterion of 80% correct, with no difference in errors to criterion for the 2 groups. Rats were then given probe trials of A-E and B-D interspersed between regular trials. Choice accuracy remained high (between 70% and 90% correct) during sessions with probe trials. In a pilot study, SHAM rats performed correctly on both types of probes, but FFX rats performed correctly only on the pair not requiring a transitive representation, pair A-E. The results suggest that the hippocampal system is involved in forming transitive inference representations, which is a property of declarative memory.

249.6

NEURAL MECHANISMS OF CONSPECIFIC DISCRIMINATION IN PIGEONS. S.Watanabe. Dept.of Psychology, Keio University. Tokyo 108,Japan

Ectostriatal lesions in pigeons have caused deficits in discrimination of arbitrary classification of natural stimuli but not in discrimination of natural concepts. In the first experiment pigeons were trained on conspecific vs non-conspecific discrimination in an operant chamber. The chamber had a TV screen connected with a floppy-video player which produced visual images of conspecific or non-conspecific on the screen. Pecking response was reinforced when conspecific appeared and was extinguished when non-conspecific appeared. Lesions in intermedial hyperstriatum ventrale (IMHV) did not cause deficits in conspecific discrimination but ectostriatal damage resulted in deficits in the discrimination. In the second experiment pigeons were trained on discrimination of individual pigeons. Ectostriatal lesions impaired this discrimination. These results suggest that the ectostriatum(which has been considered to be mammalian extrastriate cortex) has an important role in conspecific discrimination in pigeons.

249.7

NEURONAL SOMATA IN THE OCTOPUS CENTRAL NERVOUS SYSTEM ARE INEXCITABLE AND LABEL RETROGRADELY WITH DiI AND DiO. Robertson, J.D., R. Gillette, P. Lee*, S. Meadows and J. Zitz*, Duke University Marine Lab., Beaufort, NC, and Department of Physiology and Biophysics, University of Illinois, Urbana, IL.

Brains of *Octopus vulgaris*, cooled to 0°C, were removed to oxygenated saline and 1 mm slices of supra- and subesophageal lobes were placed in flowing, oxygenated saline at 12°C. Intracellular recordings were made with 3 M KCl electrodes with 20-50 MΩ resistance. Recordings were made from most cell body containing areas of the brain. Intracellular recordings were also made from isolated buccal and stellate ganglia, whose connective tissue capsules were partially removed. Action potentials and PSPs were frequently seen. However, in over 200 recordings from cells with membrane potentials of -35 to -70 mV, action potential amplitudes never exceeded 20 mV, and in most cases were <10 mV. The most likely interpretation of these results is that the octopus neuron somata are inexcitable. This resembles the situation for arthropods, and contrasts with that for generally excitable somata of other molluscs. We discuss inexcitable somata as an evolutionary adaptation of the molluscan CNS plan to the large and complex brain of the octopus.

In related experiments we injected DiI or DiO into a cerebrobrachial commissure. After 8 days, numerous large (~50-60 µm), presumptively motor cell bodies, were labeled both in the surface membranes and in 2-5 µm diameter organelles in the posterior buccal, subfrontal, subvertical and posterolateral basal lobes. The latter may represent a new motor nucleus. Numerous interspersed small unlabeled cells ~5 µm in diameter were also seen. Supp. by NSF Grants BNS-88-20409 and BNS-86-03816.

249.8

CONTINUOUS RECOGNITION MEMORY PERFORMANCE AS A FUNCTION OF HIPPOCAMPAL, PARIETAL AND MEDIAL PREFRONTAL CORTEX LESIONS. P. Jackson-Smith and R. P. Kesner, Dept. of Psychology, Univ. of Utah, Salt Lake City, UT 84112.

Using a continuous recognition memory procedure, rats were trained within a 12-arm radial maze. They were allowed sequential access to 12 arms of the maze from the center platform on each daily session. Access required that the rat orient to a cue on the clear plexiglas door of the designated arm; upon orientation, the door was opened, and latency to reach the end of the arm was measured. Of the 12 presentations, three or four of the arms were repeated, but did not contain reinforcement. Repeated arms were presented with lags ranging from 0 to 6 (from 0 to 6 different arm presentations between the first and the repeated presentation). Prior to surgery all rats received 32 training sessions, and showed long latencies at the short lags with decreasing latencies as the lag between repeated presentations increased. Latencies were short for all lags post-surgery in the group of rats receiving total hippocampus ablation (electrolytic). Following aspiration lesions of the medial prefrontal cortex, latencies at each lag decreased relative to pre-surgery performance, however, the forgetting functions remained parallel. Following aspiration lesions of the parietal cortex, sham surgery, or electrolytic cortical control lesions, there were no deficits. These data further emphasize the role of the hippocampus in mediating spatial attributes, even with no temporal delay (a lag of 0).

249.9

Effects of Stimulation of the Amygdaloid Central Nucleus (ACe) on Cortical Electroencephalographic (EEG) Activity in the Rabbit. B.S. Kapp, W.F. Supple and J.L. Doherty. Dept. Psychol., Univ. of Vermont, Burlington, VT 05405

We have recently reviewed evidence suggesting that the ACe contributes to conditioned arousal, as manifested in its contribution to the expression of a variety of arousal-indicative responses which may enhance the detection and processing of sensory information (Kapp et al. 1990). In this study the effect of stimulation of the ACe and surrounding sites on neo-cortical EEG arousal (low voltage fast activity) and heart rate was examined. New Zealand rabbits were anesthetized with alpha-chloralose, and recording electrodes were positioned on or within the frontal cortex. Electrical stimulation (1.0 sec, 100 Hz, 100-500 uA) was applied every 0.5 mm as a stimulating electrode was lowered through the dorsal-ventral extent of the ACe and surrounding regions. Although EEG changes were obtained from sites immediately adjacent to the ACe, maximal changes were obtained from sites within its dorsal region. Stimulation produced an immediate shift from large amplitude slow-waves to low voltage fast activity during and following stimulation. Maximal bradycardia was elicited from those sites eliciting maximal EEG changes.

These results are consistent with the hypothesis that the ACe contributes to the expression of responses which are indicative of heightened arousal and which may enhance sensory information processing.

249.10

A RAT MODEL OF MEDIAL-DIENCEPHALIC AMNESIA: NONRECURRING-ITEMS DELAYED NONMATCHING-TO-SAMPLE. **D.G. Mumby* and J.P.J. Pinel. Dept. of Psychology, University of British Columbia, Vancouver, B.C., Canada V6T 1Y7.**

Medial-diencephalic amnesia has been modelled in monkeys using the nonrecurring-items delayed nonmatching-to-sample (DNMS) paradigm; monkeys with mediodorsal thalamic lesions perform poorly on this task. We tested rats with mediodorsal thalamic lesions on a new version of DNMS that was designed to mimic the monkey DNMS task. Lesioned rats were impaired whether they were trained pre- or post-operatively. Lesioned rats trained postoperatively took longer to reach a criterion of 17/20 correct trials on two consecutive sessions than did sham-operated rats; pretrained rats receiving mediodorsal thalamic lesions took longer to re-attain this criterion following surgery than did sham-operated rats. Lesioned rats scored significantly lower than sham-operated rats with delays of 4 s, 15 s, 60 s, 120 s, and 300 s. Intact rats and sham-operated rats performed at levels comparable to those commonly reported for monkeys. These results suggest that there is considerable continuity among humans, monkeys, and rats in the neural basis of recognition memory, and in so doing, they illustrate the potential of the rat model in its investigation.

249.11

RATS WITH HIPPOCAMPAL REMOVALS CAN LEARN SIMPLE, CONDITIONAL AND TACTILE DISCRIMINATIONS USING ODOR AND TACTILE CUES. I. Q. Whishaw and J. Tomie*. Dept. Psychology, Univ. of Lethbridge, Lethbridge, Alberta, Canada T1K 3M4

A number of theories suggest that the hippocampus has a selective involvement in learning and memory such that it is not involved in simple stimulus-response learning but is involved in acquiring conditional and configural tasks. We examined the effects of hippocampal removal on acquisition and retention of simple, conditional and configural tasks in a series of novel tasks using tactile and olfactory cues. The hippocampus proper was removed using the neurotoxins kainic acid and colchicine. Hippocampal damage had minimal effects on retention and acquisition of the three kinds of problems. These results suggest that whereas the hippocampus may have a selective role in certain forms of learning, this involvement may be restricted to only certain sensory domains or conjunctions. Alternatively, the hippocampus may control a class of movements that are required for the solution of certain kinds of problems irrespective of their stimulus conjunctions.

249.12

TASTE AVOIDANCE, BUT NOT AVERSION, LEARNING IN RATS LACKING TASTE CORTEX. M.R. Orr* and S.W. Kiefer. Department of Psychology, Kansas State University, Manhattan, KS 66506.

Rats with ablations of the gustatory cortical area (GC) were compared to control rats in the acquisition of a conditioned taste aversion to either sucrose (Exp. 1) or sodium chloride (Exp. 2). Following aversion training, the rats were tested for taste reactivity to determine the palatability of the conditional taste stimulus in a test not involving consumption. In Experiment 1, control rats rapidly learned to avoid sucrose but showed only weak negative reactivity (e.g., gapes). The GC rats reduced sucrose consumption but the aversions were weaker than those found in control rats. The GC rats also failed to display any negative reactivity. In Experiment 2, control rats displayed rapid aversion learning to the sodium chloride solution. These rats also displayed strong aversive reactivity to the sodium chloride solution. GC rats also learned to avoid the sodium chloride solution (six of eight rats reached a learning criterion of 0.0 ml consumed) but did not show any negative reactivity to the taste. The results demonstrate that GC rats are capable of learning to avoid consumption of a taste but that the conditioning does not entail a hedonic or palatability shift of the conditional stimulus as it does in control rats.

249.13

PERFORMANCE ON A SPATIAL TASK FOLLOWING LESIONS TO THE REGION OF THE MAMMILLARY BODIES. V. Sziklas and M. Petrides. Dept. of Psychology, McGill University, Montreal, Quebec, Canada H3A 1B1.

In the present study, rats were first trained on an eight-arm radial maze. They were subsequently given lesions confined primarily within the area of the mammillary bodies and the supramammillary nucleus(MB-SM), the hippocampus (H), or a control operation (OC). Postoperatively, the animals were tested on this task under two conditions. In the first condition, the rats were not confined to the center platform between choices; in the second, they were confined for a 15 sec period before being allowed to make a choice. Rats with lesions to the H were impaired in both conditions in comparison with the OC animals, whereas those with lesions to the MB-SM were not.

These findings indicate that restricted lesions within the MB-SM region may not be sufficient to impair performance on the radial maze under the conditions tested. In an earlier study (Saravis et al., Europ J Neurosci, 1990, in press), extensive lesions within the region of the mammillary bodies that damaged not only the MB-SM area but invaded adjacent nuclei impaired severely performance on this task.

250.1

BASOLATERAL AMYGDALA LESIONS DISRUPT TASTE-POTENTIATED ODOR AVERSION LEARNING WITHOUT AFFECTING SIMPLE ODOR DISCRIMINATION LEARNING. T. Hatfield[1], M. Henegar*[2], P. Graham*[1], and M. Gallagher[1]. [1]Department of Psychology, [2]School of Medicine, The University of North Carolina, Chapel Hill, NC 27599

The role of the basolateral amygdala (ABL) in taste-potentiated odor aversion learning was studied in rats. Simple taste aversion learning readily occurs when a novel taste is paired with illness: this learning can withstand long delays between the taste (CS) and illness (US). In contrast, it is difficult to establish a conditioned aversion with odor/delayed illness pairings. However, aversion to an odor can be established at long CS-US delays by presenting the odor in compound with a taste during training: subsequent testing with the odor alone then reveals a robust aversion. Rats with ABL neurotoxic lesions induced by N-methyl-D-aspartate were significantly impaired in acquiring taste-potentiated odor aversion learning. At the same time, these lesions had no effect on the acquisition of a simple taste aversion. A second experiment was conducted to assess whether ABL damage merely impaired olfactory processing. A within-subject design was used to examine taste/odor potentiation and simple odor discrimination learning with a counterbalanced set of three olfactory cues. Animals with ABL lesions that were significantly impaired in acquisition of the taste-potentiated odor aversion in this experiment were unimpaired in odor discrimination learning. Thus animals with ABL damage can learn a simple association between either a taste or an odor that is paired with an effective US, but have a selective deficit for taste/odor potentiation learning.
Supported by a Ford Foundation Predoctoral Fellowship to TH, NIMH grant MH35554 and a NIMH RSDA (KO2-MH00406) to MG.

250.2

EFFECTS OF FORNIX LESIONS ON RAT'S PERFORMANCE OF A NONSPATIAL CONTINUOUS NONMATCHING TO SAMPLE WORKING MEMORY TASK. M.J. Pontecorvo, D.B. Clissold, R.M. Hudson* and D.S. Olton. NOVA Pharmaceutical Corp., Baltimore, MD 21224 and Johns Hopkins University, Baltimore, MD 21218.

The present study examined the effects of fornix lesions on rat's performance of an operant, nonspatial working memory task. Rats had previously been trained (Pontecorvo and Clissold, Neurosci Abstr, 14:101.2) to remember across an intertrial interval (2.5, 10, 20 sec) which of two possible stimuli was presented on the previous trial. The rats were reinforced for responding on one lever if the present trial stimulus matched the previous stimulus and reinforced for a response on a second lever if the present stimulus differed from the previous. Performance of rats receiving Sham lesions (n=4), was contrasted with that of rats receiving bilateral radio frequency lesions producing (histologically determined) Complete (n=4) or Partial (n=5) destruction of the fornix. Rats with Complete lesions showed a significant, but transient, delay dependent reduction in choice accuracy (e.g., during post-operative week 1, A' at the 2.5, 5 and 10 sec intervals was .98, .93 and .91 for the Sham group vs .97, .95 and .89 for the Partial group and .93, .90, and .82 for the Complete group). By post-operative week 3, no differences in choice accuracy could be observed. Complete lesions also produced an increase in the probability of both trial and intertrial responding that persisted throughout the duration of the study. It remains to be determined whether the relatively modest accuracy reducing effects of the fornix lesions in the present study should attributed to the substantial training these rats received prior to the lesion, or to a relatively limited involvement of the septal-hippocampal system in performance of this task.

250.3

PATH ANALYSIS OF LONG-TERM HABITUATION EFFECTS ON THE AUDITORY SYSTEM. A.R. McIntosh and F. Gonzalez-Lima. Dept. Med. Anatomy, Texas A&M Univ., College Station, TX 77843.

Techniques for mapping brain metabolic activity hold some important advantages over traditional neuroscience methods. For example, 2-deoxyglucose autoradiography reveals the activity of the whole rat brain, allowing for simultaneous examination of changes in entire neural systems. In spite of this, however, an analytic method which enables data to be quantified from a systems approach is lacking. The purpose of this study was to use path analysis to model the changes in auditory system 2-DG uptake in relation to long and short-term habituation of the acoustic startle reflex (Gonzalez-Lima et al, Brain Res., 489:67, 1989). Path analysis is a method to assess the relative strengths of directional links between elements in a system. The models revealed changes in the flow of information from the lemniscal to the extra-lemniscal system as a function of habituation. Probable sites in the system where modifications to the auditory processing took place were the superior olivary complex and the inferior colliculus. This analytic approach may prove to be a valuable tool in the exploration of the mechanisms through which functional activity in neural systems is altered during sensory processing, learning, and memory. Supported by NIMH grant R01 MH43353.

250.4

CONFIGURAL ASSOCIATIONS OF AUDITORY STIMULI ARE NOT IMPAIRED BY FIMBRIA-FORNIX LESIONS. A. Baker, R. Wax*, and M.L. Shapiro, Department of Psychology, McGill University, Montreal, Quebec H3A 1B1.

Configural association (CA) theory predicts that the hippocampal system is needed when animals must remember relationships among stimuli (Sutherland & Rudy, 1988, *Psychobiology 17*:157). Negative patterning tasks exemplify CA learning, and are impaired by hippocampal lesions. Thus, a tone-light (TL) CA task that requires rats to press a bar for food when either a light or a tone was presented alone, but not to press the bar if the light and tone were presented simultaneously, is impaired by hippocampal lesions. To test the generality of CA theory, the TL task was compared to a tone-click (TC) task that substituted a clicking stimulus for the light but otherwise was identical to the TL task, and therefore was operationally a CA task. Discrimination performance was measured as the difference (d) between the number of bar presses to the compound stimulus and the mean of the single stimulus elements. Ten male S.D. rats were trained in the TL task and 10 rats were trained in the TC tasks. After 30 training sessions, the rats discriminated between the simple and compound stimuli in both the TL (d=17) and TC (d=15) tasks. Half of the rats in each group were then given bilateral fimbria-fornix (FF) lesions. After surgery, normal rats performed as well as they had pre-operatively on both tasks (TL d=22; TC d=18). FF lesions impaired performance in the TL task relative to the normal rats for the 2 week post-operative testing period (d=8). However, rats with FF lesions performed the TC task normally (d=17). Thus, hippocampal lesions do not impair all negative patterning tasks, and CA theory must be amended to predict correctly the types of associations that require the hippocampal system.

250.5

GLUTAMATE IS PRESENT IN PRESYNAPTIC TERMINALS OF THALAMO-AMYGDALA PROJECTIONS. C.R. Farb and J.E. LeDoux, Center for Neural Science, New York University, New York, NY 10003.

Projections from the acoustic thalamus to the lateral amygdala (AL) are involved in the transformation of acoustic stimuli into emotional memories. This thalamo-amygdala pathway is believed to be excitatory and may use glutamate (Glu) as a neurotransmitter. Previous ultrastructural studies demonstrated that projections arising in the acoustic thalamus and terminating in AL form predominately asymmetric or excitatory contacts with spines or small dendrites. While similar profiles are seen for Glu-containing terminals in AL, the origin of these terminals is not known. Therefore, we have attempted to determine, using anterograde transport of WGA-HRP and Glu immunocytochemistry, whether Glu is contained in AL terminals that arise from cell bodies in the acoustic thalamus. Anterograde transport of WGA-HRP from the acoustic thalamus to AL was visualized using the tetramethylbenzidine reaction. The reaction was stabilized with diaminobenzidine and cobalt acetate. The presence of Glu was determined using an antibody against hemocyanin-conjugated Glu, IgG linked to colloidal gold, and subsequent silver enhancement. Electron microscopic analysis demonstrated that WGA-HRP was seen predominately in axon terminals. Glu-like immunoreactivity (Glu-LI) was seen in perikarya, dendrites, axons and axon terminals. Although the majority of labeled terminals contained either WGA-HRP or Glu-LI, some terminals contained both HRP and Glu-LI. These findings demonstrate that thalamo-amygdala terminals contain glutamate and suggest that glutamate may be a neurotransmitter in this pathway. Supported by MH38774.

250.6

AMYGDALA LESIONS DISRUPT EXPLICITLY CUED AND CONTEXTUAL FEAR CONDITIONING. R.G. Phillips, L. Romanski, J.E. LeDoux, Center for Neural Science, New York University, New York, NY 10003.

Rats "freeze" when exposed to a tone previously paired with footshock (explicitly cued fear conditioning) or when placed in an apparatus where shock was administered (contextual fear conditioning). A recent report suggests that explicitly cued but not contextual fear conditioning is dependent upon the amygdala. However, conditioning to explicit and contextual cues was evaluated using different test procedures. In the present study, we used a paradigm whereby the rate of acquisition of conditioned fear responses to explicit and contextual cues could be monitored using a single procedure. Rats were given blocks of 2 conditioning trials, during which a tone (800 Hz, 80 dB, 20 sec) was paired with footshock (0.6 mA, 0.5 sec), on three consecutive days. Freezing during the 20 sec preceding the first tone on day 2 and day 3 was used as a measure of contextual conditioning and freezing during the first tone on day 2 and 3 was used as a measure of explicitly cued conditioning. On day 2, the rats did not freeze before the onset of the tone but did freeze during the tone. On day 3, freezing was observed both before and during the tone. Electrolytic lesions aimed for the amygdala (histology pending) reduced freezing during the tone (days 2 and 3) and during the pre-tone period (day 3) in 3 of 4 rats. Thus, explicitly cued conditioned fear responses are acquired faster than contextually conditioned responses and amygdala lesions interfere with both. Supported by MH38774.

250.7

UNCONDITIONED STIMULUS TRANSMISSION TO THE AMYGDALA. L.M. Romanski, M.C. Clugnet, F. Bordi, J.E. LeDoux, Center for Neural Science, New York University, New York, NY 10003.

Auditory fear conditioning in the rat is mediated by projections from the medial geniculate body (MGB) and adjacent areas of the posterior thalamus to the lateral nucleus of the amygdala (AL). This area of the posterior thalamus receives both acoustic and somatosensory inputs. Thus, during fear conditioning both conditioned stimulus (CS) and unconditioned stimulus (US) information may be transmitted to the amygdala from the posterior thalamus. To examine this issue, three studies were performed. First, unit discharges were recorded in AL during electrical stimulation of the posterior thalamus or during electrical stimulation of the contralateral hindpaw in rats anesthetized with chloral hydrate. Many units responded to thalamic stimulation. Response latencies varied between 4 and 12 ms. Many but not all of the identified units also responded to hindpaw stimulation. Response latencies to hindpaw stimulation varied between 12 and 25 ms. Second, in some studies, after identifying units in AL that responded to hindpaw stimulation the MGB was lesioned. Hindpaw stimulation no longer evoked unit responses in AL. Third, the MGB was lesioned unilaterally and after 1 week responses to hindpaw stimulation were tested. Responses were not found in the AL on the side of the MGB lesion but were found in the AL on the side of the intact MGB. These data suggest that during fear conditioning both the auditory CS and the somatic US may be transmitted to the AL by way of the MGB and/or adjacent areas of the posterior thalamus. Supported by MH38774.

250.8

IN THE RAT, RF LESIONS OF THALAMUS AND FORNIX PRODUCE DIFFERENT PATTERNS OF IMPAIRMENT ON A DELAYED NON-MATCHING TO SAMPLE (DNMTS) TASK. R.G. Mair, D.M. Lacourse*, S. Koger, and G.D. Fox*. Dept. Psychol., Univ. New Hampshire, Durham, NH 03824.

Eighty Long-Evans rats were given extensive pretraining (2400 to 3700 trials) on a DNMTS task. In Exp I, 50 rats were given one of 5 treatments: RF lesions of midline thalamus (MT), bilateral (BIL) thalamus at 1 mm from midline, mammillary bodies (MB), combined MT-MB, or sham surgery. The BIL group was substantially impaired, while MT, MB, and MT-MB groups performed normally at all delays. In Exp II, 16 rats were given precommissural fornix (PF) lesions and compared to 8 sham controls. The PF impairment varied with delay, being largest at moderate delays (9-12s). The BIL deficit was more severe than PF and was apparent at short delays.

250.9

DEPLETION OF CORTICAL NE IN RATS BY 6-OHDA PRODUCES MINIMAL IMPAIRMENT ON A PRETRAINED DELAYED NON-MATCHING TO SAMPLE (DNMTS) TASK. S. Koger, G.D. Fox*, D.M. Lacourse*, and R.G. Mair. Dept. Psychol., Univ. New Hampshire, Durham, NH 03824

Following pretraining on a spatial DNMTS task, rats received injections of either 6-OHDA (N=11) or vehicle (N=5) aimed at the dorsal NE bundle. Both groups showed a comparable and significant decay in performance as delays were increased from 0 to 15 sec. Subsequent extended training at delays of 3, 6, & 15 sec (500 trials each) demonstrated a trend towards impairment in NE depleted rats at 6 sec, but not 3 or 15 sec. 6-OHDA lesioned animals also tended to respond more slowly than controls at all delays. The effects of NE depletion on this task are considerably less severe than those produced by RF lesions of thalamus (cf Mair, et al, this meeting).

250.10

DOUBLE DISSOCIATION OF ITEM AND ORDER RECOGNITION MEMORY FOLLOWING LESIONS OF THE HORIZONTAL NUCLEUS OF THE DIAGONAL BAND OF BROCA AND THE NUCLEUS BASALIS MAGNOCELLULARIS. D.L. Johnson and R.P. Kesner. Dept. of Psychology, Univ. of Utah, Salt Lake City, Utah 84112.

Rats were trained on an 8-arm radial maze on either a spatial item or spatial order recognition task. In the spatial item recognition task, on every trial animals were allowed to visit 5 new arms followed by a test between an arm visited on that day and an unvisited arm. A win-stay rule was required to receive additional reinforcement. In the spatial order recognition task, the animals visited 8 locations followed by tests between 1-2, 4-5, or 7-8 choice orders. In order to receive additional reward, the animal had to choose the arm that occured earlier in the sequence.

After the animals displayed better than chance performance, they received ibotenic acid lesions of the horizontal nucleus of the diagonal band of Broca (HNDB) or the nucleus basalis magnocellularis (NBM).

Following HNDB lesions, performance on spatial order recognition remained intact, but performance on spatial, item recognition fell to chance levels. NBM lesions produced a deficit in spatial order recognition, but had no effect on spatial item recognition. Thus, there appears to be a double dissociation between HNDB and the NBM, implying a clear functional dissociation among components of the basal forebrain.

250.11

EFFECTS OF X-IRRADIATION-INDUCED HIPPOCAMPAL GRANULE-CELL HYPOPLASIA ON MEMORY-BASED LEARNING AND LEARNED PERSISTENCE IN THE INFANT RAT. J.L. Diaz-Granados*, P.L. Greene, T.J. Schallert, & A. Amsel. Dept. of Psychology & Inst. for Neuroscience, University of Texas, Austin, TX 78712.

Electrolytic hippocampal insult in infant rats disrupts the acquisition of patterned alternation (PA; Lobaugh et al., Behav. Neurosci., 103:1159, 1989), a kind of memory-based learning and the partial reinforcement extinction effect (PREE; Lobaugh et al., Behav. Neurosci., 99:46, 1985), an indicant of learned persistence. Similar results have been shown with exposure to fetal and/or postnatal ethanol (Wigal & Amsel, Behav. Neurosci., 104:116, 1990).

We examined the effects of x-irradiation-induced hippocampal insult in rat pups on both PA and the PREE. Neonatal rats were exposed to varying doses of x-irradiation from postnatal day (P) 2 to P15, resulting in differential agenesis in postnatally arising granule cells of the hippocampal dentate gyrus. Controls were sham-irradiated. Rat pups were tested at P16-17 for PA and at P20-21 for the PREE. X-irradiated subjects showed behavioral deficits on both schedules, PA and the PREE. Also, the high-irradiation groups displayed greater running speeds in both effects. Correlations between cell loss and behavior were significant. These results are further evidence that the appearance of learned behavioral effects such as these is related to the development of the hippocampus. Supported by NSF grant BNS-8609877.

250.12

CONDITIONED AND UNCONDITIONED EFFECTS OF VARYING NOISE SPECTRAL FREQUENCIES ON THE ACOUSTIC STARTLE REFLEX IN RATS. S. Campeau and M. Davis. Dept. of Psychology and Psychiatry, Yale Univ. Sch. of Med., 34 Park St., New Haven, Ct 06508.

The intensity and spectral frequency of background noise have marked effects on the amplitude of the acoustic startle reflex. Low frequency noise elevates, whereas high frequency noise depresses, the amplitude of the startle reflex (Gerrard & Ison, JEP:ABP, 16:138, 1990). Based on these unconditioned effects of background noise on acoustic startle, it was important to examine how effective different auditory frequency bandwidths would be in the expression of fear as measured by the potentiated startle paradigm (enhancement of startle amplitude in the presence vs the absence of a stimulus previously paired with footshock). This was especially interesting because pure tone auditory stimuli often lead to unreliable potentiation of acoustic startle.

Rats (10 per group) were given 20 paired presentations of a 3.2 sec, 70 dB, 0-1, 1-10, 10-20, or 0-20 kHz noise coterminating with a 0.5 sec, 0.6 mA footshock. Additional groups were given the same number of stimuli in a specifically unpaired procedure. All animals were tested 2 days later with 100 startle-eliciting noise-bursts in the presence or absence of the auditory conditioned stimulus (CS). The 0-1 and 1-10 kHz stimuli produced 100 % startle potentiation in virtually every animal. The 0-20 and 10-20 kHz stimuli produced less than 40 % potentiation. No startle amplitude changes were obtained in unpaired or untrained groups.

These results suggest that the expression of fear-potentiated startle using an auditory CS interacts with the unconditioned effect of auditory stimuli on the acoustic startle reflex. Experiments investigating the role of the medial geniculate nucleus in the mediation of potentiated startle using an auditory CS are under way (Ledoux et al., Neurosci., 17:615, 1986).

250.13

WITHDRAWN

250.14

DIFFERENCES IN SPATIAL LEARNING AND MEMORY ABILITIES FOLLOWING MEDIODORSAL VERSUS POSTERIOR THALAMIC LESIONS IN RAT: S. W. Henderson* and P.J. Langlais. Dept. of Psychology, SDSU and Research Svc., VAMC, San Diego, CA.

Rats recovered from acute pyrithiamine induced thiamine deficiency (PTD) have cognitive and memory deficits and lesions within the mediodorsal (MD) nucleus, the intralaminar (IL) nuclei and the posterior nuclear group (Po) of the thalamus. To determine the behavioral importance of these thalamic lesions, spatial learning and memory were examined following discrete lesions of MD or Po while sparing IL.

Male Long-Evans rats with bilateral electrolytic lesions confined to MD (n=10) or Po (n=14) were studied. Three weeks following surgery, the two lesion groups and a sham-operated control group (CT, n=11) were trained on a food reinforced nonmatching-to-sample T-Maze task. The initial performance (% correct) of the MD group was significantly lower than both the CT and Po groups. By the 4th day of training all groups were performing > 90% correct. Subsequent testing at inter-run delays of 60-600 seconds demonstrated no significant group differences in performance. These data suggest that: i) subtotal destruction of MD but not of Po results in an initial spatial learning impairment; ii) lesions restricted to MD or Po do not impair delay-sensitive spatial working memory; and iii) damage of IL nuclei and/or fibers coursing in this region are responsible for memory deficits observed in recovered PTD rats. (Supported by VA Medical Research and SDSU Funds).

NEUROTOXICITY: PNS AND RETINA

251.1

ACETYLCHOLINESTERASE ANTIBODIES CAUSE PREGANGLIONIC IMMUNOSYMPATHECTOMY. S. Brimijoin, V. A. Lennon, and P. Hammond. Depts of Pharmacology and Immunology, Mayo Clinic, Rochester MN 55905.

Systemic administration of monoclonal antibodies to neural acetylcholinesterase (AChE) caused selective and permanent, complement-mediated destruction of presynaptic sympathetic fibers in adult rats. Ptosis, hypotension, bradycardia, and postural syncope appeared within hours and lasted for months after a single intravenous injection. In the superior cervical ganglion, AChE activity disappeared from the neuropil but not from the nerve cell bodies; choline acetyltransferase activity (ChAT) and ultrastructurally defined synapses were also lost. Similar effects occurred in stellate, thoracic, lumbar, and coeliac ganglia, and in the adrenal medulla. Electrical stimulation of preganglionic sympathetic fibers failed to evoke end-organ responses, but direct ganglionic stimulation was effective. Parasympathetic function was preserved. Motor performance and the ChAT content of muscle also remained normal. This unique model of cholinergic autoimmunity represents a new tool for autonomic physiology. (Supported by grants NS 18170 and NS 15057).

251.2

BOTULINUM NEUROTOXIN TYPE A AT pH 5.0 BREAKS DOWN AT ABOUT THE MID-POINT OF HEAVY CHAIN. B. R. DasGupta and W. Tepp*. Food Research Institute, University of Wisconsin, Madison, WI 53706.

The 150 kDa botulinum neurotoxin (NT) binds to receptors on the cholinergic presynaptic membrane; the C-terminal half the 100 kDa H chain is presumed to have the receptor binding site, endocytosis brings the NT into the closed vesicles within the nerve cells. After the endocytic vesicles become acidified, the N-terminal segment of the H chain is thought to form channels (FEBS Lett. 226, 115) that allow a portion of the NT to pass through the membrane into the cytoplasm. The 50 kDa L chain on reaching the cytosol inhibits neurotransmitter release (J. Biol. Chem. 264, 10354). The L chain could enter the cytosol by itself or with a segment of the H chain following reduction of a -S-S- that links the L and H chains or a peptide cleavage of the H chain away from its N-terminus. We find that the dichain type A NT exposed to pH 4.2 or 5.0, 0.05 M ammonium acetate, 8°-25°C begins to break down within 5 min yielding N100 kDa fragment(s) i.e. L chain-S-S-N-terminal half of the H chain, and a N40 kD major band. The N-terminal residues of the N40 kDa fragment, Pro-Ile-Asp-(Trp/Lys)-Asn-Gln-Ile-Gln-Leu-Phe-Asn- align with the receptor binding segment of tetanus NT beginning at its residue #915 (Pro). The five underlined residues identical to tetanus (EMBO J. 5, 2495) show 45% homology. Funded by NIH, NS17742 and NS24545.

251.3

BOTULINUM NEUROTOXIN TYPES A AND E CLEAVED WITH PEPSIN GENERATES ~45 kDa C-TERMINAL FRAGMENTS. J. A. Gimenez*, B. R. DasGupta and A. W. Clark. Food Res. Inst. & Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

Botulinum neurotoxins (NT) are proteins composed of ~50 kDa L and ~100 kDa H chains, that correspond to the N- and C-terminal segments of the parent ~150 kDa single chain. The NT binds via H chain to the receptors on the presynaptic membrane at the neuromuscular junctions; the L chain inhibits release of the neurotransmitter after its entry into the secretory cells. The N-terminal half of the H chain forms channels in lipid bilayer membrane and is believed to form channels on the neuronal cell membranes for the translocation of the L chain. The ~50 kDa C-terminus is presumed by analology with tetanus NT to contain the receptor binding site. We have digested the antigenically distinct type A and E NTs with pepsin (50-100:1 w/w, pH 4.2 and 6, 25°C) and isolated the C-fragments in one chromatographic step. The fragment from type A, although single band by SDS-PAGE, is heterogenous; the major sequence beginning with X.E.Y.I.K.N.I- aligns with residue #874 (Glu) of tetanus NT (EMBO J. 5, 2495). The first 48 residues of the pure C-fragment from type E NT, F.K.R.I.K.S.S.S.V.L.N. M.R.Y. K.N.D.K.Y.V.D.T.S.G.Y.D.S.N.I.N.I.N.G.D.V.Y.K.Y.P.T.N.K.N. Q.F.G.I.Y-, align with tetanus NT beginning at residue #877 (Asp). The 12 underlined residues identical to tetanus show 25% homology. Funded by NIH (NS17742 and NS24545).

251.4

ACRYLAMIDE AND 2,5-HEXANEDIONE INHIBIT GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE IN SYNAPTOSOMES. M. I. Sabri. Oregon Health Sciences University, Portland, OR 97201

Repeated exposure of rats to the neurotoxins acrylamide (AC) or 2,5-hexanedione (HD) results in blockade of fast axonal transport, distal axonal degeneration and hindlimb paralysis. The biochemical mechanism underlying axonal degeneration is not understood. Speculating that blockade of fast axonal transport results due to reduction in chemical energy (ATP), early investigations examined the effects of the neurotoxins on glyceraldehyde-3-phosphate dehydrogenase (GAPDH), an enzyme on which fast axonal transport is known to depend. Since GAPDH was studied in nerve tissue homogenates containing a heterogeneous population of cells, the sensitivity of this enzyme in the neuronal (axonal) compartment could not be selectively determined. The present study determines the effect of AC and HD on GAPDH activity and ATP levels in highly purified synaptosomes from rat forebrain. Purity of synaptosomes was assessed by enzyme markers and electron microscopy. Synaptosomes synthesized ATP (1.15 ± 0.16 nmol/mg protein) from glucose and iodoacetate (2.5-5.0 mM), a powerful inhibitor of GAPDH, blocked ATP synthesis by over 80%. Incubation of synaptosomes with AC or HD caused a concentration dependent inhibition of GAPDH; approximately 60% and 40% loss of GAPDH was observed after 2 h incubation with 10 mM AC and HD, respectively. The effect of AC and HD on ATP levels and fast and slow axonal transport in this model is under study. Supported in part by NS 19611.

251.5

CORRELATION OF ORGANELLE CONTENT IN SENSORY NERVE ENDINGS WITH CHANGES IN FAST AXONAL ORGANELLE TRANSPORT IN CHRONIC ETHANOL-FED RATS. J.A. McLane, J.A. McNulty*, M.B. Atkinson* and A.C. Breuer. Hines VA Hospital, Hines, IL 60141, Loyola Univ. Sch. of Med., Maywood, IL 60153, and Cleveland Clinic Foundation, Cleveland, OH 44195.

Exposure to neurotoxic substances often leads to a dying back peripheral neuropathy. Acrylamide, n-hexane, BPAU, and streptozotocin diabetes produce alterations in fast axonal transport (FAT) accompanying the dying back neuropathy. We have demonstrated the time-course of changes in FAT that result from chronic ethanol feeding (Soc. Neurosci. Abstr. 15:142, 1989). In order to examine the morphological effect that these changes in FAT have on peripheral nerves, we have examined the content of organelles in sensory nerve endings of muscle spindles from rats fed ethanol for 1 to 5 months. From electron micrographs (1) volume densities and (2) area densities of organelles, and (3) the density of "contacts" between nerve ending and myocyte were estimated and correlated with changes in FAT. An early period of cellular damage was characterized by a reduced retrograde FAT frequency and an increase in the concentration of organelles in the nerve terminals. A later compensatory phase was evidenced by an increase in the rate of retrograde FAT, a return of FAT frequencies to normal, and a return of organelle concentrations to normal. These findings suggest that the neurons are capable of altering both speed and frequency of organelle traffic to maintain an equilibrium organelle concentration in the nerve ending.

251.6

CONSEQUENCES OF CHRONIC LOW-DOSE EXPOSURE TO THE CARBAMATE PESTICIDE ALDICARB FOR RAT TRIGEMINAL VIBRISSA AFFERENTS. B.G. Klein, W.C. McCain*, L. Engr*, M. Ehrich*, & M. Farage-Elawar*. Dept. of Biomed. Sci., VA-MD Reg. Col. Vet. Med., Virginia Tech, Blacksburg, VA 24061.

The literature regarding effects of chronic low-dose exposure to carbamate pesticides is limited & equivocal. To address this deficiency, we examined trigeminal (V) primary afferents innervating C1 vibrissa follicles, at the periphery or in the V ganglion, after chronic aldicarb exposure. Rats received daily intragastric doses of 0.2 mg/kg of aldicarb in a corn oil vehicle (treated) or corn oil vehicle alone (control). After 100-106 days, 7 treated & 8 control rats received an injection of the fluorescent tracer true blue (TB) into the C1 vibrissa follicle. Following formalin perfusion, 25 um V ganglion sections were cut horizontally in a cryostat. After 110-114 days, 7 treated & 7 control rats were perfused with paraformaldehyde-glutaraldehyde. C1 vibrissa follicles were dehydrated, osmicated, embedded in plastic & cut at 1 um. Labelled ganglion cell profiles were counted at 250X under episcopic fluorescence & myelinated vibrissal nerve axons were counted at 1250X under brightfield oil immersion. Comparing treated rats with controls, no significant change was observed in the number of myelinated C1 vibrissal nerve axons or in the number of ganglion cell profiles labelled by TB injection of C1 follicles. Support: VA Tech Creative Match Grant & Col. Vet. Med..

251.7

RAPID AXONAL TRANSPORT IN EXPERIMENTAL ETHYLENE OXIDE NEUROPATHY. H.Nagata*, K.Arasaki, N.Ohkoshi*, I.Kanazawa*, T.Nakanishi*, A.Ohnishi*(§) Dept. of Neurol., Inst. of Clinical Med., Univ. of Tsukuba, Tsukuba, Ibaraki 305, Japan. (§)Dept. of Neurol., Univ. of Occupational & Environmental Health, Kitakyusyu 807, Japan

Ethylene oxide (EO), widely used as sterilant gas, causes peripheral neuropathy in human due to chronic occupational exposure. To elucidate the pathogenesis of this neuropathy, we tested axonal transport velocity and amount of transported intrinsic enzyme in peripheral nerves using experimental model of this disorder. The rats (tests) were subjected to six-hour exposures of EO in a chamber at a concentration of 500 ppm three times a week for fifteen weeks. Rapid axonal transport and quantitative histological alterations of peripheral nerves were studied. After ^{35}S-methionine injection in the dorsal root ganglion, the velocity of rapid anterograde axonal transport of radioisotope labeled protein was measured and a 33% reduction of the velocity was detected in the neuropathic rats compared with control rats exposed to filtered room air ($p<0.01$). In separate experiment, sciatic nerve was ligated at middle thigh and after 3 hour interval, the nerve was dissected. Acetylcholine esterase (AChE) activity in proximal portion of the ligation was measured and calculated as accumulation rate per hour (transport amount). 18% of decrease in the accumulation was detected in test rats compared with controls. Thus, both of axonal transport velocity and amount were decreased in the neuropathic rats. On the other hand, in morphometric studies, histological changes were very mild. Therefore, a decrease of the velocity and transport amount of anterograde axonal transport under the mild histological abnormalities of the peripheral nerve may be playing a causative role in the development of distal axonal neuropathy due to chronic EO exposure.

251.8

USE OF A FUNCTIONAL OBSERVATIONAL BATTERY TO EVALUATE PYRIDOXINE-INDUCED ACUTE TOXIC SENSORY NEUROPATHY L.G. Shell*, J.B. Musser*, B.S. Jortner, K. Dyer* Virginia-Maryland Regional College of Veterinary Medicine, Virginia Tech, Blacksburg, VA 24061.

The Functional Observational Battery (FOB), a series of home-cage and open field observations and physiological and sensorimotor measurements, has been used to evaluate neurotoxicants in laboratory rats (Moser V.C., et al. Fund. Appl. Toxicol. 11:189, 1988). We used a modified FOB to assess pyridoxine-induced sensory neuropathy. Young adult male Sprague-Dawley rats were given 600 mg/kg pyridoxine hydrochloride ip twice daily for 4 days. The FOB was done prior to dosing (day -2) and 2 days after the last dose (day 6) in control (n=12) and treated(n=13) groups. No significant differences were found between the groups on day -2. Significant differences between the groups on day 6 included: weight loss (gms) 337.6 $\pm$ 12.4 vs. 227.6 $\pm$ 10 (mean $\pm$ SEM, control values given first); rearing (numbers) 9.8 $\pm$ 1.5 vs. 2.2 $\pm$ 0.5; seconds on rotorod 21 $\pm$ 3.0 vs. 2.5 $\pm$ 0.1; pelvic limb grip strength 0.52 $\pm$ 0.03 vs. 0.29 $\pm$ 0.03 (n=5 for treated group in this test). All treated rats had gait changes such as ataxia and dragging of pelvic limbs. Neuropathological studies in treated rats showed prominent neuronopathy in peripheral sensory ganglia, progressing to neuronal necrosis. This study demonstrates usefulness of the FOB to assess pyridoxine-induced sensory neuropathy.

251.9

NEUROPATHY ASSOCIATED WITH A NUCLEOSIDE INHIBITOR OF HIV: A RABBIT MODEL OF ddC TOXICITY. M.Litwak, T.Anderson*, C.Brosnan, A.Davidovich*, and J.Arezzo. Albert Einstein Coll. of Med., Bronx, N.Y. 10461 and Hoffmann La-Roche, Nutley, N.J. 07110

The nucleoside, 2',3'-dideoxycytidine (ddC) is a potent inhibitor of HIV. In clinical trials it has been associated with a dose-dependant peripheral neuropathy. Studies in Cynomolgus monkeys have reported electrophysiological changes but little underlying neuropathology. We studied 36 New Zealand white rabbits, a control group and 5 treated groups (10, 50, 100, 150, 250 mg/kg/day), for 17 weeks. Electrophysiological measures of both motor and sensory peripheral nerves and visual evoked potentials (VEP) were taken at 0, 7, 13, and 17 wks. The VEP showed no significant changes over the course of the study. At 13 wks, sural conduction velocity (CV) was delayed by 6.1, 12.1, and 17.3% in the survivors of the 100 (N=6), 150(N=4), and 250(N=3) mg/kg dose groups, respectively. The peroneal motor CV was unchanged. At 13 wks, the 150 and 250 groups, along with 2 randomly selected controls, were sacrificed. Distal and proximal segments of the sciatic nerve and spinal roots of the treated animals showed both axonal and myelin changes, (myelin splitting, intramyelinic edema, demyelination), Schwann cell proliferation, and increase in endoneurial matrix. No CNS pathology was evident. At 16 wks, 3 of the 100 mg/kg and 1 of the 50 mg/kg rabbits showed clinical signs including: forelimb paresis, hindlimb paralysis, and muscle fasciculations. At 17 wks the sural CV was significantly delayed ($p<0.01$) in the highest dose group remaining, 100 mg/kg(N=4). The peroneal CV was delayed by 19.5 ($p<0.01$) and 29.4% ($p<0.01$) in the 50 and 100 mg/kg groups, respectively. Amplitude changes were not significant. Histopathology in the 100 mg/kg group after 17 wks showed changes identical with those of the higher dose groups at 13 wks. These findings are consistent with ddC induced toxic polyneuropathy involving both sensory and motor fibers. At higher dose levels both distal and proximal nerve segments are affected with significant myelin pathology evident.

251.10

AIDS VIRUS COAT PROTEIN-INDUCED INCREASES IN $[Ca^{2+}]_i$ AND NEUROTOXICITY ARE NOT PREVENTED BY ANTI-CD4-ANTIBODIES IN RAT RETINAL GANGLION CELLS. Evan B. Dreyer, Peter K. Kaiser, Jeffrey T. Offermann, and Stuart A. Lipton. Dept. of Neurology, Children's Hosp. & Harvard Med. Sch., Boston, MA.

Picomolar concentrations of native or recombinant coat protein gp120 from the human immunodeficiency virus type 1 (HIV-1) strikingly increased $[Ca^{2+}]_i$ and subsequently injured rat retinal ganglion cell neurons in culture (Dreyer et al., *Science* 1990;248:364). As we reported last year (Kaiser et al. *Soc. Neurosci. Abstr.* 1989;15:143), both the increase in $[Ca^{2+}]_i$ and this form of neurotoxicity could be completely abrogated by anti-gp120 but not by control preimmune serum, suggesting that the lethal effects of the purified preparations of the envelope protein were due to gp120 and not to a contaminant.

In T lymphocytes, entry of HIV-1 is mediated by gp120 binding to a surface protein designated CD4. However, in the present study, two specific anti-CD4 antibodies, at concentrations known to block effects mediated by binding to CD4 on the surface of rat T cells, did not prevent the rise in $[Ca^{2+}]_i$ or the neuronal injury induced by gp120. These findings suggest that both the increase in $[Ca^{2+}]_i$ and the deleterious effects engendered by gp120 in neurons may not be mediated via binding to the CD4 molecule; an alternative mechanism must be sought.

251.11

FORMIC ACID INHIBITS CYTOCHROME OXIDASE ACTIVITY IN RETINA AND IN CULTURED RETINAL CELLS J.T. Eells, T.G. Murray*, C. Rajani* and J. M. Burke*. Dept. of Pharmacology and Toxicology and Dept. of Ophthalmology, Medical College of Wisconsin, Milwaukee, WI 53226.

Human methanol poisoning is characterized by formic acidemia, metabolic acidosis and visual toxicity. Formate, the toxic metabolite in methanol poisoning, has been hypothesized to disrupt visual function by inhibiting the mitochondrial enzyme, cytochrome oxidase (c.o.) in the retina or optic nerve. The effect of formate on c.o. activity was investigated in bovine eye cup preparations and in cultured cells. Eye cups were incubated (8 hrs) in the presence of formate (1-100 mM) and c.o. activity was determined in different regions of the retina. Similar treatment protocols were followed for cultured Muller glial cells, retinal pigmented epithelium (RPE) and dermal fibroblasts. Formate inhibited bovine retinal c.o. activity with the greatest degree of inhibition (34% and 58% reduction in c.o. by 10 mM and 100 mM formate, respectively) observed in the region of the retina most proximal to the optic disk . In cultured Muller glial cells formate produced time and concentration dependent alterations in cell morphology (rounding and narrowing of cellular processes) and an 80% reduction in c.o. activity. RPE cells were less sensitive to formate toxicity exhibiting no morphological alterations and only a 15% reduction in c.o. activity. Remarkably, there was no effect on cellular morphology or c.o. activity in cultured dermal fibroblasts. These data are indicative of regional differences in formate action in the intact retina and further suggest that the tissue specificity characteristic of methanol toxicity may occur at a cellular level.

251.12

STUDIES OF THE MECHANISM OF FOLATE RETINAL NEUROTOXICITY Z.H. Zhang, J.C. Blanks, and S.R. Snodgrass, Depts. of Neurology and Ophthalmology, USC School of Medicine and Neurology Research Laboratory, Childrens Hospital, Los Angeles, CA 90027

We previously reported histological damage to frog retina caused by folic acid (FA) and quisqualate (Soc Neurosci Abstr 15:480, 1989). To study the mechanism of this neurotoxicity, we incubated frog retinal eyecup preparations in control buffer, 3 mM FA, 3 mM pterin, 30 uM nimodipine (NM), and 3 mM FA/30 uM NM for 1 hr at RT. Pterin and FA produced similar lesions including cytoplasmic and nuclear changes in inner and outler nuclear layers and ganglion cell change. The Ca^{++} channel blocker NM did not prevent FA injury and caused some damage when used alone. We studied the effect of FA on 2-deoxyglucose accumulation by the frog retina, and found 45% increase in 2-DG accumulation after treatment with 3 mM FA ($p<.05$). This stimulation was blocked by ouabain but not by NM. These data are consistent with an excitant effect of FA. We also studied retinal proteins by SDS-PAGE after incubation with $^{32}P_i$. We observed decreased labeling of a small protein (Mr 12 Kd) in retinas incubated with FA or FA + NM. This low molecular weight protein may contribute to FA membrane effects and neurotoxicity.

ALZHEIMER'S DISEASE: PHARMACOLOGY

252.1

CSF MONOAMINE AND NEUROPSYCHOLOGICAL CORRELATES IN PRESUMPTIVE ALZHEIMER AND RELATED DEMENTIA PATIENTS. S.G. Speciale, J. Trocewicz*, J. Hom*, M. Weiner* & R. Tintner*. Depts. of Psychiatry & Neurol., UT S.W. Med. Ctr., Dallas, TX 75235-9070.

A number of groups, including our NIA AD Research Ctr., are studying biochemical and psychological indices of Alzheimer's disease (AD) that might be diagnostic or track its progression. A neuropsychological battery included measures of reasoning, memory and motor skills. Cerebrospinal fluid (csf) was analyzed for norepinephrine (NE) and its metabolite, MHPG, dopamine and its metabolites, DOPAC and HVA, and the serotonin metabolite, 5HIAA, using HPLC-EC. Activities of two cholinergic enzymes, acetylcholinesterase (AChE) and butyrylcholinesterase, and protein concentrations were assayed, using spectrophotometric techniques.

Diagnostic classifications were made on the basis of interviews and testing by a neurologist, psychiatrist and neuropsychologist. At the end of the neurological examination, lumbar csf fractions were drawn (measures of the 15-20 ml fraction reported here). Several subgroups were made, the largest being AD/probable (N=46) and AD/possible (30). Other groups included multi-infarct (8), mixed (9) and other dementia (15), as well as several neurological, psychiatric and normal groups. Means were compared between subgroups and a Pearson correlation analysis carried out among variables. Normal and Parkinson groups had global ratings of 28, within the normal range, while dementia subgroups ranged from 50-72, classified as moderate to severe deficits. In general, 5HIAA was reduced in most subgroups, while MHPG, DOPAC and HVA values did not differ significantly. Both enzymes were lower in the dementia groups. Significant (<.01) correlations were found between: NE-MHPG, NE-AChE, DOPAC-5HIAA and AChE-trail making task. Supported by NIH AG-08013.

252.2

HIPPOCAMPAL EXCITATORY AMINO ACID RECEPTORS IN ALZHEIMER'S DISEASE: I. N-METHYL-D-ASPARTATE RECEPTOR COMPLEX. J. Ulas, L.C. Brunner, J.W. Geddes[1] and C.W. Cotman. Dept. Psychobiology and [1]Div. Neurosurgery, University of California, Irvine, CA 92717, USA.

The status of the N-methyl-D-aspartate receptor complex (NMDArc) in Alzheimer's disease (AD) is of great significance considering the suggestion that some of the cell loss observed in the hippocampus of AD brain may be mediated by NMDA receptors. In this study, receptor binding levels were assayed using *in vitro* autoradiography. Several regions of the hippocampus proper, dentate gyrus, and parahippocampal area were investigated in control (n=5) and AD brains (n=5) which were age and sex matched. The following components of the NMDArc were studied: 1) an NMDA-sensitive binding site ([^{3}H]-L-glutamate binding), 2) an allosteric regulatory site ([^{3}H]-glycine binding), and 3) a channel site ([^{3}H]-MK801 binding). The ratio of binding to agonist-preferring sites ([^{3}H]-L-glutamate binding sites) and antagonist-preferring sites ([^{3}H]-CPP binding sites) was also monitored. No statistically significant differences in the level of binding to any component of the NMDArc were detectable in the hippocampus proper, molecular layer of the dentate gyrus, and parahippocampal area when mean values of binding for control and AD brains were compared. However, some AD brains displayed pronounced changes in levels of binding. For example, in some AD individuals a dramatic loss of binding to every component of the NMDArc was found in the CA1 region, but only if profound (>50%) loss of neurons in this area was observed. An increase (10-60%) in the binding to the NMDArc was found only in the infragranular layer of the dentate gyrus. The data suggest that there is a correlation between pathology (cell loss) and decrease in binding to distinct sites of the NMDArc. The mechanism behind the elevation in the binding in the infragranular layer is unclear; it may reflect some adaptive changes of the NMDA receptor to diminished glutamatergic input.

252.3

HIPPOCAMPAL EXCITATORY AMINO ACID RECEPTORS IN ALZHEIMER'S DISEASE: II. NON-NMDA RECEPTORS. J.W. Geddes, L. C. Brunner, J. Ulas, and C.W. Cotman. *Div. Neurosurgery and Dept. Psychobiology, Univ. Calif., Irvine CA 92717*

There have been several conflicting reports regarding the status of excitatory amino acid (EAA) receptors in Alzheimer's disease. We have therefore evaluated in detail the status of the NMDA (Ulas et al.) and non-NMDA EAA receptors in AD, using *in vitro* autoradiography. Results to date have been obtained from five AD individuals and from five corresponding age-matched controls. For each experiment, an AD tissue sample was paired with a control sample. Binding densities were determined at both anterior and posterior levels of the hippocampal formation. Binding of [^{3}H] kainic acid (KA) and [^{3}H] amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) was evaluated using computer assisted image analysis (MCID, St. Catherines, Ontario). Corresponding sections were stained with cresyl violet, Bielschowskys silver stain, and Thioflavin S. The results obtained for KA were similar to those described previously. A 20% increase in the density of [^{3}H]KA binding was observed in the outer molecular layer of the dentate gyrus at both anterior and posterior levels of the hippocampal formation. A slight decrease in KA binding densities in CA1 was also observed, predominantly in posterior sections. Interestingly, added calcium, which in the rat blocks the high affinity KA binding sites, had little affect on the density of [^{3}H]KA binding in AD or control human tissues. With [^{3}H]AMPA, there was a marked increase (26%) in the binding densities in the infragranular zone of the dentate gyrus of AD patients, particularly in the anterior region. AMPA binding densities were decreased by an average of 24% in the CA1c region of the hippocampus, but otherwise were largely maintained. The losses in KA and AMPA receptor density appear to correlate with the extent of neuronal loss, whereas the increases in receptor density are thought to reflect neuronal plasticity.

252.4

U-80816: A NOVEL PARTIAL MUSCARINIC AGONIST. V.H. Sethy, J.W.Francis, R.R.Russell, R.J.Collins, R.F.Heier, and M.W.Moon. CNS Research, The Upjohn Company, Kalamazoo, MI 49001 USA.

RS 86 (RS) and oxotremorine (Oxt) have been investigated in humans for treatment of cognitive disorders with limited success because of the central and peripheral side effects. The narrow therapeutic index of these drugs may be due to their high intrinsic activity. Therefore, we attempted to synthesize a novel compound which binds to muscarinic receptors and has a low intrinsic activity as compared to RS and Oxt. U-80816 was one of the mostpromising compounds synthesized. The Ki's of U-80816, RS, and Oxt in (3H)-QNB binding assays were 72, 1600, and 130 nM, respectively, and in the (3H)-Oxt binding assays the corresponding values were 3.24, 15.6, and 0.54. U-80816 produced tremors with an ED50 of 2.5 mg/kg, and did not produce salivation or lacrimation. However, both RS and Oxt produced tremors, salivation, and lacrimation with ED50's in the range of 0.1 to 0.2 for the former and 1 to 2 mg/kg for the latter. U-80816 blocked Oxt-induced lacrimation. U-80816 has a significantly low intrinsic activity as compared to RS and Oxt for increasing striatal acetylcholine (Ach), for inhibiting hippocampal Ach release, and for increasing PI hydrolysis response. The results of our research with U-80816 suggest that this drug may have a desirable pharmacological profile for its use in human cognitive disorders.

252.5

THE DISTRIBUTION OF NICOTINIC CHOLINOCEPTIVE NEURONS IN THE FRONTAL CORTEX IN ALZHEIMER'S DISEASE. H. Schröder,[1,3] E. Giacobini,[1] R.G. Struble,[2] K. Zilles,[3] and A. Maelicke.[4] Depts. [1]Pharmacology and [2]Psychiatry, Southern Illinois Univ. Sch. Med., Springfield, IL 62794, [3]Dept. Anatomy, Univ. of Köln, F.R.G. and [4]Dept. Physiological Chemistry, Univ. of Mainz, F.R.G.

Studies of cortical nicotinic cholinergic receptor (nAChR) sites with radioligands show that their density is decreased in Alzheimer's disease (AD). Their cellular distribution, however, is not known. To elucidate their location, autopsy samples of human frontal cortex were incubated with the monoclonal antibody WF 6 (Fels et al., *J. Biol. Chem.*, 261:15746, 1986) directed to the α-subunit ligand binding site of the nAChR. Immunoprecipitate (IP) was shown at the light and electron microscopic level using immunoperoxidase in three groups of patients: (i) young controls [n=3; 55±5 yrs], (ii) aged controls [n=3; 73±6 yrs] and (iii) Alzheimer cases [n=6; 74±5 yrs]. Numbers of immunoreactive (ir) and cresyl-violet-stained (cv) neurons were counted in a standardized area. Layer II/III and V pyramidal neurons were preferentially labeled. Ultrastructurally, IP was distributed to postsynaptic densities. Neuron counts revealed statistically significant differences ($p<0.001$) between all groups [(i) 253±29 (mean±s.e.m.); (ii) 132±28; (iii) 28±6] for ir, but not for cv neurons. The findings point to a postsynaptic location of cortical nAChRs and a decrease of nAChR-expressing neurons in AD, not attributable to a simple cell loss. It remains to be assessed whether the reduction of receptor protein is due to cholinergic denervation or to a process intrinsic to cortical neurons. Supported by the Deutsche Forschungsgemeinschaft (Schr 283/6-1), Southern Ill. Univ. Central Res. Committee award, and R.J. Reynolds Tobacco Co.

252.6

HP 749: A PHARMACOLOGICAL PROFILE OF A THERAPEUTIC AGENT FOR ALZHEIMER'S DISEASE; I. M. Cornfeldt*, F. Wirtz-Brugger*, M. Szewczak*, R. Blitzer, V. Haroutunian, R. Effland*, J. Klein*, C. Smith. Depts. of Biological and Chemical Research, Hoechst-Roussel Pharmaceuticals, Inc., Somerville, N.J. 08876 and Mt. Sinai Medical Center, New York, N.Y. 10029.

HP 749 [N-(n-propyl)-N-(4-pyridinyl)-1H-indol-1-amine hydrochloride] was active in tests predictive of efficacy in Alzheimer's disease (AD). HP 749 reversed the amnesic effects induced by scopolamine in a passive avoidance paradigm in mice (0.02-0.63 mg/kg, s.c.) and enhanced memory retention in normal rats in a similar paradigm (0.02 mg/kg, s.c.). HP 749 enhanced the retention of passive avoidance learning in rats (0.04 and 0.08 mg/kg, s.c.) with combined cholinergic and noradrenergic deficits where physostigmine alone was not effective. HP 749 (1.25 mg/kg, i.p.) enhanced an electrophysiological correlate of cognitive processing (P300) in frontal rat brain regions. Wave VI of the brainstem auditory evoked response was increased by HP 749 (0.01-0.08 mg/kg, i.p.) and HP 749 reversed the depressant effects of hemicholinium (60 µg, icv) in this procedure. *In vitro* electrophysiological tests showed that HP 749 blocked the M-current, an effect which may enhance neuronal excitability, without effecting the I_{AHP} to the same degree. These results suggest that HP 749 should be efficacious in the treatment of AD particularly in those cases that are not responsive to cholinomimetic therapy alone.

252.7

HP 749, A POTENTIAL THERAPEUTIC AGENT FOR ALZHEIMER'S DISEASE: (II) NEUROCHEMICAL PROFILE. F.P. Huger, C.P. Smith, W.W. Petko*, P.G. Conway, R.C. Effland* and J.T. Klein*. Depts. of Biological and Chemical Research. Hoechst-Roussel Pharmaceuticals, Inc. Somerville, NJ 08876.

HP 749, [N-(n-propyl)-N-(4-pyridinyl)-1H-indol-1-amine hydrochloride], was active in several learning and memory tests, blocked the M-current in hippocampal slices and displayed adrenergic and cholinomimetic activities *in vitro* and *in vivo*. HP 749 inhibited biogenic amine uptake (NE ≅ DA > 5HT) and α_2-adrenergic receptor binding at sub-micromolar concentrations and increased electrically-stimulated [^{3}H]NE release from cortical slices at concentrations of 1 and 10 µM. This increase in stimulated [^{3}H]NE release is due to combined α_2-antagonist and uptake inhibitory properties of HP 749. At a concentration of 100 µM, HP 749 increased basal [^{3}H]NE release. HP 749 also increased brain NE turnover at doses of 10 and 30 mg/kg, but did not down-regulate β-adrenergic or $5HT_2$ receptors after chronic administration.

HP 749 inhibited the binding of various muscarinic ligands at low micromolar concentrations and stimulated phosphatidylinositol (PI) turnover in cortical slices at higher concentrations. The stimulation of PI turnover by HP 749 was apparently not receptor-mediated. HP 749 was not active as a cholinesterase inhibitor and did not increase the stimulated release of [^{3}H]ACh. The unique cholinomimetic properties of HP 749 may relate to its ion channel effects.

Evaluation of HP 749 in various *in vitro* radioligand binding assays revealed that it had negligible direct interactions with other types of neurotransmitter receptors.

252.8

HP 749, A POTENTIAL THERAPEUTIC AGENT FOR ALZHEIMER'S DISEASE: (III) PHARMACOKINETICS IN THE RAT, DOG AND MONKEY. R.S. Hsu*, J.W. Hubbard, S.M. Chesson*, E.M. DiLeo*, R.C. Effland* and J.T. Klein*. Departments of Chemical Research and Clinical Pharmacology. Hoechst-Roussel Pharmaceuticals, Inc. Somerville, NJ 08876.

HP 749 [N-(n-propyl)-N-(4-pyridinyl)-1H-indol-1-amine hydrochloride] has shown cholinergic and noradrenergic activities both *in vitro* and *in vivo*. Pharmacokinetic studies were performed in the rat (20 mg/kg), dog (10 mg/kg) and monkey (10 mg/kg). The plasma and brain samples were analyzed by an HPLC method using an analytical Hypersil phenyl column, and the concentrations of HP 749 and metabolites were quantitated by UV detection. Following oral administration, HP 749 was rapidly absorbed and metabolized, primarily to the N-despropyl metabolite (7480) in the tested animals. HP 749 reached maximal plasma levels (Cmax) of 3.2, 3.0 and 1.7 µg/ml at 0.25, 2 and 1 hr postdose for the rat, dog and monkey, respectively, then disappeared with an elimination half-life of about 2-4 hr. The bioavailability of HP 749, estimated by AUC, in the rat, dog and monkey was 9.0, 18.9 and 5.9 µg•hr/ml, respectively. The plasma concentration-time profiles of 7480 paralleled those of HP 749 in the respective species. The ratios (HP 749/7480) of Cmax are about 4.5, 2.5 and 6.5, and AUCs about 1.5, 1.5 and 2.0 for the rat, dog and monkey, respectively. In addition, the rat study showed HP 749 and 7480 penetrated extensively into the brain with kinetic profiles paralleling those in the plasma.

252.9

ACETYL-L-CARNITINE: A NOVEL APPROACH IN THE TREATMENT OF ALZHEIMER'S DISEASE

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G. Bartolomucci, Dept. Public Health, 2nd University of Rome, Italy.

Although the defect of the cholinergic neurotransmission still represents the major impairment of Alzheimer's disease, brain function and other systems of the neurochemical transmission are clearly involved in such disease as well. Moreover, a series of biological evidences suggest one consider AD as a systemic disease with a primary neurological expression. Therefore, a cholinomimetic therapy of AD should play only a pure symptomatic, although important, action leaving inadequate the impact on the progression of disease. Acetyl-1-carnitine (ALC) is a naturally occurring substance at the mitochondrial level, mainly involved in the B-oxidation of endogenous long-chain fatty acids. AlC shows cholinomimetic properties, (being structurally quite similar to Ach), together with a variety of metabolic actions able to restore in aging cells certain energetic processes. Interestingly,reviewing the overall data coming from 553 patients over 7 double-blind placebo controlled studies in AD patients, ALC demonstrated slowing down the progression of disease, modifying the course of the decay of neuropsychological (MMSE, BD) and clinical (CGI) profiles. Furthermore, the drugs was remarkably well tolerated and safe: 13.6% was the drop-out figure of ALC-treated patients, but only 12 of 272 subjects withdrew for possible adverse drug reactions, (mainly agitation), side effects involved less than 5% of the ALC treated patients.

252.10

SEROTONIN BINDING IN THE HUMAN PINEAL GLAND: AGE AND AD RELATED ALTERATIONS. D.L. Sparks and T. Landers* Sanders-Brown Center on Aging, Dept. of Pathology and the Medical Examiner's Program, Univ. of KY Medical Center, Lexington, KY 40536-0230.

It is well known that noradrenergic binding in the human pineal gland mediates melatonin production, and a recent report indicates that noradrenergic binding is altered in the pineal gland of AD patients (Jengeleski et al. Brain Res,481:378,1989). This laboratory has previously reported the initial finding of serotonin binding in the human pineal gland, and a reduction of that binding in the gland of some subjects that committed suicide (Sparks and Little, Psychiatr Res, in press). In that report, preliminary data indicates a lack of age related change in total serotonin binding (S-1) in the gland and the absence of the S-1a subtype.

In the present study we have investigated total serotonin binding, and its displacement by N-Acetyl-serotonin and the S-1a receptor inhibitor, 8-OH-DPAT, in the pineal gland of controls and AD subjects. Our non-heart disease controls were grouped according to their age at death as follows: 1) under 40 yr, N=26; 2) 40-65 yr, N=10; and 3) over 65 yr, N=6. The 9 AD subjects were all over 65 years of age.

There was no significant difference in the saturation isotherms of total S-1 binding among the control groups, and although not significant, the S-1 binding was reduced in the AD subjects. No S-1a type binding was found in the pineal gland in any subject group. N-Acetyl-5HT inhibited 5HT binding 30-35% in the two younger control groups, but was without effect in the older control group. In contrast, N-Acetyl-5HT inhibited 5HT binding 30% in the AD population.

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252.11

A HEMIN-SENSITIVE KINASE ASSOCIATED WITH THE A68 PROTEINS IN ALZHEIMER'S DISEASE. I.Vincent and P.Davies*, Depts. Pathol. and Neurosc., Albert Einstein Coll. of Med., Bronx, NY, 10461.

We have previously described (Neurobiol. Aging, in press) a second messenger-independent, casein kinase I-like activity in purified preparations of A68 from Alzheimer brain. This activity phosphorylates the A68 proteins, and in addition, was found to comigrate with A68 following active site labelling with the photoaffinity analog 8-azido-α-^{32}P-ATP. In continuing studies, we have found that limited chymotryptic cleavage of both ^{32}P and azido-ATP labelled A68 preparations yields similar labelled peptides. We have also examined the effects of various concentrations of hemin on the phosphorylation of these proteins. Starting with 1μM hemin, incorporation of phosphate into A68 is found to be inhibited. Phosphorylation of casein by the A68 preparation is also inhibited by 1μM hemin. In addition, it is found that subsequent immunoreactivity of A68 with Alz-50 on nitrocellulose blots is reduced following hemin treatment. This observation is not accompanied by a loss of protein as indicated by positive reaction with other antibodies directed against A68, and by silver staining. When A68 preparations are boiled prior to incubation with 1μM hemin, the subsequent loss in Alz-50 immunoreactivity is also observed. Hemin-agarose affinity chromatography of the A68 preparation resulted in retention of the proteins by the resin. Elution of the bound protein was achieved with 6M urea. Thus, hemin appears to directly interact with A68. Since hemin inhibits the phosphorylation of casein by the A68 preparation, it is suggested that the kinase activity of the preparation is associated with the A68 protein itself. This possibility is strengthened by the results of the proteolysis experiments described above. It is presently unclear whether the action of 1μM hemin on the kinase is of any physiological significance in Alzheimer's disease. The property of hemin sensitivity is rather unique, having being ascribed to only one other kinase, the heme regulated inhibitor of reticulocyte protein synthesis (Ranu & London, 1976,PNAS,73, 4349-4353).

252.12

BLOCKADE OF AF64A (ETHYLCHOLINE AZIRIDINIUM ION)-INDUCED MEMORY IMPAIRMENT IN RATS VIA PRE-TREATMENT WITH NICOTINE. J.A. Rosecrans*, J.R. James*, C. Stamford*, S.E. Robinson, & H.F. Villanueva, Dept. of Pharmacology/Toxicology, Va. Commonwealth Univ., Richmond, Va. 23298.

The present study investigated whether pre-treatment with nicotine (NIC) desensitizes acetylcholinergic (ACH) receptors, thus preventing the short-term memory deficits produced by the intraventricular (IVT) administration of the cholinergic neurotoxin AF64A (ethylcholine aziridinium ion). Following training on a memory task in a radial arm maze, rats were pre-treated with NIC, physostigmine (PHY), or saline via subcutaneous Alzet pumps for 14 days. On day 7 of drug infusion, rats were injected IVT with AF64A. After a recovery period of 7 days, behavioral testing was resumed. Rats were then sacrificed and ACH and choline levels were measured in the hippocampus, prefrontal cortex, striatum and pons via HPLC. Results indicate that NIC pretreatment partially attenuated the memory deficits produced by AF64A, suggesting that NIC may spare ACH neurons from neurotoxicity via receptor desensitization.
Supported NIH grant # DA04002-04 and Va. Center on Aging grant # 9003.

252.13

PERFORMANCE ON A DELAYED MATCHING-TO-SAMPLE TASK DURING A DOUBLE-BLIND TRIAL OF THA AND LECITHIN IN ALZHEIMER'S DISEASE PATIENTS

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Seven male subjects (Ss), 65-79 (mean=71.25, SD=+4.5) years of age meeting NINCDS-ADRDA criteria for probable Alzheimer's disease (AD) were enrolled in a double-blind, inpatient-outpatient tetrahydroaminoacridine (THA, tacrine) and lecithin trial. Memory measures on a delayed, 4-second, matching-to-sample task were made during baseline and THA administration periods using an interactive, computerized presentation of visual images ("X" and "O") and compared to the performance of 12, healthy, age-matched controls (Cs). AD Ss, performance was significantly different from Cs' during baseline periods for both in- and outpatient phases ($p<.001$). For THA periods, AD Ss also differed from Cs in reaction time (RT) and number correct (NC) responses ($p<.001$). AD Ss were slower in their RT and got fewer correct choices than Cs during both baseline and THA periods. However, when each patient was compared to the control group mean to compute standard Z scores, RT and NC performance associated with THA administration showed Z scores closer to the mean than did baseline measure. The anticholinesterase THA appears to produce a subtle improvement in memory retention IN AD patients.

252.14

TETRAHYDROAMINOACRIDINE (THA) REDUCES VOLTAGE-DEPENDENT CALCIUM CURRENTS IN RODENT SENSORY NEURONS.

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Tetrahydroaminoacridine (THA) is a centrally active anticholinesterase that may be useful in the palliative treatment of patients with Alzheimer's Disease. THA is also known to have effects on voltage-dependent K^+ and Na^+ currents in a variety of neurons. There is no information available on the effects of THA on neuronal voltage-dependent Ca^{2+} currents.

We studied the effects of THA on the T, N and L Ca^{2+} current components of acutely dissociated nodose ganglion neurons from 6-10 day old rats using the whole cell patch clamp technique. Recording electrodes contained (in mM): CsCl 140, CsOH 30, Hepes 10, EGTA 10, ATP 5 and GTP 0.1 (pH 7.2-7.3, ~300 mOsm). Neurons were bathed in (in mM): choline Cl 67, TEA 100, glucose 5.6, KCl 5.3, $CaCl_2$ 5.0, $MgCl_2$ 0.8, HEPES 10 (pH 7.35, ~320 mOsm). THA (500 nM-250 μM) was applied by passive diffusion from a blunt-tipped micropipette positioned 50 μm from the cell soma before and during voltage step commands. THA was tested on 18 neurons. THA reversiby reduced T, N and L Ca^{2+} currents in a concentration-dependent manner and was more potent in reducing T current. THA reduced peak T current by 14% at 500 nM and by 56% at 100 μM. THA had no effect on N and L currents at 500 nM but reduced the peak currents by 5% at 10 μM and by 100% at 100μM. Recovery of current magnitude was complete by 1 min (T) and 4 min (N and L). These results suggest that an additional mechanism of action of THA in central neurons is the reduction of voltage-dependent Ca^{2+} currents. *NIH NINCDS #1 T32 NS07222 and an American Academy of Neurology Research Fellowship Award (KMK), NS 01019 and NS 19613 (RAG) and DA04122 (RLM).*

252.15

RELATIONSHIP BETWEEN DOSE OF ARECOLINE AND COGNITIVE PERFORMANCE IN PATIENTS WITH DEMENTIA OF THE ALZHEIMER TYPE. K.C. Raffaele, P.P. Morris*, J.V. Haxby*, S.I. Rapoport, T.T. Soncrant. Laboratory of Neurosciences, National Institute on Aging, NIH, 10/6C103, Bethesda, MD 20892.

The finding of a cholinergic deficit in dementia of the Alzheimer type (DAT) has led to therapeutic attempts to increase brain acetylcholine action. Although cholinergic precursor therapy generally has proven inefficacious, some improvements following cholinesterase inhibitor or cholinergic agonist therapy have been reported. In the current pilot study, the effect of arecoline, a direct muscarinic agonist, on cognitive performance of DAT patients was evaluated. Three patients with mild or moderate dementia (Folstein Mini-Mental scores 25, 17 and 21) were given arecoline by continuous intravenous infusion for two weeks. Arecoline was administered in a series of increasing doses; cognitive testing was performed at five dose levels: 1, 4, 16, 28 and 40 mg/day. Eight tests were given, examining a range of cognitive functions. Significant improvement across subjects ($P < 0.05$) was found on two measures of verbal function, Stroop Words Read (4 mg/day) and FAS Verbal Fluency (28 mg/day). Thus, in DAT non-memory cognitive function may be more sensitive to cholinergic enhancement or may improve at different dose levels than does memory function.

252.16

MU OPIATE RECEPTORS IN ALZHEIMER'S DISEASE: IN VIVO QUANTIFICATION BY C-11 CARFENTANIL AND PET. H.-W. Mueller-Gaertner*, H.S. Mayberg, C. Meltzer, L. Tune*, J. Brandt*, H.N. Wagner*Jr, R.F. Dannals*, A.A. Wilson *, H.T. Ravert *, J.J. Frost. The Johns Hopkins Medical Institutions, Baltimore, MD 21205

The neurodegeneration of Alzheimer's Disease(AD) is associated with changes in many pre- and post-synaptic neurochemical markers. Metabolic and blood flow imaging in AD demonstrate characteristic reductions in temporo-parietal cortex, but the chemical specificity of these changes is low. Improved chemical specificity, and possibly improved sensitivity in detecting early disease may be achieved by imaging selected pre- and post-synaptic chemical markers.

Postmortem AD studies demonstrate a 50% decrease in mu opiate receptors in the amygdala, a brain region associated with early and severe pathological changes in AD. Accordingly, PET quantification of mu opiate receptor binding with C-11 carfentanil was performed in six AD patients and four age-matched control subjects. Imaging planes passing through the center of the amygdala and the long axis of the temporal lobe were selected by MRI. Quantification of C-11 carfentanil studies in the AD subjects (mean Mini-Mental score 17) demonstrated a decrease in mu opiate receptor binding in cingulate (18%), temporo-parietal cortex (24%), parietal cortex (22 %) and amygdala (30%). Mu opiate receptor binding in the thalamus, the frontal and temporal lobe and the caudate nucleus did not differ from normal controls.

These results demonstrate reduced mu opiate receptor binding in vivo most markedly in the amygdala in AD patients. The relationship of these findings to decrements in neurocognitive functions, such as memory, is being explored. Recovery coefficients and MRI-based atrophy correction methods are also being applied in the analysis of this data. Reduced amygdala mu opiate receptors may be an early and specific marker of AD.

253.1

IGG, IGA, AND IGM CLASS SERUM ANTIBODIES AGAINST PERIPHERAL NERVOUS SYSTEM MYELIN IN GUILLAIN-BARRÉ SYNDROME: IMMUNOBLOT STUDIES. H. Bernheimer, H. Regele*, G Suchanek*¶, M Schmidbauer*, and B. Schwerer*, Neurological Institute, Univ. of Vienna, and ¶Brain Research Institute, Austrian Academy of Sciences, Vienna, A-1090, Austria.

Antibodies against peripheral nervous system (PNS) antigens are thought to play a pathogenetic role in Guillain-Barré Syndrome (GBS), an inflammatory demyelinating disease of the PNS. Serum samples of GBS patients (n=11) and controls (n=15) were tested for the occurrence of IgG, IgA, and IgM class autoantibodies against myelin on Western blots of PNS myelin proteins, which had been separated by sodium dodecylsulfate-polyacrylamide gelelectrophoresis. In each Ig class, immunostaining with GBS and control sera, respectively, gave very similar antigen binding patterns. However, incidence as well as intensity of positive immune reactions in GBS were different from controls: incidence of IgA antibodies was higher in the GBS than in the control group, and more intense immunostaining by IgA and IgM antibodies was observed with GBS than with control sera at equal serum dilution. Our results suggest that higher levels of autoantibodies against PNS myelin antigens seem to occur in GBS patients than in controls; on the other hand, no evidence for the occurrence of antibodies against a GBS-specific myelin antigen in sera from GBS patients was obtained in the present studies. (Supported by Otto-LOEWI-Fellowship K0011-MED and Project P6438M, Austrian Science Research Fund).

253.2

CEREBROSPINAL FLUID (CSF) INTERFERON-G (IFN-G IS INCREASED TROPICAL SPASTIC PARAPARESIS (TSP). William A. Sheremata (Miami), D.E. McFarlin, (Bethesda, MD) University of Miami, PO Box 016960, Miami, FL 33101.

Central nervous system (CNS) pathology in TSP includes demyelination with mononuclear infiltrates of white matter and meninges, but mechanisms of CNS damage have not been elucidated. Using a sensitive radio-immunometric assay we found that IFN-g, a potent macrophage activator, is present in normal CSF, but not serum, and levels increase with immune activation. We have, therefore, studied 70 specimens (paired serum and CSF stored @ -70^{0}C) from our HTLV-1 seropositive TSP patients and 178 specimens from MS, AIDS and others with non-inflammatory degenerative CNS disease (DND). Serum IFN-g values (U/ml^3) were 0.13 $\pm$ 0.21 in TSP; 0.01 in MS; 0.06 $\pm$ 0.11 in AIDS; and 0.12 $\pm$ 0.22 in DND. Corresponding CSF values (U/ml^3) were 0.59 $\pm$ 0.56 in TSP, 0.18 $\pm$ 0.12 in MS; 0.35 $\pm$ 0.11 in AIDS and 0.10 $\pm$ 0.04 in DND. IFN-g values in TSP are significantly higher than other MS ($p < 0.01$) and DND ($p < 0.01$). Systemic infection in advanced DND may explain raised some serum values but not decreases in CSF. Elevations of IFN-g in TSP are consistent with immune activation by retovirus. Correlation of CSF IFN-g with neopterin content suggests that IFN-g is physiologically activating CNS macrophages in vivo and is important in lesion pathogenesis.

253.3

ALTERED PATTERNS OF NUTRIENT INTAKE IN HIV-1+ SUBJECTS WITH PYRIDOXINE (VITAMIN B_6) DEFICIENCY. G.Shor-Posner, J.Javier, R.Beach*, E.Mantero-Atienza* and M.Baum*. Biopsychosocial Center For Study Of AIDS, Univ. Miami Sch. of Med., Miami, Fl. 33101.

Our studies have indicated a high prevalence (35%) of pyridoxine deficiency during the early stages (CDC Stage III) of HIV infection. To determine the possible impact of B_6 status on dietary patterns, we examined nutrient intake in HIV+ males (n=36) at baseline (Time 1) and 26 weeks later (Time 2). Whereas B_6 deficient subjects (n=11) and individuals with normal B_6 status (n=25) consumed a similar proportion of protein, carbohydrate and fat at Time 1, distinct differences were noted at Time 2. In contrast to individuals with normal B_6 status, who consumed 16% of their intake as protein and 51% as carbohydrate, B_6-deficient subjects ate significantly less protein (13.6%, $p<0.002$) and more carbohydrate (57%, $p<0.03$); percent fat intake was similar.

As pyridoxine is involved in the synthesis of serotonin, which appears to have a role in the modulation of appetite for protein and carbohydrate, the B_6-deficient pattern of intake may reflect a decreased level of serotonin. This may be of particular importance since patients with AIDS appear to exhibit disturbances in the various serotonin/tryptophan pathways that possibly contribute to the neurological symptoms associated with HIV infection.

253.4

NUTRITIONAL ABNORMALITIES ASSOCIATED WITH HIV INFECTION. J.Javier, G.Shor-Posner, E.Mantero-Atienza*, R. Beach* and M.Baum* Univ. Miami Sch. of Med., Miami, Fl. 33101.

Nutritional status may be an important cofactor in determining the course of HIV disease progression. Nutritional assessment of a cohort of asymptomatic HIV infected homosexual males (CDC Stage III, n=102), indicates that HIV seropositive (+) individuals experience multiple abnormalities relatively early during the course of their disease and demonstrate a decline, over time, despite adequate levels of intake. Changes in overall nutritional status, as measured by blood levels, revealed that the percentage of normal nutritional status individuals decreased from 31% at the baseline evaluation to 23% at the second and 17% at the third six-months visit.

Up to 67% of the subjects had at least one nutritional deficiency and 36% of the population had multiple abnormalities at the initial evaluation. Although HIV negative (-) subjects (n=23) exhibited similar proportion of nutritional deficiency, the types of abnormalities differed between the two groups. Deficiencies in vitamins A, C, and E, which are particularly important for immune function, were observed in HIV(+), but not HIV(-) subjects. These findings indicate that the homosexual male population has a high prevalence of nutrient deficiencies, which may influence immune status as well as susceptibility to HIV infection.

253.5

STAGES IN HIV-1 LIFE CYCLE IN NEURAL CELLS. Y. Mizrachi, M. Shahabuddin*, M. Zeira*, G. Li* E.Golub* D.J.Volsky*, Molec. Virol Lab. Columbia Univ. St Luke's/Rsvelt Hspt, New York, NY 10019.

HIV-1 infection of neural cells is characterized by restricted viral expression and replication and absence of cytopathic effect. Using ^{125}I viral envelope glycoprotein (gp120), a quantitative fusion assay (fluorescence dequenching technique) and viral detection methods we have analyzed stages in HIV-1 infection of neural cell lines. We have found that neural cell infection is initiated by a specific binding of gp120 to a cellular receptor, followed by a fusion process which can be inhibited by purified gp120 in both neural and T cells. Soluble CD4 interfered in viral fusion with CD4 bearing T cells but not with virus neural cell fusion. HIV-1 neural cell fusion was restricted to neurotrophic viral isolates. Viral entry could be deduced from the detection of virally transcribed DNA thirty minutes after infection using PCR. While most non-human cells tested were not permissive to HIV-1 expression following transfection with infectious viral clones, similar levels of viral protein or viral LTR activity could be found in human cells of neural or T lymphocyte origin. We conclude, that neurotropic isolates can enter neural cells and following an initial permissivity for viral replication the neural cell restrict viral production.

253.6

LOW AFFINITY, LONG TERM BINDING OF HIV TO NEURAL CELL LINES. F. J. Denaro, Texas Tech University Health Sciences Center, Lubbock, TX.

The premise that HIV can infect neural cells is an important one that needs careful examination. In the present study HIV and an HIV-Amphotrophic variant, (HIV-A) was used to infect continuous neural cell lines. Because the HIV-A has a different capsule, it can infect cells without the need for CD_4 binding. These two viruses produce two strikingly different patterns of P-24 production. By day 3 the HIV-A is producing a massive infection as assayed by P-24. The high level of P-24 production remained high throughout the 4 months of the experiment. There was no sign of cytopathology. In contrast HIV produced a P-24 pattern which may be attributed to virus which has stuck to the cells and is eventually freed into the media. This sticking of HIV to the cells did not result in a productive infection. However, even after the passage of one month and a negative P-24 assay, HIV was detectable by co-culture with WBC's. One conclusion is that HIV can bind to the cells for long periods and not result in a productive infection. Perhaps this may be one important mechanism in the pathology of the CNS in patients with AIDS.

253.7

MECHANISMS OF HIV-1 INFECTION IN HUMAN AND NON-HUMAN NEUROBLASTOMA CELL LINES. H. Kulaga, A.J. Adams*, M. Coggiano*, Y. Shen*, L. To*, P.M. Sweetnam. NovaScreen®, a Division of Nova Pharmaceutical Corp., Baltimore, MD 21224; NPB, NIMH, WASH. D.C. 20032

Neuroblastoma lines were exposed to lymphotropic (LAV) and neurotropic (BR; SF-2) HIV-1 isolates at high multiplicities of infection. Cultures were tested for reverse transcriptase (RT) activity, production of p24 antigen, detection of viral nucleic acids and alteration of cell surface markers by flow cytometric analyses. The non-human neuroblastoma line NCB-20 and the human glioma HTB-17 were shown to be permissive to neurotropic viral stocks, although only low levels of RT were produced during infection. All other criteria indicating productive viral replication including protein and nucleic acid analyses were positive. Addition of soluble CD_4 or anit-CD_4 monoclonal antibody to these cultures did not inhibit infection in these cell lines. Immunogold labeling indicated that NCB-20 binds GP120. However, NCB-20 did not bind anti-human CD_4 monoclonal antibodies. These data indicate that HIV-1 infection of glioma neuroblastoma lines is possible but may proceed through a receptor other than CD_4.

253.8

GP120-INDUCED RETARDATION OF BEHAVIORAL DEVELOPMENT IN NEONATAL RATS: PREVENTION BY PEPTIDE T. J.M. Hill[1] and D.E. Brenneman[2] [1]Peptide Design L.P., Germantown, MD 20874, [2] LDN, NICHD, Bethesda, MD 20892.

In vitro studies with murine hippocampal cultures have indicated that purified HIV envelope glycoprotein, gp120, induced significant neuronal death which was prevented by peptide T, D-Ala peptide T amide (Brenneman *et. al., Nature* 335,639, l988; *Drug Dev. Res.* 15,361,l988). The purpose of the present study was to determine if gp120 would influence behavioral development when administered to neonatal rats, and to establish if peptide T prevented gp120-induced deficits. Sprague-Dawley rats received daily injections of 100 µl of one of the following: gp120 (0.3 nM), peptide T (3 µM), gp120 (0.3 nM) and peptide T(3µM) or saline, from birth to day 14. Observations of 14 developmental milestones/behaviors were made daily without knowledge of treatment group. The following were significantly delayed by gp120 treatment: Surface righting, air righting, negative geotaxis, grasping and fore and hindlimb placing. Peptide T co-treatment prevented the delay in behavioral development induced by gp120. The following were not influenced by gp120 treatment: Eye opening, corneal reflex, auditory startle, crossed extensor reflex, and cliff aversion. Systemic administration of gp120 significantly retarded the development of multiple, complex motor behaviors, whereas many simple reflexes and developmental milestones were apparently unaffected. These data further support the beneficial action of peptide T in preventing gp120-induced neural deficits and suggest that peptide T may be useful in the treatment of pediatric AIDS.

253.9

UNIQUE GP120 BINDING SITES IN HUMAN BRAIN CELLS. M.R. Kozlowski, P. Sandler*, P.-F. Lin*, R. Datema*, A. Watson*. Departments of Screening and Biochemical Research and Virology Bristol-Myers Squibb Company, Wallingford, CT 06492-7660 and Oncogen, Seattle, WA 98121.

A growing body of evidence demonstrates that HIV, the causative agent of AIDS, infects brain cells. It is unclear, however, whether the initial event in this infection is the same in brain cells as in lymphocytes: binding of the viral envelope glycoprotein, gp120, to the cell surface CD4 antigen. The present study examines binding of radio-iodinated recombinant HIV-1 gp120 (^{125}I-rgp120) to U-373 (human glioblastoma), U-138 (human glioblastoma), and CEM (human lymphoblastoma) cells.

Binding of ^{125}I-rgp120 to CEM cells (2h at 37C in PBS/BSA/glucose) was inhibited by anti-CD4a antigens (OKT4A, G17-2), Aurintricarboxylic Acid (ATA), and Evans Blue (EB). The anti-CD4 antibody, OKT4, and the dye, Aurin, were less active. In contrast, binding to U-138 and U-373 cells was poorly inhibited by OKT4A and G17-2, and showed no preference for OKT4A over OKT4 as a binding inhibitor (Table I). ^{125}I-rgp120 binding to these cell types was, however, inhibited to the same extent as in CEM cells by ATA and EB, with Aurin again being less active.

These results suggest that brain cells contain gp120 binding sites different from those found in lymphocytes.

253.10

SPREAD OF REOVIRUS SEROTYPE 3 INTO THE CNS FOLLOWING ENTERIC INOCULATION. L.A. Morrison*[1], R.L. Sidman[2], and B.N. Fields*[2]. Depts. of Microbiology and Molecular Genetics[1] and Neuropathology,[2] Harvard Med. Sch., Boston, MA 02115.

Infection of the central nervous system (CNS) by an enteric neurotropic virus was examined in newborn mice inoculated perorally with reovirus serotype 3, strain clone 9 (T3C9). Spinal cord and brain were dissected from formalin- and sucrose-perfused animals at various times after inoculation. Serial 30 µm cryostat sections were incubated with antireovirus antiserum and stained by the immunoperoxidase method to reveal presence of viral antigen. Neurons of the parasympathetic dorsal nucleus of the 10th cranial (vagus) nerve were specifically stained 4-5d post-inoculation. At this time no other stained cells were observed in the spinal cord, or within the brain. Staining of this nucleus was dependent on route of entry. By 6-7d post-inoculation, viral antigen was present in multiple nuclei of the brain; in sensory, motor, and sympathetic neurons of the spinal cord; and in the meninges of the spinal cord. The results indicate that reovirus T3C9 spreads to the CNS from the gastro-intestinal tract via nerves, initially the vagus para-sympathetic fibers. Second, reovirus T3C9 spreads trans-synaptically within the CNS. Third, viremia may play a role in later dissemination to, and promotion of infection in, the CNS.

256

SYMPOSIUM. RECAPITULATION OF DEVELOPMENTAL MECHANISMS IN NEURODEGENERATIVE DISORDERS. M. P. Mattson, Univ. of Kentucky (Chairperson); A. Matus, Friedrich Miescher-Inst.; K. S. Kosik, Harvard Med. School; J. W. Geddes, Univ. Calif. Irvine; C. W. Cotman, Univ. Calif. Irvine.

Both growth and regression of neurons may occur in neurodegenerative disorders (NDs). This symposium addresses current views on the plasticity and vulnerability of neuronal cytoarchitecture by presenting data on cellular and molecular mechanisms that sculpt neuronal cytoarchitecture adaptively during brain development and plasticity, and destroy neuroarchitecture in NDs such as Alzheimer's disease. Three systems that control neuroarchitecture will be featured: 1) The neuronal cytoskeleton, with a focus on microtubules and microtubule-associated proteins (MAPs). Recombinant DNA techniques are revealing how cytoskeletal proteins regulate growth-related plasticity and adult stabilization of neural circuitry, and how their disregulation leads to aberrant neuroarchitecture and loss of function. 2) Proteases and protease inhibitors. Proteins such as ß-amyloid and protease nexins can influence neurite outgrowth; altered expression or processing of these proteins may contribute to the cytoarchitectural correlates of NDs. 3) Cellular signaling systems including growth factors, excitatory amino acids (EAAs), and intracellular calcium. By influencing the neuronal cytoskeleton, these systems play key roles in regulating growth cone behaviors and synaptogenesis during development. Imbalances in EAA and growth factor systems resulting in a loss of calcium homeostasis may underlie the breakdown of neural circuitry in NDs. The presentations emphasize the links between neural development and disease, and forward the hypothesis that many features of NDs are the result of reactivated or aberrant developmental mechanisms.

257

SYMPOSIUM. GALANIN: MULTIDISCIPLINARY STUDIES. **S.F. Leibowitz, The Rockefeller University (Chairperson); A. Rokaeus*, Karolinska Institute; T.J. McDonald*, University Hospital; T. Hokfelt, Karolinska Institute; V. Chan-Palay, University Hospital.**

The peptide galanin has received increasing attention with respect to its potential physiological role and pathophysiology in the central and peripheral nervous systems. This symposium will review our current knowledge of this peptide, in terms of its gene expression, anatomical projections, structure-function relationships; its actions on endocrine, autonomic, feeding and pain systems; and its role in learning, memory and dementia. Ake Rokaeus will examine the structure, regulation and expression of the galanin gene, the regulation of its mRNA, the processing of the precursor protein, and the expression of galanin-like immunoreactivity and a galanin message-associated peptide. Thomas McDonald will describe his research on the structure-function relationships, morphological aspects and the pathophysiological actions of galanin in inhibiting insulin secretion from the endocrine pancreas and altering smooth muscle function in the gastrointestinal tract. Sarah Leibowitz will present results pertaining to the endocrine and behavioral effects of galanin in the brain and will describe pharmacological and neurochemical studies relating central galanin to monoamine neurotransmitters, circulating steroids, nutritional state and circadian rhythm. Tomas Hokfelt will focus on two galanin-containing systems in the brain: the cholinergic forebrain neurons, where galanin may have a role in learning and memory, and also primary sensory neurons, where galanin along with other peptides may exist to modulate pain. Victoria Chan-Palay will describe findings pertaining to changes in galanin, relative to other neurotransmitters, in the forebrain of humans with Alzheimer's dementia.

258.1

EFFECTS OF PERIRHINAL CORTICAL LESIONS ON VISUAL RECOGNITION MEMORY IN RHESUS MONKEYS. M. Meunier, E.A. Murray, J. Bachevalier, and M. Mishkin. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

The rhinal cortex (Rh) includes both the entorhinal cortex (ERh) which occupies the medial bank of the rhinal sulcus and the ventral cortex medial to it, and the perirhinal cortex (PRh) which occupies the lateral bank of the rhinal sulcus. Earlier work showed that monkeys with Rh (ERh+PRh) removals had a severe visual recognition memory deficit (Murray et al., 1989, Soc. Neurosci. Abstr., 15: 342) as measured by the delayed nonmatching-to-sample (DNMS) task with trial-unique objects. As a first step to determine the relative contributions of PRh and ERh to this deficit, we have now examined the behavioral effects of lesions restricted to the PRh. Naive rhesus monkeys were trained on the DNMS task with 10s between sample presentation and choice test until they reached the criterion of 90 correct responses in 100 trials. They then received a bilateral PRh ablation and, after a 10-14 day recovery period, were retrained on the DNMS task with 10s delays. When the monkeys reattained criterion, they were given a performance test that taxed their recognition memory by requiring them to remember either single objects for increasingly longer periods of time (30, 60, or 120s) or increasingly longer lists of objects (3, 5, or 10). Whereas controls relearned DNMS immediately, monkeys with PRh ablations required about as many trials to relearn as the monkeys with Rh lesions (PRh, $\bar{X}$ = 470; Rh, $\bar{X}$ = 540). On the performance test, by contrast, monkeys with PRh ablations scored 78% correct responses, which was lower than controls (92%), though significantly higher than monkeys with Rh lesions (68%). These preliminary results indicate that PRh alone makes a substantial contribution to visual recognition memory, but that other relays exist for transmitting sensory information to medial temporal and medial diencephalic limbic structures.

258.2

AMYGDALAR INTERACTION WITH THE MEDIODORSAL NUCLEUS OF THE THALAMUS AND THE VENTROMEDIAL PREFRONTAL CORTEX IN STIMULUS-REWARD ASSOCIATIVE LEARNING IN MONKEYS. E.A. Murray and D. Gaffan, Lab. of Neuropsychology, NIMH, Bethesda, MD 20892 and Dept. of Experimental Psychology, Oxford University, Oxford OX1 3UD, U.K.

Behavioral results from studies of recognition memory in monkeys have led to the suggestion that the medial temporal limbic, medial thalamic, and ventromedial prefrontal cortical areas are part of an integrated memory circuit. Because the amygdala, in particular, contributes to learning about food reward, we tested the above hypothesis as it relates specifically to the amygdalo-thalamo-frontal circuit and stimulus-reward association. Accordingly, cynomolgus monkeys were trained on a series of 2-choice visual discriminations between colored stimuli presented on a monitor screen. The feedback for correct choice was the delivery of food. The rate of learning new problems was assessed before and after surgery in a total of 16 monkeys. Three groups of 3 monkeys received bilaterally symmetrical ablations of either the amygdala and subjacent cortex (A), the medial portion of the mediodorsal nucleus of the thalamus (MD), or the ventromedial prefrontal cortex (VF). Whereas before surgery each group learned with roughly 10% error rates, after surgery each learned with 30% error. Seven additional animals received an amygdalar ablation in one hemisphere and removal of either the mediodorsal thalamus or the ventromedial prefrontal cortex in the other hemisphere, a procedure designed to disconnect the amygdalo-thalamo-frontal pathway. The disconnection groups showed a significant impairment, but the effect of the disconnection surgeries was significantly milder than that of the symmetrical ones; before surgery each group learned with error rates of roughly 10%, but after surgery each learned with error rates of about 17%. The results indicate that although A, MD, and VF appear to be functionally related, they do not form a single, tightly-linked functional pathway.

258.3

EFFECTS OF CORTICAL LESIONS ON VISUOSPATIAL PROBLEM-SOLVING. L.A. Miller. Psychology Dept., Montreal Neurological Institute, 3801 University St., Montreal, Quebec, CANADA, H3A 2B4.

In a recent study, Miller & Tippett (in preparation) found that patients who had invasive, right-hemisphere parietal, central, or frontal-lobe lesions were impaired on a Matchsticks Test of adaptive flexibility. The test required subjects to demonstrate as many ways as possible of removing a designated number of sticks from different geometric figures in order to achieve specified resultant shapes. In the present study, 10 patients who underwent cerebral excisions for the relief of epilepsy were assessed on the same task in order to determine whether lesions limited to the cortex are sufficient to cause a deficit. It was found that patients with right parietal removals tended to make many errors. Furthermore, only the patient whose right frontal lobectomy included the orbital region (and not the two with right frontal parasaggital excisions) demonstrated a selective impairment in the ability to change response strategy. The latter finding is interpreted as evidence of the lack of behavioural flexibility caused by inferior frontal-lobe damage.

258.4

IMPAIRMENTS IN MEMORY FOR SERIAL ORDER FOLLOWING LESIONS OF THE PRIMATE FRONTAL CORTEX. M. Petrides. Montreal Neurological Institute and Dept. Psychol. McGill Univ., Montreal, Quebec, Canada.

Monkeys with lesions restricted to different parts of the dorsolateral frontal cortex were tested on a task developed to assess memory for the order of occurrence of a series of visual stimuli. Three monkeys had sustained bilateral excisions of the mid-dorsolateral frontal cortex (cytoarchitectonic areas 46 and 9), three monkeys had excisions of the posterior region of the dorsolateral frontal cortex (areas 8 and rostral 6), and another four animals served as the normal control subjects. Monkeys with excisions within the mid-dorsolateral frontal cortex were severely impaired, whereas animals with lesions of the posterior dorsolateral frontal cortex performed as well as the normal control subjects. All animals had previously been tested on a visual recognition memory task (delayed non-matching to sample). The two groups of operated animals were not significantly different from the normal control group on this task. The above findings indicate that the primate mid-dorsolateral frontal cortex is a critical component of a neural circuit underlying the monitoring of the order of occurrence of a series of stimuli.

258.5

RETROGRADE AMNESIA: TEMPORAL GRADIENT IN LONG-TERM MEMORY FOLLOWING DAMAGE TO THE HIPPOCAMPAL FORMATION IN MONKEYS. S. Zola-Morgan, L.R. Squire, VA Med Center, San Diego and Dept. of Psychiatry, UCSD Sch of Med, La Jolla, CA 92093

Patients with hippocampal damage have loss of premorbid memory (retrograde amnesia) that is greater for the recent past than the more remote past. The interpretation of this finding depends on the precise shape of the performance curves, which cannot be determined with certainty using the tests that are available to assess remote memory retrospectively in humans. We have assessed retrograde amnesia in monkeys with bilateral lesions of the hippocampal formation (the H^+ lesion). Monkeys were trained on different sets of 20 two-choice object discrimination problems at 16, 12, 8, 4, and 2 weeks prior to surgery (a total of 100 discrimination pairs). Two weeks after surgery, memory was assessed by randomly presenting a single trial of each of the 100 pairs. Unoperated monkeys (N=7) exhibited forgetting, ranging from 79% correct for object pairs learned 2 wks earlier to 70% correct for object pairs learned 16 wks earlier. The H^+ group (N=11) exhibited temporally-graded retrograde amnesia (2 wk=62%; 4 wk=64%; 8 wk=65%; 12 wk=72%; 16 wk=67%). Importantly, older memories were remembered significantly better than recent memories (analysis of linear trend: $p=.01$). These findings suggest that the hippocampal formation is initially involved in memory storage and retrieval and that its role gradually diminishes with the passage of time.

258.6

LESIONS OF PERIRHINAL CORTEX, BUT NOT LESIONS OF THE AMYGDALA, EXACERBATE MEMORY IMPAIRMENT IN MONKEYS FOLLOWING LESIONS OF THE HIPPOCAMPAL FORMATION. R.P. Clower, S. Zola-Morgan, and L.R. Squire. VA Med Ctr., San Diego and Dept. of Psychiatry, UCSD Sch of Med, La Jolla, CA 92993.

Studies in our laboratory have suggested that severe memory impairment in monkeys associated with large medial temporal lobe lesions (the H^+A^+ lesion) results from damage to the hippocampal formation and adjacent, anatomically related cortex (e.g., perirhinal and parahippocampal cortex), and not from conjoint hippocampus-amygdala damage. We have evaluated memory impairment in a group of monkeys with bilateral lesions of the hippocampal formation, parahippocampal cortex, and perirhinal cortex (the H^{++} lesion, N=3). Using the delayed nonmatching to sample task, the H^{++} group was compared to four previously studied groups: H^+A^+ (N=4), H^+ (hippocampal formation and parahippocampal cortex, N=8), H^+A (H^+ lesion plus amygdala, N=3), and N (normal, N=6). All operated groups had comparable H^+ damage, and all were impaired on the task (mean score for 10-min delay: N=82%, H^{++}=54% H^+A^+=67%. The impairment associated with the H^{++} lesion was as severe as that following H^+A^+ lesions, and significantly more severe than the impairment following H^+ or H^+A lesions. These findings suggest that damage to the perirhinal cortex, not damage to the amygdala, contributes to the severe impairment that follows H^+A^+ lesions.

258.7

NUCLEUS ACCUMBENS LESIONS SELECTIVELY IMPAIR SPATIAL BUT NOT VISUAL OR MOTOR REVERSAL LEARNING IN MONKEYS *(MACACA FASCICULARIS)* C.E. Stern^ and R.E. Passingham*, Dept. of Exp. Psych., S. Parks Rd., Oxford, U.K., OX1 3UD. ^Present address: Dept. of Physics, Univ. of CA at Irvine, Irvine, CA, 92717.

The nucleus accumbens (NA), which receives inputs from limbic structures and projects to the motor system, may be important for the integration of emotion and motivation with motor behaviour. Because the NA receives inputs from the amygdala and hippocampus, tasks which are known to be disrupted by lesions to these areas in monkeys were tested on animals with lesions of the NA. In addition, a motor reversal task, in which the animals were taught to turn or pull a handle, was used to test whether these animals were capable of associating movements with reward.

Twelve monkeys (*Macaca fascicularis*) were used in the present study. Six of these received ibotenic acid lesions of the NA. One group (3 NA, 3 control) was taught a visual discrimination task pre-operatively and performed visual and spatial discrimination and reversal tasks post-operatively. A second group was taught the motor reversal task. Results indicate that ibotenic acid lesions of the NA do not impair the ability to learn visual discriminations ($F(1,4)=0.2$) or visual reversal ($F(1,4)=0.2$) paradigms. Initial spatial learning (prior to the first reversal) was spared ($t=1.59$, $df=4$), however, these same animals were found to be impaired on spatial reversal learning ($F(1,4)=14.1$, $p=0.02$). Results of the motor reversal task revealed no impairment in the ability of NA lesioned animals to initially learn the task ($t=0.75$, $df=4$) or to perform the motor reversals ($F(1,4)=0.98$).

Our results on visual and spatial tasks mimic results obtained following hippocampal, but not amygdala, lesions in monkeys (Jones and Mishkin, 1972). In addition, these results challenge theories which suggest that the NA is important for general behavioural flexibility and response selection. Instead, we suggest that the NA may play a more specific role in the association of spatial cues with movement and reward.

258.8

THE ROLE OF LIMBIC AND PREFRONTAL CORTICAL AREAS IN CONCEPT FORMATION AND IMMEDIATE MEMORY IN RHESUS MONKEYS. J. Pizlo, E.A. Murray and M. Mishkin. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

Macaques with combined amygdalo-hippocampal (AH) removals can master trial-unique delayed nonmatching-to-sample (DNMS) provided the delays are 10 seconds or less. Presumably, correct performance under these circumstances requires formation of the concepts (or categories) familiar/novel or same/different with the aid of immediate memory. If removal of inferior prefrontal cortex (IF), another major target of the occipitotemporal visual system, is added to AH removal, monkeys fail DNMS even with delays shorter than 10 s (Weinstein et al., Soc. Neurosci. Abst, 14:1230, 1988), suggesting that IF and AH are important for concept formation, immediate memory, or both. Here we analyzed the role of IF and AH in a concept formation task not requiring immediate memory. On each trial, two trial-unique pairs of objects were presented for choice, a like pair of objects (same), one of which was always baited, and an unlike pair (different), neither of which was baited. Following attainment of criterion (90 correct responses in 100 trials) the animals received the combined IF and AH surgery in two stages, in counterbalanced order, with interoperative retraining. The monkeys took progressively longer to relearn new sets of same-different discriminations at each stage (mean trials: 85 pre-op, 470 post-op 1, 925 post-op 2), but each did relearn. There was no effect of lesion order. The results suggest that IF, AH, or both make an essential contribution to immediate visual memory, and a substantial but not essential contribution to formation of the concepts same/different, familiar/novel, or both.

258.9

LACK OF MEMORY IMPAIRMENTS FOLLOWING BASAL FOREBRAIN LESIONS IN MONKEYS. M.L. Voytko, D.S. Olton, R.T. Richardson, G.L. Wenk and D.L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205.

The basal forebrain (BF) may play a role in mnemonic processes. The present study pursued this issue by examining monkeys with extensive damage of the BF on a battery of behavioral tasks that assessed various forms of learning and memory. Five cynomolgus monkeys were preoperatively trained in a computer-controlled apparatus on the following tasks: delayed nonmatching-to-sample (0- to 60-second delays and list length of three); delayed response (0- to 10-second delays); single object discriminations; and 24-hour concurrent object discriminations. Retention of this test battery was examined immediately before surgery and again two weeks postoperatively. With the aid of nuclear magnetic resonance (NMR) imaging, ibotenic acid injections were made in the BF in two of the five monkeys; the three remaining monkeys were operated controls. Postsurgical NMR images indicated successful placement of injections within BF sites. Lesions in the BF had minimal influence on the performance of monkeys on the behavioral tasks. To investigate these preliminary findings, we are currently presenting monkeys with more difficult versions of the tasks as well as new tasks.

258.10

EFFECTS OF NEOSTRIATAL LESIONS ON VISUAL HABIT FORMATION IN RHESUS MONKEYS. J. Wang*, T. Aigner, and M. Mishkin. Lab of Neuropsychology, NIMH, Bethesda, MD, 20892.

Monkeys with bilateral lesions of the amygdala and the hippocampus are impaired on tests of one-trial visual recognition memory, but are still able to learn a series of object discriminations, a test of habit formation, even when intertrial intervals are as long as 24 hrs. By contrast, animals with bilateral lesions of inferior temporal cortex (area TE) are impaired on both tests, suggesting that visual object discrimination learning is mediated by a TE-nonlimbic pathway. Because area TE has extensive fiber connections with the the tail of the caudate nucleus and the ventral putamen, we investigated whether lesions of these regions would produce selective impairment of object discrimination learning. Four female rhesus monkeys were shown 20 pairs of test objects during each daily session. Within each pair, one object was baited, the other unbaited. The 20 pairs were shown only once each session, and the left-right position of the baited objects was varied pseudorandomly across sessions. After each animal reached the criterion level of 90% correct choices on the first set of objects, ibotenic acid was injected bilaterally into the tail of the caudate nucleus and the adjoining ventral putamen using stereotactic coordinates derived from magnetic resonance imaging, which was also used to verify the accuracy of the lesion postoperatively. Subsequently, the animals were tested on 2 additional sets of objects. For each animal, the number of trials required to reach criterion was more than twice that for the initial set of objects. When tested on a visual recognition task (delayed nonmatching-to-sample), however, the animals performed as well as control animals. This pattern of impairment strongly suggests that visual recognition memory and visual habit formation are mediated by separate pathways and that the tail of the caudate nucleus and the ventral putamen are important components in the latter.

258.11

EFFECTS OF ENTORHINAL, PARAHIPPOCAMPAL, OR BASAL FOREBRAIN LESIONS ON RECOGNITION MEMORY IN THE MONKEY. L. L. Beason, M. B. Moss, and D. L. Rosene. Dept. of Anatomy, Boston Univ. Sch. Med., Boston MA 02118.

The basal forebrain and temporal lobe are two areas of the brain that have been identified as possible neuropathological substrates of the marked anterograde memory impairment which characterizes the early stages of Alzheimer's Disease (AD). To determine if damage to either of these areas produces memory impairments that parallel those seen in AD, we administered a recognition memory test (Delayed Recognition Span Task-DRST), that has been used extensively in the assessment of patients with AD, to rhesus monkeys with bilateral lesions of the basal forebrain (BF) and to normal control monkeys (N). Their performance was compared to that of animals that received lesions of the entorhinal cortex (EC) or lesions immediately outside the rhinal sulcus including perirhinal cortex, rostromedial area TE and the posterior parahippocampal gyrus (PHG). The DRST assesses the capacity of a subject to identify a new stimulus among an increasing array of stimuli, adding one new stimulus at a time until the subject commits the first error, yielding a "recognition span". The recognition span scores of monkeys with lesions of the basal forebrain did not differ from that of normal controls. In contrast, monkeys in group EC and group PHG, though not significantly different from each other, had significantly lower span scores relative to the BF or N groups. The findings suggest that damage within the temporal lobe, rather than in the basal forebrain, may contribute to the anterograde memory impairment in AD. Supported by NIH grants AG04321 and NS16841.

258.12

UNILATERAL AND BILATERAL ELECTRIC STIMULATION OF MEDIAL TEMPORAL LOBE IN THE MONKEY. J.L. Ringo Dept. of Physiology, U. of Rochester Med Ctr, Rochester, NY, 14642.

Electric stimulation at 33 Hz was applied in a pseudo random pattern of 0.2 ms pulses to electrodes implanted in medial temporal lobe, in monkeys doing a visual delayed-matching-to-sample task. Stimulation in the sample or the match period drove performance to near chance levels.

In split chiasm monkeys, stimulation to just one hemisphere during the sample presentation and to just the other hemisphere during the match choice produced chance performance even when the view was, in each case, through the eye contralateral to the stimulation. With both sample and match presented to the same side, stimulation to the contralateral side created only moderate deficits.

Unilateral stimulation during the sample presentation was used to asymmetrically bias the engram to the side opposite the stimulation. The subsequent normal performances when presenting the match choice through what had been the stimulated side suggested that interhemispheric access to memory on the other side is excellent. Such access was good through either the anterior commissure or the splenium.

258.13

STEREOTAXIC LESIONS OF THE HIPPOCAMPUS IN MONKEYS USING MAGNETIC RESONANCE IMAGING: DETERMINATION OF COORDINATES AND ANALYSIS OF THE LESIONS.

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The cynomolgus monkey has served as a valuable animal model for studies of human learning and memory. As part of our research directed at understanding how selective damage to the hippocampal formation affects learning and memory, we have developed two noninvasive anatomical techniques based on current magnetic resonance imaging (MRI) technology.

1. Determination of stereotaxic coordinates for individual monkeys: This approach was developed to address the problem of using a standardized brain atlas with monkeys whose brains vary considerably in size and shape. Prior to surgery, four small, glass beads filled with a copper sulfate solution (0.1M) were affixed to the monkey's skull. Monkeys were then placed in a nonmetallic stereotaxic headholder, and scanned using a high-resolution, inversion-recovery pulse sequence (1.5T magnet, head coil, TR=1500, TE=25, TI=708, 3mm interleaved slices) that produced high quality coronal images of the hippocampus along its full rostrocaudal extent. The glass beads (which have high signal intensity) were used both as reference points for establishing the coordinates for stereotaxic surgery and as landmarks during the surgery itself. In this way, individual lesion coordinates were used for each monkey.

2. Noninvasive postoperative analysis of lesions: The MRI protocol described above was modified in two ways in order to carry out postoperative analysis of the lesions. We administered a contrast enhancing agent (gadolinium, 0.2 ml/kg IV) and we used a scanning plane perpendicular to the long axis of the hippocampus. The images permitted preliminary verification of the extent and accuracy of the lesions and were useful in determining whether animals should undergo long term behavioral testing. Thus, MRI technology can be used to improve lesion techniques and to permit more efficient use of animal subjects.

258.14

LESIONS OF INFERIOR TEMPORAL AREA TE IN INFANT MONKEYS PRODUCE REORGANIZATION OF CORTICO-AMYGDALAR PROJECTIONS.

M.J. Webster, L.G. Ungerleider and J. Bachevalier. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

Object recognition memory is severely impaired in monkeys that receive area TE lesions as adults but only mildly in those that receive the same lesion as infants. To determine the possible mechanisms mediating this sparing of function, we have examined the development of cortico-amygdalar connections in normal and brain-damaged monkeys. We reported earlier that area TEO in normal infants projects transiently to the lateral basal nucleus (LB), a projection that is partially retained in adults that had received TE lesions as infants. Other differences in the connections of TEO in the infant-operated as compared to the adult-operated monkeys were heavier projections to the claustral nucleus of the amygdala (CA) and a new projection to the lateral nucleus (L). The connectional pattern in the adult-operated monkeys was identical to that in normal adults. Thus, only after TE lesions in infancy, a small proportion of the projection from TEO to LB is retained, whereas that to CA intensifies and possibly sprouts into L to occupy the terminal zone vacated by TE. Sparing of memory function in adult monkeys that receive brain damage in infancy may thus be due to preservation of transient connections and axonal sprouting.

EXCITATORY AMINO ACIDS: RECEPTORS VI

259.1

DISSOCIATION OF METABOTROPIC AND IONOTROPIC EXCITATORY AMINO ACID (EAA) RECEPTOR EFFECTS *IN VIVO* USING *TRANS-* AND *CIS-*(±)1-AMINO-1,3-CYCLOPENTANEDICARBOXYLIC ACID (ACPD).

D.D. Schoepp, B. G. Johnson, C. R. Salhoff*, and C. C. Hillman, Jr.*, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

The *trans-* and *cis-* isomers of ACPD were characterized for 1) their ability to stimulate metabotropic EAA receptors coupled to phosphoinositide hydrolysis in slices of the rat hippocampus, 2) their relative affinities at ionotropic EAA receptors in rat brain membranes, and 3) their ability to produce *in vivo* excitatory or excitotoxic effects in rats. *trans*-ACPD was 13 times more potent as an activator of phosphoinositide hydrolysis (EC_{50} 28.0 ± 2.8 μM) than as a displacer of NMDA (^{3}H-CGS-19755) receptor binding (IC_{50} 374 ± 78 μM). In contrast, *cis*-ACPD was 27-times more potent in inhibiting ^{3}H-CGS19755 binding (IC_{50} 3.3 ± 0.4 μM) than it was in stimulating phosphoinositide hydrolysis (EC_{50} 89.4 ± 6.4 μM). Displacement of ^{3}H-AMPA or ^{3}H-kainate binding to rat brain membranes by *trans-* or *cis-*ACPD was negligible (IC_{50}s > 500μM). When administered i.p. to neonatal rats, *cis-*ACPD was 8-times more potent than *trans-*ACPD in producing convulsions (ED_{50}s 12 and 100 mg/kg, respectively). *Cis-* or *trans-*ACPD convulsions were prevented by LY233053, a competitive NMDA receptor antagonist. When infused unilaterally into the rat striatum, 0.5 μmoles of *cis-*ACPD greatly reduced choline acetyltransferase (ChAT) activity (74 ± 6 % decrease). However, infusion of 1 μmole *trans-*ACPD did not significantly reduce ChAT activity (7.6 ± 4.7 % decrease). In contrast to *cis-*ACPD, *trans-*ACPD is a selective metabotropic EAA agonist *in vitro*. *Trans-*ACPD does not produce excitotoxicity *in vivo*, and its convulsant effects only occur at high doses and can be antagonized by an ionotropic NMDA receptor antagonist. Thus, unlike ionotropic EAA receptors, excitatory and excitotoxic effects do not appear to be a consequence of directly activating the metabotropic EAA receptor.

259.2

INTRACELLULAR CALCIUM REGULATES RESPONSES TO NMDA IN HIPPOCAMPAL NEURONS. H.Markram* and M.Segal. Center for Neuroscience, The Weizmann Institute, Rehovot, Israel.

We investigated the role of $[Ca^{2+}]_i$ in the regulation of NMDA receptor activation. Intracellular recordings were obtained from CA1 neurons in the rat hippocampal slice. NMDA, applied ionophoretically, potentiated subsequent voltage or current responses to NMDA, by more than two fold. This auto-potentiation was reduced when intracellular Ca^{2+} was chelated with BAPTA. A BAPTA-sensitive rise in $[Ca^{2+}]_i$ could be detected electrophysiologically as an enhancement of the AHP that persisted for at least 20s after the membrane response to NMDA had recovered. Increasing $[Ca^{2+}]_i$ using the Ca^{2+} ionophore, A23187, or by evoking intracellular release using either IP3-containing micropipettes, or following UV irradiation of cells loaded with a photolabile IP3, caused a 2- to 3-fold potentiation of responses to NMDA. These increases were suppressed in BAPTA-loaded cells. We conclude that $[Ca^{2+}]_i$ plays a significant role in regulating the activation of NMDA receptors.

259.3

EXTRACELLULAR ZINC ANTAGONIZES THE EFFECTS OF NMDA, QUISQUALATE AND AMPA ON INTRACELLULAR CALCIUM (CAi) IN CULTURED CHICK CORTICAL NEURONS.

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Zinc has been localized within axon terminals of central neurons and may be released during excitatory amino acid (EAA) neurotransmission. Micromolar concentrations of zinc have previously been shown to attenuate NMDA receptor-mediated excitation in cultured cortical neurons. Cai was determined using Fura2 loaded 8d chick embryo neurons after 2d in culture. EAA alone elevated Cai in the order of kainate (KA)>NMDA>AMPA>quisqualate (QA) . Also, both AMPA and QA desensitized the Cai response to KA. Zinc (10-1000 μM) antagonized both the effects of NMDA alone (38-88%) and QA/AMPA-induced KA desensitization (19-85%) compared to control. However, at a concentration of 100 μM, zinc augmented the Cai response to QA (63%) or KA (41%) alone. These results corroborate previous findings with respect to the effects of zinc on NMDA-induced excitability, but suggest that significant interactions also occur with non-NMDA sites.

259.4

PROPERTIES OF SPONTANEOUS MINIATURE SYNAPTIC CURRENTS IN CHICK SPINAL CORD CULTURES.

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We have examined the amplitude, shape, and frequency of spontaneous "miniature" inward synaptic currents (mepsc's) recorded from chick spinal neurons maintained in culture for 2-14 days. Recordings were made using tight-seal whole cell voltage clamp, with an electrode solution containing KCl or CsCl as the major ions. 1 μM TTX, 100 μM Cd^{++}, 100 μM bicuculine and 2 μM strychnine were included extracellularly. Mepsc's recorded with 1 mM bath Mg^{++} at -60 mV were eliminated by 20 μM CNQX and were markedly reduced in amplitude by 1 μM CNQX, indicating that they are produced by non-NMDA receptors.

Mepsc amplitude varied over a wide range, from 4 pA to >120 pA. No relationship was observed between the rise time and fall time of the events or between the rise time and amplitude. Events evoked in small (ca. 20 μm) regions of dendrite, close to the cell body, by focal application of black widow spider venom, produced mepsc's which also varied widely in amplitude. These data suggest that the variation in mepsc size cannot be due to cable decay. Statistical analyses indicate that the largest event were not due to the coincident release of several smaller quanta. In the presence of TTX and Cd, it is unlikely that the large mepsc's are associated with inward Na or Ca currents.

Following local superfusion of venom, mepsc's appeared in bursts, perhaps corresponding to the action of venom at different synaptic sites. Interestingly, bursts differed in the average amplitude of their mepsc's. Considering the uniformity of the mepsc's rise times, we suspect that variation in mepsc size is due to variation in postsynaptic receptor density. Supported by NS07821.

259.5

SELECTIVITY OF AMINO ACID TRANSMITTERS ACTING AT NMDA AND AMPA RECEPTORS EXPRESSED IN XENOPUS OOCYTES M. C. Curras and R. Dingledine Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, 27599

The neurotransmitter candidates, L-aspartate, L-cysteine sulphinic acid (L-CSA), L-glutamate, L-homocysteic acid (L-HCA) and quinolinate (Q) were compared for potency, efficacy and selectivity at N-methyl-D-aspartate (NMDA) and AMPA (non-NMDA) receptors in Xenopus oocytes injected with rat brain mRNA. Steady-state inward currents were measured at -60 mV in response to agonist application. Selective activation of NMDA receptors was achieved by deleting Mg and including 3-10 μM glycine in the perfusion medium, and by applying ligands in the presence of 30 μM quisqualate, which blocks the AMPA receptor and desensitizes the oocyte's Ca-dependent Cl current. Under these conditions, the potency sequence was L-glutamate (EC50=2.2 μM) > L-aspartate (13 μM) = L-HCA (13 μM) > L-CSA (59 μM) > > Q (>7200 μM). All amino acids tested had similar efficacy, i. e., 1.2-1.5 that of NMDA. Hill coefficients were greater than 1 for all agonists except L-HCA (0.6), which might be explained by heterogeneity of NMDA receptors expressed.

To study agonist activity at AMPA receptors, glycine and quisqualate were omitted and 1 mM Mg was included to block NMDA receptors. Ca-dependent Cl currents activated by L-glutamate were prevented by inclusion of 0.4 M EGTA in the electrodes. All amino acids were less potent at AMPA than at NMDA receptors; potency ratios for activation of AMPA receptors were: L-glutamate (EC50=11 μM) > > L-HCA (430 μM) > > L-CSA (3300 μM). L-aspartate and Q produced little or no inward current up to 10 mM, i. e. were inactive at AMPA receptors. The efficacy of all amino acids was only 5-10% that of kainate, presumably due to severe desensitization of the AMPA receptor by the natural agonists. We conclude that all endogenous amino acids tested have substantially lower affinity for AMPA receptors than for NMDA receptors. The most selective agonist for the NMDA receptor is L-aspartate, whereas only L-glutamate has substantial activity in μM concentrations at AMPA receptors.

259.6

INTERACTION OF Mg^{2+} AND PHENCYCLIDINE IN USE-DEPENDENT BLOCK OF NMDA CHANNELS. M.V.L. Bennett, J. Lerma[1], and R.S. Zukin, Albert Einstein Coll. Med., Bronx, NY 10461, [1]Instituto Neurobiologia "S. Ramon y Cajal", 28002-Madrid, Spain.

The interaction between Mg^{2+} and phencyclidine (PCP) in blocking open N-methyl-D-aspartate (NMDA) channels was studied in *Xenopus* oocytes injected with rat brain mRNA. PCP block, measured as reduction in current under voltage clamp at -60mV, developed within seconds in the presence of agonist, but prior application of PCP had little effect. Upon removal of PCP and NMDA, recovery of responsiveness to NMDA was very slow until NMDA was again applied; recovery occurred gradually during NMDA application. Evidently PCP was "trapped" in the closed channels; thus the degree of PCP block could be measured as the decrease in the early phase of the response elicited by the second NMDA application, as well as by the reduction in steady state current in the presence of NMDA and PCP. By both measures the IC_{50} for PCP block was ca. 120nM. Application of Mg^{2+} with PCP and NMDA reduced the fraction of channels blocked by PCP as determined by increase in the responses to subsequent test applications of NMDA. Interaction between Mg^{2+} and PCP was competitive; 0.5mM Mg^{2+} caused a 2-4 fold increase in the IC_{50} value for block of NMDA responses by PCP with no decrease in maximum degree of block. In the presence of NMDA, Mg^{2+} speeded recovery from PCP block, suggesting that Mg^{2+} reduced reblock of NMDA channels by PCP that had escaped from open channels. These findings indicate that Mg^{2+} and PCP compete with each other and cannot occupy the channel simultaneously. Since depolarization is likely to reduce Mg^{2+} block more than PCP block, neural activity is likely to modulate actions of PCP and related drugs.

259.7

DEVELOPMENTAL REGULATION OF NMDA RECEPTORS BY MAGNESIUM AND GLYCINE N. W. Kleckner and R. Dingledine. Dept. of Pharmacology, Univ. of North Carolina, Chapel Hill, 27599.

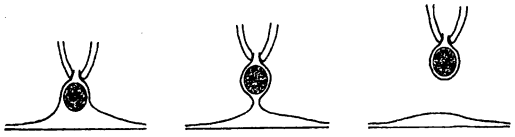
Divalent cations play an important role in NMDA receptor regulation. While Ca^{2+} permeates the channel, Mg^{2+} blocks it in a voltage-dependent manner, so that in the presence of Mg^{2+} membrane depolarization facilitates current flow into neurons through this channel. To examine whether NMDA receptor subtypes exist at different developmental stages, divalent cation block of NMDA receptors was studied in oocytes injected with mRNA isolated from hippocampi of 1-2, 7-8, 14-15 day, or 12 week old (adult) rats. Agonist-induced currents were recorded in the presence of Mg^{2+} or other divalent cations. For NMDA receptors from adult rats, the EC_{50} of Mg^{2+} block increased by e-fold (2.718-fold) for every 15 mV increase in membrane potential between -70 and -50 mV. The Mg binding site was calculated to be 81 % of the way through the membrane electric field. Mn^{2+} and Ni^{2+} were weak voltage-dependent blockers of the NMDA receptor, whereas Zn^{2+} and Cd^{2+} were non-voltage-dependent blockers. NMDA receptors from 1-2 day old rats were significantly more sensitive to block by Mg^{2+} over the entire range of membrane potentials tested (-90 to -30 mV) than receptors from 14-15 day old rats ($P<0.03$). The EC_{50} values for Mg^{2+} block at -60mV were 12.7, 18.2, 27.4, and 18.8 μM for 1-2 day, 7-8 day, 14-15 day and adult receptors. The voltage-dependence of Mg^{2+} block did not change with developmental age. The sensitivity of NMDA receptors to glycine, but not NMDA, may also vary with developmental age. The glycine EC_{50} values were 0.24, 0.23, 0.35 and 0.54 μM ($n=5$) for receptors from 1-2 day, 7-8 day, 14-15 day and adult rats. These studies suggest that different subtypes of NMDA receptor may be present that vary in their sensitivity to block by Mg^{2+} and activation by glycine.

259.8

ACTIVATION AND DESENSITIZATION OF NMDA RECEPTORS IN OUTSIDE-OUT PATCHES W. Sather, J.F. MacDonald and P. Ascher. Lab. Neurobiologie, Ecole Normale Supérieure, 46 rue d'Ulm 75005 Paris

We have discovered a method for preparing "macro-patches". The key step in the preparation of such patches is to maintain suction on the patch pipette after the rupture of the membrane leading to the whole cell configuration. The suction leads to a slow displacement of the nucleus towards the pipette tip. Then, when the pipette is pulled away, the plasma membrane wraps around the nucleus, and eventually reseals around it when it separates from the enucleated cell body. The "nucleated patch" behaves as a classical outside-out in every respect, except that high concentrations of NMDA can open simultaneously hundreds of channels.

Using this new configuration we have analyzed the concentration dependence of the peak and steady-state current induced by NMDA in the presence of a saturating concentration of glycine (10 μM). The apparent dissociation constant is about 50 μM for the peak current, 5 μM for the steady-state current. This suggests that, as in the case of the nicotinic receptor, the desensitized state of the NMDA receptor has a higher affinity for the agonist than the "resting" state.



259.9

EVIDENCE THAT KAINATE RESPONSES AND RAPIDLY-DESENSITIZING QUISQUALATE RESPONSES ARE MEDIATED BY THE SAME RECEPTOR. D. K. Patneau, C. A. Winters*, L. Vyklicky Jr.* and M. L. Mayer. Unit of Neurophysiology and Biophysics, LDN, NICHD, NIH, Bethesda, MD 20892.

Responses to quisqualate and kainate are dramatically different. Quisqualate produces a rapidly and strongly desensitizing response; kainate, a weakly-desensitizing response. Both ligands have been suggested to act at the same receptor. Quisqualate blocks kainate responses, apparently by cross desensitization (Kiskin et al, 1986); however, at high concentrations kainate can overcome the antagonist action of quisqualate, suggesting a competitive interaction (O'Brien & Fischbach, 1986). We will present kinetic evidence that kainate and quisqualate do act at the same receptor.

Our experiments used fast perfusion techniques during whole cell recording from cultured mouse hippocampal neurons voltage-clamped at -60 mV. We made equilibrium and kinetic measurements of kainate responses during competitive antagonism by several quisqualate-like agonists. Over a 1000-fold range, antagonist equilibrium potency followed the same order of affinity as that for activation of quisqualate-like responses: quisqualate>AMPA>L- glutamate>L-homocysteic acid>L-cysteic acid. The dose of quisqualate which blocked 50% of receptors available for activation by kainate (0.9 μM) was similar to the IC_{50} for desensitization of quisqualate responses by a prepulse of agonist (1.1 μM). Kinetic data provided further evidence that kainate and quisqualate compete for the same receptor. Kainate responses normally exhibit fast activation (τ <10 msec) but in the presence of quisqualate activation is at least 10x slower. Likewise, the rapid desensitization of responses to quisqualate is approximately 5-fold slower in the presence of kainate.

Our data suggest a model for a single receptor with at least two states: quisqualate-like agonists exhibit higher affinity for the desensitized state, and antagonize the kainate response by binding to the desensitized receptor; kainate has a much lower affinity for the desensitized state and therefore acts as a full agonist.

259.10

POLYAMINES POTENTIATE NMDA RESPONSES OF RECEPTORS EXPRESSED IN *XENOPUS* OOCYTES. J.F. McGurk, R.S. Zukin and M.V.L. Bennett. Albert Einstein Col. Med., Bronx, NY 10461

In *Xenopus* oocytes injected with adult rat brain mRNA, spermine markedly increased responses to N-methyl-D-aspartate (NMDA) with glycine. Conductances were increased by up to three-fold with no change in reversal potential. Onset and recovery were rapid (<0.5 sec., limited by speed of bath application). Spermine alone did not produce a current or change the membrane resistance. Spermidine also potentiated NMDA responses; putrescine did not. Spermine potentiation increased with concentration (apparent K_d ~ 40 μM, Hill coefficient ~ 2.0) and then declined above 250 μM. The magnitude of potentiation depended on the glycine concentration, but not the NMDA concentration. At saturating levels of glycine (10 μM) and NMDA (250 μM), 250 μM spermine increased the peak response approximately 2-fold versus control. At a lower glycine concentration (0.1 μM) and 250 μM NMDA, 250 μM spermine potentiated the peak response about 3-fold. Neither the K_d nor Hill coefficient for spermine were effected by glycine or NMDA concentration. Concentration-response curves for NMDA in the presence of saturating levels of spermine and glycine showed that spermine increased the maximum response, but not the K_d or Hill coefficient. On the other hand, in addition to increasing the maximum current, spermine with 250 μM NMDA produced a leftward shift in the concentration-response curve for glycine, reducing the K_d (from ~ 930 to ~ 330 nM). Moreover, desensitization was reduced at low glycine concentrations. These results suggest that polyamines may enhance NMDA responses by an "uncompetitive" interaction at the glycine site.

259.11

A Model of the Polyamine Recognition Site of the NMDA Receptor. C.Romano, K.Williams, R.Seshadri*#, M.Israel*# and P.B.Molinoff. Depts. of Pharmacology, U. of PA., Phila., PA and , U. of TN.#, Memphis,TN.

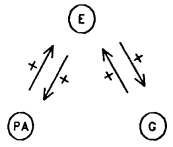
The polyamines spermine and spermidine have been shown to modulate the NMDA receptor acting at a specific polyamine recognition site. Polyamine **agonists** increase binding of [^{3}H]MK-801 above the maximal seen with L-glu + gly, **inverse agonists** decrease binding of [^{3}H]MK-801, and both of these actions are blocked by polyamine **antagonists**. The interactions of linear diamines and triamines with the NMDA receptor were examined. Binding of [^{3}H]MK-801 was measured in the presence of 100 µM L-glu + gly in the presence or absence of 10 µM spermine. Short diamines such as putrescine and cadaverine have been shown to be polyamine antagonists. As length increased, the diamines inhibited the binding of [^{3}H]MK-801 even in the absence of spermine. The inhibition (observed at 1mM) increased with diamine length. As this inhibition has been shown to be blocked by a polyamine antagonist, these compounds are acting as inverse agonists. The short triamine diethylenetriamine (TA[2,2]) was a polyamine antagonist. Longer triamines are polyamine agonists, most of which exhibited a bell-shaped concentration-effect curve, with high concentrations stimulating less than intermediate concentrations. With increasing chain length, this inhibitory region of the curve became more pronounced. The longest triamines tested, TA[3,11] and TA[3,12], did not stimulate the binding of [^{3}H]MK-801 and inhibited binding even in the absence of spermine. It is proposed that there are four amine interaction points, three in an **activation locus** and one in a **remote locus**. Polyamine agonists interact with all three sites in the activation locus and thereby enhance the binding of [^{3}H]MK-801. Polyamine antagonists interact at only two sites in the activation locus and are therefore competitive antagonists. Longer polyamines can interact with a site in the activation locus and the site at the remote locus. This leads to negative modulation that can be blocked by antagonists. This model can explain the bell-shaped concentration-response to long-chain triamines as well as the inverse agonist actions of long-chain diamines. (Supported by USPHS GM34781, NCI-CA37802, and a grant from ICI Pharmaceuticals.)

259.12

THE NMDA RECEPTOR COMPLEX: INTERACTIONS AMONG GLUTAMATE, GLYCINE AND POLYAMINE SITES. J.Lehmann, H. Canton* and F.C. Colpaert*. Neurobiology Division, Fondax-Groupe de Recherche Servier, Puteaux, France.

Agonists of three individual recognition sites - glutamate (E), glycine (G), and polyamine (PA) - promote opening of the cation channel associated with the NMDA receptor, reflected by increased [3H]MK-801 binding. These actions are not, however, independent.

Stimulating the E-site increased the affinity (2-3 x) and maximal effect (Emax; 5x) of glycine, and the affinity (10x) and Emax (2x) of spermidine. Stimulating the G-site increased the affinity (6x) and Emax (4x) of glutamate, but not the Emax and only slightly the affinity of spermidine. Stimulating the PA site increased the affinity (4x) and Emax (7-10) of glutamate, and increased the affinity of glycine (3x) but not its Emax.



Thus of the six possible interactions among the three sites, only four are robust. Specific geometry of the NMDA receptor complex is predicted by these data.

SENSORY SYSTEMS—VISUAL CORTEX: EXTRASTRIATE CORTEX

260.1

FACTORS AFFECTING RESIDUAL VISUAL SENSITIVITY IN A CASE OF HEMIANOPIA. L. Weiskrantz, Department of Experimental Psychology, University of Oxford, Oxford, OX1 3UD, England. J. L. Barbur and A. J. Harlow, The City University, Northampton Square, London, EC1V 0HB, England. (SPON: European Brain and Behaviour Society).

A well-studied "blindsight" subject (G.Y) with visual cortex damage was tested in his hemianopic field with temporally modulated sine-wave and square-wave gratings. Both the spatial and temporal parameters could be Gaussian-weighted. Detection as a function of spatial frequency, contrast, temporal modulation frequency, grating size, and the slope of the temporal and spatial Gaussian functions was investigated systematically, using a two-alternative forced-choice procedure with monitoring of eye fixation position. The most important parameters for this subject were found to be the slope of the temporal Gaussian function and the size and contrast of gratings. With optimum parameters the subject could reliably achieve a score of 100 percent correct in his "blind" field. The results are consistent with earlier studies of this subject, especially his ability to respond to moving stimuli, and also may account for why negative results had been reported for him when particular parameters were used.

260.2

CONFORMAL MAPPING OF THE VISUAL PATHWAY. George J. Carman. Salk Institute, San Diego, CA 92138.

The topographic organization of successive stages of the visual pathway can be regarded as a series of transformations of the visual field. Several investigators have attempted to model these transformations using variants of the complex logarithmic function, first proposed by Fischer (*Vis. Res.* **13**: 2113, 1973), but thus far none have been able to account for the full variation in observed topography. Here I report the determination of a composition of conformal mappings which successively transform the visual field into the topographic representations observed within the LGN and cortical areas V1 and V2 of the macaque. When applied to the hemifield with perimeter, this composition of mappings also produces an approximation to the typical shape observed for each stage of the visual pathway. Limitations of earlier efforts are overcome by modelling the observed topography of LGN (Connolly and Van Essen, *J. Comp. Neurol.* **226**: 544, 1984) by the first components of this composite mapping. Subsequent components reproduce numerous features of the known topography of V1, such as the anisotropic representation of the meridia, the central "bowing" of the isoeccentric lines, and the peripheral "convergence" of the isopolar lines (Van Essen et al., *Vis. Res.* 24: 429, 1984; Tootell et al., *J. Neurosci.* **8**:1531, 1988). Remarkably, the same mapping which produces V1 when applied to LGN also generates V2 when applied to V1. The significance of this composition of conformal mappings for the functional organization of the visual pathway will be discussed.

260.3

INDIVIDUAL AXONS (LABELED WITH *PHASEOLUS VULGARIS*) PROJECTING FROM AREA V2 TO V4 IN THE MACAQUE. K.S. Rockland. Dept. of Anatomy, Boston Univ. Sch. of Medicine, Boston, MA. 02118.

Injections of the anterograde tracer *Phaseolus vulgaris*-leucoagglutinin were made in area V2 in order to demonstrate the detailed configuration of axon arbors terminating in area V4. As of this date, 7 axons have been reconstructed in 2 monkeys. All of these axons had multiple arbors (usually 2-3); these varied in size but typically measured 150-200µm in diameter. Scanning of isolated arbors confirmed the impression that these are restricted in size. Our reconstructions showed that arbors of a given axon diverged over distances of 0.75-2.0mm. Some arbors were concentrated in layer 4, but other terminations (even belonging to the same axon) were targeted instead to both layers 3 and 4. Consistent with their origin from pyramidal-type neurons, terminal boutons were spinous in shape. Axon trunks frequently branched in the white matter; axons could also travel 2-3mm in layers 3, 5, or 6 of the gray matter of area V4 before giving off terminal arbors. Perhaps because of our small sample, these axons seemed to vary widely in terms of specific numbers, size, shape, and laminar distribution of terminal arbors. Compared with axons projecting from area V1 to V2, however, those projecting from V2 to V4 share at least two characteristics; that is, they have multiple arbors distributed over relatively large distances, and each arbor is restricted in size. (Supported by EY07058)

260.4

CORTICAL CONNECTIONS OF THE PRIMARY VISUAL AREA, V-I, OF THE GREY HEADED FLYING FOX (*PTEROPUS POLIOCEPHALUS*): EVIDENCE FOR MULTIPLE EXTRASTRIATE CORTICAL FIELDS. Leah A. Krubitzer and Mike B. Calford, Dept. Physiol. and Pharm. University of Queensland, QLD 4072 Australia

The cortical connections of the primary visual area (V-I) were investigated in the flying fox by placing single or multiple injections of anatomical tracers into V-I. In all cases, the cortex was flattened and sectioned tangentially so that areal patterns of connections could be appreciated. Sections containing transported tracer were reconstructed and superimosed on sections stained for myelin or reacted for cytochrome oxidase. Connections of area 17 with area 18 were discontinuous and formed patches across the rostrocaudal extent of this field. Area 17 was also connected with a darkly myelinated oval of cortex just rostral to area 18 (a possible homologue of the middle temporal visual area, MT, of primates), and a more lightly myelinated strip of cortex just rostral to the medial portion of area 18 (a possible homologue of the dorsomedial visual area, DM, of primates). Injections of multiple tracers into different parts of V-I in the same animal, helped us determine the topographical organization of these extrastriate cortical fields.

Because of the unique position of the megachiropteran bat in mammalian evolution (a close relative of primates), information obtained in this species could provide a better understanding of the evolution of neocortex in primates. Our results indicate that some extrastriate cortical fields identified in the primate, such as MT and DM, may also be present in other members of the Grandorder Archonta. This suggests that some cortical fields found in extant primates may be more primitive than other cortical fields. The bat is also of interest because it is a flying mammal, and its expanded visual cortex may contain features related to this behavioral specialization.

260.5

CORTICAL ACTIVATION IN HUMANS DURING VISUAL AND OCULOMOTOR PROCESSING MEASURED BY POSITRON EMISSION TOMOGRAPHY (PET). C. L. Colby and T. Zeffiro. Lab. Sensorimotor Research, NEI and Human Motor Control Section, NINDS, NIH, Bethesda, MD 20892.

In order to determine the relative involvement of different cortical areas in the performance of visuomotor tasks, we have measured changes in regional cerebral blood flow under 6 conditions: rest with eyes closed; fixation; sinusoidal pursuit; leftward pursuit; rightward pursuit; and saccade. Visual stimuli were presented on a computer monitor in total darkness and in each of the latter 4 conditions target size, total excursion, and cycle time were held constant. Eye movements were continuously monitored by EOG recordings. Measurements were obtained in healthy volunteers (n = 6) with Positron Emission Tomography (Scanditronix 2048-15B scanner) utilizing 33 mCi injections of H_2O^{15}.

The strongest cortical activation during both pursuit and saccadic eye movements was localized to the frontal eye fields. The FEF are located in the lateral portion of the motor strip, adjacent to the cortical region maximally activated by hand movement. This was confirmed in one experiment in which left and right hand stimulation replaced the left and right pursuit conditions. Additional zones of activation were observed in striate, extrastriate and parietal cortex. In frontal and occipital areas, eye movement tasks were associated with stronger activation than fixation alone.

260.6

FUNCTIONAL FIELDS IN THE HUMAN VISUAL CORTEX INVOLVED IN THE ANALYSIS OF COLOUR, FORM, AND BINOCULAR DEPTH INFORMATION, REVEALED BY POSITRON EMISSION TOMOGRAPHY (PET)
B. Gulyás, P. E. Roland, S. Stone-Elander*, and S. Holte*
PET Section, Karolinska Institute and Hospital, Box 60500, S-10401 Stockholm, Sweden

The regional cerebral blood flow (rCBF) in 9 normal young male volunteers was measured with PET, while the subjects were performing visual tasks related to a reference state as well as to the detection of colour, form, and depth (functional states). ^{15}O-butanol served as tracer and the changes in the radioactivity in the brain were monitored with an eight-ring Scanditronix PC2048-15B positron emission tomograph. Each PET measurement was preceded by a magnetic resonance (MR) scan. The head fixation used in the MR and PET scans was identical.

Both the MR and PET images were transformed to a computerized brain atlas (CBA) system. The individual images were transformed in size and shape into a standard brain (Bohm et al., *Acta Radiol. Suppl.* 369(1986):449-452). The images corresponding to the reference state were subtracted from those of the functional states. These "subtraction" images were then averaged over the 9 subjects. Correspondingly, Student-*t* images were also created in order to display significant differences between images of the reference and functional states. With the help of the CBA, the rCBF changes in the brain during different visual activations were quantified and localized.

Stimulation with colour markedly activated fields along the posterior bank of the medial inferior part of the parieto-occipital sulcus and anterior to the collateral sulcus. In addition, a small part of the precuneus and the posterior superior part of the cingular gyrus were activated. Stimulation with form caused activation in the medial part of the inferior temporal gyrus, as well as in the posterior superior part of the cingular gyrus and in the left frontal eye field. Stimulation with binocular depth cues resulted in the activation of fields around the striate cortex in a ring-like manner; as well as a strong activation in the superior medial part of the occipito-parietal gyrus.

260.7

EFFECTS OF V4 LESIONS ON VISUAL DISCRIMINATION PERFORMANCE AND ON RESPONSES OF NEURONS IN INFERIOR TEMPORAL CORTEX. R. Desimone, L. Li*, S. Lehky, L.G. Ungerleider, and M. Mishkin. Lab. Neuropsychology, NIMH, Bethesda, MD 20892.

Two Macaca mulatta were trained to maintain fixation while performing delayed match-to-sample discrimination tasks with extrafoveal stimuli. The location of the task stimuli varied among the four visual quadrants from trial to trial; on a given trial, the sample and test stimuli were presented in the same quadrant. Following acquisition, the lower field representation of area V4 in one hemisphere was ablated. In the quadrant corresponding to the lesion, as compared to the control quadrants, luminance discrimination was nearly normal, but discrimination thresholds for color and orientation matching were highly elevated and performance on pattern and texture matching was moderately impaired. Inferior temporal neurons, recorded in one monkey performing the task, clearly responded to stimuli presented in the quadrant corresponding to the lesion, but the responses were smaller and less discriminative than those to stimuli presented in the control quadrant of the same hemifield. The results suggest that V4 is an important but not the sole source of both color and form information to the temporal cortex.

260.8

CODING OF ORIENTATION BY INFERO-TEMPORAL NEURONS STUDIED IN THE DISCRIMINATING MONKEY. G.A. Orban and R. Vogels. Laboratorium voor Neuro- en Psychofysiologie, K.U.Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium.

Single cells (n=497) and multiunit activity (n=254) were recorded in infero-temporal cortex (IT) of a monkey extensively trained to perform fine orientation discriminations. The task of the monkey was to discriminate between a sequence in which two gratings presented in succession were identical and a sequence in which they differed in orientation. The orientation of the first grating was randomly changed between trials and the orientation difference was 15 deg in the standard condition. Thirty-two percent (157/497) single cells were driven by the grating presentation , the other cells either did not react or were inhibited. Sixty-seven percent (171/254) of the multiunit recordings responded excitatorily to the grating. Enough valid trials were collected in 103 responsive cells to estimate the 4 parameters determining the neuronal discriminative capacity in this task. Median orientation bandwidth was 66 deg (quartiles 43 and 99 deg). Median response strength (average firing rate) was 22 spikes/sec (quartiles 13 and 41 spikes/sec). Median normalized variance (variance divided by response strength) was 2.47 (quartiles 1.29 and 3.92). Median latency was 100-120 msec (quartiles 80-100 msec and 140-160 msec). Comparison of these characteristics with those obtained in V1 in the same animal suggests that on average orientation representation is not more accurate in IT than in V1.

In 23 cells we attempted to manipulate the level of attention by increasing the difficulty of the task. This was done by reducing the orientation difference from 15 to 7 deg. In 4 out of 23 cells this increased the slope of the tuning curves. This suggests that in IT the accuracy of the orientation representation can be improved by 'attention' as reported for V4 by Spitzer et al. (*Sci*, 240:338-340, 1988).

260.9

EFFECTS OF TASK RELATED STIMULUS ATTRIBUTES ON INFERO-TEMPORAL NEURONS STUDIED IN THE DISCRIMINATING MONKEY. R. Vogels and G.A. Orban. Laboratorium voor Neuro- en Psychofysiologie, K.U.Leuven, Campus Gasthuisberg, B-3000 Leuven, Belgium.

Single cells were recorded in infero-temporal cortex (IT) of a monkey performing a same-different orientation discrimination task (Orban & Vogels, *Soc.Neurosci.Abstr.* 1990). In order to asses the influence of order of presentation we calculated an index (S1-S2)/(S1+S2) which ranges from -1 to 1 depending on whether the cell only responds to the second grating stimulus (S2) or only to the first stimulus (S1). The distribution of this index for IT cells (n=116) was significantly broader than the distribution obtained in the same animal in V1 (n=78). This shows that in this sequential discrimination task the order of presentation influences IT cells more than V1 cells.

A number of cells fired in the interval between the successive grating presentations. In many instances these cells also responded to the grating presentation, but a number of cells fired only in the interval. With few exceptions, this delay activity was orientation tuned. Finally a number of cells fired much more when the second stimulus differed from the first one than when it did not. This difference in response could not be accounted for by the orientation or the order of presentation of the stimulus. This suggests that a number of IT cells encode whether or not the second stimulus differs in orientation from the first. These results indicate that IT cells do not only encode the physical attribute of the stimulus but are also influenced by task related attributes of the stimulus.

260.10

RIGHT INFERO-TEMPORAL CORTEX PET ACTIVATION DURING OBJECT RECOGNITION TASKS IN HUMANS. M.Corbetta, F.M.Miezin, P.T.Fox, S.M.Dobmeyer, S.E.Petersen. Dept. Neurol. and Neurol.Surgery, McDonnell Ctr. Higher Brain Function, and Mallinckrodt Inst. of Radiology, Washington Univ. School of Med., St.Louis, MO 63110; Neuroradiol. Sect., Johns Hopkins Univ., Baltimore; Dept. Neurol., Univ. of Iowa, Iowa City.

Normal subjects were studied with PET activation methods during visual recognition tasks of objects defined either by a simple feature (i.e. color) or by a feature conjunction (i.e. color by orientation). Four stimuli were simultaneously flashed for 100 msec at 1Hz around the fixation point. Subjects were instructed to search for a specific target (e.g. a red square among non-red squares, or a red bar oriented 90° among red and non-red differently oriented bars), and target probability was manipulated (50% vs 5%). One group had to report by key-press (overt task) the presence or the absence of the target. Another group had to monitor the probability of target occurrence, and report it only after PET data collection (covert task). Accuracy in all conditions was above 90%. PET subtraction images of blood flow change indicated that right infero-temporal cortex was more active when many targets (as compared to few targets) were presented in both simple feature and conjunction recognition tasks. In the overt task the activations were localized in posterior infero-temporal cortex (at or near area 37 of Brodmann). In the covert task the activations were located more anteriorly on the middle temporal gyrus (at or near area 21). Bilateral activations in the overt task were also found in primary visual cortex, lingual and fusiform gyri. These results support the notion of functional asymmetry in humans for object recognition operations, and suggest multiple functional subdivisions in human infero-temporal cortex.

260.11

WITHDRAWN

260.12

A NEURAL ANALOGUE IN MACAQUE LATERAL INTRAPARIETAL CORTEX (LIP) OF THE END-POINT UP-SHIFT OF MEMORY-GUIDED SACCADES MADE IN THE DARK. R.M. Bracewell, S. Barash and R.A. Andersen, Dept. Brain & Cog. Sci., MIT, E25-236, Cambridge, MA 02139.

We have previously shown that memory-guided saccades tend to show a systematic constant error: their end-points are shifted upwards with respect to the location of the target (Gnadt et al., Soc. Neurosci. Abst. **13**: 1090, 1987). Here we report an analogue in the activity of area LIP neurones recorded whilst rhesus monkeys performed an identical delayed saccade task. We determined the preferred directions of individual neurones during the various phases of the task: visual (V), memory-related planning (M), and saccadic (S). In general, the vertical components of the M and S preferred directions were shifted upwards with respect to the V preferred directions. The up-shift was much greater for the S than the M activity. On the other hand, the horizontal components of the V, M and S preferred directions did not vary systematically. These results suggest that the up-shift in memory saccade end-points is largely due to the sensorimotor transformations underlying the production of such saccades, and not due to sensory processing.

CALCIUM CHANNELS I

261.1

MULTIPLE FORMS OF INACTIVATION OF THE N-TYPE CALCIUM CURRENT. *Mark R. Plummer, Max Kanevsky* & Peter Hess**. Department of Cellular & Molecular Physiology, Harvard Medical School, Boston, MA 02115.

Calcium currents in mammalian neurons are subject to several forms of voltage- and current-dependent inactivation, and these properties have become important criteria in the characterization of different types of calcium channels. This is especially true for the N-type calcium channel where the time course of inactivation during a test pulse and the reduction of current at relatively positive holding potentials are the main physiological means of distinguishing N-type from L-type calcium current. However, inactivation of the N-type current has been shown to vary widely from one recording to the next, making a quantitative analysis of inactivation problematic. To understand better the sources of this inactivation, we have used a variety of voltage protocols and ionic substitutions to separate voltage-dependent from current-dependent inactivation. We have found that voltage-dependent inactivation develops slowly and is responsible for the observed holding potential dependence of the N-type current whereas the current-dependent inactivation produced by either calcium or barium acts on a more rapid time scale and may contribute to the variability of inactivation during a test pulse.

261.2

PHARMACOLOGY OF N-TYPE CALCIUM CHANNEL MODULATION IN SYMPATHETIC NEURONS. *Ann R. Rittenhouse, Mark R. Plummer, Max Kanevsky* & Peter Hess**. Dept. of Cell. & Molec. Physiol., Harvard Med. Sch., Boston, MA 02115.

Calcium currents in rat sympathetic neurons are modulated by a variety of neurotransmitters and second messengers. Controversy exists, however, over which type of calcium channel is affected. By analyzing tail currents carried exclusively by dihydropyridine (DHP) sensitive L-type calcium channels, we found that α_2-adrenergic and muscarinic agonists, the neuropeptide NPY, and GTP-γ-S reduced selectively the DHP-insensitive component of current which corresponds to N-type current in these cells. For example, 100 μM norepinephrine (NE) inhibited peak current by 38% while having minimal effects (< 5% reduction) on the L-type tail current. Clonidine (100 μM), which has no effect on chick DRG and frog sympathetic neurons, decreased peak calcium current while phentolamine (10 μM) and yohimbine (10 μM) reversed the inhibition produced by 1 μM NE. In addition, 100 μM acetylcholine (ACh) or bethanechol, and 100 nM NPY also reduced peak inward current by 29%, and 27%, respectively, with no significant effect on L-type current. Incubation with pertussis toxin eliminated the responses to both ACh and NE, confirming the involvement of GTP-binding proteins. Extracellular application of the protein kinase C activator OAG (50 μM) reduced the peak current by 57% but, unlike all other substances tested, also reduced the L-type tail current by a comparable amount. Thus muscarinic and adrenergic modulation in these cells appears to target selectively the N-type calcium current and involve inhibitory G-proteins.

261.3

NEUROTRANSMITTER MODULATION OF N-TYPE Ca CHANNELS BY SHIFTS BETWEEN MODES OF GATING. K.R. Bley, D. Lipscombe & R.W. Tsien. Department of Molecular & Cellular Physiology, Stanford University, Stanford, CA 94305.

We have investigated the mechanism by which α-adrenergic and peptidergic agents inhibit Ca channels in frog sympathetic neurons. Norepinephrine (NE) or LHRH decrease N-type Ca channel activity via a G-protein, but not through readily diffusible second-messengers or H-7-sensitive protein kinases, consistent with inhibitory effects on K^+-evoked transmitter release (Lipscombe et al. 1989; Bley & Tsien, 1990). In cell-attached recordings (110 mM Ba), the open time distribution of N channels displays two components, τ_{fast} = 0.3 ms and τ_{slow} = 1.7 ms at -10 mV. With improved frequency resolution, we find that these components arise from two different patterns of N-type channel gating: one with a relatively low open probability (p_o<0.04 at -10 mV) and larger unitary amplitude (~0.9 pA), and the other with a higher channel open probability (p_o ~0.3) and smaller unitary amplitude (~0.7 pA). Switching between the two modes is relatively slow and occurs even in one-channel patches. In the "low p_o" mode, the channels require at least 30 mV more depolarization to achieve the same opening probability as in the high p_o mode (cf. Bean, 1989, Plummer et al. 1989). We find that inclusion of 30 μM NE in the patch pipette greatly decreases the relative proportion of "high p_o" sweeps and increases the percentage of "low p_o" sweeps but leaves the pattern of gating within each mode unchanged. Thus, the average current carried by the N-type channel is strongly but not completely inhibited.

To observe reversible changes in N channel kinetics in the same patch, we have turned to outside-out patch recordings. Ca channel activity can persist for more than one hour with standard whole-cell internal solutions in the pipette. Moreover, N- and L-type Ca channels retain activation and inactivation properties similar to those in cell-attached recordings. We have seen instances of reversible modulation of N-type channel activity by LHRH and NE, and irreversible inhibition by ω-conotoxin.

261.4

CALCITONIN-GENE RELATED PEPTIDE(CGRP) INCREASES THE N-TYPE CALCIUM CURRENT AND ACETYLCHOLINE(ACh) RELEASE IN VAGAL SENSORY(NODOSE) NEURONS VIA A PERTUSSIS TOXIN(PTX)-SENSITIVE MECHANISM. J.W. Wiley[1], R.A. Gross[2] and R.L. Macdonald[2], Depts. Internal Medicine[1] and Neurology[2], Univ. of Michigan Medical Center, Ann Arbor, MI.

CGRP is present in primary sensory neurons and may have important regulatory functions. Because neurosecretion is associated with calcium entry via voltage-gated channels we tested: 1. Whether CGRP affects calcium current(s) and classical neurotransmitter release in nodose neurons and 2. Whether the peptide's mechanism of signal transduction involves guanine nucleotide binding (G)-proteins. Whole cell patch clamp studies were performed on acutely dissociated nodose ganglion neurons from 7 - 10 d rats. Calcium currents were recorded using external and internal media which blocked sodium and potassium currents. Nodose neurons exhibited T, L and N-type calcium current components. CGRP (1 - 1000 nM) had no effect on isolated T and L current components (n=12) but produced a concentration-dependent increase in the combined N/L current in 24/36 of cells evaluated. CGRP (1 μM) increased the combined N/L current by 24 $\pm$ 4% (n=15) which was blocked by pretreatment with PTX (100 ng/ml for 12 h, n=12). CGRP did not alter the voltage-dependence of the current-voltage relation and the peptide increased the peak current by the same magnitude when neurons were held at V_h= -60 or -90mV. CGRP (1 μM) produced a 22 $\pm$ 5% increase in ^{3}H-ACh release over basal levels (n=12) which was reduced by 76 $\pm$ 8% (n=8) after pretreatment with PTX (250 ng/ml for 3 h). In summary, CGRP selectively increased the N current component and acetylcholine release in nodose neurons via a PTX-sensitive mechanism.

261.5

TIME COURSE OF NEUROTRANSMITTER EFFECTS ON VOLTAGE-DEPENDENT CURRENTS OF BULLFROG SYMPATHETIC NEURONS. Stephen W. Jones. Dept. Physiol. & Biophys., Case Western Reserve Univ., Cleveland, OH 44106.

Several transmitters inhibit the N-type calcium current and the M-type potassium current in bullfrog sympathetic neurons. G proteins appear to be involved, but the receptor-channel coupling mechanism is otherwise unknown. Since the timing of the effects constrains potential mechanisms, a rapid (~100 msec) flow tube perfusion system was used to apply agonists to isolated cells, under whole-cell voltage clamp. High concentrations of low affinity agonists (salmon LHRH or muscarine) were used to speed receptor kinetics.

Part of the effect develops and recovers rapidly, limited partially or entirely by the solution exchange time. However, much of the effect is slower (> 1 sec). An even slower leak increase is sometimes seen. This suggests that more than one mechanism may be involved in receptor-channel coupling.

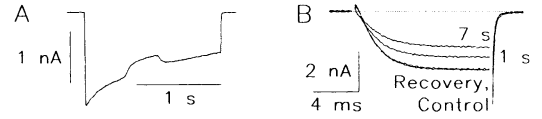


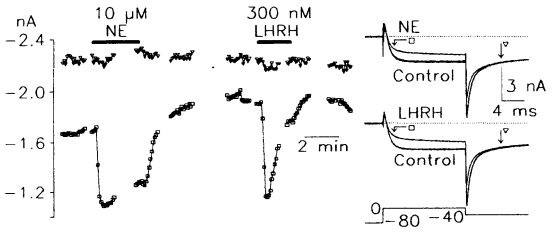
Figure: 2 μM Salmon LHRH was applied for ~0.4 s during a long voltage step (*A*) or for 30 s with brief test steps every 2 s, with maximal effect at 7 s (*B*). *A* and *B* are from the same cell. Calcium currents (2 mM Ba^{2+}) for steps to -10 mV from -80 mV.

261.6

DOES LHRH OR NOREPINEPHRINE AFFECT L-TYPE CALCIUM CURRENT IN FROG SYMPATHETIC NEURONS? Keith S. Elmslie & Stephen W. Jones, Dept. of Physiology & Biophysics, Case Western Reserve University, Cleveland, OH 44106

Whole-cell calcium current in bullfrog sympathetic neurons is mainly comprised of N-current (~90%), which can be reduced by neurotransmitters (e.g. chicken II luteinizing hormone-releasing hormone (LHRH) and norepinephrine (NE)).

We have examined the effects of NE and LHRH on cells exposed to ω-conotoxin (to reduce N) and +202-791 (to enhance L). Under these conditions current at 0 mV is a mixture of N & L, while >80% of the slow tail current at -40 mV is L-current. NE and LHRH reduce current at 0 mV (33.4% ± 11.9%) (s.d., n = 6) and the fast tail, but not the slow tail (3.3% ± 3.3%). In several cells the slow tail was slightly enhanced (~8%). Thus, LHRH and NE do not appear to significantly inhibit L-current in these cells.



261.7

DYNORPHIN A REDUCTION OF NEURONAL CALCIUM CURRENT IS ENHANCED BY CYCLIC AMP-DEPENDENT KINASE.

R.A. Gross, H.C. Moises@, M.D. Uhler*# and R.L. Macdonald, Dept. of Neurology, @Physiology and the #Mental Health Research Institute, U of Michigan, Ann Arbor, MI 48104.

Dynorphin A (DYN), a κ-selective opioid peptide, reduces neuronal adenylate cyclase (AC) activity and reduces voltage-dependent calcium currents, but the pathway by which it affects channel activity is not known. We tested the effect of DYN on the T, N and L calcium current components of acutely-dissociated rat nodose ganglion neurons and compared its effect to that of the catalytic subunit of cyclic AMP-dependent kinase (AK-C). We reasoned that if a reduction in AC activity were required for DYN-induced reduction of neuronal calcium current, this effect would not be apparent in the presence of AK-C.

We recorded whole cell calcium currents from acutely-dissociated nodose ganglion neurons from 7-10 d rats. The bath medium contained (mM): 10 Hepes, 67 choline, 100 TEA, 5.6 glucose, 5.3 KCl, 0.8 $MgCl_2$, 5 $CaCl_2$ (pH 7.3); the recording pipette (0.5-1.25 MΩ) contained 10 Hepes, 140 CsCl, 10 EGTA, 5 ATPMg, 0.1 GTP or GTP-γ-S. DYN selectively reduced N current in a reversible, naloxone-sensitive manner. The effect was blocked by pretreatment with pertussis toxin and mimicked by GTP-γ-S. In the presence of AK-C, run-down of N and L currents was prevented and the effect of DYN was greater than in the absence of AK-C. These results suggest that the acute reduction of calcium current by DYN was mediated by activation of G_i- or G_o-type G proteins, independent of an effect on the AC-cyclic AMP system, and that phosphorylated channels were preferentially inhibited by DYN. *Supported by NIH NS01019 & NS19613 (RAG) DA03365 (HCM) and DA04122 (RLM).*

261.8

THE EFFECTS OF PDAc, H-7 AND GM-1 ON THE HIGH THRESHOLD SLOWLY INACTIVATING Ca CURRENT IN THE RAT HIPPOCAMPAL SLICES. N. Agopyan, P. Miu & K. Krnjević, Anaesthesia Res. Dept., McGill University, Montreal, Que., Canada.

Protein kinase C (PKC) activators, block the slow AHP mediated by the Ca dependent G_K. Since a supression of Ca currents may explain the inactivation of Ca dependent G_K we investigated the effects of a) phorbol 12,13-diacetate (PDAc), a PKC activator, and b) H-7 and GM-1, both PKC inhibitors (Hidaka et al. 1984, Biochem, 23, 5036; Kreutter et al. 1987, J. Biol. Chem. 262,1633; Cimino et al. 1987, Acta Physiol. Scand. 130, 317), on the high threshold Ca current recorded with CsCl-filled electrodes at V_H -50 mV in hippocampal slices (at 32°) superfused with ACSF, containing TTX (1 μM), TEA (10mM), Cs (4 mM) and 4-AP (0.5 mM).

PDAc (10 μM) induced a persistent inward current, and increased the high threshold Ca current (62 ± 20 %) as well as the leak conductance (29 ± 9 %). H-7 (30 μM) induced an outward current and reduced the high threshold Ca current by 85%. Like trifluoperazine, which acts as a dual PKC/Ca-calmodulin dependent kinase inhibitor, GM-1 (0.1 μM) induced a persistent inward current and reduced the high threshold Ca current (24 ± 4 %). All of the effects were reversed 30 min after the application ended. During a concomitant application of GM-1 or H-7 the effects induced by PDAc were not observed.

In conclusion, in contrast to observations obtained from acutely dissociated hippocampal neurones (Doerner et al. 1988, J.Neurosci. 8,4069) our data show that activation of PKC enhances the high threshold Ca current.

Supported by SAVOY foundation and MRC of CANADA.

261.9

EVIDENCE FOR TWO MODES OF Ca CHANNEL REGULATION BY PKC IN HIPPOCAMPAL NEURONS. D. Doerner & B.E. Alger, Dept. of Physiol., Univ. of MD Sch. Med., Baltimore, MD 21201

Previous studies have implicated protein kinase C (PKC) in the regulation of Ca channels in hippocampal neurons. We have now compared the effects of synthetic diacylglycerols, DiC_8 and OAG, with those of phorbol dibutyrate (PDBu) on both whole-cell Ca channel current (I_{Ba}) and single Ca channels and find evidence of two modes of Ca channel regulation by PKC. High-voltage-activated I_{Ba} is predominantly suppressed by PKC activators in whole-cell recordings, although an initial, transient enhancement is frequently observed in cells treated with OAG (20-300 μM), or DiC_8 (1-200 μM), but not PDBu (0.05-5 μM). The suppressant effects of DiC_8 and PDBu are reversible with washing and blocked by dialysis with the peptide pseudosubstrate inhibitor (19-36 PKCI, n=11).

Diacylglycerols or PDBu caused an enhancement (3-10 min) followed by depression of the activity of single L-type Ca channels in cell-attached patches in 15/23 cases, although persistent increases (3/23) and monophasic decreases (3/23) were also seen. Similar results were obtained in preliminary experiments with carbachol (0.5-1 mM, n=5), an agonist that activates PKC in hippocampal neurons. Both enhancement and suppression of Ca channel activity appear to be due to PKC, possibly reflecting the activation of different isomers. Modulation of spontaneous synaptic activity in cultured neurons following DiC_8 application qualitatively resembles Ca channel modulation and is under study.

261.10

INACTIVATION OF HVA CALCIUM CURRENTS IN GRANULE CELLS FOLLOWING KINDLING-INDUCED EPILEPSY: THE Ca^{2+}-BUFFERING ROLE OF CALBINDIN-D_{28K} (CaBP). G. Köhr, C.E. Lambert* and I. Mody, Dept. of Neurology & Neurol. Sci., Stanford Univ. Sch. of Med., Stanford, CA.

In granule cells (GCs) of the dentate gyrus, kindling-induced epilepsy results in a progressive loss of a neuron-specific cytoplasmic high affinity calcium binding protein (Calbindin-D_{28K}; CaBP). The exact function of CaBP in neurons is unknown, but it has been postulated to serve as an effective high-capacity intracellular Ca^{2+} buffer.

We have shown immunohistochemically that acutely dissociated kindled neurons have low levels of cytoplasmic CaBP and consequently might have lost their calcium buffering capacity. We have investigated this hypothesis by examining the Ca^{2+}-dependent inactivation of Ca^{2+} currents in acutely dissociated GCs from hippocampal slices of control and commissurally kindled (stage 5) rats. Maximal HVA Ca^{2+} currents could be evoked from holding potentials of -50 mV to command potentials of +10 mV. These were: 216.4±54.8 pA (mean±SD; n=10) in control GCs and 202.1±53.6 pA (n=11) in kindled GCs. Conditioning voltage steps to +10 mV induced inactivation of a subsequent test Ca^{2+} current evoked 50 ms later. In control GCs the test Ca^{2+} currents inactivated by 37.4±11.9%. In kindled GCs the inactivation was significantly increased to 58.3±8.3%. The recovery from inactivation of the Ca^{2+} currents was slower in kindled than in control GCs. The decay of HVA Ca^{2+} currents evoked by 1 s voltage steps was more rapid in kindled GCs than in controls. When neurons were loaded with the Ca^{2+} chelator BAPTA, Ca^{2+} current inactivation was prevented in both control and kindled neurons. These results strongly support the Ca^{2+} buffering role of CaBP in GCs and its involvement in the regulation of HVA Ca^{2+} current inactivation.

Supported by the EFA, NIH and DFG.

261.11

DOPAMINE SELECTIVELY INHIBITS HIGH-THRESHOLD CALCIUM CURRENTS IN RAT LACTOTROPHS. D. Janigro, G. Maccaferri* and J. Meldolesi*. Dept. of Pharmacology, Istituto Scientifico San Raffaele, Milano, ITALY.

Calcium currents were investigated in rat lactotrophs. Patch-clamp experiments, using the whole cell configuration mode, were performed on cells that had been kept in culture for different periods of time. Calcium currents were elicited (after blockade of potassium currents with intracellular CsCl and extracellular TEA) by depolarizing voltage steps from a holding potential of -100 mV. In cells cultured for one day, currents showed properties typical of high-threshold, L-type channels, while cells cultured for three days appeared to express both high-threshold and low-threshold, T-type channels. Application of dopamine (10 uM) reduced the L-type component. This effect was abolished by pretreatment of the cell with the D_2 inhibitor, l-sulpiride, and pertussis toxin. In contrast it was unaffected by 8-bromo-cAMP. We conclude that both L- and T- type calcium currents are expressed in rat lactotrophs. Their ratio can, however, vary depending on the culturing conditions, and only one of them (the L-type) is blocked by dopamine, possibly via a G_i protein-mediated interaction of channels with the activated D_2 receptor.

261.12

LARGE PRE-DEPOLARIZATIONS, BUT NOT TRAINS THAT MIMIC ACTION POTENTIALS, REVERSE INHIBITION OF I_{Ca} BY 5-HT. N.J.Penington, J.S.Kelly and A.P.Fox* Univ. Chicago, Dept.Pharm/Phys, Chicago, Il. 60637. & Dept. Pharmacol. Univ. Edinburgh U.K.

5-HT_{1A} receptor activation inhibits I_{Ca} in acutely isolated adult rat dorsal raphe neurons (Penington & Kelly, Neuron, 1990). The effect is voltage dependent with little inhibition at positive potentials. The rate of activation of I_{Ca} is slowed (the channels may go from a "reluctant" gating state, to a "willing" gating state at positive potentials Bean, Nature, 1989). A pre-pulse to +80 mV for 150 ms, followed by a 10 ms return to the holding potential (-100 mV) completely reversed the 5-HT mediated inhibition of I_{Ca} elicited during a test pulse to -10 mV ; similar results were obtained with LHRH inhibition of frog sympathetic neuron I_{Ca} (Elmslie & Jones Soc. Neurosci., 1989). Trains of voltage clamp steps designed to mimic action potentials in DR neurons were applied to see if they could also reverse the action of 5-HT. In 10 μM 5-HT (which inhibited I_{Ca}) a test depolarization to -10 mV from a H.P. of -60 mV was followed by a train of 21 voltage jumps to +30 mV, each 5 ms in duration applied every 60 ms. The train was followed 60 ms later by another test jump to -10 mV to measure I_{Ca}. The train reversed the effect of 5-HT by only 4.6%. This finding suggests that under physiological conditions raphe neurons cannot significantly reverse the effect of 5-HT inhibition of I_{Ca} simply by firing faster. Periods of unusual excitation produce effects which do not persist long enough to alter the effect of 5-HT on subsequent action potentials. Supported by the Wellcome Trust.

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS V

262.1

PIONEERING GROWTH CONES IN THE EMBRYONIC ZEBRAFISH BRAIN. S.W. Wilson, and S.S. Easter Jr. Department of Biology, University of Michigan, Ann Arbor, MI 48109.

By 24 hours post-fertilisation axons in the embryonic zebrafish brain have established a simple stereotyped scaffold of axon tracts (Wilson et. al. Development 108:121, 1990). Using neuroanatomical tracing techniques combined with light and electron microscopy, we have examined the development of one of these early tracts, the dorsoventral diencephalic. We find that it is pioneered at a precise time in development by the growth cone of a single neuron present in an identified location. The soma of this neuron is located in the anlage of the pineal and extends a growth cone which courses superficially and ventrally, between the end feet of neuroepithelial cells located along its pathway. These same cells have processes which envelop the axon, but we see no indication of any specialisation ahead of the growth cone. Growth cone filopodia extend in many directions, including deep into the brain, occasionally inserting into the neuroepithelial cells. Midway down the diencephalon the growth cone encounters neurons and axons within the tract of the postoptic commissure (TPOC), at which point it invariably turns rostrally. It fasciculates with these TPOC axons and passes contralaterally through the postoptic commissure.

Supported by SERC/NATO (BRF8217) to S.W.W. and NIH (EY00168) to S.S.E.

262.2

MULTIPLE MECHANISMS OF GROWTH CONE GUIDANCE IN THE ZEBRAFISH BRAIN. A.B. Chitnis & J.Y. Kuwada. Dept. Biology & Neurosci. Prog., U. of Michigan, Ann Arbor, MI 48109.

The early zebrafish brain contains a simple scaffold of longitudinal tracts connected by commissures. Growth cones of neurons in the nucleus of the posterior commissure (nuc PC) follow a stereotyped pathway within the scaffold. Nuc PC growth cones extend ventrally along the PC to the anterior tegmentum where a number of tracts intersect. In principle the growth cones could extend along any of these tracts, but always turn posterior to descend in the tract of the postoptic commissure (TPOC) to the hindbrain without sending collaterals into the other tracts. To identify mechanisms that guide the nuc PC growth cones, we have manipulated embryos to see how nuc PC growth cones behave following elimination of the TPOC (n=46). In the absence of the TPOC nuc PC growth cones follow inappropriate tracts in 55% of embryos. This suggests that cues associated with the TPOC help guide nuc PC growth cones, and that nuc PC growth cones are not inhibited or prevented from entering other tracts. However, since in 45% of embryos nuc PC growth cones did follow their normal pathway despite the absence of the TPOC, other cues in the tegmentum, independent of the TPOC, may also be capable of guiding nuc PC growth cones. Thus multiple mechanisms may operate simultaneously to insure that all nuc PC growth cones follow their appropriate pathway. (Supported by NIH, MOD, and UM.)

262.3

ALTERATION OF MOTOR NERVE PATHWAYS BY ECTOPIC FIN BUDS IN THE JAPANESE MEDAKA FISH. H. Okamoto & J.Y. Kuwada, Dept. of Biology, Univ. of Michigan, Ann Arbor, MI 48109.

The pectoral fin muscles of the Japanese Medaka fish are normally innervated by motor axons from segments 1-4. During embryogenesis these axons extend toward the fin bud and converge to form a plexus at the base of the fin bud in S2. Nerves in more posterior segments follow a pathway parallel with the segmental borders of the axial muscles. Previously we showed that the fin bud is necessary for outgrowth by the motor nerves of S1-4 to the base of the fin bud by ablating the fin bud. In such embryos motor nerves from S1-4 extended along a pathway parallel to the segmental borders of the axial muscles much like in the more posterior segments. This suggested that the motor growth cones in S1-4 normally extend to the base of the fin bud because they are attracted to it. We have now confirmed this hypothesis by transplanting fin buds to ectopic sites. When fin buds were transplanted to more posterior segments, motor nerves from S1-4 extended to the base of ectopic buds. In these cases the S2 motor nerve which normally extends laterally and S3 and S4 motor nerves which normally extend anteriorly instead all extended posteriorly. One possible mechanism for the attraction of motor growth cones by the fin bud is a long distance cue emitted by the fin bud. (Supported by Toyobo Biotechnology Foundation, NIH, MOD, and UM.)

262.4

GROWTH CONES OF ECTOPIC MOTONEURONS SELECT NORMAL PATHWAYS IN EMBRYONIC ZEBRAFISH. C. L. Gatchalian and J. S. Eisen. Institute of Neuroscience, University of Oregon, Eugene, OR 97403.

The growth cones of identified primary motoneurons in embryonic zebrafish follow cell-specific pathways and innervate non-overlapping regions of muscle. To learn whether the motoneurons are able to select their normal pathways when removed from their original positions we labeled CaP, the first primary motoneuron to extend a growth cone, and transplanted it to an ectopic location outside of the spinal cord in unlabeled host embryos. We found that the growth cones of most of the surviving transplanted CaPs (23 of 27) extended along the normal cell-specific CaP pathway into the ventral muscle. This result suggests that the pathway itself may provide cues that guide the CaP growth cone. To learn whether these putative guidance cues are generally recognized by other growth cones, we transplanted labeled primary sensory Rohon-Beard neurons to the same location. In contrast to transplanted CaPs, 8 of 8 surviving transplanted Rohon-Beard cells elaborated a network of processes that projected in many directions, but showed no preference for the CaP pathway. These results suggest that primary motoneurons and primary sensory neurons are guided by different pathway cues. Supported by the NIH, NSF, and Proctor and Gamble Company.

262.5

CIRCUMFERENTIAL GUIDANCE CUES FOR PIONEER GROWTH CONE MIGRATION IN GRASSHOPPER EMBRYONIC LIMB BUDS. T. P. O'Connor, L. Gorodezky*, A. Torojan-Raymond* and D. Bentley, Dept. of Mol. and Cell Biol., University of California, Berkeley, CA 94720.

Axonal growth cones in a variety of vertebrate and invertebrate systems appear to be guided by molecular information available at the basal surface of ectodermal epithelium or neuroepithelium. In embryonic grasshopper limb buds, for example, the Til pioneer afferent neurons arise at the limb tip and their growth cones migrate proximally to the CNS along a stereotyped pathway defined by cues provided by the ectodermal epithelium and nascent 'guidepost' neurons derived from it. The epithelium is a two dimensional surface with a proximo-distal axis and an orthogonal circumferential dorso-ventral axis. While most analyses to date have addressed the nature of proximo-distal guidance, many cell and growth cone migrations in both the anterior and posterior limb compartments include oriented segments along the dorso-ventral axis. We report three kinds of experiments which indicate that ventrally polarized guidance information is present in the epithelium. (1) selective elimination of specific cells by timed heat shocks indicates that ventral re-orientation of growth cones is independent of the presence of ventrally located guidepost neurons; (2) time-lapse video of double labeled pioneer growth cones and ventral guidepost cells indicates that the ventral growth cone turns are initiated before contact with guideposts; (3) microsurgical manipulation of limbs so that growth cones are presented with a binary choice indicates that ventral epithelial cells can mediate re-orientation. Double-labeling of neurons and compartments shows that growth cones from both compartments collect near the ventral compartment boundary and turn proximally toward the CNS along a series of cells at the ventral-most region of the anterior compartment.

262.6

PATTERNED SPECIFICATION OF EPITHELIAL SUBSTRATE OCCURS PRIOR TO PERIOD OF AXON OUTGROWTH IN THE DEVELOPING WING OF *DROSOPHILA*. S.S.Blair and E.Rulifson*. Dept. Zoology, Univ. Wisconsin, Madison, WI 53706.

During metamorphosis of *Drosophila*, sensory neurons arise within the wing imaginal disc and project axons over the disc epithelium towards the CNS. These axons follow specific routes, establishing a stereotyped pattern of nerve bundles. Previous work strongly suggests that the cues responsible for guiding these axons are found within the epithelial substrate; however, the wing epithelium appears morphologically uniform during this period. We have therefore begun using the "enhancer trap" technique to examine patterns of differential gene expression in the epithelial substrate. Preliminary results (using trap lines kindly provided by Drs. Carroll, Laughon, and Ganetzky) indicate that complex patterns of gene expression are present in the wing epithelium prior to the period of axon outgrowth. Moreover, many of the patterns observed define portions of the normal axon pathways.

Work is underway to develop tests for the possible roles of known and unknown gene products in defining these pathways. We will report in particular upon a variation upon the mitotic recombination technique which uses beta-gal expressing inserts to mark clones in the developing wing, allowing the examination of the effects of lethal mutations during axon outgrowth.

262.7

GROWTH CONES IN DROSOPHILA EMBRYOS SHOW STEREOTYPIC MORPHOLOGY AND BEHAVIOR. A. Chiba, T.N. Chang, M.E. Halpern & H. Keshishian, Dept. of Biology, Yale Univ., New Haven, CT 06511

The neuromuscular synapses in *Drosophila* embryos are highly stereotypic. By characterizing individual motoneurons and the dynamics of growth cone movements in mid-embryogenesis (st. 15-16), we find that each growth cone shows a specialized response as it contacts its appropriate target. A specific set of motoneurons are consistently labeled when the motor nerve terminals on a muscle fiber are backfilled with the vital dye DiI. While DiI labeling demonstrates the cell body locations of all motoneurons, the detailed morphology of individual neurons is revealed through intracellular dye-filling with Lucifer yellow. From nerve SNb, motoneuron RP1 (i.e. MN6-7) innervates muscle fibers 6 and 7. Two other motoneurons innervate muscle fiber 13 through the same nerve; the soma of MN13a is ipsilateral and in the same segment as its target, while the soma of the MN13b which innervates the same fiber is contralateral and one segment anterior. The locations and relative development of all SNb motoneurons are demonstrated by anti-HRP staining. Double labeling of dye-filled motoneurons shows that their growth cones exit the nerve from characteristic sites to grow directly to their target muscle fibers where they differentiate anatomically distinct synapses. We are able to visualize growth cone behavior *in situ* in living filleted embryos by time-lapse video microscopy and DiI injection. CNS injection labels one or a small ensemble of growth cones at the growing tip of nerves SN and ISN. These growth cones are capable of extending filopodia of various lengths (2-12 μm) and sampling the surfaces of muscle fibers adjacent to the nerve, including those which are not normal targets. These results indicate that motoneurons are matched to individual target muscle fibers, and that synaptogenesis in *Drosophila* may involve specific cell-cell recognition events between growth cones and their targets.

262.8

DEVELOPMENT OF SITE-SPECIFIC SYNAPSES BY THE DROSOPHILA MOTONEURON RP1 INVOLVES STEREOTYPIC FILOPODIAL CONTACTS DURING EMBRYOGENESIS M.E. Halpern & H. Keshishian. Dept. of Biology, Yale Univ., New Haven, CT 06511.

Each muscle fiber in the bodywall of *Drosophila* larvae is innervated in an anatomically stereotypic fashion. During embryogenesis, these synapses arise through the selective projections of motoneurons onto specific muscle fibers. The motoneuron RP1 establishes a precise synaptic connection on the posterior portion of the two longitudinal skeletal muscle fibers 6 & 7. The target muscle fibers are generated between stages 12-15. We find that intra and intersegmental dye coupling has ended and myoblast fusion is complete when synapses are formed. Thus, fibers 6 and 7 are differentiated by the time the RP1 growth cone contacts their surfaces. Video imaging of intracellular dyefills of RP1 reveals the behavior of its growth cone as it differentiates at the target cells. The growth cone exits the CNS at stage 15, and projects along nerve branch SNb to the cleft between the adjacent fibers 6 & 7. Here it transiently sprouts a pair of anteriorly directed filopodia, one on each of the two fibers. Other filopodia make characteristic projections that contact and are retracted from neighboring fibers. The RP1 growth cone grows posteriorly along the internally facing surfaces of the target fibers 6 & 7, where it divides to establish synaptic endings on both fibers. By comparing living, staged neurons using digital optical imaging we find that filopodial projections, rather than being random, are arrayed in stereotypic patterns during the development of the synapse. These contacts generate the basic morphology of the RP1 ending over a period of 2 to 3 hrs during embryogenesis. Our results suggest that larval innervation patterns are a consequence of precise filopodial projections from exploring growth cones in response to highly localized cues on the target cell surfaces. Supported by grants from the NIH and the March of Dimes.

262.9

MUTATIONS AFFECTING AXONAL OUTGROWTH AND GUIDANCE OF THE MOTOR NEURONS IN THE NEMATODE C. ELEGANS. S. S. SIDDIQUI . Lab. of Molecular Biology, Toyohashi University of Tech., Toyohashi 440, Japan.

I have previously reported anti-tubulin monoclonal antibodies that preferentaially stain specific sets of neurons in the nervous system of the nematode C. elegans (Society of Neurosci. Abstr. 1989), including mAb 2-28-33, that stains 26 GABA immunoreactive neurons in C. elegans. These are six DDs, thirteen VDs, four RMEs, and three single neurons, RIS, AVL, and DVB. The DDs and VDs are known to be the inhibitory motor neurons, located along the ventral nerve cord of the animal. We have screened "uncoordinated" (unc) mutants of C. elegans, defective in locomotion, immunocytochemically using 2-28-33, on wholemount "squash" preparations, and identified mutants in 15 genes that show abnormal staining pattern of the DD and VD neurons. Mutants in 11 genes(unc-5, unc-6, unc-13, unc-34, unc-40, unc-51, unc-62, unc-69, unc-71, unc-73, and unc-76) affect the outgrowth and process placement of the dorsalward axons of the DD and VD neurons. Mutants in unc-30, lack the staining of the axonal processes, but the DD and VD cell bodies stain to a variable degree; whereas, mutants in unc-25 fail to stain both the cell bodies and the axonal processes. In mutants of two genes (unc-59, and unc-85), that are known to affect cytokinesis, multiple DDs and VDs are stained. Most of the mutants listed above also affect the axonal growth and guidance of other neuron classes.

262.10

ACCUMULATION OF LAMININ AND MICROGLIAL CELLS AT SITES OF INJURY AND REGENERATION IN THE C.N.S. OF THE LEECH. L. Masuda-Nakagawa*, K.J. Muller, B. Sasse* and J.G. Nicholls. Pharmacol. Dept., Biocenter, Basel University, 4056 Basel, Switzerland; Dept. of Physiol. & Biophys., Univ. of Miami Med. Sch., Miami, FL 33101

Profuse sprouting of leech neurons occurs in culture when they are plated on a substrate consisting of laminin molecules extracted from extracellular matrix that surrounds the C.N.S. To assess the role of laminin as a potential growth promoting molecule in the animal, its distribution was compared in intact and regenerating C.N.S. by light and electron microscopy, using a monoclonal anti-laminin antibody (206) and conjugated secondary antibodies. In frozen sections and electron micrographs of normal leech nervous system the label was restricted to the connective tissue capsule surrounding the connectives that link ganglia. Immediately after the connectives had been crushed the normal structure was disrupted but laminin remained in place. Two days after the crush, axons began to sprout vigorously and microglial cells accumulated in the lesion. At the same time labelled laminin molecules were no longer restricted to the basement membrane but appeared within the connectives in the regions of neurite outgrowth. The distribution of laminin at these new sites within the C.N.S. was punctate at two days, but changed over the following two weeks; the laminin became aggregated first as fibrils and then as condensed streaks running longitudinally along the connectives beyond the lesion. The close association of regenerating axons with laminin in electron micrographs suggests that it may promote neurite extension in the C.N.S. of the animal as in culture.

Supported by grants from the Swiss National Fund (J.G.N.) and USPHS NS 20607 (K.J.M.).

262.11

DIFFERENTIAL GROWTH OF *APLYSIA* NEURONS R2 AND L10 ON RUQ TARGET CELLS IS ASSOCIATED WITH THEIR CAPACITY TO FORM SYNAPSES. D. Hawver and S. Schacher. Dept. Pharm., Ctr. Neurobiol. & Behav., Columbia CPS & NYS Psych. Inst., New York, NY 10032.

Previous studies of *Aplysia* neurons L10 and RUQ in co-culture revealed that regenerating L10 neurites tend to avoid growing on RUQ target cells, due in part to the failure of L10 growth cones to fasciculate with RUQ neurites. This avoidance may help explain the paucity of L10-RUQ chemical synapses despite proper matching of ACh in L10 with AChR on RUQ. To determine whether this avoidance of RUQ is specific to L10, we used fluorescent dye injections to examine the pattern of neurite outgrowth from cholinergic neuron R2 in the presence of RUQ. In 14 of 17 cases, 2-8 major R2 neurites appeared to alter direction so as to converge upon the RUQ soma and major axon. All R2 cells showed growth of neurites in the RUQ area, often characterized by extensive branching on the soma and fasciculation along the major axon and neurites of the target cell. 12 of these R2-RUQ pairs were tested electrophysiologically for chemical connections, and 7 showed a 1-5 mV response in RUQ to a train of 3-5 spikes evoked in R2. Thus, R2 differs strikingly from L10 in its ability to form synapses with RUQ as well as in its propensity to grow on the RUQ target cell. Preliminary observations of early interactions show that, unlike L10 growth cones, R2 growth cones have a marked tendency to fasciculate with RUQ neurites. This suggests that the differences in the mature patterns of neurite outgrowth from R2 and L10, and the differences in synapse formation, may arise from selective fasciculation of initial contacts.

INVERTEBRATE LEARNING AND BEHAVIOR III

263.1

A MATHEMATICAL MODEL OF PROTOZOAN HABITUATION. D. C. WOOD. Dept. of Behavioral Neuroscience, Univ. of Pittsburgh, Pittsburgh, PA 15260.

Seven of the 9 parametric characteristics used to operationally define metazoan habituation have been observed when the contractile protozoan Stentor habituates to repeated mechanical stimuli. This behavioral habituation is correlated with a progressive decrement in mechanoreceptor potential amplitude. This decrement results from a increase in the voltage dependence of the mechanoreceptor channel conductance which reduces the number of channels opened by a mechanical stimulus. The mechanoreceptor channel modification occurs only when action potentials and contractions are elicited.

This simple form of learning has been mathematically modeled employing only conventional psychophysical (Steven's Power Function) and electrophysiological (Boltzmann equation and Ohm's law) formulations. The parameters employed in these equations and their variances were experimentally determined. The feedback modification of mechanoreceptor channels by action potentials or contractions and their recovery from it were presumed to be first-order processes. Using this model, data demonstrating 5 of the parametric characteristics of habituation were closely fit by varying only the 2 rate constants of the feedback processes. The other 2 characteristics could be fit with small extensions to the model.

263.2

DISSECTING MALE-MATING BEHAVIOR IN *C. ELEGANS* K. Liu, Y. Hajdu*, and P. Sternberg*. HHMI, Div. of Biology, 156-29, Caltech, Pasadena, 91125.

C. elegans naturally occurs in two sexual forms, self-fertilizing hermaphrodites, and males, which must mate in order to produce progeny. Male mating behavior comprises a number of discrete (but not discreet) steps: males first respond to hermaphrodites by backing along its length, turn around the head and/or tail if necessary, locate the vulva, insert their spicules, and inject sperm. The copulatory structures and their associated neurons in the male tail appear to mediate this behavior. To understand the underlying neuronal circuitry, we are taking two approaches. First, to identify genes which specifically affect the participating neurons, we have isolated mutations which affect mating. Details of this screen were described previously (Liu, K. and Sternberg, P., Soc. Neurosci., 1989). To date, we have isolated 22 mutants defective in the steps outlined above, 19 of which exhibit defects at a single step. For example, 11 mutants are blocked at the level of spicule insertion. The fact that these steps are independently mutable suggests that separate neural components mediate these steps. Our second approach is to systematically ablate the different sensory structures specific to the male to ascertain their roles in mating behavior. Preliminary results indicate redundancy in the system. For example, the "hook" normally mediates the location of the vulva, as its ablation results in male initially unable to perform this step. However, after a few unsuccessful attempts, the spicules appear to be able to partially compensate for this defect. This redundancy in the system may explain why it has been difficult to isolate mutations which act early in the behavioral pathway.

263.3

FACTORS AFFECTING HABITUATION AND RECOVERY FROM HABITUATION IN *C. ELEGANS* C. H. Rankin and B. S. Broster*, Department of Psychology, University of British Columbia, Vancouver, B.C. V6T 1Y7

Although habituation is the simplest and most ubiquitous form of learning the biological mechanisms of habituation are still incompletely understood. In these behavioral experiments we have investigated factors that affect the rate of habituation, the degree of habituation and the rate of recovery from habituation in a simple reflex circuit in *C. elegans*. One objective of these experiments was to determine the behavioral rules governing habituation; hypothesized cellular mechanisms of habituation must take these behavioral rules into consideration. In *C. elegans* the rate of habituation, amount of habituation and time to recover to baseline response levels are affected by the rate of stimulation. Habituation is deeper and more rapid, and recovery from habituation is more rapid, if stimuli are delivered at short interstimulus intervals than if they are delivered at long interstimulus intervals. The rate of recovery from habituation is dependent upon the interstimulus interval used and not upon the amount of habituation, nor upon the number of stimuli received. For example following habituation with a 5s ISI worms recover within 10 min, however after the same number of stimuli at 60s ISI worms took more than 30 min to recover.

These behavioral experiments show that the rules for habituation in *C. elegans* are similar to those observed in a large variety of species. Thus *C. elegans* is an appropriate model system in which to investigate biological mechanisms underlying habituation.

263.4

MODELS OF LEARNING WITHOUT DETECTABLE SYNAPTIC PLASTICITY IN THE LEECH. S.R. Lockery and T.J. Sejnowski. Salk Institute, La Jolla, CA 92037.

Studies of neural mechanisms of learning have focussed on large changes at identified synapses. However, learning in even simple reflexes may be distributed over many synapses. We used a model network of the local bending reflex in the leech to investigate the distribution of synaptic plasticity underlying habituation in a parallel processing system. The model comprised 4 sensory, 20 inter-, and 8 motor neurons. Synaptic connections were trained by the recurrent backpropagation algorithm until the model reproduced the amplitude and timecourse of synaptic potentials recorded intracellularly from motor neurons in response to sensory cell stimulation in non-habituated preparations. This "naive" network was then "habituated" by retraining it until the amplitude of each synaptic potential was 40% of the non-habituated level. The training algorithm was allowed either to increase or decrease the strength of a connection. Final connection strengths in the model network were inferred from the heights of simulated synaptic potentials when the presynaptic neuron was stimulated with a standard current pulse. Comparison of connection strengths in naive and habituated networks revealed that habituation was distributed across all connections in the model, with the largest changes in the connections between sensory and interneurons. However, even these changes were small--on average, less than 1 mV. This result raises the possibility that substantial changes in behavior can occur in the absence of easily detectable changes in synaptic strength.

263.5

CLONING OF A NEURAL CELL ADHESION MOLECULE FROM *APLYSIA* THAT IS MODULATED IN THE SENSORY NEURONS IN RESPONSE TO 5-HT. M. Mayford*, F. Keller, A. Barzilai, S. Schacher, & E.R. Kandel. Ctr. Neurobiol & Behav., Columbia Univ., & HHMI, NY, NY 10032

Both long-term sensitization training and long-term facilitation of the *Aplysia* sensory-motor connection induced in culture by application of serotonin (5-HT), result in structural changes in the sensory neurons and in the motor neurons of the gill-withdrawal reflex. Barzilai et al. (Neuron 2, 1577, 1989) found that a number of proteins are altered in their level of expression in the sensory neurons within one hour after exposure to 5-HT. Among these are four of 100-150 Kd M.W. that are reduced in their rate of synthesis within one hour after exposure to 5-HT. These proteins cross-react with a monoclonal antibody (mAb) that is specific to an *Aplysia* neuron specific cell adhesion molecule. Immunofluorescent studies with the mAb suggest that within one hour after the addition of 5-HT these antigens are also reduced on the membrane surface of cultured sensory neurons. We have obtained a cDNA clone from an *Aplysia* central nervous system lambda gt11 library that expresses a protein which cross reacts with the mAb. Preliminary sequence data suggests that this protein may be a member of the immunoglobulin class of cell adhesion molecules. The down regulation of these adhesion molecules may be a necessary early component of the structural changes associated with long-term sensitization and facilitation.

263.6

REGULATION BY 5-HT OF NEURAL CELL ADHESION MOLECULES IN THE NERVOUS SYSTEM OF *APLYSIA*. F. Keller, A. Barzilai, S. Grant*, M. Mayford*, I. Winicov*, S. Schacher, & E.R. Kandel. Ctr. Neurobiol & Behav., Columbia Univ., & HHMI, NY, NY 10032.

Among proteins in the sensory neurons of *Aplysia* that are transcriptionally regulated by 5-HT, a neurotransmitter involved in long-term facilitation (A. Barzilai, Neuron 2, 1577, 1989), five proteins have their rate of synthesis down-regulated. We now have characterized four of these proteins using monoclonal antibodies (mAb) and have found that they are related glycoproteins (100-150 kD M.W.), specific to neurons, that are enriched in the neuropil of ganglia and in neurites and growth cones in culture. In addition, these proteins are expressed in pregastrulation embryos. In cultures of sensory neurons, mAb against the proteins perturb axon fasciculation and increase the number of fine diameter neurites. Preliminary sequence data suggest that they are adhesion molecules of the immunoglobulin superfamily (M. Mayford et al., Abstract this meeting). Because long-term facilitation is associated with structural changes in sensory-motor synapses, the down-regulation of adhesion molecules may be one step of a growth program activated by 5-HT.

We are now investigating the mechanisms by which the proteins are regulated by 5-HT. We find that sensory neurons release into the culture medium proteins crossreacting with the mAb recognizing the membrane-bound species. This is consistent with the finding that 5-HT appears to down-regulate the expression of the proteins on the cell surface.

263.7

SEARCH FOR ADDITIONAL SITES OF ASSOCIATIVE INTERACTIONS DURING ACTIVITY-DEPENDENT FACILITATION IN *APLYSIA* SIPHON SENSORY NEURONS. T.W. Abrams Dept. of Biology, Institute of Neurological Sciences, Univ. of Pennsylvania, Phila., PA 19104.

During conditioning of the withdrawal reflex in *Aplysia*, synaptic transmission from sensory neurons (SNs) of the CS pathway is strengthened through activity-dependent presynaptic facilitation. One site at which SN activity and the accompanying Ca^{2+} influx, triggered by the CS, enhance the SNs' response to facilitatory transmitter, released by the US, is the dually-regulated Ca^{2+}/calmodulin-sensitive cyclase. In addition, activity and Ca^{2+} could enhance the facilitation response by other mechanisms downstream from the synthesis of cAMP. Using light induced release of cAMP from "caged cAMP" we are attempting to identify downstream sites of convergence. In these experiments, a UV light flash was given either immediately after a train of 5 spikes in a SN (Paired) or 4 sec after a spike train (Unpaired). Paired activity did not increase the spike broadening response to the cAMP transient. We are now attempting to identify downstream sites of activity-dependent interactions in the second facilitatory process responsible for reversing depression, a process distinct from the modulation of the S-K^+ current spike broadening. Spike broadening lasts less than 2 min after a flash. At times at which spike broadening has decayed but reversal of synaptic depression persists, preliminary experiments indicate there is no effect of paired activity on the facilitatory effects of light induced cAMP release. This suggests the cyclase may be the major site of stimulus convergence during this form of associative synaptic plasticity.

263.8

FMRFAMIDE ACTS IN PARALLEL PRE- AND POSTSYNAPTICALLY, VIA ARACHIDONIC ACID, TO INHIBIT THE NEURAL CIRCUIT OF THE SIPHON WITHDRAWAL REFLEX OF *APLYSIA*. K.J. Belkin and T.W. Abrams. Dept. of Biol. & Inst. of Neurol. Sci., Univ. of Penn., Phila, PA 19104

Electrical shock to the tail produces transient inhibition of the siphon withdrawal reflex of *Aplysia*, followed by sensitization. At least part of this inhibition is mediated by the neuropeptide FMRFamide (Mackey et al., 1987). FMRFa activates arachidonic acid (AA) production in siphon sensory neurons (Piomelli, et. al., 1987), causing an opening of S-K^+ channels (Belardetti, et. al., 1987), thereby inhibiting the synaptic connection of sensory neurons to motor neurons. We found that FMRFa exerts a parallel inhibitory effect postsynaptically on LFS siphon motor neurons. A puff of 2 uM FMRFa onto the cell body of an LFS neuron blocks its spontaneous firing. The response to FMRFa is biphasic, consisting of a transient depolarization followed by a prolonged hyperpolarization. The hyperpolarizing response is accompanied by an increase in membrane conductance, and has a reversal potential of -75 to -85 mV, which shifts in a depolarizing direction with elevated $[K^+]_o$. This suggests the hyperpolarizing response may be at least partially due to an increase in a K^+ current. A puff of 30 uM AA onto the cell body causes hyperpolarization, but does not mimic the early depolarization caused by FMRFa. 100 uM 4-bromophenacyl bromide, a phospholipase A2 inhibitor, blocks the hyperpolarizing, but not the depolarizing, FMRFa response. Thus, FMRFa acts via a single second messenger, both pre- and postsynaptically in sensory and motor neurons, to inhibit the siphon withdrawal reflex.

263.9

STRUCTURAL REMODELING OF SENSORY NEURONS ACCOMPANIES FMRFAMIDE-INDUCED LONG-TERM DEPRESSION OF SENSORIMOTOR SYNAPSES OF *APLYSIA* IN CULTURE. S. Schacher and P. G. Montarolo,* Ctr. for Neurobiol. & Behav., Columbia University CPS and NYS Psych. Inst., New York, NY 10032.

Repeated applications of FMRFamide produce long-term depression of synapses established in cell culture between sensory and motor cells isolated from the abdominal ganglion of *Aplysia*. To determine whether the synaptic depression is accompanied by changes in sensory cell structure, low light epifluorescent video microscopy was used to image sensory cell neurites apposed and adjacent to the major axons of motor cell L7 both before and 24 hours after a) control treatment, b) one 5 min application (1X) of FMRFamide (1 μM), or c) 4 applications of FMRFamide (4X) at 25 min intervals. EPSPs measured before and after treatment confirmed previous results; little or no change with controls (0 ± 4.2%, N = 17) or with 1X treatment (-8 ± 3.2%, N = 5), and a large decrease with 4X treatment (-39 ± 5.7%, N = 19). Both control and 1X treatments were accompanied by minor changes in sensory cell structure: -1 ± 2.6% and -2 ± 2.8%, respectively, in the number of varicosities; 1.5 ± 0.6 and 1.6 ± 0.8, respectively, in the number of neurite retractions. In contrast, 4X treatment resulted in a -26 ± 3.4% decrease in varicosities, and 5.7 ± 0.9 neurite retractions. This loss of structure was not cell wide, however, since the number of sensory neurite extensions in areas adjacent to the motor axons was not affected by treatment. Because varicosities of sensory cells apposed to the axons of L7 contain transmitter release sites, these results are consistent with the idea that the loss of varicosities and the retraction of neurites by sensory cells contribute to long-term synaptic depression evoked by FMRFamide.

263.10

IN VIVO FIRING PATTERN OF THE FOOD AROUSAL NEURON CPR IN *APLYSIA*.

T. Teyke*, K.R. Weiss and I Kupfermann.
Center for Neurobiology & Behavior, Columbia University and NYS Psychiat. Inst., New York, NY 10032.

The cerebral neuron CPR has appropriate input and output characteristics to function as a command element for the food-induced arousal state, which modulates feeding in *Aplysia*. We recorded extracellularly from the cerebral-pedal connective (which has the axon of the CPR) in free moving animals in order to correlate CPR activity with the behavior of the animal. Following the in vivo recordings, the ganglion was removed and the CPR was stimulated in order to characterize the extracellular spike on the basis of spike parameters, such as amplitude and rise time. These data were then used to identify the spike of the CPR in the previously obtained recordings in the freely behaving animal. When the animal was quiescent, or during locomotion, the CPR was not active, and only a few spikes occurred during head withdrawal and changes of the position of the head. A strong increase in activity of the CPR occurred during the appetitive head lifting behavior which was evoked by stimulating the animal with seaweed. 'Spontaneously' occurring appetitive head lifting responses were also associated with a strong increase in CPR activity. CPR activity remained elevated as long as the animal kept its head up, and decreased when the animal returned its head to the substrate. The spike frequency during head lifting was about 10 spikes/sec, which is a rate which produces clear effects on the activity of neurons involved in food arousal responses (e.g., the Metacerebral Cell). The indication that CPR activity is strongly correlated with the head lifting behavior of the animal provides additional support for the notion that the CPR operates as a command element for the food-arousal state in *Aplysia*.

263.11

NEURAL ORGANIZATION OF PREDATORY BEHAVIOR IN *Pleurobranchaea californica*. R. Gillette. Department of Physiology & Biophysics, University of Illinois, Urbana, IL 61801

Predatory feeding and avoidance behaviors are integrated by motivational state and learned recognition of dangerous prey in the opisthobranch *Pleurobranchaea*. Locomotion, orienting, and sensory avoidance are regulated by the functional state of the neural network of feeding behavior. Skin secretion of sulfuric acid in response to noxious stimuli both repels other predators and potentiates *Pleurobranchaea*'s own avoidance behavior via nociceptive paths. The mechanisms of food-avoidance learning, and feeding and avoidance behaviors indicate a simplest connectionist scheme for integration of complex behavior. Briefly, weaker food stimuli simultaneously stimulate systems driving locomotion, orienting, and aversive turns. Stronger stimuli excite feeding. The feeding network has essentially two excited states: (I) oscillatory, driving feeding or rejection movements, and (II) non-oscillatory, in which it is locked up in the proboscis retraction phase of its cycle. State I suppresses locomotion and aversive sensory paths, while state II inhibits orienting pathways and releases locomotion. State transitions are core gulated by motivation and learning.

SECOND MESSENGERS III

264.1

THE LOW MOLECULAR WEIGHT FORM OF STIMULATORY G-PROTEIN ALPHA SUBUNIT (Gsα) IN MOUSE BRAIN IS RESISTANT TO CHOLERA TOXIN-INDUCED ADP-RIBOSYLATION. **James P. Whelan, Paula L. Hoffman* and Boris Tabakoff. DICBR, NIAAA, Bethesda, MD 20892.** **We have previously found quantitative differences in the ratio of the two major forms (46 kD and 52 kD) of the α subunit of Gs (Gsα), the stimulatory guanine nucleotide binding protein, in various mouse brain regions using Western blotting (Whelan et al., FASEB J. 4:A1119 (1990)). The level of the 52 kD form was similar in cortex, hippocampus, cerebellum and striatum, while the level of the 46 kD protein varied dramatically among brain areas, being highest in striatum and lowest in cerebellum. Cholera toxin-induced ^{32}P-ADP-ribosylation of membranes from each brain area demonstrates that the 52 kD form of Gsα is ribosylated more efficiently than the 46 kD form in vitro. This difference may be due to conformational factors, or, alternatively, the 46 kD form of Gsα may be endogenously ADP-ribosylated in vivo which reduces further ribosylation by cholera toxin in vitro. Utilizing a sensitive avidin-biotin-peroxidase detection system for Western blots, we have identified doublet bands of both forms of Gsα in untreated brain membranes, which may reflect endogenous ADP-ribosylation (electrophoretic migration is altered by cholera toxin-induced ADP-ribosylation under our conditions). In the 46 kD doublet, the majority of the protein appears as the slower (putatively ribosylated) band, while in the 52 kD doublet, the majority of the protein migrates as the faster (non-ribosylated) band. Basal (no stimulation) adenylate cyclase activities in brain membranes correlate positively with the relative amounts of the 46 kD form in each region. Since ADP-ribosylation activates Gs, it is possible that preferential ribosylation of the 46 kD form may account for this correlation. Studies under way using ADP-ribosylarginine hydrolase may provide a more direct evaluation of endogenous ribosylation.**

264.2

SPECIFICITY OF APLYSIA PROTEIN KINASE C ISOZYMES W.S. Sossin, T.C. Sacktor and J.H. Schwartz. Center for Neurobiology and Behavior. Howard Hughes Medical Institute, Columbia University College of Physicians & Surgeons, New York, NY 10032.

Aplysia sensory neurons express several isozymes of protein kinase C (PKC). We find that these isozymes have different requirements for translocation and activation, suggesting that a specific isoform(s) might be responsible for the enhanced release of transmitter at sensory-to-motor synapses (Sacktor and Schwartz, PNAS 87: 2036, 1990).

One difference between isozymes is the ability of phorbol ester (TPA) alone to translocate PKC. While some kinase activity is translocated to neuronal membranes by TPA, the *Aplysia* β_1 isozyme, assayed by Western blotting, is not. This isozyme is translocated to neuronal membranes by Ca^{2+}, and TPA reduces the Ca^{2+} concentration required for translocation. Another difference is the dependence of PKC activity on Ca^{2+}. A kinase similar to nPKCε, a Ca^{2+} independent isozyme, can be inferred from cDNA sequence information (Kruger, PhD thesis, Columbia Uiversity, 1990). A TPA-inducible, Ca^{2+}-independent activity remains after removal of Ca^{2+}-dependent activity from extracts of *Aplysia* neurons by precipitation with phosphatidylserine in the presence of Ca^{2+}. This PKC preferentially phosphorylates a synthetic peptide related to the *Aplysia* nPKCε pseudosubstrate sequence. We are using these procedures for distinguishing *Aplysia* PKC isozymes to determine the enzyme(s) involved in the presynaptic facilitation underlying behavioral sensitization.

264.3

DEAFFERENTATION INCREASES PROTEIN KINASE C-β-LIKE IMMUNOREACTIVITY (PKC-β-LI) IN THE RAT SUPERIOR CERVICAL GANGLION (SCG). R.Roivainen*and J.Koistinaho Dept. of Public Health, Univ. of Tampere, P.O. Box 607, SF-33101 Tampere, Finland

Protein kinase C is a Ca^{2+}/phospholipid-dependent enzyme, which plays an important role in neuronal signal transduction. We have studied immunohistochemically the expression of PKC-β-subtype in the rat SCG at different time points after preganglionic denervation. In unoperated and sham operated ganglia as well as in ganglia 1 h postoperation (p.o.), the neuronal perikarya showed only faint PKC-β-LI. One day p.o. both the number of immunoreactive neurons and their staining intensity had increased. Two days p.o. the maximal staining was seen. Seven days p.o. the staining intensity was decreased, but it was still above the control level. At 14 days p.o., no difference in the PKC-β -LI between the operated and sham operated ganglia was observed anymore. At light microscopic level, the subcellular distribution of PKC-β -LI was not changed at any time point after denervation. The results suggest that the preganglionic innervation is a factor regulating the amount of PKC contained in rat SCG neurons.

264.4

IN SITU HYBRIDIZATION LOCALIZATION OF BRAIN INOSITOL TRISPHOSPHATE RECEPTOR. C.A. Ross, S.K. Danoff, C. Donath, A. Ullrich, S.H. Snyder. Johns Hopkins Univ. Sch. of Med., Dept. of Neuroscience, Baltimore, MD 21205 and Max Planck Institute for Biochemistry, Martinsreid, FRG.

The inositol trisphosphate receptor (IP_3R) releases calcium from the endoplasmic reticulum when phosphoinositide hydrolysis is stimulated by hormones or neurotransmitters. We have performed in situ hybridization analysis of expression of IP_3R using oligonucleotide probes based on the mouse IP_3R cDNA sequence (Furuichi et al, Nature 342, 32, 1989) or a 1.8 kb rat IP_3R fragment generated by PCR. Highest levels of expression were in cerebellar Purkinje cells, cerebral cortex, hippocampus, and striatum, as previously shown by receptor autoradiography. In the thalamus, highest levels were in the medial dorsal nucleus. In addition, transcripts were present in basal forebrain, pontine nuclei, and raphe complex. Using a cDNA for 1.5 kb of the 5' region of the human IP_3R as a probe, high levels of expression were found in human cerebral cortex, hippocampus, and Purkinje cells of the cerebellum.

264.5

FUNCTIONAL REGULATION OF PURIFIED AND RECONSTITUTED IP3 RECEPTOR . C. D. Ferris, R. L. Huganir, R. J. Mourey, and S. H. Snyder. Dept. of Neuroscience, The Johns Hopkins Univ. Sch. of Medicine, Baltimore, MD 20205.

IP3 receptor has been purified from rat brain and smooth muscle membranes and localized to portions of the endoplasmic reticulum including the nuclear envelope. The IP3 receptor is a glycoprotein of Mr , in the native state, by gel filtration, of 1000 kD; it is a homotetramer with a subunit size of 260 kD. Recently, we reported the functional reconstitution of the purified receptor from rat cerebellum and demonstrated that the single receptor protein contains both the IP3 recognition site and the associated calcium ion channel (Ferris, C. D., et al., Nature 342, 84-87). We now demonstrate a potent and selective allosteric regulation of calcium flux by ATP and other adenine nucleotides. ATP (or any of its non-hydrolysable analogues) enhances IP3 stimulated calcium flux by 50% at low micromolar doses; this effect is reversed at doses from 100uM to 1mM. This regulation is selective since it is not observed with guanine or other nucleotides, and we speculate that this regulation may participate in calcium oscillations. In reconstituted vesicles the IP3 receptor is stoichiometrically phosphorylated by protein kinase A. Phosphopeptide mapping indicates that a single peptide is phosphorylated on a serine residue and this phosphorylation appears to functionally regulate the reconstituted receptor. Also, magnesium ions potently inhibit IP3 stimulated calcium flux at micromolar doses; this regulation may also have physiologic significance. Purified IP3 receptor from smooth muscle can also be reconstituted using the same approach as that employed from the brain receptor. The reconstituted smooth muscle receptor appears very similar to the brain receptor in most of its properties.

264.6

LOCALIZATION OF TWO FUNCTIONALLY DISTINCT NON-MITOCHONDRIAL CALCIUM POOLS IN RAT BRAIN A.Verma and S.H.Snyder , Johns Hopkins Sch. of Medicine ,Dept. of Neuroscience

CNS neurons contain intracellular Ca2+ stores capable of accumulating and releasing Ca2+. We used ATP-dependant 45 Ca2+ transport in fresh frozen sections and microsomes to characterize and localize functionally distinct non-mitochondrial Ca2+ stores in rat brain.Two Ca2+ stores are distinguished by their permeability to the oxalate anion and by sensitivity to thapsigargin (Tg) and inositol-1,4,5-trisphosphate (IP3).Active 45Ca2+ accumulation in the absense of oxalate saturates by 60 min. and is enriched in the brainstem, thalamus, cerebral cortex, and inferior colliculus. Oxalate strongly stimulates 45Ca2+ uptake into a pool which is not saturated by 3 hrs. and is concentrated in cerebellar and cerebral cortices, hippocampus, striatum, basal forebrain nuclei, and olfactory bulb. Tg potently and selectively inhibits 45Ca2+ uptake into the oxalate permeable pool with an IC50 of ~30nM and maximal inhibition at 100 nM.45Ca2+ uptake in the absense of oxalate is insensitive to [Tg] upto 10 uM. IP3 (10 uM) also selectively mobilizes Ca2+ from the oxalate permeable Ca2+ pool. Autoradiography reveals that while Tg inhibits oxalate stimulated 45Ca2+ uptake in all brain regions IP3 only mediates Ca2+ release from oxalate permeable stores which also contain IP3 receptors. Thus one brain Ca2+ accumulating pool is permeable to oxalate and inhibited by Tg while another displays neither of these properties. The IP3 sensitive Ca2+ pool is a subset of the Tg sensitive Ca2+ pool.

264.7

CHARACTERIZATION AND PARTIAL PURIFICATION OF A MEMBRANE-ASSOCIATED INS 1,4,5-P_3 5'-PHOSPHATASE FROM RAT CEREBELLUM. S.K. Danoff, W.A. Theibert, E.A. Davis*, R.K. Barrow* and S.H. Snyder. Dept. of Neuroscience, Johns Hopkins Univ., School of Medicine, Baltimore MD 21205

The rat cerebellar Ins 1,4,5-P_3 receptor has been purified (Suppatapone, S. et al, JBC, 263:1530, 1988) and reconstituted (Ferris, C. et al., Nature, 342:87, 1990) and has been shown to act as a calcium channel. In cerebellum, the membrane-associated $InsP_3$ 5'-phophatase represents ~90% of the enzymatic activity leading to inactivation of $InsP_3$. This enzyme is stimulated by physiological concentrations of free calcium (ED_{50}=~400nM). The protein has been highly enriched using conventional ion exchange and $InsP_3$ affinity chromatography.

264.8

EFFECT OF ELECTROCONVULSIVE SHOCK (ECS) ON PHOSPHOSPHOINOSITIDE TURNOVER IN RAT BRAIN. G.N. Pandey, S.C. Pandey, L. Isaac and J.M. Davis Illinois State Psychiatric Institute, Chicago, IL 60612

It has been shown that that alpha$_1$-adrenergic and serotonin (5HT$_2$) receptors stimulate phosphoinositide breakdown and inositol phosphate formation in rat brain. Up and down regulation of these receptors in general produce corresponding changes in phosphoinositide breakdown. Chronic administration of ECS in rats produces upregulation of alpha$_1$-adrenergic and 5HT$_2$ receptors in rat cortex. In order to examine if changes in alpha$_1$ and 5HT$_2$ receptors are associated with changes in the phosphoinositide signalling system, we studied the effect of ECS on norepinephrine- (NE) and serotonin- (5HT) stimulated inositol-1-phosphate (IP$_1$) and total inositol phosphate formation in rat cortical slices. Rats were treated with ECS (75mA/0.2 second) once daily for 14 days, and were sacrificed 24 hours after the last shock. Cortical slices were labeled with [^{3}H]-inositol for 1 hour at 37^{o}C. After washing, slices were stimulated with NE (10^{-4}M) and 5HT (10^{-4}M). It was observed that 5HT-stimulated total inositol phosphate and [^{3}H]-IP$_1$ formation were significantly higher in the brains of ECS-treated rats than in the brains of control rats. However, NE-stimulated [^{3}H]-inositol phosphate formation, although higher in the brains of ECS-treated rats, was nonsignificantly different from control rats. Thus, our results indicate that changes in receptor number induced by ECS are in general accompanied by corresponding changes in the phosphoinositide signal transduction pathways.

264.9

PAF ANTAGONIST DECREASES *fos* EXPRESSION IN RAT HIPPOCAMPUS INDUCED BY SINGLE SEIZURE **V.L. Marcheselli,* J.P. Doucet,* and N.G. Bazan, LSU Eye Center and Neuroscience Center, New Orleans, LA, 70112.**

Neuronal stimulation activates the expression of immediate-early transcriptional regulatory proto-oncogenes in the central nervous system. The convulsants pentylenetetrazol (Metrazole) and picrotoxin, as well as electroconvulsive shock (ECS), have been demonstrated to induce a rapid increase of *fos* mRNA expression in hippocampal neurons. One consequence of experimental convulsion is accumulation of the bioactive etherphospholipid platelet-activating factor (PAF). Ginkgolide PAF antagonist BN 52021 reduces cellular damage in the brain after ischemia/reperfusion. In SH-SY5Y neuroblastoma cells, PAF induces rapid transcriptional activation of *fos* and *jun* proto-oncogenes, an effect reversed by BN 52021 (*J Neurosci Res, 24:558-566, 1989*). We seek to correlate immediate-early gene expression with ECS-induced PAF synthesis. Here we demonstrate that BN 50730, a potent competitive PAF antagonist for high-affinity intracellular binding sites (*J Biol Chem 260:9140-9145, 1990*), partially inhibits the *fos* transcription that is induced by ECS in the rat hippocampus. Brain cortex also reflects the same effect but to a lesser extent. Drugs were injected intraperitoneally 30 min before ECS and did not alter the seizure intensity. Total cellular mRNA was isolated 60 min after ECS and analyzed by Northern and slot blotting techniques. Unlike BN 50730, BN 52021 had no effect on *fos* mRNA levels, which suggests a correlation with ECS-induced *fos* expression and intracellular high-affinity PAF-binding sites. These results suggest a role for PAF as an intracellular messenger involved in seizure-induced immediate-early gene expression in brain. **Supported by NIH grant NS 23002.**

264.10

EFFECTS OF CLONIDINE AND OTHER AGENTS INTERACTING WITH IMIDAZOLE RECEPTORS ON SECOND MESSENGER SYSTEMS AND CATECHOLAMINE RELEASE IN ADRENAL CHROMAFFIN CELLS. S. Regunathan, M.J. Evinger, M.P. Meeley and D.J. Reis, Div. of Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021.

In brain clonidine (CLON), an imidazole, binds to a novel class of imidazole-preferring (IM) as well as to α_2-adrenergic receptors. In adrenal chromaffin cells, where it inhibits the carbachol-induced release of catecholamines (CA), CLON binds only to IM receptors. We sought to determine whether the signal transduction mechanism of IM receptors differs from α-2 receptors (inhibition of adenylate cyclase) and its relation to CA release in cultured (72h) bovine chromaffin cells. CLON did not change: (a) basal or GTPγs- (50μM; 5-fold) or carbachol-(100μM; 3-fold) stimulated PI turnover;(b) basal nor forskolin-stimulated levels of cAMP (3μM; 2-fold). However, CLON elicited a concentration-dependent (EC_{50}; 5μM), slow (peak response at 15 min), 50% increase in cGMP production not potentiated by the phosphodiesterase inhibitor IBMX, and suggesting a direct intracellular action of the drug. Other agents binding to IM receptors (naphazoline, oxymetazoline, idazoxan, rilmenidine, clonidine displacing substance) and norepinephrine, failed to alter cGMP. All agents binding to IM receptors inhibited, by 40%, the carbachol-mediated release of CA's, although CLON was the most potent one (about 60%). We conclude that the signal transduction mechanisms coupled to IM differs from adrenergic receptors and IM receptor occupancy may inhibit the release of adrenal medullary CA's.

264.11

THE PROTEIN KINASE C PHOSPHORYLATION SITES OF THE β_2-ADRENERGIC RECEPTOR CONTRIBUTE TO THE CROSS-TALK BETWEEN SECOND MESSENGER SYSTEMS. M. Bouvier, N. Guilbeault* H. Bonin* Department of Biochemistry, University of Montreal, Montreal, Quebec, H3C 3J7, Canada.
Modulation of the β-adrenergic stimulated adenylyl cyclase activity by hormone known to stimulate the phosphatidyl inositol (PI) hydrolysis pathway has been widely reported. These observations were taken as evidence of the existence of a cross-talk between the second messengers generating systems. Protein kinase C (PKC) phosphorylation of various components of the adenylyl cyclase stimulatory pathway have been proposed to contribute to this process. The β_2-adrenergic receptor has been shown to be a substrate for the PKC. In the present study, we addressed directly the role of the receptor phosphorylation by PKC in this regulatory pathway. Using site directed mutagenesis, the potential PKC phosphorylation sites of the human β_2-adrenergique receptor were destroyed by substituting alanine residues for the serines 261, 262, 345 and 346. This mutation completely abolished the phorbol ester induced phosphorylation of the receptor expressed in chines hamster fibroblasts. Phorbol ester treatment induces a significant increase in β-adrenergic stimulated adenylyl cyclase activity in membranes derived from cells expressing either wild type or mutated receptor. This increase was accompanied by a rightward shift in the EC_{50} of the β-agonist isoproterenol to stimulate the adenylyl cyclase in cells expressing wild types receptor but not in cells expressing the receptor devoided of PKC phosphorylation sites. Similarly, the potency of isoproterenol to stimulate cAMP accumulation in phorbol ester treated cells was significantly increased in cells expressing the mutated receptor. These results therefore suggest that phosphorylation of the β_2-adrenergic receptor by PKC decrease the potency of the receptor to stimulate the adenylyl cyclase even in the presence of an increased maximal response.

264.12

IDENTIFICATION OF A NOVEL SIGNAL TRANSDUCTION PATHWAY OF THE 5-HT1A RECEPTOR *IN VITRO*. Yafang Liu and Paul R. Albert. Dept. of Pharmacology and Therapeutics , McGill University , 3655 Drummond Str., Montreal, PQ, Canada, H3G 1Y6.

We used the mouse Ltk$^-$ cell line, which lacks 5-HT1A receptors, as a model to examine the second messenger system of the cloned receptor. The rat 5-HT1A receptor gene transcription unit was transfected into this cell line and the LZD-7 subclone, expressing 100,000 5-HT1A receptors/cell, was isolated. In LZD-7 cells, 5-HT stimulated two separate phases (fast phase and slow phase) of increase in $[Ca^{++}]_i$ in a dose-dependent manner (EC50 =3 nM). The fast phase of 5-HT-induced change in $[Ca^{++}]_i$ was due to influx of calcium since1 mM EGTA or 5 µM nifedipine blocked this phase. The slow phase was due to alteration of intracellular calcium storage or buffering and was resistant to EGTA and nifedipine. Pretreatment of LZD-7 cells with pertussis toxin (10 ng/ml) inhibited totally both phases. TPA blocked separately the slow phase (0.1-1 nM) and fast phase of influx of calcium (5-10 nM). Forskolin (0.2-10 µM) or 8-Br cAMP (1-5 mM) blocked partially the fast phase of calcium influx, but completely abolished both phases at the lowest concentrations when 0.1-1 nM TPA was added. The effects of TPA were completely blocked by 24h pretreatment with 500 nM TPA to downregulate protein kinase C. These results suggest that the 5-HT1A receptor is coupled by pertussis toxin-sensitive G proteins to an alternate transduction pathway in LZD-7 cells. Receptor activation increases PIP turnover, and may stimulate different protein kinase C isoenzymes, which presumably regulate the opening of distinct calcium channels. There was interaction between protein kinase A and protein kinase C, which in turn synergistically modulated influx of calcium (MRC, Canada).

SENSORY SYSTEMS—DEVELOPMENT AND PLASTICITY I

265.1

DEVELOPMENT OF METABOLIC ACTIVITY PATTERNS IN KITTEN SOMATOSENSORY CORTEX. S.L. Juliano, W. Ma, and D.E. Eslin*. Dept. of Anatomy, USUHS, Bethesda, MD 20814.

The visual cortex of the cat exhibits a number of changes in anatomy and physiology indicative of a "critical period" during development. No comparable changes have yet been observed in cat somatosensory cortex. To begin analysis of cortical activity and anatomical relationships during the development of cat somatosensory cortex, kittens 3-5 wks of age were studied to evaluate their responses to somatic stimulation. To assess cortical activity, each kitten received a somatic stimulus during a 2-deoxyglucose (2DG) experiment. The evoked pattern of activity in the somatosensory cortex of kittens aged 3-4 wks was distinctly different from that found in adults or in 5 wk old kittens. The metabolic patterns in the younger animals were less distinctly patch-like and appeared more diffuse than in the adult. Evoked activity in 3 & 4 wk old kittens was 40-55% above background in density and localized in the center of the cortex only. Adult patches of activity are densest in the central layers, but extend into layers III & II, occasionally into layer V, and are 60-90% above background in density. Stimulus-evoked metabolic activity in the 5 wk old kittens was nearly adult-like and occurred in relatively dense (60% above background) patches that extended into layers III & II. Comparison of the metabolic patterns with Nissl-staining indicated that in the 3 wk old kittens, layers V & VI could be easily distinguished, but layers II-IV were not separable into distinct entities as they are in the adult. In 4 wk old kittens, cortical layers II-IV were apparent, but not as clearly so as in the adult. The metabolic activity patterns in kittens appear to parallel the laminar development in somatosensory cortex. These morphologic and functional characteristics may correspond to developmental changes in visual cortex that indicate a critical period of development. Supported by NS-24014.

265.2

TWO VIEWS ON FORMATION OF OLFACTORY GLOMERULI BASED ON NUMERICAL STUDY OF OLFACTORY NEURONS, GLOMERULI AND MITRAL CELLS IN RAT, HAMSTER AND RABBIT. E. Meisami, Physiol. Dept., Univ. of Illinois, Urbana, IL 61801.

Numerical study of the three main relay/integrative elements in the olfactory system [olfactory receptor neurons (ORN), olfactory glomeruli (OG), mitral cells (MC)] in three altricial mammals with varying gestation periods (hamster 16 d, rat 21 d, rabbit 30 d) reveals that at birth, of all three elements, only MCs have reached adult values. The GLs and ORNs increase significantly postnatally, the increase being more pronounced for ORNs than for GLs and more marked in rat and hamster than in rabbit. The ORN increase includes both immature and mature neurons, resulting in increase in surface density, per counts of receptor knobs. The postnatal ORN increase occurs across all epithelial zones, particularly in posterior and lateral zones. If GLs are counted in sections staining their meuropil, instead of Nissl sections where periglomerular cell assemblies are counted, more GLs are seen in the neonatal bulb, i.e., less marked postnatal increase. Two hypotheses on glomerular formation and connectivity in the afferent olfactory pathway is suggested: 1) Early arising ORN fibers make contact with all MCs and establish the total number of GLs at an early stage; some of the early GLs may be too small to be seen; 2) Some GLs form by birth; others from postnatally as new ORNs arrive in the bulb and find targets preoccupied.

265.3

EARLY CHANGES IN TACHYKININ-LIKE IMMUNOREACTIVITY IN PRIMARY SOMATOSENSORY CORTEX OF ADULT SQUIRREL MONKEYS FOLLOWING NERVE INJURIES. C.G. Cusick. Dept. of Anatomy, Tulane Univ. Medical School, New Orleans, LA 70119.

Following nerve injuries in adult mammals, functional changes occur in the spinal cord, brainstem, thalamus, and primary somatosensory cortex. The purpose of the present study was to investigate changes in neurotransmitters that might occur within the intrinsic cortical circuitry in response to nerve injury. Squirrel monkeys (Saimiri sciureus) were deeply anesthetized with ketamine hydrochloride and Ace-promazine, and the median and ulnar nerves were unilaterally transected and ligated in the distal forearm. Nine-10 days later, the animals were euthanized with Nembutal and the somatosensory cortices were processed for immunocytochemistry using a monoclonal antibody raised against the carboxyl-terminal end of the tachykinin peptide substance P (NC1/34HL, Sera Labs). Contralateral to the nerve injuries, the numbers of cells containing tachykinin-like immunoreactivity were reduced by about 35% in layer IV of the area 3b hand representation, compared to equal lengths of layer IV in the area 3b face representation. Since many tachykinin immunoreactive cells in layer IV of monkeys colocalize GABA, the results suggest that cortical inhibitory neurons rapidly modify their content of neuroactive peptides in response to nerve injury.

265.4

EFFECTS OF THALAMOTOMY AT BIRTH UPON TRIGEMINAL BRAINSTEM CELL NUMBER, SIZE AND DISTRIBUTION. M.F. Jacquin, N.L. Chiaia, C.A. Bennett-Clarke, N. Hobart* & R.W. Rhoades. Dept. of Anat. & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104, & Dept. of Anat., Med. College of Ohio, Toledo, OH. 43699.

Neonatal thalamic destruction deprives many trigeminal (V) brainstem neurons of their target and axotomizes many of these cells. Such lesions disrupt normal cell patterning in V nucleus principalis (PrV), yet we have no information about how they affect cell number in any portion of the V brainstem complex. To address this issue, unilateral right thalamotomy was performed (by electrocautery) at birth in 6 rats. They were sacrificed on postnatal day 6, and 10 micron thick paraffin sections of the brainstem were stained with cresyl violet. Four of the 6 cases had complete thalamic lesions and cell (nucleoli) number, cell diameter and V brainstem nucleus cross-sectional areas were sampled for left and right PrV and subnucleus interpolaris (SpVi). Relative to the control right side, cell number in left PrV was reduced by 59.1 $\pm$ 7.3% (mean $\pm$ SD), PrV transverse area was reduced by 20.5 $\pm$ 6.6%, cell density was reduced by 48.1 $\pm$ 13.2%, and average cell diameters were increased by 35.8%. All of these effects were statistically reliable. In left SpVi, however, no reliable changes were observed. Mean cell number, transverse nuclear area, and cell density were +7.0%, -4.9% and +14.7%, respectively, of the values obtained on the control right side. These data indicate that thalamotomy reduces cell number in PrV, but not in SpVi. The change in PrV cell size distribution likely reflects the selective preservation of PrV cells that project to non-thalamic targets and are known to have larger diameters. Support: DE07734, DE07662, and DE08971.

265.5

AXON NUMBERS IN SPARED SUPRAORBITAL VIBRISSAE FOLLICLE NERVES FOLLOWING INFRAORBITAL NERVE SECTION AT BIRTH. P.H. Young, D.S. Zahm, J. Golden and M.F. Jacquin. Dept. of Anatomy and Neurobiology, St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Prior studies indicate that the central projections of ear (zygomaticofacial) and eye (supraorbital) vibrissae afferents occupy a supranormal transverse area in the rat trigeminal brainstem complex following neonatal infraorbital nerve injury (Renehan et al., JCN 289:493, '89; Waite, Soc. Neurosci. Abstr. 15:92, '89). Enlarged central projections from these spared vibrissae could be due to central and/or peripheral sprouting, central arbor expansion and/or displacement, and/or preservation of an immature projection pattern. Here, we tested the hypothesis that central axon reorganization reflects an abnormal innervation of the spared vibrissae follicles. Six rats received left infraorbital nerve sections at birth; 3 were sacrificed on postnatal day 7 (PND-7), the other 3 on PND-17. Left (L) and right (R) supraorbital vibrissae follicle nerves were processed for EM analysis and montages of each nerve were made at 4300X. At PND-17, there were 166 ± 12 and 156 ± 38 axons in individual L and R nerves, respectively (mean ± SD). The numbers of myelinated axons were 110 ± 4 (L) and 110 ± 33 (R); unmyelinated axon counts were 56 ± 11 (L) and 46 ± 7 (R). No L-R comparison reached statistical significance. At PND-7, there were 122 ± 6 and 118 ± 9 axons in the L and R nerves. The numbers of myelinated axons were 99 ± 1 (L) and 97 ± 6 (R); unmyelinated axon counts were 23 ± 6 (L) and 21.3 ± 6 (R). Again, L-R differences were not significant. Thus, the enlarged central representation of the supraorbital vibrissae does not reflect the maintenance of an immature peripheral innervation pattern. These data also suggest that regenerate infraorbital axons do not grow into supraorbital vibrissae follicles. Support: DE07734, DE07662, NS23805.

265.6

DEVELOPMENT OF THALAMOCORTICAL RESPONSES IN BARREL CORTEX OF EARLY POSTNATAL MICE A. Agmon, D.K. O'Dowd and E. G. Jones Department of Anatomy and Neurobiology, California College of Medicine, Irvine, CA 92717

Layer IV of the rodent somatosensory cortex has discrete cytoarchitectonic structures, called barrels, that receive vibrissae input in a one-to-one manner from the ventrobasal (VB) nucleus of the thalamus. Barrels are first apparent on the third postnatal day, however it is not known when thalamocortical synaptic connections are first established. In order to examine this question we stimulated VB and recorded postsynaptic potentials and currents in a slice preparation that preserves functional connectivity between the thalamus and the barrel cortex (Agmon and Connors, Neurosci., in press). We used the whole cell recording technique as applied to thick slices (Blanton, LeTurco and Kriegstein, J. Neurosci. Meth. 30, 203-210, 1989). Prior to barrel formation, 0-2 days postnatal, some cells in layers V/VI received strong monosynaptic EPSP's. In animals older than 3 days, in which barrels could be visualized, cells in both deep layers and in layer IV received monosynaptic EPSP's. In animals 10 days old or older we frequently encountered disynaptic IPSP's in addition to monosynaptic EPSP's. Supported by NS08364 and NS21377.

265.7

RECEPTOR CELLS DIFFERENTIATE FROM SUPPORTING CELLS IN DEVELOPING ELECTRORECEPTOR ORGANS. H.A. Vischer. SIO, Neurobiol. Unit, UCSD, La Jolla, CA 92093.

South American, gymnotiform glassknife fish of the genus Eigenmannia were induced to spawn under laboratory conditions. In order to visualize developmental patterns, animals of various ages were either injected with 0.5-2μl 5-bromo-2'deoxyuridine (BrdU) or an eqivalent amount of [^{3}H]-thymidine and processed for immunohistochemistry (marked cells are labelled with rhodamine) or autoradiography, respectively. In addition, BrdU-labelled specimens were counterstained with the nuclear stain Hoechst 33342. Ultrastructural analysis was performed by transmission electron microscopy (TEM). First primordial electroreceptor cells can be recognized within the striatum germinativum of the epidermis. In all cases, an unmyelinated axon of the afferent lateral line nerve lies underneath these cells but does not penetrate the basal membrane, which shows a significant indentation and strong apical vascularization in this area. The primordial cells undergo rapid cell division (t<2hrs) and differentiate into two cell layers: the prospective basal supporting cells and the apical receptor cells which exclusively arise from supporting cells (8hrs<t<12hrs). At this time, the afferent fiber penetrates the basal lamina and forms synaptic contact with newly differentiated receptor cells.
Supported by a grant from SNSF.

265.8

QUANTIFICATION OF RADIAL GLIA AND RADIAL DENDRITES IN BARREL CORTEX AFTER WHISKER REMOVAL IN NEONATAL MICE: INITIAL APPROACHES WITH IMAGE ANALYSIS. J.E. Crandall, D. Butler*, and S.A. Tobet. Department of Developmental Neurobiology, E.K. Shriver Center, Waltham, MA 02254.

We have described a spatial pattern of radial glia and radially oriented dendrites that emerges in the mouse barrel cortex at a similar time to that of the cellular pattern of barrels (Crandall *et al.*, Dev. Brain Res., In press). Both glia and dendrites appear to be more densely distributed toward the walls than the hollows of individual barrels. Six days after row C whisker removal on P1, serial tangential sections were immunolabeled simultaneously with monoclonal antibodies specific for MAP2 (neuronal dendrites and somata) and RC2 antigen (radial glia). Microscopic images were analyzed interactively using an IBAS 2000 workstation. Measuring numbers of elements rather than the optical density of individual elements minimized histochemical processing differences. Unreliable feature extraction, based on inspection of overlay images, averaged less than 1% of the total element number. The density of radial glia and radial dendrites was higher in the barrel walls compared to the hollows ipsilateral to the side of whisker removal. The density of radial dendrites in the walls was higher than in the hollows in the barrels sampled adjacent to the deafferented region as well. In contrast, the density of radial glia was similar in barrel walls and hollows. The ability to accurately measure large numbers of neural profiles allows analysis of changes in populations of interactive neuropil elements.
Supported in part by the NIH (NS24386, HD 20327 and HD 04147).

265.9

DEVELOPING BARREL PATTERNS SHOWN BY SEROTONIN, LECTIN AND CYTOCHROME OXIDASE IN MICE

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Peanut lectin binding (PNA), cytochrome oxidase histochemistry (CO) and antibody binding to serotonin (5-HT) were used to identify emergent barrels in the postnatal mouse SI cortex. Littermates from several litters were prepared with each method to determine the relative time of appearance of the whisker- barrel pattern. The infraorbital nerve (IO) was sectioned surgically at birth (P0) to alter the barrel pattern. PNA barrel pattern is seen first on P4, declining by P10; CO pattern is first seen on P3, still rising on P10; 5-HT pattern is first seen on P3, mostly disappearing by P10. IO lesioned animals all had similar altered patterns of staining where barrels should have been. No barrel pattern appears before the clustered endings from VB. We conclude that all three markers follow the organization set by fibers from the thalamus. The exact role played by molecules binding PNA and 5-HT in the developing whisker map, if any, has yet to be determined.

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265.10

GLYCOCONJUGATE BOUNDARIES OUTLINE BARRELS THAT FORM IN VISUAL CORTEX TRANSPLANTED TO THE BARRELFIELD OF SI CORTEX. B.L. Schlaggar and D.D.M. O'Leary Depts of Neurosurgery and of Anatomy & Neurobiology, Washington Univ Sch Med, St Louis, MO 63110

The lectin, peanut agglutinin (PNA), reveals in developing rodent cortex a pattern of glycoconjugate "boundaries" that outline the barrels of primary somatosensory (SI) cortex. This transient pattern is unique to the SI barrelfield and is reported to precede the emergence of barrels (Cooper & Steindler 1986 J Comp Neurol 249:157; McCandlish et al. 1989 Exp Br Res 77:425). It is hypothesized that the lectin binds a framework of glycosylated molecules intrinsic to SI and responsible for establishing the barrel pattern (Steindler et al. 1989 Dev Biol 131:243). We have tested this hypothesis by transplanting E17 visual cortex to the prospective barrelfield of SI cortex in newborn rats and processing on P8 alternate sections with PNA to reveal any glycoconjugate pattern, and for AChE to mark ventrobasal thalamic afferents and Nissl to show the distribution of neurons. In layer 4 of normal P8 visual cortex processed in the same way, PNA binding is uniformly absent while the AChE stained geniculocortical input and the distribution of neurons are uniformly dense. However, within layer 4 of transplanted visual cortex (prelabeled with vital dye and ^{3}H-TdR) rings of PNA binding are present in a pattern that parallels a pattern of dense patches of AChE staining coincident with aggregations of neurons. These patterns are complementary, closely resemble those in normal SI cortex, and maintain continuity across borders between the host and transplanted cortex. Our results indicate that thalamocortical input molds the pattern of glycosylated molecules defined by lectin binding and that this molecular framework does not initiate the formation of individual barrels nor specifies the stereotypic pattern of barrels. We conclude that neither barrels nor the complementary glycoconjugate boundaries are predetermined in SI cortex and that thalamocortical input is a primary agent in the differentiation of area-specific features characteristic of the adult neocortex.

265.11

PLASTICITY IN THE MOUSE WHISKER-TO-BARREL PATHWAY: EFFECTS OF PERIPHERAL DEPRIVATION IN NEONATES ON THE FUNCTIONAL MAP IN ADULTS; A DEOXYGLUCOSE STUDY.

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Lesions of whisker follicles in newborn mice result in an altered morphological whisker-map in the barrel region of the sensory cortex. We studied the effects of such perinatal lesions on functional maps in the whisker-to-barrel pathway of the mice in adulthood. The functional maps were obtained with the autoradiographic [^{14}C]deoxyglucose (DG) method for the determination of local cerebral glucose utilization ($lCMR_{glc}$). From 7 albino mice the follicles of left whiskers C1-3 were removed on the day of birth. After 3 months, the lesioned mice and 7 age-matched control animals were injected with DG, and left whiskers B1-3 & D1-3 were stimulated. Brain sections were cut tangential to the pial surface through the cortex and transverse through the brainstem. In the control mice, $lCMR_{glc}$ was increased in two discrete areas of the barrel cortex contralateral to stimulation: barrels B1-3 & D1-3. In the lesioned mice, the two areas of high $lCMR_{glc}$ were fused by an area of lower, yet elevated $lCMR_{glc}$. Nissl and cytochrome oxidase (CO) staining showed that barrels C1-3 had not developed; the vacant territory was invaded by enlarged barrels of rows B & D, by C4 and by Y. Increased $lCMR_{glc}$ covered the full extent of enlarged barrels B1-3 & D1-3. No such fusion could be found in the trigeminal brainstem. Ipsilateral subnuclei caudalis and interpolaris showed, as in normal mice, two separate and distinct areas of increased $lCMR_{glc}$. In the territory where the removed whiskers are normally represented, CO activity was diminished, and segmentation was absent. This area was not filled in by adjacent segments.

265.12

BARREL SIZE IN MOUSE SOMATOSENSORY CORTEX INCREASES AFTER EARLY EYE REMOVAL. J.P. Rauschecker, S. Kröger* and B. Tian*. NIH Animal Center, National Institute of Mental Health, Poolesville, MD 20837 and Max-Planck-Institut für biologische Kybernetik, Tübingen, FR Germany.

Visual deprivation leads to compensatory changes in the auditory system of cats, which can be demonstrated on the physiological (Rauschecker & Harris, 1983), anatomical (Rauschecker & Aschoff, 1987), and behavioral (Rauschecker & Kniepert, 1988) level. Cats deprived of vision also have significantly longer vibrissae than normal controls (Rauschecker, Egert & Hahn, 1987). We therefore became interested in whether visual deprivation induces also modifications of the central somatosensory system. Since rodents have an anatomically distinct somatosensory cortex (the "barrel-field", Woolsey & Van der Loos, 1970), we decided to tackle this question in mice rather than cats.

Twenty pigmented mice were unilaterally or bilaterally enucleated 1 week after birth. After 3 months, flatmounts of cortex were produced, fixed in formalin, and cut into 50 μm thin sections. Cytochrome oxidase staining was used to reveal the barrels in somatosensory cortex. They were then traced with a camera-lucida system and their areas measured with a Zeiss planimeter. It was found that the mean size of 362 barrels in 5 mice after binocular enucleation was 57.1 mm^2/1000 while that of 520 barrels in 7 normal mice from the same litters was 51.8 mm^2/1000 ($t=3.78$; $p<0.001$). When barrel size was compared directly in the two hemispheres of 8 monocularly enucleated mice, mean area was larger by more than 10% on the side contralateral to the enucleated eye ($t=2.81$; $p<0.005$). Our results indicate that a substantial rearrangement of the somatosensory system is generated crossmodally by visual deprivation.

265.13

PERIPHERALLY EVOKED CORTICAL RESPONSES IN NEONATAL RAT SI BARREL FIELD FOLLOWS A GRADIENT OF DEVELOPMENT. C.A. McCandlish, R.S. Waters, and C.X. Li*. Dept. of Anatomy and Neurobiology, Univ. of Tennessee, Memphis, Col. of Medicine, Memphis TN 38163.

Previously, we reported that the peroxidase conjugated lectin (PNA) binds differentially to SI barrel sides/septa during the first postnatal week. The representation of the face barrel subfield is first detected by postnatal day 4 (PND-4), and is followed by the representation of the forepaw subfield on PND-5. These results led us to hypothesize that neurons in the face subfield may be responsive to peripheral natural or electrical stimulation before neurons in the forepaw and hindpaw subfields.

To test this hypothesis, neonatal rat pups between PND-3 through PND-10 were anesthetized with Nembutal (40 mg/kg ip) and placed in a head-holding apparatus. A midsagittal slit was made in the skin overlying the skull and the skin retracted. The cisterna magna was opened. The bone overlying the sensori-motor cortex contralateral to the head-holding apparatus was removed and the dura opened. A single micropipette filled with 2M NaCl or carbon-fibre electrode was inserted into the cortex and natural or electrical peripheral stimulation was used to evoke single and multiple-unit responses from SI cortex. Following sensory mapping, rats were sacrificed, hemispheres removed and sectioned in a tangential or coronal plane, and the tissue processed using PNA-histochemistry. Using these techniques, the following results were obtained:

1. Evoked cortical responses were elicited in the developing face barrel subfield as early as PND-4.
2. Evoked cortical responses in the forepaw barrel subfield were elicited by PND-5, while evoked responses in the hindpaw barrel subfield appeared shortly thereafter.

These results suggest that the face barrel subfield is functionally intact prior to the forepaw and hindpaw, corroborating our previous report of differential development of the SI barrel field in neonatal rats. (Supported by NSF Grant BNS 88-02766.)

TRANSMITTERS IN INVERTEBRATES III

266.1

TISSUE DISTRIBUTION, PARTIAL PURIFICATION, AND MODULATION OF THE GASTRIC MILL BY A CRUSTACEAN CHOLECYSTOKININ-LIKE PEPTIDE. G.G. Turrigiano, L. Ogden*, A. Van Wormhoudt*, and A.I. Selverston, Dept. of Biology, UCSD, La Jolla, Ca 92093.

The output of the gastric mill neural circuit is highly flexible, and can be modulated by a number of neuropeptides, including mammalian cholecystokinin (CCK) (Turrigiano and Selverston, J. Neurosci. 9:2486, 1989). There is evidence that an endogenous CCK-like peptide is responsible for the feeding-induced activation of the gastric mill in intact lobsters (Turrigiano and Selverston, Nature, in press, 1990), but it requires high concentrations of the mammalian peptide to activate the gastric mill. We therefore wished to isolate and characterize the effects of the endogenous CCK-like peptide. To this end we have made methanol extracts of several lobster tissues known to contain CCK-like immunoreactivity, including the stomatogastric ganglion, the pericardial organs, the eyestalks, and the haemolymph, and partially purified these extracts by reverse phase chromatography. Five peaks immunoreactive for CCK were fractionated in this way, and were common to all of these tissues. An additional two peaks were present in the stomatogastric ganglion extracts.

The physiological effects of the five peaks from pericardial organs were tested on the isolated stomatogastric nervous system. One peak, designated crustacean CCK E (C-CCK E) was found to modulate the activity of the gastric mill, in concentrations estimated to be between 10^{-8} and 10^{-10} M. This peptide can initiate cycling in a quiescent gastric mill, and can intensify ongoing gastric mill activity. The effects are dose-dependent, and can be blocked by the specific CCK antagonist proglumide, which also blocks the effects of mammalian CCK, and blocks the feeding-induced activation of the gastric mill in intact lobsters.

We are currently using an endoscope to test whether peptide C-CCK E can activate the gastric mill when injected into the hemolymph of intact lobsters, as is the case for mammalian CCK8 (see abstract by M. Boyle, G. Turrigiano, and A.I. Selverston), and to determine whether its behavioral effects are consistent with its effects on the isolated stomatogastric nervous system.

266.2

FMRFAMIDE-IMMUNOREACTIVITY IN THE CRAYFISH NERVOUS SYSTEM. A. J. Mercier, V. TeBrugge* and I. Orchard, Dept. of Biol. Sci., Brock University, St. Catharines, Ont., L2S 3A1, and Dept. of Zoology, University of Toronto, Toronto, Ont., M5S 1A1.

Previous work (eg. Kobierski et al, J. Comp. Neurol. 266: 1-15) suggests that FMRFamide-related peptides act as neurohormones in crustacea. The present study was undertaken to determine whether FMRFamide-related peptides are present in Procambarus clarkii. Immunostaining with a commercial antibody to FMRFamide revealed reactive cell bodies and processes within each ganglion and two brightly stained axons traversing almost the entire length of the nerve cord. Immunoreactive varicosities were present in the nerve cord and in the pericardial organs (PO's). In addition, the antibody stained 5-6 axons that exit the sixth abdominal ganglion via the intestinal nerve and form an immunoreactive plexus of varicosities on the hindgut.

The presence of FMRFamide-related material in all the above sites was confirmed and quantified with radioimmunoassay. The PO's had the highest levels of immunoreactivity; pooled PO's from one side of one crayfish contained the equivalent of about 64 pmol of FMRFamide. Extracts of the PO's were fractionated by HPLC using a C18 column and a Phenyl column with Acetonitrile gradients. Immunoreactive material co-eluted with two identified peptides from lobster PO's, with the sequences TNRNFLRF-NH_2 and SDRNFLRF-NH_2 (Trimmer et al, J. Comp. Neurol. 266: 16-26), and other peaks were also present. Immunoreactive fractions increased the rate and amplitude of contraction of isolated crayfish hearts, as did SDRNFLRF-NH_2. The results support a neurohormonal role for FMRFamide-related peptides. Supported by NSERC Canada.

266.3

ONLY TWO OF THE FOUR ACCESSORY NEURONS OF THE CRAYFISH MRO ARE GABAergic: PHENOTYPING NEURONS BY COMBINING BIOCYTIN BACKFILLS WITH IMMUNOCYTOCHEMISTRY. B. Mulloney and W.M. Hall*. Zoology and Neurobiology, Univ. California, Davis CA 95616.

The Muscle Receptor Organs in the abdomen transduce information about body position (Alexandrowicz, 1967). Each MRO is innervated by four Accessory neurons that are thought to use GABA to inhibit the MRO (Kuffler and Edwards, 1958; Leise et al., 1987). So we were surprised that the number of axons that labeled with GABA antiserum, four, in nerves innervating MROs in *Pacifastacus leniusculus* was less than the six predicted by the assumption that all Accessory neurons were GABAergic (Mulloney and Hall, 1990). To resolve this discordance, we examined the transmitter phenotype of each Accessory neuron.

We used a polyclonal antibody (Hoskins, et al., 1986) to label all GABAergic neurons in abdominal ganglia whose Accessory neurons had been filled with biocytin (Horikawa and Armstrong, 1988) by axonal backfilling (Iles and Mulloney, 1971). The cell bodies of the Accessory neurons lie in the ganglion posterior to the MRO they innervate; backfills of the nerve to an MRO fill only those four cell bodies in the next posterior ganglion. Wholemount preparations of these ganglia were analyzed and photographed using fluorescent optics: biocytin-filled neurons were visualized with Streptavidin-Texas Red, the antibody-labeled neurons with an FITC-conjugated secondary antibody. When all four Accessory neurons were filled clearly, two of them also labeled with antiGABA. In the same ganglia, the other two were not labeled by the antibody. We conclude that only two of these neurons use GABA as a transmitter; the transmitter phenotype of the rest is unknown.

266.4

SEROTONIN INDUCES PLATEAU PROPERTIES IN A STOMATOGASTRIC MOTONEURONE BY A MIXED CONDUCTANCE DECREASE AND INCREASE MECHANISM. Q. Kiehn and R. Harris-Warrick. Sect. of Neurobiology and Behavior, Cornell Univ., Ithaca, NY 14853.

Stretch activated sensory cells synapse on neurones in the stomatogastric ganglion (STG) in crabs (Katz, P. & Harris-Warrick, R., *J. Neurophysiol.*, 62:571, 1989). These cells, called gastro-pyloric receptor cells (GPR cells) contain acetylcholine and serotonin (5-HT). In the DG cell, a gastric mill motoneurone, GPR activity evokes cholinergic nicotinic EPSPs and induces bistable plateau properties. The transmitter responsible for the plateau induction was uncertain. We have examined the transmitter regulation and the underlying ionic basic of the plateau induction.

When nicotinic and muscarinic receptors were blocked by d-tubocurarine (0.1mM) and scopolamine (0.1mM), stimulation of GPR cells evoked a slow depolarization in DG. This sometimes exceeded the threshold for triggering a plateau. In other cases the plateau could be initiated by a brief depolarizing current pulse. A brief puff of 5-HT (10μM-1mM) onto the STG neuropil mimicked the non-cholinergic GPR plateau enabling effects in DG. The GPR- and 5-HT-elicited slow depolarization decreased upon hyperpolarization and was accompanied by an apparent increase in input resistance. The 5-HT-evoked depolarization, apparent increase in input resistance, and plateau induction persist in TTX (0.1μM-1μM). The plateau was, however, blocked in low (0-25%) Ca^{2+} and reduced in normal Ca^{2+} and 10mM Co^{2+}, leaving a slower, smaller depolarization. Under these conditions, 5-HT caused a decrease in the input resistance rather than an increase. The depolarization was practically eliminated in low Ca^{2+} and low Na^{+} (50%).

We conclude that 5-HT is responsible for the GPR-evoked plateau properties in DG. The plateau potential appears to be Ca^{2+}-dependent, while the induction process seems to involve both a conductance decrease and a conductance increase mechanism. Supported by NIH#NS17323

266.5

NEUROSECRETORY CELLS IN THE LOCUST CORPORA CARDIACA ARE BORN THROUGHOUT DEVELOPMENT AND MATURATION. S Kirschenbaum and M O'Shea. Cell Biology Lab, London Univ (RHBNC), UK.

The glandular lobes of the corpora cardiaca (CC) in the locust S gregaria synthesise adipokinetic hormones (AKH). Throughout post-embryonic development the total amounts of AKH peptides increase while the relative amounts change dramatically. To study the cellular mechanisms by which peptide amounts might be regulated, we provided cells of the CC with bromodeoxyuridine (BUdR) in vivo and in vitro. BUdR incorporated into glandular lobe cells was visualised by immunocytochemical methods allowing us to localise DNA replication in the CC. Replication is associated with cell division indicated by the presence of labelled cell pairs rather than isolated cells which would indicate replication leading to polyploidy. Pairs of new cells observed throughout the glandular lobe resemble the existing CC cells. Cell division takes place throughout development including the adult stage when the animals become sexually mature. During a 16-hour period the cell number in adult glands increases by approximately 5%. In CCs cultured in vitro BUdR labelling is observed indicating that new cells arise from within the CC rather than from cells which might form elsewhere. Double labelling studies in which we localised peptide hormone precursor and BUdR indicate that new cells arise from undifferentiated cells of the CC that do not express peptides.
Supported by NATO and SERC.

266.6

TWO SMALL mRNAs PRODUCE THE THREE DIMERIC PROHORMONES OF AKH I & AKH II AND ARE TRANSLATIONALLY REGULATED. M O'Shea, M-F Schulz-Aellen*, S Hekimi* and J Fischer-Lougheed*. Cell Biology Lab, Univ of London (RHBNC), UK.

The neuropeptides adipokinetic hormones (AKH I and AKH II) are processed from 3 dimeric prohormones called P1, P2 & P3. These are produced, following removal of signal sequences, by the oxidation of cysteines contained in two small proteins, pre-proAKH I and pre-proAKH II, translated from two small mRNAs. Both mRNAs are expressed in all neurosecretory cells of the locust (S. gregaria) corpora cardiaca (CC) - a peptide factory much like the ELH-producing bag cells of Aplysia. Relative amounts of AKH I and AKH II and the three AKH-Precursor-Related-Peptides (APRPs) change dramatically post-embryonically. Early AKH I to II ratio is approximately 1:1 and later in mature adults almost 5:1. Dimer formation occurs rapidly in the ER and amounts of each formed can be explained solely by the relative synthetic rates of the two subunits. Differential production of subunits could depend on the relative amounts of the 2 mRNAs (transcriptional control) or by the regulation of protein synthesis from similar amounts of the 2 mRNAs - indicating translational control. Experiments measuring translation in the CC and in cell-free assays, and Northern analysis to measure levels of the two mRNAs suggest that the production of precursor subunits is regulated translationally.
Supported by SERC and Ciba Geigy.

266.7

ROLE OF CENTRAL AND PERIPHERAL PEPTIDE RELEASE IN THE CONTROL OF ECDYSIS BEHAVIOR IN MANDUCA SEXTA. R.S. Hewes and J.W. Truman. U. of Washington, Seattle, WA 98195.

Insects perform highly specialized ecdysis behaviors at the end of each molt to shed the old exoskeleton. Physiological and behavioral events at ecdysis are coordinated by a unique, 62-amino acid neuropeptide, eclosion hormone (EH). Although EH was thought to trigger ecdysis via the circulation, the recent discovery in Manduca sexta of a set of EH-containing neurons that project throughout the CNS as well as into the periphery prompted an evaluation of the roles of centrally versus peripherally released peptide.

Although blood-borne EH is sufficient to trigger ecdysis, it is not necessary. Removal of the peripheral EH release site (the proctodeal nerves) eliminated circulating EH but did not interfere with the subsequent ecdysis behavior in prepupae. In addition, antidromic stimulation from the proctodeal nerve stumps (following proctodeal nerve removal) elicited precocious ecdysis, and preliminary immunohistochemistry has revealed arborizations and varicosities of the EH cells in each of the ventral ganglia.

The presence of blood-borne EH is necessary, however, for activation of a peripheral target (the Verson's glands) responsible for secretion of the cuticle cement layer at ecdysis. EH therefore is released both within the CNS to act on central targets and also into the blood to act on targets in the periphery. (Supported by NIH grant GMD7108-14 (RSH) and NSF grant DCB8615627 (JWT)).

266.8

STAGE SPECIFIC EXPRESSION OF FMRFamide-LIKE IMMUNOREACTIVITY IN MOTONEURONS OF THE TOBACCO HORNWORM, *MANDUCA SEXTA*, IS MEDIATED BY STEROID HORMONES. J.L. Witten and J.W. Truman. Dept. of Zoology, U. Washington, Seattle, WA 98195.

FMRFamide-like immunoreactivity (FLI; antiserum provided by Drs. E. Marder, Brandeis U. and B. Trimmer, U. Oregon) was localized to identified motoneurons in abdominal ganglia of the moth where the peptide(s) may function as a co-transmitter. The expression is present in motoneurons only during the larval stage. A gradual decline in the number of immunoreactive motoneurons occurs during metamorphosis and is not due to cell death but is temporally correlated to changes in the normal ecdysteroid titers.

The transition from larva to pupa is effected by two pulses of ecdysteroids, a small commitment peak (CP) followed by the larger prepupal peak (PP). To examine the role of these steroid peaks in the changing transmitter expression, we developed a novel *in vivo* ganglia culture system. Ganglia from specific larval stages, V+2(prior to the CP), W+0 (after CP) and W+3 (after PP) were implanted into a steroid-free environment, the abdomen of diapausing pupae. The results suggested that the CP regulated the decline in FLI. These conclusions were also supported by ecdysone infusions administered to mimic the CP or PP peaks. This is in contrast to the regulation of morphological changes in motoneuron dendritic arbors that occur during metamorphosis which are regulated by the prepupal rise in steroids. Supported by NIH NS13079(JWT).

266.9

TRANSMITTER SWITCH BY IDENTIFIED INSECT PEPTIDERGIC NEURONS IS CONTROLLED BY THE STEROID HORMONE ECDYSONE. N.J. Tublitz and P.K. Loi*. Inst. of Neurosci., U. Oregon, Eugene, OR 97403.

Recent work has demonstrated that mature neurons can alter their transmitter phenotype in vivo, yet little is known about the mechanisms controlling this biochemical plasticity. The moth, Manduca sexta, contains 4 individually identified neurosecretory cells that differentially express two neuropeptides post-embryonically. In larvae, these cells predominantly secrete one peptide, Cardioacceleratory Peptide$_2$ (CAP_2), whereas in pupae and adults, another peptide, bursicon, is produced. Because this transmitter switch occurs during metamorphosis, we tested the hypothesis that this CAP_2-to-bursicon conversion is regulated by the same hormonal mechanisms controlling metamorphosis, specifically flucuations in the levels of the steroid hormone, ecdysone.

To precisely determine the time of this switch, individual 4 cell clusters were microdissected from animals at various stages and assayed for both CAP_2 and bursicon contents. Our results revealed that the levels of the two peptides followed independent time courses. CAP_2 decreased in two discrete steps, with an initial drop seen at wandering followed by a slower decline over the next few days. In contrast, bursicon was not detectible until pupal ecdysis, after which there was a rapid accumulation. Results from ligation experiments implicated ecdysone as the causal factor responsible for these changes, with the CAP_2 decline regulated by the 'commitment' pulse of ecdysone whereas bursicon levels were affected by the 'prepupal' ecdysone peak . These conclusions were confirmed by ganglion implantation manipulations in which pre-metamorphic ganglia containing these cells were implanted into host animals at key stages prior to pupation and assayed for both peptides when the hosts were midway through adult development. These results are consistant with the idea that this transmitter switch is under the control of the insect steroid hormone, ecdysone.

266.10

Molecular and Genetic Studies of *Drosophila* Glutamic Acid Decarboxylases. F.R. Jackson, S.J. Kulkarni,* K.A. McLaughlin,* L.M. Newby,* and B.J. Walker.* Worcester Fndn. Exptl. Biol., Shrewsbury, MA 01545.

We previously reported molecular analyses of a *Drosophila glutamic acid decarboxylase* (*Gad*) gene mapping to chromosome region 64A3-5 (Jackson, F.R., *Soc. Neurosci. Abstr.* 14:30, 1988). Additional studies reveal that this chromosomal locus contains a pair of tandemly duplicated genes, one of which corresponds to the previously characterized *Gad* gene. We are presently engaged in cDNA sequencing studies to determine the similarity between the two genes. Concurrent immunoblotting studies have revealed the presence of at least three *Drosophila* proteins (43 kDa, 57 kDa, and 76 kDa) that cross react with anti-mammalian GAD antisera. The expression of these proteins appears to be developmentally and spatially regulated: the apparent ratio of the 57 kDa and 76 kDa forms changes between the pupal and adult stages, while the 43-kDa protein is detected in adult bodies (presumably due to expression in the thoracic ganglion), but not in adult heads.

As a corollary to these molecular analyses, we have initiated genetic studies of *Drosophila* GAD proteins. To produce *Gad* mutations, we have induced mutations in chromosome region 64AB that cause morphological phenotypes or result in lethality. Presently, we have isolated a minimum of 28 mutations, one of which maps to region 64A3-5 and is a candidate *Gad* mutation.

266.11

A GENETIC ANALYSIS OF THE *FMRFamide* NEUROPEPTIDE GENE IN *DROSOPHILA*. M.A. O'Brien and P.H. Taghert. Depart. of Anatomy & Neurobiology, Washington Univ. Sch. of Med., Saint Louis, MO 63110.

We are using *Drosophila* molecular genetics to investigate the functions of modulatory neuropeptides. The gene of interest encodes many diverse FMRFamide-related sequences; several features indicate that the >13 deduced peptides will have multiple functions. An analysis of expression throughout postembryonic development, using *in situ* hybridization, revealed that the gene is persistently expressed in different neuron types. Furthermore, stage-specific gene expression was displayed by certain neurons. We are in the process of creating mutations for the gene so that we may investigate the development and physiology of animals that lack its expression. Our first step has been to generate deficiencies in 46C, the cytological locus for the gene. Because the loss of a single copy of the *FMRFamide* gene would likely be invisible (behaviorally), we began with a strain containing an *Adh* (*Alcohol dehydrogenase*)-bearing P element inserted in the 46C-D region (from J. Hirsh, Harvard University). Flies were X-irradiated and progeny screened for loss of *Adh* gene function by selection on pentynol and pentenol. From ~43,000 progeny screened, 5 *Adh*$^-$ stocks were identified. These *Adh* revertants were further analyzed for the loss of adjacent genomic regions by cytological analysis, complementation tests and Southern blot analysis. The data indicated that in 1 stock the *FMRFamide* gene had been deleted. This strain, hemizygous for the *FMRFamide* gene, does not display any abnormalities. Because the severity of the null phenotype for the gene is not known, current efforts to generate new alleles include both a lethal screen, as well as using antibodies to screen for the absence of peptides in the case of a non-lethal phenotype. This genetic analysis should complement traditional pharmacological and physiological analyses of such modulatory neuropeptides.

266.12

REGULATION OF THE *FMRFamide* GENE IN *DROSOPHILA*. L. E. Schneider and P. H. Taghert. Dept. of Anatomy and Neurobiology, Washington University Medical School, Saint Louis, Mo 63110.

We have examined the expression of the *Drosophila FMRFamide* neuropeptide gene using *in situ* hybridization and immunocytochemical methods. Results from the two methods reveal that gene expression is restricted to a stereotyped subset of neurons in the CNS: ~60 in larval stages and ~100 in adults. The pattern of *FMRFamide* transcription develops gradually, beginning at ~70% of embryonic development, and the initial differences in steady-state levels of transcript are maintained. During metamorphosis, the basic pattern of transcription is retained, although a few hybridization signals are lost and new signals appear in several regions, including the optic lobes (MA O'Brien, LES and PHT, unpublished). We are interested in the molecular mechanisms that regulate these complex patterns of expression, including the cell-specific distribution of transcripts, the different times of developmental onset, the different steady-state levels of transcripts, and the postembryonic changes that occur during adult development. Many of these mechnanisms are likely to occur at the level of transcription and, therefore, to identify potential gene regulatory regions, we have compared the sequence of regions upstream of the *FMRFamide* gene between two *Drosophila* species, *D. melanogaster* and *D. virilis*. Several small regions that display sequence conservation are candidate DNA elements for the control of *FMRFamide* gene transcription. Currently, the function of these and other potential *cis*-regulatory regions, in directing cell-specific neuropeptide gene expression, is being studied *in vivo* with P element transformation methods.

PAIN MODULATION: PHARMACOLOGY III

267.1

MU-OPIOIDS INHIBIT TWO TRANSIENT CALCIUM CURRENTS INACTIVATED BY VOLTAGE, BUT SPARE A CURRENT INACTIVATED BY Ca^{++}. J.E. Schroeder, P.S. Fischbach*, M. Mamo*, and E.W. McCleskey*, Department of Cell Biology and Physiology, Washington University, St. Louis, MO 63110.

Three types of Ca current exist in adult rat dorsal root ganglia neurons. Low threshold T channels may influence firing threshold and bursting behavior. High threshold Ca current is composed of sustained and transient components. Availability of the latter depends on the rest potential and the prior electrical activity whereas the former seems to provide a baseline level of voltage-dependent Ca^{++} entry that is lost only when intracellular [Ca^{++}] rises.

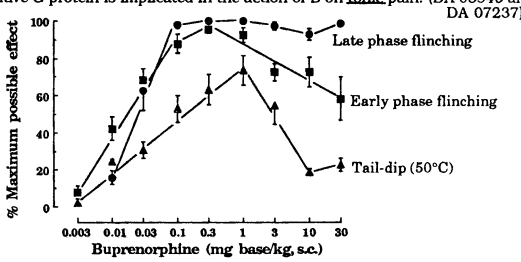
We report now that activation of opioid receptors by 100nM-1000nM DAGO, an enkephalin analog selective for the mu receptor, inhibits T and high threshold transient currents and spares the sustained current. Both the DAGO-sensitive and DAGO-insensitive current components are sensitive to 10μM omega-conotoxin, a calcium channel blocker.

10μM naloxone, an opioid antagonist, abolishes the DAGO effect fully. Surprisingly, either 500nM nor-binaltorphimine, a kappa receptor antagonist, or 10μM β-funaltrexamine, an irreversible mu antagonist, partially block the effect. When DAGO and dynorphin A, a kappa agonist, were applied to the same cells, DAGO consistently inhibited the Ca current more. This and the observation that dynorphin A does not inhibit T channels indicate that DAGO is not exerting its effects solely at kappa receptors. Others have reported that mu and kappa receptor activation cause increases in K and decreases in Ca currents, respectively. In addition to effects on K channels, our results indicate mu opioids inhibit multiple types of Ca channels in rat sensory neurons.

267.2

A PERTUSSIS TOXIN-SENSITIVE MECHANISM IS ASSOCIATED WITH THE ENHANCED EFFICACY OF BUPRENORPHINE IN A TONIC PAIN MODEL. H. Wheeler-Aceto* and A. Cowan. Department of Pharmacology, Temple University School of Medicine, Philadelphia, PA 19140.

In the rat tail-dip test buprenorphine (B) gives a bell-shaped dose-response curve, with low efficacy characteristic of 'agonist-antagonist' analgesics. We have compared this dose-response profile with that obtained in the rat paw formalin test (Wheeler-Aceto et al., Pain 40: 229,1990). B attained maximum efficacy against the acute phase (0-10 m post-formalin) of the response but increasing doses were associated with reduced efficacy. However, full efficacy was maintained against tonic pain (20-35 m post-formalin). The demonstration of three distinct DRC's with B depending on the nociceptive stimulus employed is in contrast to morphine which gives linear, full efficacy DRC's in all three tests. Despite the fact that pretreatment with PTX (1 μg i.th. 80-90 h prior to testing) markedly enhances formalin-induced nociception in both phases of the response, close to maximum efficacy was still achieved by B. The shape of the DRC against acute pain was unchanged; however, higher doses of B no longer maintained full efficacy against tonic pain. A PTX sensitive G protein is implicated in the action of B on tonic pain. (DA 03945 and DA 07237)



267.3

Contribution of Supraspinal Mu and Delta Opioid Receptors to Analgesia in the Rat. Y.O. Taiwo, C.A. Miaskowski and J.D. Levine* Neuroscience, UCSF, Box 0724, San Francisco, CA 94143

The relative contribution of different opioid receptor classes in supraspinally mediated analgesia has remained controversial. In this study, we evaluated the antinociceptive effects of intracerebroventricularly injected selective mu- (DAMGO), delta- (DPDPE) and kappa- (U50,488H) opioid agonists (5fg-5ng) on mechanical nociceptive threshold using the paw-withdrawal test. DAMGO and DPDPE, but not U50,488H produced dose-dependent increases in mechanical nociceptive thresholds. The antinociceptive effect of DPDPE, but not DAMGO, was inhibited by the selective delta antagonist ICI- 174,864 (3ug), suggesting that the actions of the agonists are mediated by different receptors. In addition, we observed that a low-analgesic dose of DPDPE (50fg) coadministered with sequentially increasing doses of DAMGO produced analgesic synergy. We conclude that mu and delta but not kappa opioid receptors contribute to supraspinal opiate-induced analgesia in the rat. Further examination of the contribution of supraspinal opioid receptors to analgesia, using other selective opioid antagonists, are currently in progress.

267.4

ORGANIZATION OF SOME FOREBRAIN INPUTS TO THE MIDBRAIN PERIAQUEDUCTAL GRAY. M. Ennis, T. Rizvi, M. Shipley and M. Behbehani. Depts. of Anatomy and Physiology, Univ. Cinti. Coll. Med., Cincinnati, OH 45267.

The midbrain periaqueductal gray (PAG) has been implicated in diverse functions such as analgesia, autonomic regulation, sexual behavior and defense\escape responses. Anatomical studies on the substrates of such functions have largely focused on connections of PAG with brainstem and medullary nuclei. The extensive input to PAG from forebrain structures has been largely unexamined. Here, we used axonal tracing to examine inputs to PAG from cortex, central nucleus of the amygdala (CNA) and the medial preoptic area (MPO).

PAG receives heavy input from medial prefrontal and lateral cortex (i.e. insular and perirhinal cortex). Anterograde labeling from both cortical regions was distributed along the entire rostrocaudal extent of PAG. Inputs from CNA and MPO also project along the entire rostrocaudal axis of PAG, however, in contrast to medial and lateral cortex, connections between PAG and CNA and MPO are strongly reciprocal. These forebrain regions gave rise to different patterns of terminal labeling in PAG.

Preliminary results indicate that at least some individual neurons in these 3 forebrain groups project to multiple rostrocaudal levels of PAG. These results indicate that PAG receives heavy and highly organized inputs from medial and lateral cortex, CNA and MPO. Projections from these forebrain areas gives rise to distinct longitudinal input columns that span the length of PAG. (Supported by PHS Grants NS20463, NS24698 and HL08097).

267.5

INTRATHECAL OPIATES AND α_2 AGONISTS PRODUCE A SYNERGISTIC ANTINOCICEPTION. M.H. Ossipov, E. Messineo*, R. Lozito*, J. Green*, S. Harris* and P. Lloyd*, Anaquest/BOC Health Care, 100 Mountain Ave, Murray Hill, NJ 07974, U.S.A.

The spinal antinociceptive interaction of morphine, fentanyl, and meperidine with clonidine is described. Male Sprague-Dawley rats received fixed ratios of clonidine to fentanyl (10:1), meperidine (1:3), or morphine (10:1) by i.t. injection through implanted catheters terminating at the level of the lumbar cord. Antinociception was assessed in the tail-flick test before (Control) and several times after (Post) drug injection. A 7 sec cut-off time prevented tissue damage. Data were converted to % maximal possible effect (%MPE) by: [(Post - Control)/(7 - Control)] x 100. The A_{50} doses (producing 50 %MPE) for clonidine, fentanyl, morphine, and meperidine are 44 µg, 1.2 µg, 1.4 µg, and 432 µg, respectively. For combinations of fentanyl, morphine, and meperidine with clonidine, the total drug A_{50} values are 1.06 µg, 0.82 µg, and 35 µg, respectively. These values are significantly less than those predicted if the interaction was purely additive; thus, isobolographic analysis indicates a synergistic interaction between α_2 adrenergic and opiate agonists. A pharmacokinetic effect is unlikely since fentanyl, unlike morphine and meperidine, does not produce an active metabolite yet still produces a synergistic effect with clonidine.

267.6

CONDITIONED AUTOANALGESIA: BLOCKADE BY INJECTIONS OF MORPHINE INTO THE ACCUMBENS NUCLEUS. R. F. Westbrook*, J. Harris*, A. J. Good* and G. Paxinos. School of Psychology, Univ. New South Wales, Sydney, NSW, Australia 2033.

Under some conditions, exposure to a heated floor aversively conditions associated cues and renders the rat analgesic when re-exposed to that heated floor. This aversive conditioning fails to occur when morphine-naive or morphine tolerant rats are given peripheral injections of the drug in combination with exposure to the floor on the first exposure (Greeley, J. and Westbrook, R.F., Q. Jl exp. Psychol., in press). The present experiment demonstrates a comparable failure of aversive conditioning when morphine is centrally administered on the first exposure to the heated floor. Specifically, unilateral injection of morphine into the accumbens nucleus (15µg in 1µl) did not provoke analgesia, but did block the aversive conditioning otherwise accruing from the initial exposure to the heated floor. Morphine blockade of aversive conditioning was reversed by peripheral injections of naloxone (5mg/kg), indicating an opioid inhibition of the processes which give rise to aversive conditioning and its resultant analgesia.

267.7

OPIOID AND NICOTINIC PROCESSES IN MEDULLARY HYPERALGESIA. W. R. Martin and S. Parvini*, Department of Pharmacology, University of Kentucky, Lexington, KY 40536.

Rats were prepared with an indwelling guide cannula which allowed the placement of a chemotrode in the mid 4th ventricle (4th V). The responses to ip and 4th V (0.5 or 1 ul) administered EKC, U-50,488 (U), morphine (M), (-)-nicotine (N), naltrexone (Na), and mecamylamine (Me) were studied in female Sprague Dawley rats (ca 250 gr) using a low intensity tail flick reflex (LITFR) with a mean control latency of ca 20 sec. EKC, U, and M administered ip produced a dose related increase in the latency of the LITFR whereas they produced a dose related decrease in the latency of the LITFR when administered into the 4th V. High dose (40 ug) 4th V N also produced hyperalgesia. Na and Me prolonged the latency of the LITFR when administered ip and into the 4th V and antagonized the hyperalgesic effect of EKC and N when administered ip. These data confirm the existence of a medullary hyperalgesic center and the role of both mu and kappa opioid and nicotinic mechanisms in its function. The analgesic effects of 4th V Na and Me may be a consequence of their antagonizing spontaneous activity of 4th V nicotinic and opioidergic hyperalgesic mechanisms. (Research was supported by the Tobacco and Health Research Institute, Univ. of Kentucky).

267.8

GABA VESICLE-CONTAINING DENDRITES: A CRITICAL ELEMENT IN PROCESSING SENSORY INPUT IN THE PRIMATE DORSAL HORN. **S. M. Carlton and E. S. Hayes*. Univ. of TX Medical Branch & Marine Biomedical Institute, Galveston, TX 77550.**

Several lines of evidence support a role of gamma-aminobutyric acid (GABA) as a major inhibitory transmitter in the mammalian nervous system. High concentrations of GABAergic cells in the dorsal horn of the spinal cord suggests it plays an integral role in the processing and modulation of sensory input. The purpose of this study was to identify the neurocircuitry of this system and determine how it related to dorsal horn elements. Three monkeys (M. fascicularis) were perfused with mixed aldehydes, the lumbar enlargement removed, cut on a vibratome (25µm), and immunostained with anti-GABA (1:1:000). The tissue was embedded in plastic, thin sectioned and analyzed at the EM level.

The majority of the input to GABAergic neurons occurred on the dendrites, not cell bodies. Furthermore, numerous GABAergic dendrites, containing synaptic vesicles, were observed presynaptic to labeled and unlabeled cell bodies and dendrites and to unlabeled spine heads and axon terminals; however, they were postsynaptic to primary afferents and other GABAergic dendrites. These data suggest that GABAergic dendrites play a role in feedforward and feedback inhibition in the spinal cord. Supported by NS11255, NS27910 and Bristol Myers-Squibb Corp.

267.9

POTENTIATED ANTINOCICEPTIVE EFFECTS IN A MODEL OF VISCERAL PAIN BY INTRATHECAL COADMINISTRATION OF AN ALPHA-2 ADRENOCEPTOR AGONIST AND A 5-HT-1B OR 5-HT-2 RECEPTOR AGONIST. R.M. Danzebrink and G.F. Gebhart, The University of Iowa, Iowa City, Iowa, 52242.

The interaction between adrenoceptor and serotonin receptor agonists in the spinal cord was evaluated in the present study following i.t. coadministration of clonidine (alpha-2 adrenoceptor agonist) with DOI or RU-24969 (5-HT-2 or 5-HT-1B receptor agonists, respectively). Colorectal distension (CRD) evokes a pressor response which was monitored before and after i.t. coadministration of receptor selective drugs; inhibition of the pressor response to CRD was indicative of antinociception. Supra-additive antinociceptive effects were produced following the i.t. coadministration of subanalgetic doses of clonidine with DOI and similarly with clonidine and RU-24969. The potentiated antinociceptive effects produced following the i.t. coadministration of DOI with clonidine were antagonized by i.t. pretreatment with methysergide but not by yohimbine or phentolamine and the effects produced following i.t. coadministration of clonidine with RU-24969 were antagonized by phentolamine but not by prazosin, yohimbine or methysergide. These results suggest modulation of descending 5-HT-containing bulbospinal neurons by descending NE-containing neurons and provide evidence for an important interaction between spinal alpha-2 and 5-HT receptors in the modulation of visceral nociceptive transmission.

267.10

THE NOCICEPTIVE RESPONSE TO IV 5HT IN RATS IS MEDIATED BY DUAL ACTIVATION OF $5HT_2$ AND $5HT_3$ RECEPTOR SUBTYPES. S.T. Meller, S.J. Lewis, M.J. Brody and G.F. Gebhart, Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

This study examined the specific receptor subtypes mediating the nociceptive response to IV 5HT. 5HT produced a dose-dependent (3-96 µg/kg, IV) inhibition of the tail-flick (TF) reflex (ED50=24 µg/kg) and complex cardiovascular responses. With doses greater than the ED50, complex pseudaffective responses were evoked (flattening of the ears, closing of the eyes to slits, contraction of the facial musculature). The ED75 dose of 5HT produced a passive avoidance behavior significantly different from saline. While α-methyl 5HT and 2-methyl 5HT produced dose-dependent (3-192 µg/kg, IV) $5HT_2$ and $5HT_3$ receptor-mediated cardiovascular responses, neither drug inhibited the TF reflex, produced pseudaffective responses or a passive avoidance behavior. However, a 1:1 combination of these agonists (3-192 µg/kg, IV) not only produced a cardiovascular profile similar to that produced by 5HT, but also produced a dose-dependent inhibition of the TF reflex (ED50=80 µg/kg) and the same profile of pseudaffective behaviors found with 5HT. Rats administered with the ED75 combination dose also showed a passive avoidance behavior. These results clearly support the hypothesis that the noxious character of IV 5HT requires dual activation of $5HT_2$ and $5HT_3$ receptor subtypes.

267.11

LIDOCAINE BLOCKS Aδ AND C FIBER INJURY DISCHARGE WITHOUT ALTERING NERVE CONDUCTION. M.B. MacIver and D.L. Tanelian. Anesthesia Dept., Stanford Univ. Sch. of Medicine, CA 94305.

Low serum concentrations of lidocaine (2 to 13 µg/ml) relieve both acute and chronic pain in humans. Conduction block of nerve fibers has not been demonstrated at these low concentrations. The present study investigated lidocaine's effects on discharge activity of acutely injured Aδ and C fiber nociceptors using an *in vitro* preparation of rabbit cornea. Corneas were maintained at normal temperature (35 °C) and intraocular pressure (18 mm Hg) and a standard 3 mm^2 abrasion was produced. The resulting injury discharges were recorded from nerve fibers using a glass suction electrode. Nerve fiber conduction velocity was measured following electrical stimulation using bipolar tungsten microelectrodes. Lidocaine produced a concentration-dependent depression of injury-induced action potential discharge. At 2.0 µg/ml, discharge frequency was significantly reduced by 18+/-3.2 % (SD, n=5; $p<0.005$ ANOVA) and a concentration of 20 µg/ml produced nearly complete block (98+/-2.5 % depression). The time course for lidocaine-induced depression was comparable to times for pain relief in patients (20 min), and full recovery was observed following 30 min of wash. The depression of discharge did not involve conduction block since electrically evoked action potentials could be elicited at concentrations up to 250 µg/ml. The results indicate that nerve endings are more sensitive to lidocaine than are axons, perhaps due to a lower Na+ channel density in sensory terminals and concomitant reduction in discharge 'safety factor'.

Supported by the Parker B. Francis Foundation and NIH.

267.12

DESYNCHRONIZATION OF THE EEG FOLLOWING LOW-DOSE MORPHINE. K.Grasing and H.Szeto*, Department of Pharmacology, Cornell University Medical College, New York, NY 10021.

We have previously shown that treatment with the opioid blocker naloxone can increase delta wave activity in the EEG of opioid-naive rats, if administered at the onset of active periods. This finding is suggestive of an excitatory effect of endogenous opioid peptides on level of arousal, that leads to a less alert EEG when an opioid antagonist is administered. To further investigate the hypothesis that opioids can result in a more alert level of arousal, we measured the effect of morhpine on the EEG when administered to rats during active periods.

Rats were prepared with chronic jugular catheters and lectrocortical electrodes, and EEG was analyzed on-line y fast fourier transformation. Morphine was administered is an intravenous infusion over one hour, two hours following the onset of darkness, at doses of 0.1, 1.0, and 10.0 mg/kg-hr. The 10.0 mg/kg-hr dose resulted in ncreased total spectral power, with periods of delta wave activity. In contrast, 1.0 mg/kg-hr was followed by periods of sustained decreases in total power, without a predominant frequency. This desynchronized EEG is similar to the EEG pattern that occurs in rats at the onset of darkness. In addition to sedating effects that occur at relatively high doses, low doses of morhpine cause a desynchronized EEG that is suggestive of a more alert psychological state.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CEREBELLUM I

268.1

OPTICAL IMAGING OF PARALLEL FIBER BEAM CHANGES TO APPLICATION OF GLUTAMIC ACID IN THE RAT CEREBELLAR CORTEX IN VIVO. S.A. Elias, H. Yae*, T.J. Ebner, Depts. Neurosurgery and Physiology, Univ. of Minnesota, Mpls., MN 55455.

Changes in the spatial patterns of neuronal activity in response to drugs are difficult to examine with conventional electrode recording. This study examines the effects of topically applied drugs on Purkinje cell responsiveness to parallel fiber (PF) input with optical imaging to measure amplitude and spatial changes. Rats were anesthetized with ketamine/xylazine, the cerebellum exposed and placed under an epifluorescence microscope and stained with the dye RH795. The imaging detector was a Photometrics CCD camera (14 bit A/D, 576X384 pixels). A PF beam was elicited with a tungsten electrode and the field potential monitored. Application of 0.5mM glutamate increased the amplitude of the imaged PF beam, whereas 5-50µM quisqualate (QQ) reduced the width and beam amplitude, which may be due to activation of inhibitory interneurons. The optical beam was not significantly affected by N-methyl-D-aspartate (NMDA) but was abolished with 50µM kainate (KA). δ-D-Glutamylaminomethyl-sulfonic acid (QQ and KA antagonist), D-O-phosphoserine (KA antagonist), and kynurenic acid (NMDA and KA antagonist) reduced the imaged beam. It would appear that the optical signal is post-synaptic in origin and that KA and maybe QQ are the glutamate receptors activated by the PF input. Supported by NIH Grant #RO1-NS-27210.

268.2

SPATIAL SPREAD OF VOLTAGE TRANSIENTS IN CEREBELLAR PURKINJE CELLS

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The information processing capability of Purkinje cells is thought to critically depend on a segregation of specific conductances, together with an electrotonic non-compactness.
We applied multisite optical recordings using voltage sensitive dyes to current- and voltage-clamped Purkinje cells to investigate these properties in cerebellar slice cultures. Voltage transients induced by intrasomatic current injection through the recording electrode were monitored optically from somatic as well as dendritic sites. Pharmacological blockade of potassium conductances resulted in an increased amplitude and a decreased rise-time of the dendritic signals.
These observations suggest that, under control conditions, part of the current spread between soma and dendrites is shunted by potassium conductances. We conclude that voltage signals are more or less independently integrated at the somatic and dendritic membrane of the Purkinje cell while the electrical coupling between compartments is controled by potassium conductances.

268.3

SET-RELATED UNIT ACTIVITY IN THE PRECENTRAL CORTEX AND CEREBELLAR NUCLEI OF PRIMATE. L. Germain, Y. Lamarre and M.-T. Parent, CRSN, Université de Montréal, Montréal, Québec, Canada H3C 3J7

In a two-choice instructed delay paradigm a set-related cell is defined as a unit modulated during the delay between the instruction and the go-signal. In order to study the activity of cerebello-cortical pathways during an instructed immobile waiting period a monkey was trained in the following task. After a fixed 500 msec control period, the monkey was given an auditory instruction as to the movement to make (400 Hz tone for extension, 1000 Hz for flexion). The duration of the tone was 400 msec which was followed by a delay period of 500-1500 msec. The GO-signal at the end of the delay period was a small torque perturbation of the elbow. The biceps and the triceps were never observed to contract during the delay as shown by EMG recording. A total of 177 task- related cells were isolated and recorded during 100 trials each. In the motor cortex (MI) 30 the premotor cortex (PM) 64 the dentate nucleus (D) 47 and the interpositus (I) 36 cells were recorded. A significant (paired t-test, $p \leq 0.005$) change of the firing rate during the instructed delay as compared to the control period was demonstrated for 64% of PM cells 62% of D cells 55% of I cells and 49% of MI cells. In several trials a movement in the wrong direction was performed during recording of a cell. The analysis of these trials shows that the pattern of activity of set related cells is related to the direction of the intended movement and not to the auditory instruction. These results suggest a conribution of cerebello-cortical pathways in the preparation for movements. (Supported by MRC Group Grant in neurological sciences).

268.4

VISUAL PONTINE PROJECTIONS TO THE CEREBELLAR HEMISPHERES OF MACAQUES. M. Glickstein, B. Mercier*, J. Steiff, C. Legg* and J. Voogd*, University College London, Gower St. London WC1E 6BT, England and Erasmus University, 3000 DR, Rotterdam, Netherlands.

Extrastriate areas MT, MST and adjacent visual areas of the parietal lobe project to the dorsolateral region of the pontine nuclei. Cells in this region of the pons respond vigorously to appropriate visual targets. In this study small WGA-HRP injections were made into target zones in the cerebellar cortex. Injections into either dorsal or ventral paraflocculus lead to retrogradely labelled cells bilaterally in the dorsolateral pontine nuclei. Injections into the paramedian lobe result in labelled cells which border and extend into this region. The bilaterality of pontocerebellar projections may account for the paradoxical sparing of visuomotor performance in monkeys deprived of cortical connections between visual and motor areas. These data help to establish the principle cerebellar targets of each of the extrastriate cortical areas projecting to the pontine nuclei.

268.5

UNIFORM CONDUCTION TIMES OF CLIMBING FIBERS DETERMINED AT DIFFERENT FOLIAL DEPTHS USING A MULTIPLE ELECTRODE RECORDING PARADIGM. I. Sugihara, E. J. Lang, R. Llinas Dept of Physiology, New York University Medical Center, 550 First Avenue, New York, NY 10016.

The functional organization of olivo-cerebellar system has been studied using multiple electrode recording of complex spikes (CSs) in Purkinje cells. In a previous study it was found that simultaneous firing of CSs occurred in organized rostro-caudal bands of Purkinje cells on a given folium (Sasaki et al., *European J. of Neurosci.* 1: 572-586, 1989).

Here we measured the conduction times of the olivo-cerebellar fibers by recording the Purkinje cell CS responses to olivary tract stimulation at the brainstem in anesthetized adult rats. In 104 CS units recorded from the surface of the Crus IIa folium, the latency of the onset of the direct CS response ranged from 3.4 to 4.6 ms, however most latencies were close to 3.9 ms (3.92 ± 0.28 (mean $\pm$ SD, n=104)).

The conduction time of olivary fibers projecting to the Purkinje cells in the deep portions of the cerebellar folium (Crus IIa) was also measured. It ranged 3.4 - 5.0 ms, also close to 3.9 ms in latency (3.93 ± 0.28, n=26). It was nearly the same for the deep as that of the superficial portion of the folia, although the deep fibers are up to 20 % shorter than the superficial ones. Multiple electrode experiments in which electrodes measured CSs from the deep portion of the folium indicated that they tend to fire simultaneously with CSs in the follial surface and also with an approximate rostro-caudal organizations. These results indicate that the conduction velocity of the olivo-cerebellar systems seems to be organized such as to generate a synchronous activation of Purkinje cells independent of their location on a given folium.

268.6

INTERPOSITUS DISCHARGE DURING REACHING A.R. Gibson, K.M. Horn, P.L.E. van Kan. Barrow Neurological Institute, Phoenix, AZ, 85013

Single units in the interpositus nucleus of the cerebellum fire at high rates when a monkey reaches out and grasps a raisin. The goal of the present experiment is to understand what elements of reaching are important for eliciting discharge. The experimental paradigm separated the reaching motion into a coordinated limb movement with and without a grasping component. We trained monkeys to operate a lever device that described an arm trajectory similar to that of a reach. To date, 50 forelimb interpositus units have been tested during device operation and during reaching. Many units showed vigorous phasic firing when the monkey released the device handle and retrieved a raisin from a remotely opened box positioned near the end point of the lever trajectory, but few units fired well during device operation. The beginning and end points of the reach are the same as those of lever operation, and we conclude that the hand component of a reach is important for interpositus firing. Individual units show a distinctive pattern of firing during the raisin reach indicating that they serve different aspects of the movement. Some units fire discrete bursts at particular movement phases while others fire throughout the entire movement. We hypothesize that the interpositus is important for coordinating hand use with movements involving the entire forelimb.

268.7

COMPARISON OF SIMPLE AND COMPLEX SPIKE ACTIVITY IN IDENTIFIED SAGITTAL ZONES OF THE CAT CEREBELLUM DURING PERTURBATION OF THE LOCOMOTOR CYCLE USING A MULTIUNIT RECORDING TECHNIQUE. T.M. Kelly, F.J. Rubia*, F. Kolb*, J.D. McAlduff*, and J.R. Bloedel. Div. of Neurobiology, Barrow Neurological Institute, Phoenix, AZ 85013.

These experiments compare the characteristics of simple and complex spike activity in identified sagittal zones during modifications of the locomotor cycle and test the hypothesis that the information processing in each zone relates differently to the kinematics and EMG activity of the animal's forelimb during corrective movements. The study was performed in acutely decerebrated cats capable of locomoting on a treadmill. Two five-electrode arrays, each positioned in an electrophysiologically identified zone, were used to simultaneously record the activity of up to 10 Purkinje cells. Initially, the cat was conditioned to modify its gait in order to avoid a bar interjected into the forelimb's trajectory on successive step cycles. Once the animal acquired this behavior additional modifications in gait were generated by either changing the height of the bar or the speed of the treadmill.

The data support the view that neuronal interactions in several of the sagittal zones are uniquely associated with corrective changes in gait. Furthermore, these changes in activity are not restricted to a period immediately following the onset of the perturbation. Rather, in many trials, the modulation of simple and complex spike activity was most dramatic as the animal acquired and subsequently performed the new strategy during periods of prolonged perturbation. The data suggest that neuronal activity in these zones is principally related to characteristics of the movement and its performance rather than only with the acquisition phase of the task. (Supported by NIH Grant NS21958)

268.8

VARYING THE SET OF RECEPTIVE FIELDS CHANGES WHICH OF THE STIMULUS ARRAYS EVOKE THE SAME ENSEMBLE RESPONSE G. McCollum, R.S. Dow NSI, Good Samaritan Hosp. & Med. Ctr., Portland, OR 97209

Stimulus arrays (complex stimuli considered as multipositional, multisensory combinations of simpler stimuli) are transformed by the nervous system into ensemble responses (response activity of many neurons). This talk will examine a construction of temporally simple ensemble responses assuming simple unions of single-cell responses in cat tactile receptive fields in cerebellar climbing fibers in the anterior lobe and paramedian lobule.

Complex stimuli may be reduced in the nervous system in order to extract the specific information required. To achieve such a reduction, different stimulus arrays may evoke the same ensemble activity, so that the information contained in the difference is lost.

Stimulus arrays that evoke the same ensemble response are response-equivalent. The set of all stimulus arrays is divided into equivalence classes according to available ensemble responses. The population of receptive fields determines the set of available ensemble responses, and therefore how the set of stimulus arrays is divided into equivalence classes.

The equivalence relation will be treated algebraically. When the set of stimulus arrays is mapped onto the set of ensemble responses and back again, the equivalence relation is expressed as a closure on the set of stimulus arrays.

268.9

PURKINJE CELL COMPLEX SPIKE MODULATION DURING VOLUNTARY MOTOR LEARNING IN THE PRIMATE. C.L.Ojakangas, T.J.Ebner, Depts. Neurosurgery and Physiology, Neuroscience Grad. Program, Univ. of Minnesota, Mpls., MN 55455.

The climbing fiber system's role in voluntary motor behavior during learning remains unclear. Two rhesus monkeys performed a two-dimensional, visually-guided arm movement, placing a cursor in target boxes using a manipulandum. When the relationship (gain) between cursor and hand position was altered, the animals gradually adapted to the imposed error and matched their arm movements to the new gain. Primates adapt to a novel gain over 100-200 trials by gradually scaling peak tangential velocity and movement amplitude while keeping time until peak velocity constant. Of 89 identified Purkinje cells, 43% showed complex spike modulation which increased significantly during learning. The increase occurred early in the movement (within the first 400 ms) with a mean of 233% +/- 167% of control ($p < .0005$, T test). Of these, 46% showed no subsequent peak following adaptation. For 35% of all P-cells the complex spike discharge increased during corrections later in the movement. Of these cells, 75% did not show this increase once corrections were eliminated. In summary during the adjustment to a novel condition, P-cell complex spike discharge is engaged, suggesting a role for the climbing fiber system in the scaling and calibration of the metrics of voluntary movements. Supported by NIH Grant #5R01-NS-18338.

REGULATION OF AUTONOMIC AND RESPIRATORY FUNCTIONS

269.1

RETICULOSPINAL SYMPATHOEXCITATORY NEURONS MODULATE THE PRESSOR REFLEX TO MUSCULAR CONTRACTION. R.M. Bauer and T.G. Waldrop, University of Illinois, Urbana, IL 61801

Previous studies have shown that many neurons in the ventrolateral medulla (VLM) with discharge related to sympathetic nerve activity demonstrate increased firing frequency during hindlimb muscular contraction (MC). These results suggest that sympathoexcitatory neurons in the VLM participate in the pressor reflex evoked by MC. In the present study extracellular single unit activity was recorded in anesthetized cats to determine if spinally projecting VLM neurons show altered firing patterns during MC. Hindlimb MC was induced by electrical stimulation of L7 and S1 ventral roots. Units were tested for antidromic activation by spinal stimulation in the intermediolateral nucleus (T2 or T5). VLM unit discharge was also related to cervical sympathetic nerve discharge or the cardiac cycle with a computer averaging program. Most VLM neurons (78%) which were activated antidromically displayed cardiovascular related discharge. Arterial pressure and the firing rate of most VLM units (64%) increased during MC. Nearly all of the reticulospinal VLM neurons (92%) with cardiovascular related discharge were excited by MC. Most VLM neurons unaffected by spinal stimulation or unrelated to cardiovascular rhythms were unaffected by MC. This suggests that reticulospinal VLM neurons with cardiovascular related discharge participate in the pressor reflex evoked by muscular contraction.

269.2

CONVERGENCE OF DIFFERENT SENSORY INPUTS ONTO RETICULOSPINAL NEURONS IN VENTROLATERAL RETICULAR FORMATION. A.R. Evans and R.W. Blair. Dept. Physiol. & Biophysics, Univ. Okla. Health Sci. Ctr., Oklahoma City, OK, 73190.

The ventrolateral medullary reticular formation (VLRF) contains pools of neurons involved in cardiovascular, respiratory, and nociceptive functions. Few studies have examined whether neurons in VLRF respond to different sensory inputs. The goal of this study was to determine response characteristics of VLRF reticulospinal (RS) neurons to sympathetic, vagal, somatic, baroreceptor, auditory, and visual stimuli. Twelve cats were anesthetized with α-chloralose (40 mg/kg). Extracellular potentials were recorded from 21 neurons in VLRF whose axons were antidromically activated from the T_3 segment of the spinal cord. Mean conduction velocity was 28.6 M/s. Eleven neurons (52%) were excited by electrical stimulation of the left stellate ganglion. Two neurons (11% of 17 tested) were inhibited by vagal afferent stimulation, and three were excited. Ten of 19 (53%) and four (20%) of 20 neurons tested were excited by auditory and visual inputs, respectively. Eleven (59% of 19 tested) had somatic receptive fields. Three neurons were excited by decreases in blood pressure, and/or inhibited by increases in pressure, and one was the reverse, indicating that they received baroreceptor input. Nine neurons (60%) responded to stimuli from at least two sensory modalities. We conclude that RS neurons in the VLRF integrate afferent information arriving from different sources of sensory input. (Supported by NIH grant HL29618).

269.3

CARDIOVASCULAR EFFECTS OF NEUROTENSIN IN VENTROLATERAL MEDULLA AND NUCLEUS AMBIGUUS. J. Ciriello and T. X. Zhang, Dept. of Physiology, Univ. of Western Ontario, London, Canada, N6A 5C1.

The ventral medulla has been shown to be extensively innervated by neurotensin containing fibers. In this study the cardiovascular effects of microinjecting neurotensin at sites throughout the ventrolateral medulla (VLM) and nucleus ambiguus (AMB) region were investigated in chloralose-anesthetized, paralyzed and artificially ventilated male Wistar rats. Microinjections (20-50 nl) containing 100-250 pmol of neurotensin elicited a decrease in mean arterial pressure (89 ± 9 mmHg) when the injections were made at sites from the junction of the A1/C1 region rostrally into the C1 region. In addition, decreases in heart rate (45 ± 5 bpm) were elicited when the injection sites were located in AMB. The cardiac slowing was not affected by spinal cord transection at the level of C1-C2 (51 ± 9 bpm), but was abolished by ipsilateral vagotomy. The depressor response was reduced by approximately 60% following spinal cord transection and by 40% in spinal cord intact vagotomized rats, suggesting that a component of the depressor response was secondary to the vagal bradycardia. These data suggest that neurotensin containing fibers and terminals innervating the ventral medulla are components of a neuronal circuit controlling vasomotor tone and cardiac function.
(Supported by MRC of Canada).

269.4

EFFECT OF PARAVENTRICULAR NUCLEUS OF THE HYPOTHALAMUS ON PLASMA RENIN ACTIVITY IN NEUROGENIC HYPERTENSION. T. X. Zhang and J. Ciriello, Dept. of Physiology, Univ. of Western Ontario, London, Canada, N6A 5C1.

We have previously shown that paraventricular nucleus of the hypothalamus (PVH) lesions alter the development and maintenance of the hypertension resulting after aortic baroreceptor denervation (ABD) in the rat. This study was done to determine if changes in plasma renin activity (PRA) were associated with the hypertension after ABD and if PVH contributed to these changes in PRA. Rats were instrumented with arterial cannulae for the recording of arterial pressure (AP) and the removal of blood samples. After ABD, the rats received either sham or bilateral electrolytic lesions of PVH. AP was significantly elevated (30-50 mmHg) in all rats after ABD. Similarily, PRA was increased (11.6 ± 0.6 ng/ml/h) after ABD compared to pre-ABD levels (7.5 ± 0.8 ng/ml/h). PVH lesions significantly reduced the elevated AP and PRA (3.6 ±1.1 ng/ml/h) to levels not different from pre-ABD levels. On the other hand, sham lesions of PVH did not alter the elevated AP and PRA (12.9 ± 0.9 ng/ml/h) after ABD throughout the experimental period. These data suggest that ABD results in an increased activity of PVH neurons which in turn increase sympathetic drive to the kidney which results in an increase the release of renin that likely contributes to the development and maintenance of neurogenic hypertension.
(Supported by Heart and Stroke Fdn. of Ontario).

269.5

NEUROTOXIN LESIONS OF THE PARAVENTRICULAR NUCLEUS (PVN) ATTENUATE HORMONAL AND CARDIOVASCULAR RESPONSES TO STRESS. M.F. Callahan, W.K. O'Steen, K.A. Gruber*, and M. Morris. Depts. of Medicine, Anatomy and Physiology and Pharmacology. Bowman Gray School of Medicine of Wake Forest University, Winston Salem, NC 27103.

Electrolytic lesions of the PVN or antagonism of vasopressin/oxytocin receptors attenuates tachycardia responses to stress. We have sought to determine whether damage to PVN cell bodies would attenuate stress responses.

Male Sprague-Dawley rats received neurochemical lesions of the PVN(5.0ug ibotenic acid/0.5ul/nucleus) or vehicle injection then intravenous and arterial catheters. Rats were subjected to a mild footshock stress and measures of arterial pressure, heart rate, plasma oxytocin and vasopressin were taken prior to, immediately after and five minutes after the stress. Two days later each rat received i.v. hypertonic saline (150ul/100g body wt. 18% NaCl sloution). Plasma samples were taken at baseline and five minutes post infusion. Brain sections were evaluated histologically for lesion placement and extent.

PVN lesions attenuated the tachycardia (78+8bpm -v- 131+11 bpm in controls) and oxytocin response to stress (400+91% increase -v- 2070+418% in controls). Plasma vasopressin failed to increase in response to stress with no differences seen between the two groups. In addition, the plasma oxytocin response to osmotic challenge showed no differences between the groups. Thus the lesion specifically attenuated plasma oxytocin and tachycardia responses to stress.

269.6

NEURAL PATHWAY MEDIATING VASOPRESSIN (AVP) RELEASE FOLLOWING SPLANCHNIC OSMORECEPTOR ACTIVATION. M.S. King* and A.J. Baertschi, Neuroscience Prog., Univ. of Virginia, Charlottesville, VA 22908.

To determine the central neural pathway by which splanchnic osmoreceptors stimulate AVP release, bilateral electrolytic lesions of selected brain areas were performed 6 days prior to gastric infusion of hypertonic saline through a naso-gastric tube in conscious rats (598 mOsm/kg, 2ml/4min). The most effective lesions were located in the locus sub-coeruleus (sub-LC), approximately 1 mm below the locus coeruleus. Bilateral sub-LC lesions (n=9) attenuated the AVP response to hypertonic gastric infusion by 61.6% ($p<.05$), as compared to sham-lesioned controls. Bilateral lesions of the locus coeruleus (n=4), periaqueductal gray (n=5) and lateral parabrachial nuclei (n=5) were ineffective. Lesions of the median preoptic area (n=7) nonsignificantly decreased the AVP response by a maximum of 38.0%, suggesting that this area may play a minor role in this response. The sub-LC lesions interrupted a catecholaminergic pathway, as indicated by diminished DBH immunostaining in the SON and PVN following the lesion. This suggests that a catecholaminergic pathway, which traverses the sub-LC area, is necessary for the stimulation of AVP release following activation of splanchnic osmoreceptors. [Supported by NSF BNS-8819877].

269.7

EPISODES OF PRIMARY CORONARY VASODILATION DURING PERIODS OF SLOW WAVE SLEEP INTERRUPTED BY EEG DESYNCHRONIZATION. **LW Dickerson*, AH Huang*, BD Nearing*, RL Verrier. Georgetown University Medical Center, Department of Pharmacology, Washington, D.C. 20007.**

We have previously observed sympathetically mediated surges in heart rate (HR) and coronary blood flow (CBF) with little or no change in mean systolic blood pressure (SBP) during REM sleep in dogs. In the present study, significant increases in CBF were preceded by transient asystole during slow wave sleep (SWS). Five beagles were instrumented with electrodes for polysomnography, ECG and arterial SBP, and with Doppler probes around the circumflex coronary artery to record CBF. We selected samples for analysis which were characterized by an increase in CBF occurring during deep slow wave sleep interrupted by desynchronization of the EEG and by a pause in heart rhythm of at least 1.3 seconds (1.3-6.1 sec). The rate-pressure product (HR x SBP) is the standard index of cardiac metabolic demand. *P<0.05

	SWS Pre-Asystole	SWS Post-Asystole
CBF (mean kHz shift/min)	**84 ± 8.0**	**113* ± 7.9**
HR x SBP (bpm x mmHg)	**12,128 ± 1028**	**12,786 ± 705**

We conclude that the CBF increases are not due to cardiac metabolic demands but instead to primary (neurogenic) coronary vasodilation, resulting from enhanced vagal discharge indicated by the associated asystole. The characteristic, brief desynchronization of SWS suggests that this phenomenon may represent the coronary vascular component of arousal/orienting responses.

269.8

EFFECTS OF GASTRIC VAGAL FIBER STIMULATION ON SINGLE NEURONS IN NUCLEUS ACCUMBENS IN THE CAT. C.S. Yuan* and W.D. Barber. Dept. of Anatomy, College of Medicine, Univ. of Arizona, Tucson, AZ 85724.

It has been suggested that the nucleus accumbens (NA) may serve as a functional interface between the limbic and motor systems (FASEB J., 4:A977, 1990). We conducted studies on anesthetized cats to evaluate gastric vagal input to NA. Unitary responses were recorded extracellularly in NA during electrical stimulation of gastric vagal branches serving the proximal stomach. The nerves were stimulated with a paired-pulse (10 msec interval), 0.3 msec in duration, 300 μA at a frequency of 0.5 Hz. In some experiments, the left greater splanchnic nerve was also stimulated. A total of 102 orthodromic gastric vagally-evoked unitary responses were recorded bilaterally from the NA. The mean latency of the unitary discharges in NA was 395 ± 44.3 msec (range 296-525 msec), while the mean latency in the medial and lateral hypothalamic gastric vagally-evoked unitary responses, recorded in our previous experiments, was 370 msec. Eighty eight units (87%) showed phasic responses while 14 tonic discharges (13%) had excitatory or inhibitory responses to gastric vagal input. Spontaneously discharging neurons recorded in NA were fewer in number than recorded in the hypothalamus. Convergent input between gastric vagal and greater splanchnic fibers upon single accumbens neurons was also observed. Our present data demonstrated that NA also receives visceral input from the proximal stomach. The gastric vagally-evoked unitary responses and convergent input from the splanchnic fibers suggested that the NA may play a role in integration of gastric signals concerned with the ingestive process. (Supported by USPHS Grants DK 36289 and DK 35434).

269.9

FREQUENCY OF RESPIRATORY PAUSES IN INFANTS WHO DIE OF THE SUDDEN INFANT DEATH SYNDROME. V.L. Schechtman, R.M. Harper, A.J. Wilson* and D.P. Southall*. Brain Research Inst. and Dept. of Anatomy & Cell Biology, UCLA, Los Angeles, CA 90024; Dept. of Med. Phys. & Clin. Engineering, Univ. of Sheffield, England; National Heart & Lung Inst., London, England.

Infants who subsequently die of the sudden infant death syndrome (SIDS) are subtly different from other infants on a variety of physiological measures including heart rate, heart rate variation, and the coordination of cardiac and respiratory systems. The frequency of respiratory pauses has been shown to be altered in infants at risk for SIDS but not in infants who succumbed to the syndrome. In this study, we assess the frequency of apneas during quiet sleep and rapid eye movement sleep in control infants and infants who subsequently died of SIDS. Sleep states were identified in 12-hr physiological recordings of SIDS victims and matched control infants. The number of respiratory pauses > 4 sec was computed for each state. SIDS victims over 1 mo of age showed significantly fewer pauses than did age-matched controls. Short respiratory pauses (< 7 sec) accounted for most of the reduction in the SIDS victims during both states. During the first month of life, the frequency of respiratory pauses in SIDS victims was comparable to that of controls. The finding that this respiratory difference exists during the second month of life, just before the period of maximal risk for SIDS, but not earlier, may have implications for the etiology of SIDS.

Supported by NICHD grants HD22695 and HD22506.

269.10

MODULATION OF RESPIRATION BY NEURONS OF THE CENTRAL NUCLEUS OF THE AMYGDALA DURING BEHAVIORAL AROUSAL. C.A. Richard, S.L. Raetz and R.M. Harper. Brain Research Institute and Department of Anatomy & Cell Biology, UCLA School of Medicine, Los Angeles, CA 90024.

The central nucleus of the amygdala (ACE) plays a role in the behavioral expression of arousal, attention and defensive reactions. Coincident with natural or ACE-induced arousal and attention to the environment are respiratory changes, e.g. apneusis and/or tachypnea. Single-pulse stimulation of the ACE results in respiratory pacing, a phenomenon confined to the aroused state. We hypothesized that a subpopulation of ACE neurons should respond to environmental stimuli and also exhibit discharge patterns related to respiration. Bundles of 65μ microwires were placed in the ACE, and EMG leads were placed in the diaphragm of three cats under pentobarbital anesthesia. After at least 1 week of recovery, recordings were performed during the presentation of exteroceptive stimuli, e.g. taps or knocks on the recording box, hand claps, light flashes or dimming of ambient light. Single unit activity was quantified for 5 sec before and after the stimuli. Respiratory relationships were assessed by linear regression of firing rate vs. total cycle time and cross-correlation analysis during epochs of 60-240 sec containing several stimuli. Fourteen cells were recorded, with 8 cells showing consistent increases in firing rate and 2 cells showing consistent decreases in discharge rate. Of these 10 cells, 7 were related to respiratory patterning. We suggest that a subset of ACE neurons that are modulated by arousal mechanisms may contribute to the respiratory changes accompanying arousal. Supported by HL-22418-13.

270.1

IS *XENOPUS* RETINAL MELATONIN-DEACETYLATING ARYL ACYL-AMIDASE RELATED TO CHOLINESTERASES? Michael S. Grace and Joseph C. Besharse, Depts. of Anatomy and Cell Biology, Univ. of Kansas Med. Ctr., Kansas City KS, and Emory Univ. Sch. of Med., Atlanta GA .

Melatonin is deacetylated to produce 5-methoxytryptamine (5MT) in the retina of *Xenopus laevis* and in the eyes of other non-mammalian vertebrates by an aryl acylamidase (AAA)-like enzyme. This pathway appears to be the sole clearance mechanism for melatonin in the *Xenopus* retina. Previous reports have shown that mammalian AAA and acetylcholinesterase (AChE) activities are inseparable. We are therefore studying the relationship between cholinesterases and the *Xenopus* retinal melatonin deacetylase. Melatonin deacetylase activity occurs in retina, pigment epithelium/choroid and pigmented skin, all of which are affected by melatonin, but is absent in brain, blood, muscle and liver. Whereas acetylcholinesterase exists in both membrane-associated and soluble forms, retinal melatonin-deacetylating activity requires detergent (0.5% Triton X-100 or 30mM octylthioglucoside) for solubilization. The AChE inhibitor eserine (physostigmine) at 100μM inhibits the the *Xenopus* melatonin deacetylase by approximately 75%, but 1mM acetylcholine (ACh) has no effect. Melatonin, 5MT and N-acetylserotonin inhibit ^{3}H-melatonin deacetylation in a dose-dependant manner. Butyrylcholinesterase and AChEs from bovine erythrocytes and electric eel hydrolyze ACh, but fail to generate 5MT from melatonin. These results suggest that the melatonin deacetylase and cholinesterase activities are not associated. Support: NIH grant EY02414 and a Sigma Xi Grant-in-Aid of Research

270.2

DOPAMINE ACTS THROUGH D2 RECEPTORS TO MIMIC LIGHT IN PHASE-SHIFTING THE CIRCADIAN CLOCK IN THE RETINA OF *XENOPUS*. G.M. Cahill and J.C. Besharse, Department of Anatomy and Cell Biology, University of Kansas Medical Center, Kansas City, KS 66103.

Melatonin synthesis in the retina of the frog, *Xenopus laevis*, is regulated by a retinal circadian oscillator. We have used a superfusion culture system to monitor rhythmicity of melatonin overflow from individual eyecups in experiments designed to identify input pathways of the circadian oscillator. The results indicate that D2 dopamine receptors are part of such an input pathway. The D2 receptor agonist quinpirole (100 nM) suppresses melatonin production and causes phase-dependent phase shifts of the melatonin rhythm that are similar to those produced by light. Specific receptor agonists and antagonists were tested for effects on the oscillator during the early subjective night, when light and quinpirole cause phase delays. Six-hour pulses of dopamine (0.1-1.0 μM) and quinpirole (10-100 nM) suppressed melatonin production and caused phase delays of 1.5-5.5 hr, but the D1 agonist SKF-38393 had no effect at 1 μM. The adrenergic agonists phenylephrine (α1), clonidine (α2) and isoproterenol (β), also had no effect at 1μM. The ~3 hr phase delay caused by 500 nM dopamine was completely blocked by the D2 antagonist eticlopride (50 μM), but was not affected by the D1 antagonist SCH-23390 (100 μM). These data, together with the finding that dopamine release in *Xenopus* retina is increased by light (Boatright et al., 1989, Brain Res. 482:164), suggest that retinal dopamine may act through D2 receptors as part of the light signal for entrainment of the retinal circadian clock.

270.3

NMDA RECEPTOR ANTAGONISTS BLOCK THE EFFECTS OF LIGHT ON CIRCADIAN BEHAVIOR IN THE MOUSE. **C.S. Colwell, R. Foster* and M. Menaker, Department of Biology, University of Virginia, Charlottesville, VA 22901.**

We report here the results of experiments designed to evaluate whether specific NMDA receptor antagonists, (+)-5-Methyl-10,11-dihydro-5H-dibenzo[a,b]cyclohepten-5,10-imine maleate (MK-801) and 3(2-carboxypiperazin-4-yl)-propyl-1-phosphonic acid (CPP), block the phase shifting effects of light on the circadian rhythm of wheel-running activity in mice. Intraperitoneal administration of (+)MK-801 as well as CPP produced a dose dependent blockade of both light-induced phase advances and delays. Neither drug, by itself, caused any consistent effect on the phase of the rhythm. These data, coupled with previous findings, indicate that excitatory amino acid receptors play an important role in the transmission of light information from the retina to the circadian system.

270.4

THE INTERACTION OF AGING AND CHRONIC LITHIUM TREATMENT ON MALE RAT ACTIVITY RHYTHMS. D.L. McEachron and N.T. Adler, Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA 19104

Twenty-four male Sprague-Dawley rats were used in this crossover study to assess the effects of chronic lithium treatment on circadian wheel-running rhythms. Rats were placed into individual cages in environmentally-controlled cabinets and initially exposed to a 12/12 light/dark cycle for 3 weeks and then to constant dim red light. The first 4 weeks were used to obtain baseline estimates of circadian period (tau). For Phase I, 12 rats were fed a lithium containing diet beginning with 7 days of 0.075% lithium carbonate (Li) followed by 7 days of 0.15% Li and then 0.3% Li for 8 weeks. Li diets contained 2.1% KCL as a buffer against Li's toxic side-effects, so that 1/2 of the remaining rats received a diet containing 2.1% KCL while the other 6 were fed normal Purina Rat Chow. At the end of Phase I, all rats received normal Rat Chow for 3 weeks. Phase II consisted of a complete crossover of the diets and lasted for 13 weeks. Although lithium treatment during Phase I lengthened tau values (a mean increase of 0.27 hrs vs. 0.09 hrs for KCL-fed rats and 0.12 for rats on the normal diet), this effect was not seen during Phase II (a mean increase of 0.11 hrs for Li-fed rats vs. 0.12 hrs for KCL-fed rats and 0.06 hrs for rats on the normal diet). In addition, 4 of 12 Li-fed rats during Phase I and 2 of 12 rats during Phase II displayed such severe disruption of normal rhythmicity that no tau values could be obtained. This disruption ceased within 2-3 days after removal from the Li diet and was not observed in rats fed the KCL or normal diets. We conclude: 1. Lithium treatment may disrupt rather than lengthen rhythms; and 2. Age or length of time in the cages reduces lithium's efficacy in altering activity rhythms.

270.5

THE EFFECTS OF TEMPERATURE ON THE CIRCADIAN OCULAR RHYTHM OF *BULLA GOULDIANA*. B.L. Bogart, G.D. Block. Biology Dept., University Of Virginia, Charlottesville, VA. 22901.

The free-running period of all circadian rhythms remains constant over a wide range of temperatures. This phenomenon is referred to as temperature compensation. The cellular mechanisms subserving temperature compensation are not yet understood.

We have recently begun studying the temperature dependency of the *Bulla* ocular pacemaker system with the goal of ultimately understanding the cellular basis of temperature compensation. The free-running period of the ocular pacemaker was found to be temperature compensated over a wide range of temperatures 12°C-25°C (Q10=1.02). The mean free-running periods were: 12°C (23.6 hrs. ±0.5 95% C.I.), 15°C (24.1±0.2), 22°C (23.8±0.3), and 25°C (23.6±0.6).

We also assessed the temperature sensitivity of the light entrainment pathway by measuring the phase angle of entrainment of eyes from animals maintained on L:D 12:12 at 12°C, 15°C, 22°C, or 25°C. We found no statistical difference in the phase angle for entrainment of eyes maintained at 12°C (ψ=-0.8 hr.), 15°C (ψ=+0.1), or 22°C (ψ=-0.5). The phase angle of eyes maintained at 25°C was highly variable and may reflect deterioration of Bulla maintained at high temperatures.

Taken together, these results indicate that the phase angle for entrainment is stable, suggesting that the entrainment pathway is relatively unaffected by ambient temperature. NS15264 to GDB.

270.6

LOW pH AND PROTEIN SYNTHESIS INHIBITORS APPARENTLY STOP THE MOTION OF A NEURONAL CIRCADIAN PACEMAKER S.B.S. Khalsa and G.D. Block. Department of Biology, University of Virginia, Charlottesville, VA 22901 *Supported by NS09621 & NS15264*

Retinal cells in the eye of the mollusc *Bulla* act as a competent *in vitro* circadian pacemaker. With extracellular pH at 6.9 (normally 7.8) the eyes become arrhythmic and express only a low constant frequency of impulse activity, suggesting that the pacemaker may have stopped. To evaluate this possibility, extracellular pH was lowered to 6.8 starting at CT 13 and then restored at progressively later times in 3 hour increments to separate eyes for up to 47 hrs. For pulse lengths up to 11 hr (CT 0), subsequent phases of the restored rhythms were related to the projected dawn of the previous LD cycle as for untreated control eyes. However, for longer pulses, phase was strictly a function of the end of the pulse, supporting the hypothesis that the pacemaker was stopped around subjective dawn during the treatment.

The transcriptional inhibitor dichlororibofuranosylbenzimidazole (DRB) and the translation inhibitor cycloheximide (CHX) greatly lengthen period at low concentrations (36 hr, 20μM DRB; 31 hr, 3mM CHX), suggesting that pacemaker motion may be stopped at higher concentrations. Using the same experimental paradigm as for low pH above, both DRB (100μM) and CHX (10mM) yielded data similar to that for the low pH treatments, including the critical phase specificity around subjective dawn. These data suggest that protein synthesis is also a phase specific requirement for pacemaker motion.

Although it is possible that these treatments reset the pacemaker to CT 0, rather than block its motion, at least in the case of DRB and CHX, the period lengthening argues that the pacemaker is stopped. The relationship, if any, between low intracellular pH and protein synthesis inhibition is uncertain. Their similarity in action raises the possibility that both may converge on the same dawn sensitive variable or process of the pacemaker.

270.7

REDUCED LIGHT SENSITIVITY OF THE CIRCADIAN CLOCK IN A HYPOPIGMENTED MOUSE MUTANT. M. M. Hotz, L. H. Pinto, and F. W. Turek. Dept. Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Hypopigmentation and albinism are known to affect visual pathways. Since the effects of these mutations on light information reaching the circadian clock is not known, we studied clock responses to light in C57BL/6J mice (+/+) and in C57BL/6J homozygous for the recessive mutation pink-eye dilute (p/p). Mice kept in constant darkness were given a series of four light pulses at three week intervals and the resulting phase shifts in the activity rhythm were measured. No differences in response to one hr white light at CT 16 (circadian time; CT 12 = activity onset) or at CT 0 were found, indicating that maximal clock resetting is not altered. However, five min 502 nm light pulses at CT 16 using light levels near the expected ½ saturation resulted in significantly different phase shifts ($p<.02$). At $1x10^{11}$ photons/cm²/sec, the p/p mice had a mean phase shift of 0.67 hr, while the +/+ mice had a mean phase shift of 1.27 hr. Upon transfer to constant light the period of +/+ mice was lengthened to a greater extent than that of p/p mice($p<.01$). Preliminary data from ^{3}H Proline tracing of retinal projections to the hypothalamic suprachiasmatic nucleus indicates that there is a reduced retinal innervation of this clock-containing area. This reduced innervation may underlie the decreased sensitivity of the circadian clock of p/p mice to light.

270.8

HIGH AFFINITY MELATONIN BINDING SITES IN THE DJUNGARIAN HAMSTER FETUS. Scott A. Rivkees, David R. Weaver, Steven M. Reppert. Laboratory of Developmental Chronobiology, Massachusetts General Hosp. Boston, MA 02114

Maternal melatonin readily crosses the placenta communicating time-of-day and daylength information to the fetus. To determine if melatonin may have a more widespread role during fetal development than presently recognized, we examined the distribution of high affinity melatonin binding sites in Djungarian hamster fetuses. Using [^{125}I]-2-iodomelatonin, a potent melatonin agonist, melatonin binding sites were examined at different gestational ages in whole hamster fetuses by in vitro autoradiography (total gestation length ca. 16-18 days; [125I]-2-iodomelatonin = 30 pM). At gestational day (GD) 5 (embryo size = 1.6 mm), specific binding was not detectable. At GD 8 (crown-rump length = 6 mm), specific binding was present in the nasal mucosa, the Harderian gland and the fetal adrenal region. At GD 12 and 16 (crown-rump length = 16 and 23 mm, respectively), specific binding was not observed in the the adrenal region, but was present in the nasal mucosa and Harderian gland. At GD 12, specific binding was also present in Rathke's pouch. At GD 16, specific binding was also detectable in the suprachiasmatic nuclei, pineal and pituitary glands. The presence of melatonin binding sites at very early stages of development in neural and non-neural structures, suggests that melatonin may have a greater role during fetal development.

270.9

ANDROGENIZATION OF FEMALE SLEEP-CYCLE RHYTHMICITY BY PERINATAL INJECTION OF TESTOSTERONE (I). W. Fishbein, J. Fang, S.W. Yang and S.J. Tien. Neurocognition Program, CUNY, City College & Graduate School, N.Y. 10031

We previously reported that sleep-cyclicity is sexually dimorphic in mice (SN, 1987). In addition, we showed that prenatal stress completely sex-reverses the sleep pattern of males, but not females (SN, 1987). Sleep-cyclicity is also sexually dimorphic in rats (SN, 1988). In both species the sex-linked difference in sleep is due primarily to the frequency of paradoxical sleep (PS) bouts; it appears that the normal biological timing clock controlling the interval between PS episodes runs at a different speed in males than females. As a result the total amount of PS/24 hrs. is substantially different between the sexes.

Prenatal stress produces a reduction in fetal testicular enzyme activity with an attending reduction in plasma levels of testosterone (Ward, Psychoneuroendocrin, 1984). Since testosterone might be the catalyst of the sex difference, we undertook the present experiment to determine whether female sleep-cyclicity is subject to androgenization by perinatal injection of testosterone (1,000ug/50ul/animal injected <24 hours after birth).

The baseline data replicates our previous observation; namely, control (safflower oil, 50ul) injected male rats exhibit more PS bouts than control females. Perinatal testosterone loading in males had no effect, whereas perinatal testosterone injected females display significantly more PS bouts than control females. The sleep-cyclicity of testosterone injected females was indistinguishable from the vehicle and testosterone loaded males.

The results provide strong evidence that organization of the PS-Slow Wave Sleep cycle is genetically programmed, yet remains susceptible to modification by male gonadal steriods up to the time of birth.

270.10

ANDROGENIZATION OF FEMALE SLEEP-CYCLE RHYTHMICITY BY PERINATAL OVARIECTOMY (II). J. Fang, S.W. Yang S.J. Tien, and W. Fishbein. Neurocognition Program, CUNY, City College & Graduate School, N.Y. 10031

We previously showed that sleep-cyclicity is sexually dimorphic in both mice and rats (SN, 1987, 1988). Yet one puzzling aspect of our findings is that the direction of the sex difference is just the opposite; namely, female mice have more paradoxical sleep (PS) than males, whereas male rats have more PS than females. Our previous studies employing prenatal stress in male mice (producing feminization; SN, 1987) and perinatal testosterone loading in female rats (producing masculinization; see joint abstract, Fishbein, et al.), suggests that the genetic organization of sleep-cyclicity is essentially modulated by male gonadal steroids.

However, another interpretation of these findings is that the sexual dimorphism is related to estrogen feminizing, rather than testosterone masculinizing influences. We therefore undertook the present experiment to determine whether perinatal castration (<24 hrs after birth) - orchidectomy (testosterone deficient males) or ovariectomy (estrogen deficient females) - produces alterations in sleep-cyclicity.

The baseline data replicates our previous observation; non-castrated (control) male rats exhibit more PS bouts than non-spayed (control) females. Perinatal castration of the males had no effect; the orchidectomized males were indistinguishable from the control males, whereas the spayed females display significantly more PS bouts that control females. Indeed, the ovariectomized females were indistinguishable from control and castrated males.

Taken together with our accompanying testosterone study, the results indicate that organization of the PS-Slow Wave Sleep cycle in females can be androgenized by perinatal ovariectomy (as well as by perinatal testosterone loading). The findings suggest that estrogen may also participate in the organization of sleep-cyclicity.

270.11

MATERNAL ENTRAINMENT OF HAMSTERS WITH MUTANT CIRCADIAN CLOCK. N.Viswanathan and F.C.Davis. Department of Biology , Northeastern University., Boston, MA 02115.

In mammals, a circadian pacemaker begins to function prenatally and is entrained by the rhythms of the mother. Rodents exhibit circadian rhythms of wheel-running activity even on the day of weaning (~3-4 wks postnatal) and the phases of such rhythms are similar to those of their mother's. The present study examined the maternal entrainment of hamster pups heterozygous for the τ mutation (freerunning period ~ 22 hr). Pups were born to wildtype females (~24 hr) that had been mated to homozygous males (~20 hr). Pups from each litter were weaned on postnatal days 18 and 24 and rhythms were measured in constant dim light. The freerunning period of the τ mutation was fully expressed even at weaning. Unlike results with wildtype pups, the phases of the mutant pups on days 18 or 24 were not similar to the phases of their mothers, and pups left with the mother were clearly freerunning between days 18 and 24. However, pups within a litter as well as pups in different litters were synchronized among themselves, suggesting that they had entrained to a common signal sometime earlier in development. The results indicate that: 1. the signal, most probably maternal, is a strong entraining agent; despite the period difference between mothers and pups (24 vs. 22 hrs), the pups were entrained during development, and 2. the pups began freerunning at about the same time in development, otherwise synchrony among the pups would not have been observed at weaning. Supported by NIH grant HD 18686 to FCD.

270.12

SPECTRAL SENSITIVITY OF THE EFFECTS OF LIGHT ON THE OSCILLATION OF MELATONIN RELEASE FROM CHICK PINEAL CELLS. L.M. Robertson and J.S. Takahashi. Dept. of Neurobiology and Physiology, Northwestern U., Evanston, IL 60208.

Chick pineal cells exhibit a circadian oscillation of melatonin release. The cells are photoreceptive and light has two major effects: 1) acute inhibition of melatonin release and 2) entrainment of the circadian oscillator. There are striking homologies between retinal photoreceptor cells and pineal cells. Deguchi (Nature 290:706, 1981) has shown that the acute response is mediated by a rhodopsin-like photopigment. However, previous studies have indicated that some of the cellular processes involved in retinal phototransduction are involved in the acute but not the phase-shifting effect of light. Thus, it becomes of primary importance to characterize the photoreceptors mediating entrainment and to determine whether they are different from those mediating the acute response to light. In order to characterize the photoreceptors and photopigments involved, we measured the spectral sensitivity of both responses. We utilized a flow-through cell culture system in which cells were grown in monolayer cultures on multi-well cell culture plates. A fiberoptic system underneath the plates provided the photic stimulus. Interference filters were used to provide monochromatic light and neutral density filters were used to adjust the irradiance. Cells were exposed to wavelengths of 450, 500, 550 and 600 nm at various levels of irradiance for 6 hours during the subjective night of the second cycle in constant darkness. Both the acute and phase-shifting responses increased in magnitude with an increase in irradiance at each wavelength. The maximal sensitivity for both effects was achieved with wavelengths of 450-500 nm. The sensitivity functions were similar to the Dartnall nomogram for a retinal-based photopigment with a maximum at 500 nm. Therefore, these results are consistent with the hypothesis that a visual pigment with a λ_{max} near 500 nm mediates the acute and phase-shifting effects of light on chick pineal cells.

270.13

LIGHT INTENSITY-RESPONSE (I-R) FUNCTIONS OF INTERGENICULATE LEAFLET (IGL) NEURONS IN THE GOLDEN HAMSTER AND THE EFFECTS OF CHRONIC CLORGYLINE. M.E. Harrington and B. Rusak. Dept. of Psychology, Smith College, Northampton, MA 01063 USA and Dept. of Psychology, Dalhousie University, Halifax, NS B3H 4J1 Canada.

Chronic administration of the antidepressant compound clorgyline, an irreversible type A monoamine oxidase inhibitor, alters the I-R curve for phase advance shifts of hamster circadian rhythms (Soc. Nsci. Abstr., 14:907, 1988). Ablation of the IGL also alters light-induced advance shifts. We examined whether the responses of IGL cells to changes in illumination were altered by chronic clorgyline treatment.

25 adult male hamsters were housed under a 14:10 h light:dark cycle. 18 hamsters were implanted with osmotic mini-pumps (Alzet, Model 2002) filled with either clorgyline (n = 12; 2 mg/kg/day) or vehicle (n = 6; 0.9% saline) three weeks before electrophysiological recording. Responses of single IGL cells to diffuse retinal illumination were recorded using previously published techniques (Vis. Nsci., 2:367-375, 1989). Cells showing sustained responses to illumination were recorded for 20 min of darkness, followed by an 8 min presentation of increasing light intensity (0.8 to 1000 uW/cm^2), 10 min of the highest light intensity, and 8 min of decreasing light intensity. Small lesions were placed to mark the locations of cells.

IGL cells from control and clorgyline-treated animals (n=14 in each group) showed both monotonic and non-monotonic I-R functions with a large range of thresholds. The only clear difference between the groups was that IGL cells from clorgyline-treated animals showed lower firing rates during both light and dark conditions.

These results indicate that IGL cells show a wide variation in I-R functions. Clorgyline-induced alterations in I-R curves for phase-shifting are not reflected in obvious changes in I-R functions for individual IGL cells. A general reduction in firing rates of IGL cells, perhaps resulting from altered serotonergic influence on these cells, may affect the size of light-induced phase shifts after chronic clorgyline treatment.

Supported by NSERC and MRC of Canada and NIH and Smith RDFS.

270.14

EVIDENCE FOR SEROTONERGIC REGULATION OF THE HAMSTER CIRCADIAN RHYTHM SYSTEM. L.P. Morin, J.Blanchard. Dept. Psychiatry, Health Sci. Center, SUNY, Stony Brook, NY 11794.

The densest innervation of the SCN, other than that from the retina, is serotonergic. The present work demonstrates that rhythmicity of adult male hamsters housed under constant light (LL) is markedly altered following depletion of brain serotonin.

Animals were initially housed in LD 14:10. While under this lighting condition, each was administered 5,7-dihydroxytryptamine (DHT) intraventricularly to deplete brain serotonin or given a sham procedure (CON). Surgical and histological methods were the same as described in Smale et al. (Brain Res. '90, 515, 9-19). Following recovery for about 18 days, all animals were placed in constant light (LL) conditions.

Behavior of DHT animals under LD was the same as described by Smale et al. with activity onset occurring before lights off and activity offsets occurring during the late night. LL suppressed the daily number of wheel revolutions of CON, but not DHT, animals. LL quickly lengthened tau of DHT animals (24.43 ± .04 vs 24.19 ± .05, $p<.001$). In addition, 65% of DHT animals had rhythm splitting or desynchrony within 30 days of LL vs 0% of CON ($p<.01$). The results support the view that serotonin may regulate circadian rhythm stability, particularly rhythm "splitting," under LL conditions.

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DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: MOLECULAR CORRELATES

271.1

THE B30 GANGLIOSIDE IS A SPECIFIC MARKER FOR NEURAL CREST DERIVED NEURONS IN THE DEVELOPING MOUSE. D.Y.R. Stainier*, D.H. Bilder*, and W. Gilbert. Cellular and Developmental Biology, Harvard Univ., Cambridge, MA 02138

We have previously reported (J. Neurosci. 9, 2468-85) the isolation of a monoclonal antibody, mAb B30, that recognizes a rare ganglioside specifically expressed on a small subset of neurons -including the mesencephalic trigeminal nucleus (MesV)- in the developing mouse CNS. In this study, we have characterized the B30 immunoreactivity in the developing PNS. We report that B30 is a specific marker for neural crest derived neurons. Consequently, we used B30 to follow the neuronal differentiation of neural crest cells in a serum-free chemically defined culture system. Within hours, neural crest cells migrate away from a neural tube explant on a fibronectin substrate; by 24 hours, up to 15% of them have differentiated into morphologically identifiable neurons. While both undifferentiated and differentiated crest cells express the GD3 ganglioside recognized by our mAb B33, B30 specifically stains the neural crest derived neurons, reflecting the in vivo situation. The demonstration that the B30 ganglioside is a marker for neural crest derived neurons provides the first biochemical evidence for the neural crest origin of the centrally located MesV neurons. We also show the unique biochemical specificity of mAb B30 and provide experimental evidence for the role of the B30 ganglioside in the cellular adhesion process.

271.2

CARBOHYDRATE BINDING PROTEINS IN EMBRYONIC SPINAL CORD AND BRAIN. W. Niforatos*, M.G. Hvizd*, K.L. Knepper*, R.G. Higbee, S. Nakahara*, D.G. McLone and P.A. Knepper. Division of Neurosurgery, Children's Memorial Hospital and Northwestern University Medical School, Chicago, IL 60614.

Embryonic and differentiating tissues of vertebrates contain carbohydrate binding proteins (CBP), i.e., endogenous lectins, that agglutinate trypsinized and fixed rabbit erythrocytes. Developmental studies on the changing distribution pattern of spinal cord-specific oligosaccharides have opened the possibility that there are CBPs specific for each oligosaccharide. In this study, embryonic cerebrum, cerebellum, spinal cord, heart and lung of gestation day 18 C-57 BL/6J mice embryos were microdissected, the CBPs were extracted by homogenization with 9 volumes of MEPBS containing 0.3M lactose and centrifuged at 100,000 x g for 1h. The supernatant was concentrated in Centricon-10 tubes to remove the lactose and fractionated on an HPLC size exclusion column. Each fraction was monitored using trypsinized and fixed rabbit erythrocytes and by polyacrylamide gel electrophoresis. The results to date indicate the presence of several CBPs with varying molecular weights and that the agglutination was most active in heart, although agglutination was observed in all tissues. Studies are in progress to determine the saccharide specificities and agglutination of CBPs in earlier spinal cord development.

(Supported in part by Kiwanis International)

271.3

ZEBRIN II AND O-ACETYL GD3 DIVIDE ALL CEREBELLAR PURKINJE CELLS INTO TWO DISTINCT COMPLEMENTARY SETS. N. Leclerc, K. Herrup, R. B. Hawkes, G. Schwarting and M. Yamamoto. Dept. of Dev. Neurobiology and Biochemistry, E. K. Shriver Center, 200 Trapelo Rd, Waltham MA 02254.

Increasingly, use of molecular markers has revealed heterogeneity among cerebellar Purkinje cells. One such marker is a monoclonal antibody against a 36 kD polypeptide, Zebrin II (ZII). The pattern of ZII staining defines a series of 7 parasagittal bands in each hemi-cerebellum. This pattern is perfectly congruent with the discontinuous topography of the olivocerebellar projection as well as with the histochemical zonation of 5'-nucleotidase, AChE and cytochrome oxidase enzymes.

O-acetyl GD3 is a ganglioside expressed on the plasma membrane of embryonic brain cells. Recently, an antibody directed against O-acetyl GD3 has been shown to stain adult rat Purkinje cells (Mendez-Otero et al. J. Neurosci.8: 564, 1988). Using an anti-O-acetyl GD3 monoclonal antibody called P-Path, we find that the antigen is expressed by a subset of Purkinje cells, organized in parasagittal bands. This antigenic pattern is precisely complementary to ZII. All P-Path-positive Purkinje cells are ZII-negative and vice versa. Neurological mutants that interfere with Purkinje cell placement (*reeler* and *weaver*) will be used to determine whether the binary ZII/P-path segregation is susceptible to experimental manipulation.

Supported by NIH (NS18381) and the March of Dimes (#1-1175)

271.4

MONOCLONAL ANTIBODIES AGAINST CELL NUCLEI: EXPRESSION IN MOUSE CEREBELLUM, MUTANTS, AND TISSUE CULTURE. A. M. Smith, C. R. Buck and R. J. Mullen. Dept. of Anatomy, University of Utah Sch. of Med., Salt Lake City, UT 84132.

We have generated three MAbs which are useful markers to study the development of the murine nervous system. Two of these MAbs (F41 and A18) stain the cell nuclei of neurons throughout the nervous system and the third (A61) stains both neurons and glia. MAb F41, an IgG1, intensely stains mature granule cells in the cerebellum. However, at postnatal day 10 (P10) the cognate antigen is not detected in the external granular layer (EGL) nor in migrating granule cells. At P10 the cell nuclei of Golgi type II neurons are intensely stained by F41, but Purkinje cells are just beginning to show immunoreactivity. In the cerebellar mutant weaver (*wv*), whose Purkinje cells are relatively normal, the antigen is detected. Whereas in staggerer (*sg*), where the defect has been shown to be in Purkinje cells, the antigen for F41 is not detected in the Purkinje cells. In the cerebellum at P10, A18 appears to stain just the cytoplasm of granule cells in the EGL and the Purkinje cells, while the Golgi type II neurons show intense nuclear staining. Later all three cell types show intense nuclear immunoreactivity. A18 is similar to F41 in that it stains the nuclei of *wv*/*wv* Purkinje cells but not those of *sg*/*sg*. MAb A61, an IgA, stains all cell types in the cerebellum including some glia, The immunoreactivity of the cognate antigen is sensitive to the type of fixative used with staining being either nuclear or dendritic, or both. A comparative study of the developmental expression of these three antigens will be presented and compared with A60, a previously reported antigen (Mullen, et al, Soc. Neurosci. Abstr:15:499, 1989). In addition, an immunohistochemical study of primary cerebellar cerebellar cultures and P19 embryonal carcinoma cells stimulated with retinoic acid will be presented. Immunoblot analysis has been performed for all three MAbs and recently we have isolated at least one cDNA clone for one of these antigens from a gt11 expression library. (Supported by NIH Grant EY07017).

271.5

REGULATION OF SERPINS DURING MOUSE BRAIN DEVELOPMENT
Jasti S. Rao, Riichiro Suzuki, Bokka R. Reddy and Barry W. Festoff, Department of Neurology, University of Kansas Medical Center, Kansas City, KS 66103, and Neurobiology (151), DVA Medical Center, Kansas City, MO. 64128.
Serpins inhibit the activity of several serine proteases including thrombin, trypsin and the plasminogen activators (PAs). Protease nexin I (PNI) and PA inhibitors 1,2 and 3 (PAI-1, 2 and 3) are all potential physiological inhibitors and play important roles in the development of tissues as well as in various pathological states. We report both qualitative and quantitative changes in PNI and PAI-1 serpins during mouse brain development. Cerebellum and cerebral hemispheres at embryonic day 14 and up to postnatal day 40 were studied by complex formation with labeled ^{125}I-urokinase with and without SDS. Results indicated developmental regulation of active serpins. PNI was detected in embryonic brain maximally at birth and decreased thereafter. PAI-1 had essentially the same kinetics but was present at lower levels at birth. No regional differences were detected with these methods despite the known developmental differences between cerebellum and cerebral hemispheres in the mouse. These studies suggest regulatory roles for PNI and PAI-1 during brain development.
Supported by the American Health Assistance Foundation and the Medical Research Service of the Department of Veterans Affairs.

271.6

VARIATION OF CEREBELLAR INSULIN-LIKE GROWTH FACTOR II GENE EXPRESSION IN EARLY POSTNATAL DEVELOPMENT.
K.M. Rosen, R.L. Mozell*, and L. Villa-Komaroff, Dept. Neurology, Children's Hospital, Harvard Medical School, Boston, MA 02115.
We have investigated IGF-II gene expression in early postnatal (P2-P12) mouse cerebellum to determine the levels of IGF-II mRNA in developing neurons and glia. Since earlier reports indicated high levels of expression in choroid plexus and meninges, these structures were removed prior to the analysis. Using a novel, dual S1 nuclease protection assay, we identified a peak of expression in P4 cerebellum. In granule cell precursors purified by Percoll gradient centrifugation, IGF-II mRNA levels were constant through P4 and then dropped precipitously, mirroring the decline in whole cerebellum. The yield of isolated glia was insufficient to allow definitive S1 analysis; therefore, we utilized reverse transcription followed by the polymerase chain reaction to detect IGF-II mRNA in these cells and to confirm its presence in granule cells. We detected mRNA in granule cells from all ages examined and verified the expression of IGF-II mRNA in isolated glia. One key feature of the developing postnatal cerebellum is the differentiation and migration of the granule cells. It is intriguing that the decline in IGF-II gene expression occurs just prior to the peak of granule cell migration and mitotic activity in the mouse, suggesting that IGF-II may be involved in the regulation of granule cell development.

271.7

SPECIFIC EXPRESSION OF THE GAMMA ISOFORM OF PROTEIN KINASE C IN DEVELOPING FIBER TRACTS AND DISCRETE STEM CELL POPULATIONS OF THE BRAIN.
R.Ferri and P.Levitt. Dept. of Anatomy. Med Coll of PA, Philadelphia, PA 19129.
The expression of protein kinase c (PKCγ) was examined in the developing CNS of the fetal rat using immunocytochemical analysis employing a polyclonal antibody directed against the gamma isoform. At embryonic day 12 (E12), PKCγ expression was very low throughout all areas of the brain. The first appearance of an increased expression in the prosencephalon was at E14 in developing fiber tracts, including the internal capsule and fasciculus retroflexus. The distribution in fiber tracts increases at later ages to include the corpus callosum, fornix, and anterior commissure. In addition to growing axons, PKCγ immunoreactivity was seen in cell groups within the ventricular zone along the lateral and third ventricles and in adjacent migratory regions. At E16, the forebrain contained PKCγ-stained cells in discrete foci in the ventricular and intermediate zones that give rise to the hippocampus, perirhinal and piriform cortex. This remained prominent at E18. In the diencephalon, discrete subpopulations of PKCγ immunoreactive stem cells were distributed dorsal and ventral to the medial sulcus of His, giving rise to cells of the dorsal thalamus and hypothalamus, respectively. The patterns of PKCγ expression demonstrate a precise localization in developing fiber tracts, consistent with a role for this enzyme in neurite elongation. In addition, this enzyme is produced during periods of neurogenesis and may be an early marker of stem cell populations that will ultimately reside in specific anatomical regions of the brain. (Supported by NIMH Grant MH45507).

271.8

PROMOTER ANALYSIS OF A NEURON-SPECIFIC GENE, PEP-19.
L. Sangameswaran, R. Smeyne, R. Wurzburger and J. Morgan, Department of Neurosciences, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110
PEP-19 is a neuron-specific polypeptide that is distributed in defined areas of the rodent central nervous system. PEP-19 levels, (both mRNA and protein), increase dramatically during the late maturation of the CNS, the mRNA being detectable at least four days before the protein. Within the cerebellum, the peptide is localized predominantly in Purkinje neurons. Using PEP-19 cDNA as a probe, we have investigated possible mechanisms that might control the expression of PEP-19 mRNA in the cerebellum. Analysis of the mRNA levels in neurological mutant mice and in rats after chemical lesions indicate that neither the climbing fibers from the olivary nucleus nor the parallel fibers from the granule cells influence the triggering of the PEP-19 gene.
To dissect out the regulatory elements that are responsible for the spatial and temporal expression of PEP-19 we have isolated genomic DNA clones from a mouse library. We are currently pursuing biochemical analysis of the 5' flanking region of PEP-19 gene to gain insight into specific DNA binding proteins. Comparative sequence analysis of the 5' flanking region of PEP-19 with L7, another Purkinje cell marker, revealed regions of homology. Using synthetic oligonucleotides for these regions, electrophoretic mobility shift assays are being performed. Such assays are also being done with larger DNA fragments from the 5' flank sequence. In addition, experiments where PEP-19 genomic regions fused with Lac Z have been used as constructs for transgenic mice will be discussed.

271.9

DIFFERENTIAL EXPRESSION OF THE THYMOSIN β10 GENE DURING RAT DEVELOPMENT.
D.I. Lugo, J. Hempstead* and J.I. Morgan Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110
In previous studies the expression of the thymosin β10 gene has been shown to be developmentally regulated in the brain. In addition, the expression of this gene has been shown to be regulated by retinoic acid in a variety of neuroblastoma cell lines. Here we present a detailed analysis of the expression of thymosin β10 during rat fetal/neonatal development. Steady state levels of β10 mRNA were analyzed in different areas of the fetus/neonate by Northern blot analysis and the spatial localization of the mRNA was investigated by in-situ hybridization to tissue sections. In addition, changes in mRNA level were correlated to changes in β10 peptide levels by HPLC analysis of isolated tissues. The β10 gene is widely expressed at moderate levels throughout the embryo at E13. By E15, marked regional differences in expression are apparent. High abundance of thymosin β10 mRNA is present in the brain, spinal cord, lungs, heart and gonads, while the liver contains very low levels of this transcript. Uniquely in the brain, levels persist at high abundance through birth, whereafter they decrease to adult values by P24. In parallel studies utilizing the mouse embryonic carcinoma cell line P19, the expression of this gene has been shown to increase dramatically following differentiation into a neuronal phenotype. These studies demonstrate a tissue-specific regulation of thymosin β10 gene expression during development. The elucidation of the regulation of the thymosin β10 gene may provide critical leads into the mechanisms that shape neuroembryogenesis.

271.10

SINGLE-STRANDED DNA BINDING PROTEIN IS DETECTED IN MITOTIC AND POSTMITOTIC EMBRYONIC RAT NEURONS.
R.S. Lasken, L. Lyandvert and D.J. Reis. Div. of Neurobiol., Cornell Univ. Med. Coll., NY, NY 10021.
We sought to establish whether single-stranded DNA binding proteins (SSB), essential to DNA replication in prokaryotes, are present in mitotic or postmitotic neurons of rat brain. Cerebral cortex and caudate nucleus of embryonic day (E) 13-17 were dissociated and immunocytochemically stained for SSB using antibodies to the human SSB isolated from HeLa cells (Kenny, M.K. et al., *J. Biol. Chem.*, in press). The SSB was localized in the nucleus of cells including neurons as identified by the presence of neuron specific enolase. The staining for rat SSB, using antibodies to human SSB, was competitively blocked by the human SSB protein. Furthermore, 3 different monoclonal antibodies for human SSB recognized the putative rat SSB. Double labeling for tritiated thymidine and rat SSB revealed the protein's presence in both postmitotic and actively dividing cells. Thus, this protein may play a role in nonreplicating cells in a function such as DNA packaging or RNA transcription. The ability of antibody to human SSB to recognize the rat protein suggest that a widespread occurrence of highly homologous proteins will be found among mammals. Detection of an SSB in rat neurons will allow its regulation to be investigated in normal developing tissue.

271.11

EXPRESSION OF PEP-19 AND L7 COGNATE AND TRANSGENES IN CEREBELLAR PURKINJE CELLS IN PRIMARY CULTURE. K. Schilling, J. Oberdick, R.J. Smeyne, and J.I. Morgan Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110

In previous studies two developmentally regulated genes, PEP-19 and L7, have been characterized, which, within the cerebellum, are expressed specifically in Purkinje cells. In this study, we have assessed the expression of PEP-19 and L7 in developing Purkinje cells in primary culture using immunocytochemistry and histochemistry for β-galactosidase in cultures established from a transgenic mouse carrying a L7-βGal fusion gene (Science 248, 233 1990). This approach permits us to investigate the role of epigenetic mechanisms in the regulation of these specific genes. In cultures derived from 16 day old fetal rats, neurons immunoreactive (IR) for PEP-19 are first seen after 3 days in vitro, but L7-IR cells appear only after 5 days. With ongoing cultivation, Purkinje cells develop increasingly complex neurites, which can be seen in preparations stained for PEP-19 as well as L7. At the peak of expression, PEP-19-IR neurons outnumber L7-IR cells by about twofold. In cultures derived from 16 day old fetal mice, PEP-19 and L7 are expressed about two days earlier than in rat cultures, but follow the same relative time course. Expression of the L7-βGal transgene paralleled that of the cognate L7 gene. Comparison of these results to data obtained in intact animals shows that expression of PEP-19 precedes that of L7 and the L7-βGal transgene both in culture and in vivo. This suggests that developmental expression of these genes is independent of extracerebellar input, which are absent in these cultures. Supported by the DFG (to KS, Schi 271/2-1), NRSA (to RJS, 8680-01)

271.12

EXPRESSION OF L7βGAL TRANSGENE AND L7 COGNATE GENE IS CONFINED TO PARASAGITTAL CLUSTERS IN DEVELOPING CEREBELLUM. R.J.Smeyne, J. Oberdick*, A.S.Berrebi, E. Mugnaini, and J.I. Morgan, Roche Institute of Molecular Biology, Nutley, NJ 07110 and University of CT, Storrs, CT.

In a previous study, we have shown that a fusion transgene, L7βGal, is expressed in cerebellar Purkinje and retinal bipolar cells. In this study, we compared the spatial and temporal expression of the cognate L7 gene with that of the L7βGal transgene during development in cerebellum to assess if the regulatory sequences within the transgene were sufficient to produce normal expression. E15 through P10 control and transgenic mice were perfused; then frozen sectioned or removed into buffer for in toto staining. L7βGal gene expression is first seen at E17 in 2 bilateral bands. At P0, in L7 and L7βGal staining, 3 bilateral bands are evident; the most lateral band being interrupted by on/off zones. Histochemically-reactive Purkinje cells in these bands are characterized by staining solely in the soma. At P4, 6 bilateral bands are seen, with reaction product found in the Purkinje cell soma, dendrites and axons of the transgenic animals. At P7, expression has expanded across the cerebellum so that only the most lateral parts of the hemisphere do not express either the cognate L7 or L7βGal transgene. and by P9 expression is complete. This study demonstrates that the onset of L7 cognate and L7βGal transgene expression is organized into similar and discrete bands of parasaggitally organized clusters. This suggests that the information contained in the 5' flanking sequences and introns of the L7βGal transgene are necessary and sufficient to direct normal expression.

271.13

SUBSETS OF PURKINJE CELLS AND BIPOLAR NEURONS IDENTIFIED BY THE L7 PROMOTER. J. Oberdick*, R.J.Smeyne, J.G.Corbin*, R.Wurzburger, and J.I.Morgan, Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

L7 gene expression is restricted to cerebellar Purkinje cells and retinal bipolar neurons. We have recently shown that an L7βGal transgene in mice accurately reflects the restricted distribution of L7 cognate gene expression (Oberdick et al, Science, 248: 223, 1990). A bipolar subpopulation, the rod bipolars, expresses the cognate gene (Berrebi et al, Soc.Neurosci.Abs, 1990) and the βGal staining pattern in transgenic retina is consistent with this. All Purkinje cells in adult cerebellum are positive for both L7 cognate and transgene, but during early postnatal development L7 expression is restricted to a progressive wave of discrete parasaggittal bands (Smeyne et al, Soc.Neurosci.Abs,1990). We are addressing the mechanisms responsible for establishing these biochemically distinct subpopulations of bipolar neurons and Purkinje cells. Specifically, common elements (PCE's) have been identified in the putative control regions of several Purkinje cell-specific genes. All of these elements lie within less than 1kb upstream of the L7 cap site. A deletion variant of the L7βGal transgene carrying only 1kb of upstream sequence showed reduced expression in Purkinje cells, although the primary sites of expression remained the bipolar and Purkinje neurons. The cerebellum of a 12d old mouse expressing this deleted construct had a pronounced medial-lateral gradient of βGal staining seen only at much earlier times with the full length construct. This suggests that the PCE's influence the specificity of L7 transcription, but that sequences more 5' control the levels and/or timing of transcription.

271.14

NGFI-C. A ZINC FINGER PROTEIN INDUCED BY NGF. S.D.CROSBY, J.MILBRANDT. Department of Laboratory Medicine, Washington University School of Medicine, St. Louis, MO, 63110.

Nerve growth factor stimulation of the rat pheochromocytoma cell-line PC12 results in neuronal differentiation. Within minutes of this stimulation, transcripts from a number of "immediate early" genes (IEG) (e.g. c-fos, NGFI-A, NGFI-B) which encode transcriptional regulatory proteins are increased. Using reverse transcriptase polymerase chain reaction (RT/PCR), we have isolated a new IEG, NGFI-C, from NGF-stimulated PC12 mRNA. The RT/PCR oligonucleotides were poly-dT and a degenerate consensus sequence of the Cys-Cys-His-His zinc finger. Sequence analysis of the NGFI-C cDNA reveals 3 adjacent zinc fingers which have >95% homology to the NGFI-A and Krox 20 zinc finger regions. Preliminary northern blot analysis suggests alternative splicing of the mRNA. A larger mRNA exists almost exclusively in JS-1 cells while a smaller form predominates in brain and PC12 cells, implying that the two protein products may have different functions.

DIFFERENTIATION, MORPHOGENESIS AND DEVELOPMENT: NEUROTRANSMITTERS, PEPTIDES, HORMONES

272.1

DEVELOPMENT OF LONGITUDINAL DENDRITIC BUNDLES IN RAT SPINAL CORD: ANALYSIS OF CHOLINERGIC SYMPATHETIC PREGANGLIONIC NEURONS. J.A. Markham, P.E. Phelps and J.E. Vaughn. Div. Neurosci., Beckman Res. Inst. of the City of Hope, Duarte, CA 91010.

In adult spinal cord, dendritic processes of sympathetic preganglionic neurons (SPNs) are arranged in transverse and longitudinal bundles that give rise to a ladder-like appearance in horizontal sections. Using ChAT-immunocytochemistry, Phelps and coworkers (*Soc. Neurosci. Abstr.* 15:589, 1989) have shown that transverse dendritic bundles are detectable by embryonic day 15, extend between the intermediolateral (ILp) and central autonomic (CA) nuclei by E17, and also display a distinct periodicity at this latter time. In contrast, longitudinal dendrites were not detectable at any prenatal stage. Thus, we investigated the development of the ChAT-positive SPN longitudinal dendritic bundles at early postnatal ages. At birth (P1), SPN cell clusters were only occasionally interconnected by longitudinal dendrites. Dendritic bundles were found between adjacent cell clusters more frequently by P4 and were even more numerous at P7, forming a continuous structure between adjacent cell clusters. These longitudinal dendritic bundles were found in both the ILp and CA nuclei, but the former appeared to develop earlier. The adult pattern of dendritic bundling was present by P14. When combined with previous studies, the results support the view that SPN processes in the spinal cord develop in the transverse plane embryonically, whereas growth in the longitudinal plane occurs during early postnatal life. Studies are in progress to determine possible bases for this temporal developmental difference. *Supported by NIH grant NS25784.*

272.2

DETECTION OF LOW LEVEL CHAT GENE EXPRESSION IN ADULT CHROMAFFIN CELLS AND IN THEIR EMBRYONIC PRECURSORS. D.J. Vandenbergh*and D.J. Anderson Div. of Biology, Caltech, Pasadena, CA 91125.

Chromaffin cells of the adrenal medulla and sympathetic neurons share a common precursor. Chromaffin cells maintain an ability to transdifferentiate into sympathetic neurons in response to NGF or FGF. These sympathetic neurons can be further converted to a cholinergic phenotype by CDF/LIF. We have demonstrated that the potential to express a cholinergic phenotype is reflected in an incomplete repression of the CHAT gene. Low level expression of CHAT is detected not only in adult adrenal medullary tissue, but also in MAH cells, an embryonic sympathoadrenal precursor cell line [Neuron 4, 189 (1990)]. MAH cells are able to respond to CDF/LIF by inducing CHAT mRNA levels as well as reducing TH mRNA levels. These results indicate that progenitor cells in the sympathoadrenal lineage contain an active or potentially active CHAT gene, as well as the machinery to induce this gene in response to environmental factors prior to their differentiation into either chromaffin cells or noradrenergic sympathetic neurons. Moreover, a low level expression of the CHAT gene may persist in fully differentiated chromaffin cells.

272.3

Characterization of a target derived cholinergic differentiation factor present in rat sweat glands Mahendra S. Rao*#, Paul H. Patterson* and Story C# Landis Dept of Neuroscience, Case Western Reserve Univ, Cleveland, OH 44106# and Div of Biology, Caltech, Pasadena, CA.91125.*

During normal development, the sympathetic innervation of rat sweat glands undergoes a switch from a noradrenergic to a cholinergic and peptidergic phenotype. Previous studies have demonstrated that this switch is target mediated. To characterize the molecular nature of the signal(s) involved, we have prepared low salt extracts of sweat glands. The extracts contain a soluble factor(s) which induces choline acetyltransferase activity and vasoactive intestinal peptide expression in a dose dependent fashion in cultures of sympathetic neurons. The extract also reduces the levels of catecholamines, tyrosine hydroxylase and neuropeptide Y. The cholinergic inducing activity is first detected at postnatal day 5 and is present throughout the time period in which the sweat gland innervation undergoes a phenotypic switch as well as in adult animals. An initial characterization indicates that the ChAT inducing activity is heat labile and inactivated by trypsin. It does not bind to a heparin agarose column but it does bind to an anionic column indicating that it is an acidic protein. Antibody immunopreciptation experiments with an affinity purified antibody to the N-terminal of LIF/CDF suggest that this differentiation factor is not LIF/CDF. The soluble cholinergic inducing activity present in low salt sweat gland extracts is a likely candidate for mediating the target-mediated noradrenergic to cholinergic switch seen in vivo. Supported by AHA and NINDS.

272.4

SKELETAL MUSCLE PROTEINS STIMULATE CHOLINERGIC DEVELOPMENT IN HUMAN NEUROBLASTOMA CELLS. F.G.Crawford* and J.L.McManaman. Dept. of Neurology and Div. of Neuroscience,Baylor College of Med., Houston,TX 77030

Extracts of rat skeletal muscle contain substances which stimulate the development of choline acetyltransferase (ChAT), the enzyme responsible for the synthesis of acetylcholine, in the cholinergic human neuroblastoma cell line LA-N-2. The ChAT enhancing activity co-purifies with CDF (ChAT Development Factor), the skeletal muscle factor which rescues motoneurons from naturally occurring cell death in vivo and enhances ChAT activity in embryonic rat spinal cord cultures (McManaman et al., Neuron, in press). CDF produces a 7-fold increase in the ChAT activity of LA-N-2 cells after 8 days in culture, but does not affect their growth or metabolic activity. Basic fibroblast growth factor (bFGF) and insulin like growth factor (IGF-1) also stimulate ChAT in embryonic rat spinal cord cultures. However, in contrast to the effects of CDF, bFGF produces a relatively small increase in the level of ChAT activity in LA-N-2 cultures while IGF-1 appears to be inactive. These results demonstrate that exogenous factors can stimulate cholinergic development of neuroblastoma cells and suggest that CDF may play a role in cholinergic differentiation as well as affecting motoneuron survival.

272.5

A NEUROGENIC CULTURE SYSTEM FOR CHOLINERGIC DIFFERENTIATION. M. Martinic, M.P. Lambert and W.L. Klein. Institute for Neuroscience, Northwestern University, Evanston, IL. 60208.

We have developed a system for studying differentiation of presumptive cholinergic neuroblasts in vitro. E15 rat basal forebrain cells were grown at low density with or without soluble proteins from olfactory bulb. Unsupplemented cultures died rapidly (t1/2 < 10 hrs.), and attempts to inhibit death by blocking induction of cytotoxic proteins, known to be effective for NGF-dependent peripheral neurons, were unsuccessful. Supplemented with proteins from olfactory bulb, cultures developed a predominant cholinergic phenotype (90% AChE-positive, 70% ChAT-positive); ChAT-positive cells preferentially aggregated with each other. Cultures supplemented with NGF, EGF, IGF, bFGF, or proteins from cerebellum, not a target of cholinergic forebrain neurons, did not survive. Cell survival with olfactory bulb supplement required DNA synthesis during the initial stages of culture, and on day 2 in culture, 25% of cells were still incorporating 3H-thymidine. Of these cells, 90% later became neurofilament-positive. These results show that growth factors in olfactory bulb support the terminal mitosis of cultured basal forebrain neuroblasts, with consequent enrichment of the cholinergic population. (Supported by NIH grant NS23348 to WLK)

272.6

DEVELOPMENT OF HUMAN FETAL BULBOSPINAL NORADRENALINE NEURONS IN VIVO AND IN VITRO.
E. Sundström*, P. Almqvist*, H. Pschera* and Å. Seiger. Dept. of Geriatric Medicine and Dept. of Gynecology, Karolinska Institutet, Huddinge University Hospital, S-14186 Huddinge, Sweden.

In the present study we characterise neurochemically and morphologically the development of the human bulbospinal noradrenaline (NA) neurons in vivo and in vitro. Fetuses from first trimester abortions were dissected and selected regions were either analysed for their NA content using HPLC-ED or immersionfixed for tyrosine hydroxylase (TH) immunohistochemistry. We observed an early appearance of NA in regions containing the noradrenergic perikarya with approximately 60 ng NA/g found in pons at 6.5 weeks. This concentration increased fourfold over the next five weeks. However, the total amount of NA in pons increased 30-fold owing to the increased weight of this region. A similar pattern was seen in medulla oblongata where there was only a slight increase in NA concentration but a 10-fold increase in the total amount of NA between 6 and 10 weeks. The noradrenergic innervation of the spinal cord was detectable at 6 weeks (5-15 ng/g) with a rapid increase of NA concentration during the next 5 weeks (35-50 ng/g). We were unable to resolve any clear differences between different parts of the spinal cord.

Morphological evaluation of a few selected stages and CNS regions confirmed the presence of TH-immunoreactive cells in the brain stem and nerve fibers in the spinal cord at 7.5 weeks as well as at 10 weeks of embryonic development. The TH-positive cell bodies in the lower brain stem showed a significant structural differentiation during this 2.5 week period.

Selected regions of the fetal nervous system were used to develop a culture system in which dissociated cells, grown on collagen and polylysine-coated plates, could be maintained in vitro for 6 months or more, without even brief exposure to antibiotics, specific growth factors or preconditioned media . The development of TH-immunoreactive neurons in culture was compared to that in vivo and the results will be discussed.

272.7

REGIONAL ONTOGENY OF DARPP-32 mRNA IN MOUSE BRAIN BY *IN SITU* HYBRIDIZATION. R.M. Lewis and R. Perez. Dept. NACS, Univ. Pittsburgh, Pittsburgh, PA 15261.

Dopamine stimulates the phosphorylation of the neuronal phosphoprotein phosphatase DARPP-32. Previous studies utilizing antibodies tentatively have identified regions of the brain in which DARPP-32 is enriched either in cell bodies, axons or terminals. Because we are interested in studying control of gene expression for DARPP-32, we wanted to determine the location of the DARPP-32 mRNA, and to quantify changes in mRNA levels that occur during development, and in response to specific stimuli. An existing bovine cDNA for DARPP-32 was utilized to screen a mouse brain cDNA library in λgt11. The sequence of the isolated mouse brain cDNA was highly homologous to the bovine cDNA. The mouse brain cDNA was used to synthesize an ^{35}S-labeled riboprobe. Frozen sections of mouse brain were hybridized with this probe to detect DARPP-32 mRNA. Label was quantified by counting grains in random selections of groups of cells. Conditions were adjusted such that label in the cell bodies of the caudate (which contain the highest level of DARPP-32 protein) was just below saturation. At early stages, it was possible to distinguish cells in the caudate which were highly labeled, contained no label, or contained intermediate amounts. Similar distinctions could be made for other brain regions which contained DARPP-32. Little label was detected in glial cells in fiber tracts.

272.8

POSTNATAL EXPRESSION OF TYROSINE HYDROXYLASE IMMUNO-REACTIVE NEURONS (TH-IN) IN THE CEREBRAL CORTEX AND STRIATUM OF THE MOUSE: TREATMENT WITH DISULFIRAM OR THIRAM. J. Satoh and K. Suzuki. Dept. of Clin. Neuropathology, Tokyo Metropol. Inst. for Neurosciences, Japan and Dept. of Pathology, Univ. of North Carolina at Chapel Hill, USA.

We have reported transient increase of TH-IN in the cortex on the 2nd postnatal (PN) week in mice (Satoh & Suzuki, 1988). TH-IN in the striatum have been reported in primate and rat, but their transient change has not been noticed. Using the same method, we found a TH-IN in 22 out of 60 Swiss Webster mice of PN day (P)2 to P30. They reached their peak between P12 and P16, but none was found on P60. Mice treated with Disulfiram (tetraethylthiuram disulfide), a potent inhibitor of DBH, on the 2nd PN week revealed a striking increase of cortical and a TH-IN. The treatment on the 1st or 3rd PN week showed very little effect. Emergence of TH-IN was observed in Thiram (tetramethylthiuram disulfide) treated mice. In these brains, norepinephrine decreased and dopamine increased. In the brindled, murine model of congenital copper deficiency with decreasing DBH activity, similar enhanced expression of cortical and striatal TH-IN has been reported (Satoh & Suzuki, 1989). Thus, enhanced expression of TH-IN in both Disulfiram or Thiram treated mice and the brindled could be considered due to perturbation of catecholamine system during well defined PN periods.

272.9

TYROSINE HYDROXYLASE-IMMUNOREACTIVE NEURONS IN THE CHICK HYPOTHALAMUS: LACK OF THE IMMUNOCYTOCHEMICAL DEMONSTRATION OF DOPAMINE SYNTHESIS. A.A. Romero*, A.J. Vallejos*, J.K. Lobner* and J.A. Wallace. Dept. of Anatomy, University of New Mexico Sch. of Med., Albuquerque, NM 87131.

In the chick diencephalon, numerous tyrosine hydroxylase-immunopositive (TH+) cells can be distinguished in patterns similar to that observed for DA-containing neurons in mammals. However, with the exception of certain posterior hypothalamic groups, few cells can be detected by anti-DA immunocytochemistry in tissue from untreated control chick hatchlings (Wallace, et al., Anat. Rec. 226:107A,'90). Here we examined the potential of the majority of chick hypothalamic TH+ cells to synthesize DA by pretreating hatchlings with monoamine oxidase inhibitors (MAO-I) alone, or in combination with L-DOPA (the immediate precursor to DA synthesis). With either pretreatment, the number of hypothalamic DA-immunopositive cells increased only slightly as compared to controls. Therefore, it appears that most hypothalamic TH+ cells in the chick do not possess dopa decarboxylase, or that this enzyme is present yet inactive. Supported by NIH grants MRC 1 T34-GM-08222 and GM-08319.

272.10

EFFECTS OF MUSCLE-DERIVED NEUROTROPHIC FACTORS ON DIFFERENTIATION OF IMR-32 NEURBLASTOMA CELLS. E.D.Rabinovsky, W-D Lee, and J.L.McManaman,Dept. of Neurology, Baylor College of Medicine, Houston, Tx. 77030.

The neuroblastoma cell line, IMR-32, exhibits both cholinergic and adrenergic properties. We have used IMR-32 cells to study the effects of two skeletal-muscle derived neurotrophic factors, CDF (ChAT Development Factor) and bFGF (basic fibroblast growth factor), on the development of neurotransmitter properties. Treatment with CDF increased CHAT activity in a dose dependent manner, independent of cell density. Time course studies showed that there is a 3-fold increase in the specific ChAT activity in IMR-32 cells, treated with CDF, after 6 days in culture. By contrast, CDF did not effect proliferation, metabolic activity, or the level of tyrosine hydroxylase (TH) activity. Basic FGF, on the other hand, increased cell proliferation, metabolic activity and induced TH activity 2.5-fold after 6 days in culture. However, bFGF had no effect on the CHAT activity of IMR-32 cells. These results indicate that CDF specifically induces IMR-32 cells towards cholinergic differentiation while bFGF induces adrenergic differentiation. Thus, IMR-32 cells appear to be differentially responsive to distinct neurotrophic factors, and provides a model for studying the specific intracellular events mediating the effects of neurotrophic factors on functional differetiation.

272.11

EXPRESSION OF D_1-DOPAMINE RECEPTOR BINDING SITES IN AN IMMORTALIZED MURINE CORPUS STRIATUM CELL LINE. M.S. Wainwright, B.D. Perry, P. Kontur and A. Heller. Dept. of Pharmacological and Physiological Sciences, The University of Chicago, Chicago IL 60637.

We examined the effects of a differentiating agent (n-butyrate) on expression of D_1 receptor binding sites in an immortalized cell line derived from murine CNS.

Immortalized dopaminoceptive cells were prepared by the fusion of fetal (E18) corpus striatum (CS) cells with a neuroblastoma line (N18TG2). Following treatment with n-butyrate (1 mM for 6 days), long neurites and a decrease in growth rate were observed in the CS, but not the N18 cell line. D_1 binding sites were labeled with ^{125}I-SCH 23982 (SCH) in 8 point saturation studies (0.03 to 5.0 nM) using standard techniques. In the untreated CS line the affinity (Kd) of SCH was 0.59 ± 0.28 nM with a Bmax (binding site density) of 214.9 ± 61.6 fmol/mg prot (n=3). n-Butyrate treatment did not alter SCH affinity (0.34 ± 0.15nM) but increased binding site density to 700.8 ± 135.4 fmol/mg prot (n=3). In the untreated N18TG2 line, SCH affinity was 0.22 ± 0.14 nM with a Bmax of 75.3 ± 25.5 fmol/mg prot (n=6). There were no significant changes in Kd (0.19 ± 0.07 nM) or Bmax (30.7 ± 1.6 fmol/mg prot: n=3) following n-butyrate treatment. In both lines, drugs inhibited SCH binding with a rank order of potency consistent with a D_1 receptor. In the treated CS cell line basal adenylate cyclase activity (13 pmol/mg prot/min) was enhanced by 56% (n=3) by DA (10^{-4}M) in the the presence of haloperidol (10^{-7}M). In treated and untreated N18TG2 cells, DA inhibited, but did not stimulate, adenylate cyclase activity (23% decrease from basal; n=3).

These results suggest that this CS derived cell line may serve as a model for examining mechanisms of the developmental regulation of D_1 receptor/effector systems. Supported by MH-28942 and the Brain Research Foundation.

272.12

THE INFLUENCE OF DOPAMINE NEURONS ON D_1-DOPAMINE RECEPTOR BINDING SITE DEVELOPMENT IN THREE DIMENSIONAL REAGGREGATE TISSUE CULTURE. B.D. Perry, M.S. Wainwright, L. Won, A. Heller and P. Hoffmann. Depts of Psychiatry and Pharmacological and Physiological Sciences, The University of Chicago, Chicago, IL 60637.

We examined D_1-dopamine receptor binding sites in a three-dimensional reaggregate tissue culture system containing dopaminergic (DA) cells from fetal mouse brain. DA-containing cells from rostral mesencephalic tegmentum (RMT) were grown in rotation-mediated cell culture with cells obtained from corpus striatum (CS), a target tissue for DA neurons, or from tectum (T), which is not a DA neuron target. RMT-CS reaggregates form highly organized structures in which the DA-containing cells from the RMT innervate the CS target cells and make appropriate functional connections (Kotake et al., J Neurosci 2:1307, 1982).

Reaggregates were prepared on embryonic day 14 and maintained in culture for 21 days. Three cell combinations were studied: 1) 10 x 10^6 CS cells cultured alone (CS); 2) 5 x 10^6 RMT cells co-cultured with 5 x 10^6 CS cells (RMT-CS); 3) 5 x 10^6 RMT co-cultured with 5 x 10^6 T (RMT-T). On day 21, reaggregates were harvested and membranes prepared for radioligand binding studies. ^{125}I-SCH 23982 (SCH) was used to label the D_1 sites. Eight concentration saturation studies were performed on each flask using standard techniques.

At day 21 the RMT-CS cultures expressed twice as many D_1 binding sites as the CS cultures and five times as many sites as the RMT-T reaggregates (RMT-CS: 162 ± 24.8, n=8; CS: 84 ± 8.9, n=10; RMT-T: 34.6 ± 4.5, n=8; values in fmol/mg prot ± SEM).

These findings suggest that the developing cells of the RMT play a role in the phenotypic expression of the D_1-dopamine receptor.

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272.13

CHARACTERIZATION OF THE NOREPINEPHRINE UPTAKE SYSTEM EXPRESSED BY NEURAL CREST CELLS IN CLONAL CULTURE. J-M. Zhang and M. Sieber-Blum. Dept. of Anatomy and Cellular Biology, Medical College of Wisconsin, 8701 Watertown Plank Road, Milwaukee, WI 53226.

Previous results suggested that adrenergic differentiation of neural crest cells in clonal culture is blocked by norepinephrine (NE) uptake inhibitors, such as the tricyclic antidepressant, desipramine (DMI). This observation suggested that the catecholamines present in the ventral migratory pathway, at a time it is traversed by presumptive adrenergic cells, may participate in a positive feedback mechanism that serves to enhance and stabilize expression of the adrenergic phenotype [Sieber-Blum (1989) *Dev. Biol.* 136, 372]. We report here that as in the embryo, neural crest cells in clonal culture express a high affinity NE uptake system before other adrenergic traits become evident. Uptake of radioactive NE, visualized by autoradiography, indicated that the system first appears at clonal culture day 5, i.e. 2 days before the cells start to accumulate catecholamines at detectable levels. Rare uptake-positive cells were detected as early as culture day 3. On day 5, 5/27 (18%) unpigmented colonies and 19/75 (25%) mixed colonies contained uptake-positive cells. On day 6, 9/31 (29%) unpigmented and 33/96 (33%) mixed colonies were positive, whereas by day 7, 9/32 (28%) unpigmented and 43/101 (42%) mixed colonies were positive. In experiments using a different lot of horse serum, the number of positive unpigmented colonies was as high as 64% and the number of positive mixed colonies 23% on culture day 5. The number of colonies containing uptake-positive cells was decreased by 79% in the presence of DMI. Uptake reached maximum by 5 min. The K_m was determined to be 0.8 to 4.8 µM depending on the control conditions. The data indicate that clonal neural crest cell cultures provide a useful experimental system for investigating the role of the NE uptake system in the differentiation of pluripotent neural crest cells into adrenergic cells. Supported by USPHS grant HD21423.

272.14

POSTNATAL DEVELOPMENT OF THE ALPHA2-ADRENOCEPTOR SYSTEM IN THE TREE SHREW BRAIN. G. Flügge and E. Fuchs
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In adult tree shrews (*Tupaia belangeri*), central adrenergic receptors have a very high affinity for alpha2-adrenoceptor ligands (Flügge et al. 1990). To gain insight into the maturation of the central alpha2-adrenoceptor system in these animals, we studied its postnatal development by in vitro autoradiography with the specific antagonists ^{3}H-rauwolcine and ^{3}H-idazoxan.

Alpha2-adrenergic binding sites (a2-BS) are already present at birth and their pharmacological properties are similar to those in the adult. But in contrast to the adult brain which reveals high numbers of alpha2-binding sites in rostral areas (telencephalon, diencephalon), the brain of neonates is primarily labeled in its caudal regions (myelencephalon, metencephalon) and the labeling is more diffuse, although the septum, hippocampus and the geniculate nucleus already show a high number of a2-BS immediately after birth. Especially the rhombencephalic areas bearing the anlage for the catecholaminergic cell groups A1-A7 form a strongly labeled, continuous band. Furthermore, there is a high number of a2-BS in the lateral cerebellum which can be observed during the first 2 postnatal weeks. The number of the caudal a2-BS decreases gradually while that of the rostral BS increases to reach the adult pattern of distinct nuclear a2-BS distribution around postnatal day 25.

Our results show that the central nervous alpha2-adrenoceptor system in the tree shrew is already expressed at birth when a2-BS can be detected in the caudal parts of the brain and in the limbic system. Within the first 25 postnatal days, the caudo-rostral gradient in the pattern of a2-BS changes into a rostro-caudal gradient which is characteristic for adult tree shrews.

272.15

CHRONIC EXPOSURE OF THE CEREBELLA OF NEONATAL MICE TO THE NMDA RECEPTOR ANTAGONIST, AP5, DISRUPTS PURKINJE AND GRANULE CELL DEVELOPMENT.

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Studies in the visual system have shown that NMDA receptor activation plays an important role in the establishment of mature synaptic connections. Purkinje and granule cells are more sensitive to NMDA receptor activation in neonates compared to adults, and we have begun to test the role of synaptic activity and NMDA receptor activation on Purkinje and granule cell development.

Elvax strips with or without DL-AP5 (1mM) were implanted over the cerebellum of C57BL/6 mice at P5 and the mice were then killed at ages ranging from P17 to P33. The brains of 6 AP5 treated mice, 2 mice with drug-free elvax implants, and 5 age-matched control mice were then processed for Golgi-Cox staining and counterstained with cresyl violet. In the AP5 treated mice, but not the control mice, granule cells were found positioned ectopically within the molecular layer near the elvax implant. The quantity and position of the ectopic granule cells varied. In 2 of the AP5 treated mice there were extensive tracts of ectopic granule cells at the folia surface and in the folds between folia. Morphometric analysis of Purkinje cells near the AP5 treated elvax strip suggests that their dendritic trees are significantly smaller, less isoplanar, and occupy less of the upper half of the molecular layer. These preliminary results suggest that blocking the NMDA receptor during postnatal development can interfere with granule cell migration and the elaboration of Purkinje cell dendrites.

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272.16

NMDA AND CALCIUM REGULATES THE ENKEPHALINERGIC PHENOTYPE IN DEVELOPING SPINAL CORD CULTURES

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The establishment of the neuronal phenotype is developmentally regulated by epigenetic influences like electrical activity and soluble factors. In the developing embryonic spinal cord dorsal root ganglia (SC-DRG) cultures, spontaneous electrical activity is a major factor regulating the expression of neuropeptide genes including enkephalin. Calcium ionophore or NMDA cause a significant up-regulation in the expression of the enkephalin transcripts ($mRNA^{enk}$) in developing SC-DRG neurons (Agoston et al., in prep). Here we have pharmacologically characterized the receptor specificity of calcium flux regulation of enkephalin biosynthesis and secretion in embryonal neuronal cultures. SC-DRG cells were treated with NMDA (10^{-6} to 10^{-4} M) or +202 791 (10^{-6} to 10^{-4} M) - a selective agonist of L-type voltage-sensitive calcium channles (VSCC) - prior, simultaneously or without TTX treatment. Enkephalin transcripts were analyzed by Northern blot hybridization using a complementary DNA probe. Met-enkephalin (menk) was measured in a RIA system.

In electrically active cultures NMDA treatment increased the expression of $mRNA^{enk}$ and the cellular menk content in a concentration-dependent manner. Menk secretion was slightly but significantly reduced by NMDA in active cultures but was not significantly altered in TTX-blocked cultures. The VSCC-agonist +202 791 was ineffective if it was applied simultaneously with TTX. However, 1 hour pre-treatment with + 202 791 could not only prevent the TTX caused downregulation of $mRNA^{enk}$ expression in a concentration- dependent manner but increased $mRNA^{enk}$ over the TTX treated controls. Similarly, cellular menk content was also increased as compared to the TTX-blocked control. Similar pre-treatment did not increase menk secretion compared to the TTX-treated controls. These results indicate that a) the developmental expression of the enkephalin gene is regulated by calcium through NMDA and VSCC-stimulation and b) secretion and biosynthesis of enkephalin are regulated by parallel but separate calcium-dependent intracellular mechanisms. (D.V.A. is supported by the Deuthsche Forschungsgemeinschaft)

272.17

DEVELOPMENT OF MEMBRANE RESPONSES OF EMBRYONIC RAT SPINAL CORD CELLS.

M.K. Walton, A.E. Schaffner, and J.L. Barker. Laboratory of Neurophysiology, NINDS, NIH, Bethesda, MD 20892.

The presence of functional sodium channels, GABA receptors, and kainic acid receptors was examined in dissociated embryonic rat spinal cord cells across several developmental ages using computerized digital imaging microscopy and a voltage sensitive fluorescent dye. Cervical regions of spinal cords were removed from rat embryos from embryonic days 13,15,17,19,21 (E13 to E21) and were divided into dorsal and ventral portions. The tissue was enzymatically dissociated into single cell suspensions, placed onto culture dishes and allowed to adhere for ca. 2 hours. Culture dishes were perfused with continuously flowing solutions, all with 50 nM of the voltage-sensitive oxonol dye $DiBaC_4(5)$. Voltage-sensitive sodium channels were probed for with veratridine (25 uM), GABA-A receptors were probed for with muscimol (2.5 uM), and kainic acid (50 uM) was used as a probe for non-NMDA glutamate receptors. The depolarizing response seen to each of these drugs could be eliminated by use of specific blockers of their actions, tetrodotoxin, bicuculline or CNQX, respectively.

Overall, sodium channel appearance preceded appearance of responses to muscimol, and muscimol responses appeared earlier than kainic acid responses. This delay was more pronounced in the dorsal portion than in the ventral portion cells. Development of kainic acid responses progressed to include almost all cells by E19-E21, while veratridine and muscimol responding percentages remained lower. Individual cells could exhibit one or both receptor responses without sodium channel responses. Magnitude of the response of individual cells showed an increase during the developmental period examined, particularly for the kainic acid response.

272.18

FUNCTIONAL $GABA_A$ RECEPTORS ARE CRITICAL FOR SURVIVAL AND PROCESS OUTGROWTH OF EMBRYONIC CHICK SPINAL CORD CELLS *IN VITRO*.

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Acutely dissociated chick embryonic (E) spinal cord cells are depolarized by $GABA_A$ agonists in a bicuculline sensitive manner as early as E4, as demonstrated using flow cytometry and voltage-sensitive dyes (Prasad et al.,(1989) Soc. Neurosci. Abstr.15:295). However, with the exception of some behavioral studies, little is known about the functional role of GABA during early embyrogenesis, prior to the onset of well-differentiated synapses in the spinal cord. We have investigated the effects of bicuculline, a $GABA_A$ antagonist, and muscimol, a $GABA_A$ agonist, on embryonic spinal cord cells differentiating in culture. Cells (E4.5-E9) were dissociated with papain, resuspended in MEM and serum, and plated on poly-lysine coated plastic plates. After overnight exposure to GABAergic agents, cells were fixed and stained for neurofilament-like-immunoreactivity.

Several hours were required before any visible changes between the experimental and control cultures were evident. Overnight exposure to 20-200 µM bicuculline consistently decreased cell number and neurite outgrowth relative to control cultures. No changes were seen with 2 µM, or lower, doses. The effects of bicuculline (at doses which block muscimol/GABA depolarization) were antagonized by co-incubation with 10 µM muscimol. These results suggest important if not critical roles for $GABA_A$ receptors in the survival and differentiation of chick spinal cord neurons *in vitro*.

272.19

LATE GENERATION OF GABA-ERGIC SUBPLATE NEURONS IN THE RAT.

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The subplate layer (SP) contains many GABA-ergic neurons during early prenatal cortical development (Van Eden et al.,J.C.N. 289:213-227;1990). In order to estimate the percentage of GABA-ergic neurons in the SP of the rat, timed pregnant females were injected with ^{3}H-thymidine (10 µCi/g., i.p.) at embryonic days (E) 13 and E14 (E1 is the day of insemination). Fetusses and pups were perfused at different ages between E16 and postnatal day (P) 25. Plastic sections were processed for GABA-ICC and autoradiography. After both injections at E13 and E14 heavy labelled cells were found in the plexiform primordium, the marginal zone (MZ) and the SP. E14 injections showed the highest number of heavy labelled cells in these layers in the dorsomedial frontal cortex, whereas in the ventrolateral cortex mainly cells of layer VIa were labelled. After birth these heavy labelled subplate cells were located in cortical layer VIb. No indication was found for a major decline in the number of labelled cells in the SP (layer VIb) during the postnatal period. The MZ and the SP contained the highest number of GABA-IR cells during the prenatal period. However, only very few GABA-ergic cells were labelled by ^{3}H-thymidine injections at E13 or E14. Also after birth, only a few GABA-ergic cells were labelled with ^{3}H-thymidine in the cortex. The few GABA-ergic cells that were generated at E13 or E14 were primarily situated within cortical layers V and VIa. The present data demonstrate that although a large number of the SP cells are generated on E13 and E14, the GABA-ergic population in these layers must be generated later and subsequently added to the SP.

272.20

L-GLUTAMATE MICROSPHERE STIMULATION OF THE TRIGEMINAL MOTOR NUCLEUS IN GROWING RATS.

E.L. Hamilton-Byrd*, A.J. Sokoloff, A.J. Domb*, L. Terr and K.E. Byrd. Univ. of Southern California Sch. of Dent., Los Angeles, CA 90089.

The purpose of this study was to investigate the efficacy of glutamic acid loaded microspheres for long-term stimulation of trigeminal motoneurons as a possible treatment for growth disorders of the craniofacial skeleton. 10% glutamic acid:HCl in polyanhydride, P(FAD-SA) 1:1, microspheres (106 - 250 µm dia.) were implanted in the region of the trigeminal motor nucleus (TMNu) of 11 male Sprague-Dawley rats (33 - 38 days old) using the technique of Howard et al. (J. Neurosurg., 71:105 - 112, 1989). Control rats had implants of blank microspheres or actual penetration of the empty delivery system into the target region. All rats were killed and perfused 10-14 days post-operatively. 3 rats implanted with glutamate microspheres showed pronounced skeletal changes in the snout region when compared to control rats. In vivo sustained release of glutamic acid in proximity to the TMNu can effect significant changes of the craniofacial skeleton in growing rats. Supported by NIDR grant R29 DE07380 to K.E. Byrd and Nova Pharmaceutical Corporation.

272.21

IMMUNOCYTOCHEMICAL DETECTION OF PROENKEPHALIN-DERIVED PEPTIDES IN THE CORPUS STRIATUM OF THE DEVELOPING RAT. D.D. SONG AND R.E. HARLAN, Department of Anatomy, Tulane University School of Medicine, New Orleans, Louisiana 70112.

Immunocytochemistry using antisera to the C-terminal octapeptide of synenkephalin, proenkephalin (63-70), and to methionine enkephalin (met-Enk) was performed on serial cryostat sections through the corpus striatum from embryonic day 14 (E14) through postnatal day 10 (P10).

Immunostained cells were detected in the caudate-putamen by E16 with the synenkephalin antiserum. At E18 immunostained cells became more numerous. At E20 more cells were detected in the caudate putamen; however, the intensity of staining became weaker. Also by E20 immunopositive fibers in the globus pallidus first became detectable, suggesting increased axonal transport. By the day of birth (P0) an adult-like distribution of immunoreactivity became apparent; cells could no longer be detected in the caudate-putamen while immunoreactive fibers in the globus pallidus increased in density. At subsequent ages (P5 and P10), only immunoreactive fibers in the globus pallidus could be detected. Our met-Enk antiserum was unable to detect any cells or fibers in the caudate-putamen or globus pallidus at the ages studied, except for P10. It appears that met-Enk, prior to P10, may be stored in a cryptic form, possibly as part of a larger, unprocessed precursor.

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272.22

DIFFERENTIAL MORPHOLOGICAL RESPONSES TO ECDYSTEROID OF CULTURED NEURONS FROM INSECT ANTENNAL LOBE. L.A. Oland and L.P. Tolbert, Arizona Research Labs Division of Neurobiology, University of Arizona, Tucson, AZ 85721.

The titer of the hormone 20-hydroxyecdysone (20-HE) rises dramatically during metamorphic development in insects when neurons in the antennal lobe (AL) develop their adult morphology and synaptic connections. To isolate the effect of 20-HE from other influences on the morphological development of these neurons, we dissociated neurons from early pupal ALs (prior to the rapid rise in 20-HE titer) that had not been allowed to receive input from the antenna, and exposed them to doses of 20-HE that span the range seen *in vivo*. After 2-3 weeks, we examined particular cell types that consistently appear in these cultures, some of which were described by Hayashi and Hildebrand (1990). Of the approximately 400 cells examined, a quarter could be identified as belonging to one of 8 cell types. In all cases, the basic branching pattern of the neuronal type was maintained across all doses of 20-HE. Also in all cases, neurons exposed to 1μg/ml 20-HE generally were larger, reached a higher branching order, and consistently had more robust branches than did neurons grown in the absence of 20-HE. In three of the 8 types, exposure to 10μg/ml 20-HE enhanced these effects. In three other types, there was no morphological difference between 1 and 10μg/ml. In the final group of two types, the overall robustness of the cells was less at 10μg/ml than at 1μg/ml. The 8 cell types used in this study are known to include both local and output classes of AL neurons; each of the response patterns to 10μg/ml 20-HE was seen in at least one of the cell types belonging to each class, indicating that output and local neurons cannot be segregated on the basis of their responses to 20-HE. These data suggest that *in vivo*, the morphology of different types of AL neurons may be differentially regulated by the titer of 20-HE. [Supported by NS20042.]

272.23

ONTOGENESIS OF CGRP RECEPTORS IN THE RAT CEREBELLUM. A. Rosina, S. Morara, G. Forloni* and L. Provini° (SPON : ENA). Ist. Fisiol. Centri Nervosi CNR, *Ist. Mario Negri and °Ist. Fisiol. Chim. Biol., Fac. Farm., Milano, Italy.

A quantitative autoradiographic study of the density and distribution of Calcitonin gene related-peptide (CGRP) binding sites was conducted in the rat cerebellum, in an attempt to discern what their relationship is to the postnatal morphogenesis of the cerebellar cortex. Autoradiography was performed using 0.1 nM ^{125}I-CGRP (human α-CGRP); nonspecific binding was determined on adjacent sections by the addition of 1 uM unlabeled CGRP to the labeled ligand. Adjacent sections of cerebella at postnatal day (PD) 0,3,6,9, 12,15,20,26 were examined. CGRP binding sites started to be detected in the white matter, where they steadily increased from PD 0 to 20, to decrease to adult levels at PD 26. In the molecular layer, CGRP receptors were first detected at PD 6-9 and their density increased along a caudo-rostral and medio-lateral gradient, up to PD 26. CGRP receptors binding was undetectable or very low in the external or internal granular layers respectively. These findings indicate a close relationship between CGRP receptor expression and both the growth of the Purkinje cell dendrites and the climbing stage of the olivocerebellar fibers.

272.24

APPEARANCE OF PEPTIDERGIC NEURONS IN THE DEVELOPING CHICK GUT. M.L. Epstein and K.T. Poulsen*. Dept of Anatomy, University of Wisconsin, Madison, WI 53706.

Neural crest-derived cells are found in the ileum by embryonic day (ED)6.5. We are interested in the factors regulating the expression of neuropeptides in the gut. The appearance of vasoactive intestinal peptide (VIP)- and somatostatin (SOM)-immunoreactive (IR) neurons in different regions of the gut was studied by immunostaining whole mounts of embryonic gut. The pattern of expression of these peptides in myenteric neurons showed a number of similarities. Both peptides first appeared in the region of the gizzard-proventriculus-duodenum; SOM at embryonic day (ED)4, VIP at ED5.5. Both peptides were found at later times in positions both rostral and caudal to the gizzard. By ED7.5 both peptides were found proximal to the proventriculus and in the descending loop of duodenum. Both peptides next appeared in cells in the cecum of the hindgut, although no immunoreactive cells were found in the midgut; SOM was observed at ED6.5 and VIP at ED7.5. VIP- and SOM-IR cells appear throughout the cecum and then in the terminal part of the rectum. Another similarity was that immunostained cells appear last in the ileum with VIP appearing at ED10-12 and SOM at ED15.5-17.5. Differences in the pattern of expression are also found. SOM-IR appeared in cells in the terminal part of Remak's Ganglion at ED5.5. These cells became segregated into clusters along the ganglion; cells sent fibers into the wall of the rectum and later into the ileum. No VIP-IR cells were found in Remak's Ganglion. These findings suggest that neural crest-derived cells first express peptides in the region of the gizzard of the foregut and then in the cecum of hindgut. The appearance of peptidergic cells in the hindgut before the midgut is consistent with a caudal source of neural crest-derived cells. Supported by BNS 8820658.

CELL LINEAGE II

273.1

CHARACTERIZATION OF A SUBPOPULATION OF EARLY NEURAL CREST CELLS RECOGNIZED BY THE MONOCLONAL ANTIBODY B-1A11. C.J. Langtimm and M. Sieber-Blum. Department of Anatomy and Cellular Biology, Medical College of Wisconsin, Milwaukee, WI 53226.

The monoclonal antibody B-1A11 recognizes a cell surface epitope on a small (10%) subpopulation of undifferentiated, cultured neural crest cells. The present data characterize the epitope and the immunoreactive cells. The epitope is trypsin resistant, diminished after chloroform-methanol treatment, and not detectable by Western hybridization, suggesting that it is part of a lipid rather than a protein. However, neuraminidase treatment and incubation with phospholipase C do not affect staining. The expression of the epitope is transitory. It first appears by 48 hr of culture. By 96 hr, early melanocytes and unpigmented cells resembling nerve supporting cells bind the antibody. Fully developed pigment cells are no longer immunoreactive. In older cultures, neuronal cells do not bind B-1A11, with the exception of rare sensory neurons. B-1A11 immunoreactive cells also occur in clonal cultures. Staining patterns are marked by considerable variability. By clonal culture day 4, 0% to >90% of all cells within a colony are immunoreactive in both unpigmented and mixed colonies. By day 5, this number drops to 0%-24% of positive cells per colony. In older clonal cultures, immunoreactive cells are only rarely observed. Neural crest cells from 48 hr primary explants can be enriched up to 5-fold by separation with magnetizable beads. When such purified, immuno-fluorescent cells are seeded out at clonal density, they attach, proliferate and form pigmented colonies. In whole mount stains of 48 hr embryos (stage 14; 22 somites) individual cells or small groups of 2-4 cells are located medially in mesencephalic areas as well as in somitic levels 8-12, above the triangular space outlined by two adjacent somites and the neural tube. By 96 hr of incubation, immunoreactive cells occur in groups throughout the ectoderm, suggesting their melanogenic nature. The data a) suggest that B-1A11 recognizes a differentiation antigen that characterizes cells in the melanogenic and possibly the nerve supporting cell lineages and b) show for the first time heterogeneity within a particular class of colonies in clonal culture. Supported by USPHS grant HD21423.

273.2

PATTERN OF IN VITRO DIFFERENTIATION OF HNK-1 SORTED QUAIL TRUNK NEURAL CREST CELLS G.D. Maxwell and M.E. Forbes*. Dept. of Anatomy, Univ. of Connecticut Health Center, Farmington, CT 06032.

We have continued our analysis of the spectrum of phenotypes which differentiate in tissue culture from populations of quail trunk neural crest cells isolated as HNK-1+ and HNK-1- by fluorescence activated cell sorting. We have found that tyrosine hydroxylase and somatostatin immunoreactive cells developed consistently from the HNK-1+ sorted cells. In contrast, few if any cells with these markers differentiated in the HNK-1- sorted population. These observations are in agreement with our previous work showing that catecholamine containing cells arose preferentially from the HNK-1+ sorted population (Maxwell et al. 1988 Neuron 1: 557-568). When we examined the differentiation of A2B5, E/C8, and middle molecular weight neurofilament protein immunoreactive cells, substantial numbers of these cells developed from both the HNK-1+ and the HNK-1- sorted cell populations. These results indicate that, under the in vitro conditions used in these experiments, the presence of the HNK-1 antigen(s) on early trunk neural crest cells is correlated rather selectively with the development of adrenergic cells and some of their peptidergic subsets, but not with other cell classes bearing neural markers. This work is supported by grant NS 16115 from the NIH.

273.3

THE GENERATION OF CELL DIVERSITY IN THE SUPERIOR CERVICAL GANGLION. A.K. Hall and S.C. Landis, Dept. Neuroscience, Case Western Reserve University School of Medicine, Cleveland, OH 44106

The neural crest contains multipotent cells that give rise to different types of daughter cells including neurons and glia of the PNS. In the superior cervical ganglion (SCG), principal neurons, small intensely fluorescent (SIF) cells and glial satellite cells are derived from the neural crest.

To investigate how the specific cell types found in the mature SCG were generated from their precursors, the lineage relationships and the phenotypic development of cells in the embryonic rat SCG were examined. Analysis of dissociated E14 SCG cultures with BrdU and antibodies to NF160 indicated that neuroblasts divide for several days in vitro. Retrovirus-mediated gene transfer with the beta-galactosidase marker was used to examine lineage relationships in cultures of embryonic rat SCG. Retrovirus-labelled clones from ganglia dissociated at E13, E14 or E17 contained cells of either neuronal or non -neuronal morphology. These data suggest that neuronal and glial precursors are committed before or shortly after gangliogenesis. In situ, clusters of non-neuronal cells were observed transiently at E18 and may represent the expansion of a glial precursor. These cells lacked detectable immunoreactivity for tyrosine hydroxylase (TH), fibronectin and laminin. Non-neuronal clusters were no longer observed by birth, suggesting that cells subsequently migrate and associate with principal neurons. Before the completion of neurogenesis, neuronal precursor cells contained variable levels of catecholamines, and stained brightly with antibodies for TH, but did not contain the numerous dense-cored vesicles characteristic of mature SIF cells. Individual cells with a SIF phenotype appeared at E16/18 near blood vessels, and smaller, clustered SIF cells were seen after birth. Because the embryonic SIF-like cells were larger and found individually, these cells may represent SIF precursors which divide to give rise to smaller postnatal SIF cells in groups. These studies reveal that both lineage restrictions and environmental signals are important in the generation of cell diversity in the developing SCG.

273.4

CHARACTERIZATION OF GLIAL CELL DIVERSITY IN THE NEONATAL RAT SPINAL CORD. Robert H. Miller and Vilma Szigeti. Department of Neuroscience, Case Western Reserve University School of Medicine, Cleveland, OH 44106.

Considerable evidence suggests that many regions of the vertebrate CNS contain more than one type of astrocyte, in addition to oligodendrocytes. Three different approaches were used to examine whether the developing spinal cord also contained multiple glial cell types: Single cell cloning, retroviral lineage tracing in vitro, and the generation of cell type specific monoclonal antibodies.

Single cell cloning studies demonstrated that the neonatal rat spinal cord contained six morphologically distinct glial cell populations whose precursors differed in abundance and proliferative capacity. Labeling with antibodies against glial fibrillary acidic protein showed that at least four of these populations were astrocytes. To determine if such morphologically distinct subpopulations of glial cells could be identified in a more complex cellular environment, retroviral lineage analysis was performed on complete neonatal rat spinal cord cultures. In this case, morphologically discrete labeled clones of cells were also seen. In addition, the vast majority of labeled clones contained cells with a homogeneous morphology.

Novel monoclonal antibodies generated against spinal cord glia specifically labeled the surface of subsets of astroctyes, suggesting that these cells are antigenically, morphologically and developmentally distinct.

These studies suggest that a cytoarchitecturally complex tissue such as the mammalian spinal cord may contain as many as six different types of glial cell, each of which presumably subserves distinct functions in the developing and adult spinal cord.

273.5

Cell Type-Specific Expression of Phosphomyristin C in Glial Cell Lines and the Murine Nervous System. D Hilt, T Mical, D Farrand, P Fishman, and D Kligman. **Dept. of Neurology, University of Maryland School of Medicine, Baltimore, Md. 21201**

Phosphomyristin C (PMC-also known as the 80k or the MARCKS protein) is an approximately 80kD protein kinase C (PKC) substrate. PMC is phosphorylated rapidly after PKC activation and may function as an important mediator of PKC action. PMC is expressed in a variety of tissues but the cell type-specificity of expression in the nervous system has not been well studied. Using an antisera raised against a tryptic oligopeptide from purified rat brain PMC the expression of PMC in the RT4 cell culture system was examined. By Western blot analysis it was determined that PMC is present in a bipotential precursor cell type (AC36) and in a glial daughter cell line (D6) but is not expressed in two separate neuronal daughter cell lines (B8 and E5). Conversely, GAP43, a putatively neuron-specific protein kinase C substrate in the nervous system, was expressed in the two neuronal cell lines but not in the glial cell lines. Experiments to demonstrate the phorbol ester induced phosphorylation of these two important kinase C substrates in the RT4 cell lines will be shown.

The glial cell type-specific expression of PMC in the RT4 cells suggested that in the nervous system PMC was primarily expressed in glial cells. Therefore, the distribution of PMC in the adult murine nervous system was determined by immunocytochemical techniques. Within the CNS anti-PMC antisera revealed a dense network of fine glial processes without labeling of neurons. Both astrocytes and microgila were stained in the brain and the spinal cord. Schwann cells showed consistent cytoplasmic labeling in the PNS. These results suggest that PMC may serve as an important mediator of PKC action in glial cells in the nervous system.

273.6

MIGRATION OF LHRH NEURONS FROM THE OLFACTORY PLACODE TO THE BRAIN IN THE CHICK. R. B. Norgren and M. N. Lehman. Dept. Anat. & Cell Biol., Univ. Cincinnati Med. Coll., OH 456267

Recent findings in the mouse suggest that luteinizing hormone releasing hormone-immunoreactive (LHRH-IR) neurons do not originate in the brain, but rather migrate into the brain from the olfactory placode early in development. We have examined the distribution of LHRH-IR neurons in the developing chick. Chick embryos were immersion-fixed in 4% paraformaldehyde, and horizontal cryostat sections were incubated in an antisera to LHRH (LR-1, gift of Dr. R. Benoit) and the LHRH visualized with either an avidin-biotin-HRP technique or avidin-Texas red. In E4 chick embryos, a small cluster of cells of LHRH-IR neurons were found just lateral to the brain, immediately ventral to the olfactory pit. A few labeled cells were also apparent in the epithelium of the ventral olfactory pit. In E7 chick embryos, many more labeled cells were observed. A few LHRH-IR neurons were observed in the nasal epithelium. Clusters of LHRH-IR neurons appeared to form a chain stretching along the olfactory tract and dorsal-caudally in close proximity to the medial edge of the telencephalon. In dorsal sections, a few LHRH-IR neurons were found in the brain and had labeled processes that appeared to make contact with the lateral ventricle. Our results suggest that LHRH-IR neurons arise from a cell group associated with the olfactory placode and migrate along the olfactory tract before entering the brain. We are currently examining other stages of chick embryos to substantiate this hypothesis. [Supported by NIH grant HD 21968 (MNL)]

273.7

CO-EXPRESSION OF PNMT AND NEUROFILAMENT IN ADRENAL CHROMAFFIN CELLS. G. Teitelman, M.J. Evinger and M. Ehrlich. Div. Neurobiol., Cornell Univ. Med. Coll., New York, NY 10021.

The epinephrine synthesizing enzyme phenylethanolamine N-methyltransferase (PNMT) appears at embryonic day 16 (E16) in the rat adrenal in a subset of neural crest derived chromaffin cells. *In vitro*, these cells can be induced to extend neurites containing neurofilament (NF). We sought to determine whether adrenal medullary cells may simultaneously express neuronal traits and PNMT. Rat adrenals were removed and processed for immunocytochemical localization of PNMT and NF in the same tissue section. We found that, in embryos, a significant percentage of chromaffin cells contain both markers (E16=20%; E17=40%; E20=17%). In contrast, PNMT cells of adults do not contain NF. To ascertain whether the loss of neuronal properties from mature epinephrine cells is irreversible, adult bovine chromaffin cells maintained for two weeks in glucocorticoid free media were immunostained. The cultures contained many PNMT(+) cells, some of which extended long varicose processes. Sister cultures contained many NF(+) cells. We conclude that embryonic chromaffin cells co-express adrenergic and neuronal traits and that suppression of the neuronal phenotype during maturation is reversible.

273.8

A NEURONAL INTERMEDIATE FILAMENT PROTEIN, IS EXPRESSED IN THE RAT INSULINOMA CELL LINE. M.M. Portier, M. Escurat, K. Diabali, C. Huc, A. Prochiantz, C. Bécourt* and C. Boitard* Collège de France, 75231 Paris Cedex 05 and *Service d'immunologie, 161 Rue de Sèvres, 75743 Paris Cedex 15, France.

The developmental lineage of endocrine pancreatic cells has long been a matter of controversy and two possible origins have been proposed : either endodermal or neuroectodermal. However, a study of the nature of intermediate filament proteins (IFP) expressed in the rat RIN 5F insulinoma cell line by biochemical and immunological methods reveals that these cells express neuronal IFP : neurofilaments proteins (NFP) and peripherin which has been shown to be expressed in well defined neuronal populations and particularly in every neuron from neural crest origin. However, these IFP are not expressed in the islets of Langerhans.

Interestingly, NFP and peripherin are present in the rat PC 12 pheochromocytoma cell line although they are expressed neither in the adrenal medulla nor in the tumor from which the PC 12 cell line is derived. A parallel can thus be established between the expression of IFP in the rat insulinoma RIN 5F cell line and in the rat pheochromocytoma PC 12 cell line suggesting a common neural crest origin for the cells from which these cell lines originate.

273.9

PROLIFERATION IN THE NEURAL PLATE OF THE ASCIDIAN EMBRYO: BIRTH-DATES OF CELLS IN THE LARVAL CNS. T. Bollner and I. A. Meinertzhagen, Life Sciences Centre, Dalhousie University, Halifax, Nova Scotia. CANADA B3H 4J1

The tadpole larva of ascidians possesses a dorsal tubular CNS which is derived from an ectodermal neural plate in the embryo and contains only 370 or so cells. Two contemporary accounts differ as to whether all anterior neural plate cells contribute to the CNS. Descriptive studies in *Ciona* indicate that they contribute only CNS cells (Nicol, D.& I.A.Meinertzhagen, *Dev. Biol.*, 130:737, 1988); Whereas HRP blastomere injections in *Halocynthia* imply that anterior neural plate cell rows migrate anteriorly and acquire non-neural ectodermal fates (Nishida, H., *Dev. Biol.*, 121:526, 1987). The interpretation of these differences rests upon when proliferation in the CNS actually ends. Thymidine incorporation has been reported in neural plate cells up to the tenth cleavage (Reverberi, G., et al., *Acta. Embryol.*, 3:202, 1960), too early to arbitrate. In resolution, we have examined 5-bromo-deoxyuridine (BrdU) incorporations in neural plate cells of the ascidian *Ascidia ceratodes*, using anti-BrDU immunocytochemistry. Embryos were exposed to BrdU (1 mM in sea-water) at 7hrs (38%), 14hrs (75%), or 15 hours (81%) post fertilization, or at hatching (100%), and left in the BrdU until fixation 6hrs post-hatching. Larvae labelled from 7hrs development showed immunostaining in both anterior and posterior parts of the larval CNS, as well as in the so-called neurohypophysis, progenitor of the adult neural complex. Exposure at 14 and 15hrs produced staining in the anterior CNS even though of fewer cells than in the 7hrs group. In the neurohypophysis, labelling seemed as frequent as in the earlier group. Embryos exposed to BrdU at hatching exhibited no CNS incorporations. Although it is not certain whether the labeled CNS cells are neurons, glial or ependymal cells, we conclude that cell division occurs later than 70%, currently the best previous estimation for cersation time. The similarity in labelling patterns between the 14 and 15-hr groups is possibly explained by the relatively long cell-cycle during late neural development.

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273.10

CONTROL OF SEGMENT IDENTITY IN THE LEECH EMBRYO. L. Gleizer and G. S. Stent. Graduate Group in Neurobiology, Univ. of California, Berkeley, CA 94720.

Although the 32 segments of the leech body arise from homologous sets of mesodermal and ectodermal blast cell clones, they are distinguished by characteristic sets of morphological features that reflect unique segmental identities. For instance, in *Theromyzon rude*, nephridia are present in only 14 and genital primordia in only 2 segments. To ascertain whether the segment-specific distribution of these features, which are of mesodermal origin, is attributable to a mechanism which is intrinsic to the mesodermal blast cells, their bandlet was induced to slip out of segmental register so that the mesodermal cell clones were made to take part in the formation of segments for which they are not normally destined. Such shifted mesodermal blast cells autonomously expressed their original segment identity, giving rise to segment-specific structures according to their original birth rank rather than to their new position. It appears, therefore, that the origin of longitudinal differentiation in the mesoderm is intrinsic to the mesodermal blast cell bandlet.

This procedure also allowed us to investigate whether ectodermal segment-specific features are determined by their mesodermal environment since in experimental embryos the mesoderm is shifted out of segmental register relative to the ectoderm. The results of these studies indicate that some segment-specific features of ectodermal development, such as the soma size of the identified Retzius neurons, are controlled by the segmental identity of the local mesoderm, while others, such as the presence or absence of the identified ams neurons, are not.

273.11

WITHDRAWN

273.12

FLUORESCENT LABELING OF DEVELOPING ENDOTHELIUM AND BLOOD VESSELS IN *XENOPUS LAEVIS*. C.M.Rovainen, Dept. Cell Biology and Physiology, Washington Univ. School of Medicine, St.Louis, MO 63110

The goal of this work has been to observe the behavior of single endothelial cells during angiogenesis. Clones of cells were labeled by pressure injections of tetramethylrhodamine-dextran (MW 10,000) into single blastomeres in 16-128 cell stage embryos of "albino" *Xenopus laevis*. Early tadpoles with labeled endothelial cells and blood vessels were selected for detailed sequential examinations with an epifluorescence microscope, SIT camera, and video recordings. None of the early blastomeres produced clones which consisted exclusively of vascular endothelium. Other labeled cells included muscle fibers, lymphatics, mesodermal stellate cells, circulating blood cells, gut, and some epidermis. Fluorescence was intitially cytoplasmic but later became vesicular. Small fractions of total endothelial cells were labeled. Single endothelial cells and small groups could often be recognized and followed in case histories in vessels of the tail fin, brain, external gills, and aortic arches. In established vessels the patterns of fluorescent endothelial cells typically were stable for several days and occasionally for weeks until fluorescence faded. In a few clear cases single labeled cells migrated distally in elongating endothelial sprouts. Fluorescent dextrans are suitable clonal markers for observations *in vivo* of developing cells in *Xenopus* tadpoles over days and weeks.

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273.13

DIFFERENCES IN CELLULAR LABELING BY TRITIATED PROLINE IN NEURONAL TISSUES OF DIFFERENT EMBRYOLOGICAL ORIGIN. C. Hubscher and K. J. Berkley. Dept. of Psychology, Florida St. Univ., Tallahassee, FL 32306-1051.

It has been shown that tritiated proline (^{3}H-pro) is selectively incorporated into macroglial, but not neuronal proteins throughout the brain. In dorsal root ganglia, however, ^{3}H-pro is incorporated into neuronal (ganglion cell) proteins (Contos, N., Molinari, H. H. and Berkley, K. J., *Brain Res.*, 479:172, 1989). The basis for this difference is unclear. Brain and dorsal root ganglion tissue differ not only in their embryological origin (i.e., neural tube and neural crest, respectively) and location, but also in their synaptic and cellular configurations. In order to determine if configurational rather than embryological factors accounted for ^{3}H-pro's incorporation into neuronal proteins in dorsal root ganglia, ^{3}H-pro was injected, in adult rats, into autonomic and sensory ganglia that differ in their synaptic and cellular configurations. The ganglia were then examined with light microscopic autoradiographic techniques. Included were the following: C2 dorsal root and nodose ganglia, superior cervical and pelvic ganglia. Unlike brain tissue, neurons were labeled in every ganglion. These results support the conclusion that it is the neural crest origin of the dorsal root ganglion, and not a specific configurational arrangement, that accounts for the labeling of its neurons by ^{3}H-pro. Such results further suggest that the selective incorporation of ^{3}H-pro by macroglial cells in the brain may be due to their derivation from the neural tube.

Supported by NSF grant BNS 8818657.

274.1

LTP AND PTP IN A SIMPLIFIED PIRIFORM CORTEX SLICE: EFFECTS OF APV, APB AND DIVALENT CATIONS. N. Hori, P. McCauley* and D. O. Carpenter. Wadsworth Labs, NYS Dept. Health and School of Public Health, Albany, NY 12201.

Using a simplified slice containing only apical dendrites of pyramidal neurons we have demonstrated LTP in the lateral olfactory tract (LOT)-piriform cortex pathway. Population EPSPs were evoked by 0.5 - 1.0 Hz supramaximal stimulation of the LOT. Tetanization (100 Hz for 1 sec.) induced a transient PTP (peak time 2-5 sec. post tetanus) and a stable LTP lasting greater than 2 hrs. LTP was seen in almost all preparations, and peak EPSP was increased an average of about 15%. PTP was significantly enhanced by perfusion with low Ca^{2+} high Mg^{2+} medium and local anesthetics. Aminophosphonobutyric acid (APB) (10^{-4}M) depressed the EPSP but dramatically enhanced PTP. These observations are consistent with the conclusion that PTP is presynaptic and that APB acts at a presynaptic site. LTP was unaffected by APB but was blocked by aminophosphonovaleric acid (APV) (5 x 10^{-5}M) or perfusion with a high Mg^{2+} (6.3 mM) solution. These results are consistent with the view that LTP is at least primarily post-synaptic and is dependent upon activation of NMDA receptors.

274.2

THERMAL DEPENDENCE OF TETANUS INDUCED LONG-TERM POTENTIATION IN HAMSTER HIPPOCAMPAL SLICES MAINTAINED IN 4.5 mM EXTRACELLULAR CALCIUM. M. S. Krelstein* and J. Horowitz. Animal Physiol., Univ. of Calif., Davis, CA 95616

Previously we have shown that the initial expression of long-term potentiation (LTP) is temperature dependent and is blocked at 20°C in CA1 pyramidal cells. In addition, the thermal block of LTP at low temperatures is calcium dependent, since it can be overcome if a tetanus is applied during a high calcium (4.5 mM) pulse. However, the effects of temperature on tetanus induced LTP in *maintained* elevated extracellular calcium remains to be determined. Using standard procedures (Living in the Cold, John Libbey, London, pp 245-253, 1989), in the hamster (*Mesocricetus auratus*) we measured field PSPs from the stratum radiatum in area CA1 before and after tetanizing the Schaffer collateral/commissural fibers in slices maintained in 4.5 mM calcium. We found that though tetanus induced LTP is clearly observed in slices maintained in 4.5 mM calcium at 30°C and at 25°C, LTP cannot be observed in slices maintained in 4.5 mM calcium at 20°C. However, initial triggering events are operational at 20°C, since LTP is observed relative to control responses (recorded at 24°C) when, following a tetanus at 20°C, a slice is warmed back to 24°C. [Supported by NSF grant BNS-88-19973]

274.3

DITHIOTHREITOL INCREASES THE MAGNITUDE OF LTP. D. L. Tauck and G. A. Ashbeck*, Department of Biology, Santa Clara University, Santa Clara, CA 95053.

In several neuronal preparations, currents evoked by exogenous NMDA are potentiated by treatment with the sulfhydryl reducing agent dithiothreitol [DTT] (Aizenman, et al., 1989, *Neuron* 2:1257). Since activation of NMDA receptor/channel complexes underlies long-term potentiation, we used LTP as an assay for NMDA receptor activation in rat hippocampal field CA1. Applied in the perfusate, DTT had no effect on field potentials recorded either in *stratum pyramidale* or *s. radiatum* in response to low frequency stimulation. Prior treatment with 10 μM DTT greatly increased the effects of high frequency stimulation (100 Hz for 1s). Slices exposed to DTT developed more than twice as much LTP of both the population spike and the EPSP than controls. The effect of DTT could be modulated by agents known to act at the NMDA complex. Glycine (100 nM) further potentiated the effect of DTT through a kynurenate-sensitive mechanism. APV blocked LTP although a higher concentration was required in DTT-treated slices. These results suggest that reduction of sulfhydryl groups in NMDA complexes or closely related proteins greatly increases the efficacy of synaptic transmission mediated by NMDA channels in intact tissue.

274.4

LOW CONCENTRATIONS OF NMDA BLOCK THE INDUCTION OF CA1 LONG-TERM POTENTIATION. Y.Izumi* D.B.Clifford and C.F.Zorumski. Dept. of Psychiatry, Washington Univ., St.Louis, MO63110.

N-methyl-D-aspartate(NMDA) receptors play a critical role in the development of long-term potentiation(LTP) in CA1 of the hippocampus. However, previous studies have demonstrated that pretreatment with NMDA inhibits the development of LTP produced by perfusion of conditioning solutions (Neurosci.lett.,88:201,1987), suggesting that untimely NMDA receptor activation may have an antagonistic effect on LTP-development. To extend this observation we have examined the effects of NMDA agonists on LTP produced by tetanic stimulation of the Schaffer collateral pathway. A five-minute application of NMDA(0.2-2.0μM, N=15), aspartate(5-100μM, N=20) or glutamate(50μM, N=5) reliably inhibited the development of CA1 LTP measured 20 minutes after tetanic stimulation. Quisqualate(1-5μM, N=7) had no effect on LTP. TEA(5mM) blocked the inhibitory effect of NMDA on LTP(N=2).

Preliminary intracellular recordings indicate that NMDA produced an augmentation of a synaptic afterhyperpolarization. The afterhyperpolarization may contribute to the inhibition of LTP by NMDA.

274.5

FACTORS CONTROLLING THE TIME COURSE OF SYNAPTIC POTENTIATION IN AREA CA1 OF THE HIPPOCAMPUS. R.C.Malenka. Depts. of Psychiatry and Physiology, University of Calfornia, San Francisco, CA. 94143

A variety of experimental manipulations result in a decremental potentiation of synaptic transmission distinct from LTP. Recording in the CA1 region of the hippocampal slice, I have examined some of the factors which can convert a decremental enhancement of synaptic transmission to LTP.

In a single slice, the time course of synaptic enhancement was affected by the concentration of D-APV. In high (20-40 uM) D-APV, a tetanus (50-100 hz, .5-1 sec) caused PTP lasting only 20-50 sec. In 1-2 uM D-APV, the same tetanus resulted in a decremental potentiation (3-30 min). Upon wash out of the D-APV, the tetanus induced non-decremental LTP (lasting 1 hour).

The interval between brief conditioning trains and single test stimuli evoked in independent afferents also could affect the time course of synaptic enhancement. Using a fixed number of pairings in a single slice, decremental potentiation was elicited when the tetanus followed the test stimulus by 20-40 msec whereas non-decremental LTP was induced when initiation of the tetanus preceded the test stimulus by 20-30 msec.

Using intracellular recording, a number of manipulations could control the time course of synaptic enhancement in single cells. Pairing 10 stimuli (.1-.2 hz) with different levels of postsynaptic depolarization converted a decremental (5-20 min) potentiation to LTP. Similar results were obtained by varying the timing between a brief depolarizing current pulse and afferent stimulation or by changing the frequency of a fixed number of afferent stimuli from .2 to 2-3 hz while depolarizing the postsynaptic cell to a constant level.

These findings are consistent with the proposal that the magnitude of NMDA receptor-dependent postsynaptic calcium accumulation can control the time course of synaptic enhancement. Specifically, a "moderate" postsynaptic calcium increase may cause a decremental synaptic enhancement whereas a higher, "threshold" level of postsynaptic calcium may be required to generate non-decremental LTP.

274.6

DEVELOPMENTAL CHANGES IN SYNAPTIC PROPERTIES AND ONSET OF LTP IN ORGANOTYPIC HIPPOCAMPAL CULTURES. D. Muller, and L. Stoppini. Department of Pharmacology, Centre Médical Universitaire, 1211 Geneva 4, Switzerland.

In order to study the mechanisms responsible for the onset of long-term potentiation (LTP) during the second week after birth, we have developed a simple method to maintain hippocampal slices in culture and analyse synaptic properties during the first days of culture. Hippocampal slices (450-500 μm thick) of 4-6 day old neonates were prepared. They were then placed on a poreous and translucid membrane at the interface between a regular culture medium (MEM + 25% horse serum) and an atmosphere containing 5% CO_2. In those conditions slices survive for several weeks, keep their organotypic organization and tend within a few days to flaten into a mono- or bilayer. Both extra- and intracellular recordings have been obtained at different times of culture. In all slices we observed that synaptic responses disappear within a few hours of culture. This silent period lasts for about 48 hours. At days 3-5, excitatory field potentials of 1-2 mV can again be evoked in the different hippocampal areas with a selectivity which is compatible with a preserved organotypic organization. EPSPs are characterized by a very small degree of paired-pulse facilitation, LTP is absent and there is no evidence of inhibitory responses. At days 10-12, field potentials of 3-10 mV are obtained, paired-pulse facilitation is significantly increased, LTP can be induced by high frequency stimulation and strong inhibition is present. It is concluded that the same developmental changes take place with the same time course in *in vitro* organotypic hippocampal cultures as *in vivo*. Work supported by FNRS 3.173.0.88.

274.7

ENVIRONMENTAL MANIPULATIONS CAN REPEATEDLY INFLUENCE HIPPOCAMPAL PB POTENTIATION IN THE BEHAVING RAT D.M. Diamond, M. Fleshner and G.M. Rose. Dept. of Pharmacology, University of Colorado Health Sciences Center and VA Medical Center, Denver, Colorado 80262

Exposure to a novel environment, which is stressful to a rat, interferes with the induction of primed burst (PB) potentiation (Diamond et al., *Psychobiology*, in press). In the present report, we have manipulated the stress-induced blockade of hippocampal plasticity repeatedly within animals. We hypothesized that the experimenter can influence the efficacy of PB stimulation by placing an animal into either a familiar (*facilitatory*) or a novel (*inhibitory*) environment.

We recorded CA1 population spikes in behaving rats (see Diamond et al., *J. Neurosci.*, 8:4079, 1988 for details). PB potentiation occurred when subjects had acclimated to CHAMBER 1. One week later, we recorded from the same subjects when they were in a novel environment (CHAMBER 2). PB potentiation did not occur in CHAMBER 2. When the subjects were returned to CHAMBER 1, PB potentiation again occurred. Correlative data indicate that PB potentiation was more likely to occur when placement of the animal in a recording chamber did not increase serum corticosterone levels.

These findings indicate that, as with learning and memory, hippocampal plasticity in behaving rats is acutely sensitive to environmental manipulations. Novel environments impair, and familiar environments enhance, the effectiveness of PB stimulation.

274.8

LONG-TERM POTENTIATION (LTP) INDUCED BY PATTERNED STIMULATION OF THE COMMISSURAL PATHWAY TO HIPPOCAMPAL CA1 REGION IN BEHAVING RATS. L. Stan Leung. Depts. Clin. Neurol. Sci. and Physiology, Univ. Western Ontario, London, Ontario N6A 5A5 Canada.

Patterned stimuli (typically 8 bursts of 1 pulse followed 130 msec later by 10 pulses at 100 Hz) of 0.1 ms duration and various intensities were delivered to the contralateral hippocampus while recording from the CA1 region in freely moving rats. Single-pulse responses to the commissural stimulation were recorded before and after the patterned bursts, typically during awake immobility or slow-wave sleep. The responses consisted of early population excitatory postsynaptic potentials (EPSPs), sometimes accompanied by a population spike, and late - (30-50 msec) latency potentials. Single-pulse stimulation of str. oriens of the contralateral CA1 or CA2/CA3 gave mainly basal-dendritic EPSPs that were (alvear-) surface negative and deep-positive. Single-pulse stimulation of str. radiatum of the contralateral CA1 gave mainly apical-dendritic EPSPs that were surface-positive and deep-negative. The basal-dendritic response in CA1 showed a consistent and robust LTP. Potentiation of the slope of the population EPSP was 222 ± 22% (mean ± S.E.M., 9 rats) at 30 min after the patterned bursts, and decayed exponentially with a time constant of about 5 hr. Population spikes were potentiated for a shorter duration. In contrast, patterned bursts delivered to sites evoking an apical-dendritic response in CA1 were only successful in eliciting LTP in 1 of 7 rats. Even when afterdischarges were evoked, another 14 rats showed little LTP of the apical-dendritic response. The results were confirmed using other tetanic stimuli. The difference in the LTP propensity of intrinsic hippocampal pathways may have functional implications for memory and information processing. (Supported by NS25383).

274.9

LTP INDUCED BY PATTERNED STIMULATION IN THE CA1 REGION IS DIFFERENT IN PROXIMAL AND DISTAL APICAL DENDRITES. G. Capocchi*, R. Corradetti, G. Della Torre* and M. Zampolini*. Ist. Clin. Malattie Nervose e Mentali, Univ. of Perugia, 06100 Italy.

The induction of long-term potentiation (LTP) at distal (dAD) and proximal (pAD) level of Apical Dendrites in the CA1 region was investigated in rat hippocampal slices. The stimulating and recording electrodes were placed in the stratum radiatum at pAD or dAD level. LTP was evoked by short bursts (4 pulses at 100 Hz), repeated (2-10 times) with different intervals (2-1-0.2-0.1-0.05 sec) in different slices. The results showed that: 1) in pAD but not in dAD two bursts were able to induce LTP; 2) in pAD but not in dAD an interburst interval of 0.1 sec was as effective as a 0.2 sec interval in eliciting LTP; 3) in pAD the increase in amplitude and slope of the field potential during LTP was greater ($P<0.05$) than that obtained in dAD; 4) the same differences were observed regardless the position (pAD or dAD) of the stimulating electrode.

The results suggest that the expression of LTP differs in pAD and dAD, reflecting different properties of somatic and dendritic components.

274.10

EVIDENCE THAT LTP EXPRESSION IS NOT DUE TO CHANGES IN SPINE NECK RESISTANCE. M. W. Jung, J. Larson and G. Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717.

Long-term potentiation (LTP) could be expressed by morphological changes in dendritic spines that decrease their longitudinal electrical resistance and, hence, enhance the synaptic current produced by a given amount of synaptic conductance. One prediction of this hypothesis is that synaptic current in potentiated spines should be more affected by changes in synaptic conductance than current in unpotentiated spines. This was tested by using field potential recordings of synaptic responses in CA1 pyramidal cells in hippocampal slices in response to stimulation of Schaffer/commissural inputs that either received LTP-inducing stimulation or did not. Two manipulations were used to systematically reduce synaptic conductances: reductions of extracellular Ca^{++} and partial blockade of postsynaptic receptors with 6,7-dinitroquinoxaline-2,3-dione (DNQX) or kynurenic acid. The initial slopes of the control and potentiated (56.9±11.0 % LTP) responses were reduced by 67.1±3.9 % and 68.6±2.9 %, respectively, by lowering extracellular Ca^{++} from 3.4 mM to 1 mM. The initial slopes of the control and potentiated (43.1±5.2 % LTP) responses were reduced by 66.4±4.7 % and 65.1±5.1 %, respectively, by bath application of DNQX (final concentration 1-2 μM). Finally, the initial slopes of the control and potentiated (49.4±7.8 % LTP) responses were reduced by 47.8 ± 3.3 % and 43.3 ± 3.3 %, respectively, by perfusion of 500 μM kynurenic acid. Statistical analysis revealed no significant differences in response reduction between control and potentiated pathways by each manipulation. These results suggest that LTP expression is not due to a change in the ratio of synaptic current to synaptic conductance as would be predicted by the spine resistance hypothesis.
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274.11

DEVELOPMENT OF HIPPOCAMPAL LTP IS SUPRESSED BY CALPAIN INHIBITORS. S. Del Cerro, J. Larson, M.W. Oliver and G. Lynch. Center for the Neurobiology of Learning and Memory, University of California, Irvine, CA 92717, U.S.A.

We have previously proposed that calpains (calcium-activated proteases) could play a role in the establishment of long-term potentiation (LTP). In the present study we have examined the effects of two recently synthesized inhibitors of calpains, calpain inhibitors I and II (CiI and CiII), on the development of LTP in field CA1 of rat hippocampal slices. Theta busrt stimulation (TBS) induced stable LTP (defined as a potentiation of at least 15% that did not detectably decay between 20 and 30 min. after TBS) in 80% of pathways tested in control slices. However, only 37% of slices treated with CiI (100 mM) and 40% of slices treated with CiII (100 mM) showed stable LTP by the same criterion. Neither CiI nor CiII had any significant effects on the postsynaptic responses to the high frequency stimulation used to induce LTP, indicating that the drugs did not disturb the physiological processes that trigger the effect. It appears that the calpain inhibitors impair the development of stable LTP rather than blocking its induction mechanism.

These results confirm and extend previous findings using the protease inhibitor, leupeptin, since CiI and CiII are both more potent and selective inhibitors of calpains than leupeptin. Therefore, they strengthten the hypothesis that calcium influx through NMDA receptor channels during high frequency synaptic stimulation triggers a calpain-mediated proteolytic event that is involved in the development of stable synaptic potentiation.

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274.12

ENHANCED SENSITIVITY OF METABOLOTROPIC GLUTAMATE RECEPTORS AFTER LONG-TERM POTENTIATION (LTP) IN RAT HIPPOCAMPUS. E. Aronica*, K. Reymann*#, M. Krug*#, U. Frey*#, H. Schroeder*#, C. Speciale and F. Nicoletti*, Inst. of Pharmacol. Univ. of Catania, Italy;and #Inst. of Neurobiol. and Brain Res., Acad. of Sci. of G.D.R., Magdeburg, G.D.R.

Recent studies have implicated a role for metabolotropic glutamate receptors in activity-dependent synaptic plasticity (Dudek, S.M. and Bear, M.F., Science, 246:673, 1989). We now report that stimulation of [^{3}H]inositolmonophosphate formation by ibotenate (IBO) was potentiated in hippocampal slices after tetanization of the Schaffer collateral-commissural pathway or the perforant pathway projecting to the dentate gyrus. The increased response to IBO developed slowly (after 4-6 hours), and was not observed when tetanization was performed in the presence of AP5 or AP7, which prevented LTP formation. LTP was also studied in rats repeatedly injected with LiCl, which blocks the recycling of inositol into membrane phospholipids. In these animals, tetanization of the perforant pathway induced a decremental form of LTP, that decayed within 4-5 hours. These results support a role for metabolotropic glutamate receptors in the synaptic events enabling the expression and/or maintenance of LTP.

274.13

LONG - TERM POTENTIATION (LTP) IN RAT DORSAL LATERAL SEPTAL NUCLEUS (DLSN) IS *NOT* BLOCKED BY DL - 2 - AMINO - 5 - PHOSPHONOPENTANOATE (AP5).

Fang Zheng and J.P. Gallagher, Dept. of Pharmacology & Toxicology, Univ. of Texas Medical Br., Galveston, TX 77550.

Intracellular recordings were made from DLSN neurons following orthodromic low frequency (0.1 Hz) stimulation. Synaptic potentials, including a fast EPSP, fast IPSP, and LHP were elicted focally in a coronal slice of basal forebrain. Combinations of the NMDA receptor antagonist, AP5 (50μM), and the non-NMDA receptor antagonist, CNQX (20μM), or, the non-selective excitatory amino acid antagonist, kynurenic acid (5 mM), failed to block EPSPs, completely. AP5 pretreatment increased EPSP amplitude following 0.1Hz stimuli to a value 20 to 40% above control.

LTP was induced at this synapse with high frequency trains of stimuli (100 Hz, 1s duration, at an interval of 20 sec). LTP was defined as an increase of EPSP amplitude to greater than 120% of its control value. Following tetanization, EPSP amplitudes ranged from 130 to 300% greater than control. AP5 alone or the combination of AP5 with CNQX could not block the induction of LTP. LTP could also be induced in the absence of bicuculline, demonstrating that LTP can be induced without blocking $GABA_A$ mediated inhibition in the rat DLSN. Preliminary experiments showed that L-alpha-aminophosphonobutyrate (L-AP4, 150μM) could block the induction of LTP.

We suggest that L - AP4 - sensitive - quisqualate receptors, possibly coupled to a phosphoinositol second messenger system, are necessary for the induction of LTP in the DLSN.

274.14

LONG TERM INCREASES IN THE EVOKED POPULATION SPIKE IN THE CA1 REGION OF RAT HIPPOCAMPUS INDUCED BY BETA-ADRENERGIC RECEPTOR ACTIVATION

T. V. Dunwiddie and L. R. Thomas. Dept. of Pharmacology, University of Colorado Health Sciences Center and VA Medical Center, Denver, CO 80262.

The effects of the selective β-adrenergic receptor agonist isoproterenol (ISO) were characterized in the CA1 region of the rat hippocampal slice preparation. As we have previously reported, 500 nM ISO increased the amplitude of the evoked population spike response without any corresponding effect upon the field EPSP responses. In the present studies we have demonstrated that the increase in this response is quite persistent, and is not reversed by >30 minutes of washout in the majority of the slices tested; we have termed this prolonged increase in pyramidal neuron sensitivity β-adrenergic potentiation (BAP). As with the acute effect of ISO, BAP is confined to an increase in the population spike response and not the EPSP. In input-output curves, this was clearly observed as a prolonged leftward shift in the EPSP-population spike relationship. Similar long-term increases could also be elicited by superfusion of the slices for 10 min with 20-50 μM norepinephrine (NE). Although both the acute and the long-term effects of ISO were blocked by pretreatment with timolol, a β-adrenergic antagonist, they could not be reversed by timolol once the increased response was established. However, BAP was not blocked by pretreatment with 50 μM APV, an NMDA receptor antagonist that blocks LTP and some other forms of NE-induced plasticity. Time course studies suggested that the $T_{1/2}$ for the decay of BAP was approximately 2 hours. These experiments demonstrate that NE can induce a novel and long-lasting increase in the excitability of the pyramidal neurons following relatively brief activation of β-adrenergic receptors.

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274.15

CELLULAR MECHANISMS UNDERLYING LONG-TERM BETA-ADRENERGICALLY MEDIATED POTENTIATION IN THE CA1 REGION OF RAT HIPPOCAMPUS.

M. Taylor, T.V. Dunwiddie and W.R. Proctor. VA Med. Center and Univ. of Colorado Hlth. Sci. Ctr.

We have previously demonstrated that isoproterenol (ISO) and norepinephrine (NE) can produce a persistent excitatory effect in the CA1 region of rat brain slices, which we have termed *b*-adrenergic potentiation (BAP). The purpose of the present study was to investigate the cellular mechanism(s) underlying BAP. Isoproterenol (500 nM) was found to produce a long lasting potentiation of only the population spike amplitude, which outlasted the duration of the drug superfusion by as much as an hour. Because ISO had no effect on the EPSP response, this suggested that the primary change was in the excitability of the pyramidal neurons. To test this hypothesis we examined the effects of ISO on repetitive bursting evoked by antidromic stimulation of the CA1 pyramidal neurons in medium containing 0.24 mM calcium. Under these conditions, ISO produced an increase in burst amplitude that far outlasted the duration of drug superfusion. Intracellular recording experiments demonstrated directly that some of the postsynaptic effects of ISO (e.g., the reduction in the afterhyperpolarization) were quite persistent. We also investigated the actions of cAMP analogs and forskolin to see what role they played in BAP. Both 8-bromo-cAMP (500 μM) and forskolin (1 μM) mimicked the effects of ISO on orthodromic population spike responses. Pretreating slices with 8-pcpt-cAMP (100 μM) completely blocked subsequent responses to ISO, indicating that they acted upon a common mechanism. These experiments suggest that the long-term increase in the evoked population spike response can occur in the absence of synaptic transmission and may be mediated by an increase in intracellular cAMP.

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274.16

β-ADRENERGIC RECEPTOR ACTIVATION INCREASES PHOSPHORYLATION OF SYNAPSINS I AND II AND INCREASES SYNAPTIC TRANSMISSION IN DENTATE GYRUS, BUT NOT IN AREA CA1 OF THE HIPPOCAMPUS.

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Previous studies have shown that norepinephrine (NE) or isoproterenol (ISO) enhance the slope of the field excitatory postsynaptic potential (epsp) in the dentate gyrus of the rat hippocampal formation. In contrast, NE and ISO cause no increase in excitatory transmission in area CA1 of the hippocampus. The molecular mechanism underlying this brain region-specific increase in synaptic transmission is not known. The phosphorylation of synapsins I and II, two homologous presynaptic vesicle-associated proteins, is thought to promote neurotransmitter release. We have previously observed ISO-enhanced phosphorylation of the synapsins in the dentate. The purpose of this study was to determine whether ISO-stimulated phosphorylation also occurs in area CA1 where NE has no effect on excitatory transmission. These studies were correlated with electrophysiological studies in *in vitro* hippocampal slices. Dentate and CA1 minislices were incubated for 90 min in $^{32}PO_4$ in unlabeled PO_4-free buffer followed by 10 min incubation in 1 μM ISO or control buffer. In some experiments, the ISO treatment was followed by a 30 min wash with drug-free buffer. ISO produced significant increases in the phosphorylation of the synapsins in dentate slices but had no effect on these proteins in CA1 slices. Enhanced phosphorylation was not observed in the dentate following the 30 min of ISO free wash. Similarly, electrophysiological studies showed that ISO potentiated excitatory transmission in the dentate but not in the CA1, as previously observed. Like the synapsin phosphorylation, the enhanced epsp in dentate gyrus returned to baseline levels within 30 minutes of washout of ISO. This close temporal and brain regional correlation between synapsin phosphorylation and ISO stimulation of synaptic transmission suggests that the synapsin proteins may play a role in the potentiating effect of ISO in the dentate.

274.17

MUSCARINIC MODULATION OF RAT HIPPOCAMPAL CA1 LONG-TERM POTENTIATION (LTP).

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LTP is a use-dependent form of synaptic plasticity thought to underlie some forms of information storage in the nervous system. Alzheimer's dementia (AD) is associated with deficiencies of memory and cholinergic neurons. The hippocampus is prominently involved in AD and has a role in memory storage. Thus, an abnormality of cholinergic innervation of the hippocampus may contribute to the AD memory deficit; and, if the cholinergic system modulates memory in the hippocampus, then it may modulate hippocampal LTP. Our lab previously showed that muscarine inhibits mossy fiber synapse LTP (Williams & Johnston, *Science* 1988, 242, 84). Using extracellular recordings in the rat *in vitro* hippocampal slice preparation, this study extends that previous investigation to area CA1 where the LTP induction mechanism is different. Except for one series of experiments, slices were tetanized with 1.3mV pEPSPs.

Control slices showed LTP (128±13% of pretetanus pEPSP slopes at 1 hour post tetanus, n=7). Bath applied carbachol (CCh) and muscarine (Mus) depressed pEPSPs so that in their presence, the stimulus intensity was adjusted to obtain a 1.3mV pEPSP for tetanus. To control for the increased stimulus intensity, several slices were tetanized at 1.5mV (133±8%, n=5). CCh (0.1μM), a mixed muscarinic-nicotinic agonist, enhanced LTP (160±8% 2 hours post tetanus, n=4). Atropine (0.1μM) washed in with CCh blocked the LTP enhancement (139%, n=2), and Mus alone (0.5-10μM) enhanced LTP (178±19%, n=8), suggesting that the cholinergic effect is muscarinically mediated. Atropine alone (0.1μM) did not reduce LTP (143±7.4% at 1 hour, n=6). We conclude that the cholinergic system enhances LTP in CA1 via muscarinic receptors, and any endogenous acetylcholine released during tetanus *in vitro* does not significantly affect LTP. (MH44754, AG00432)

274.18

MUSCARINIC RECEPTOR ACTIVATION BLOCKS ß-ADRENERGIC-INDUCED SYNAPTIC PLASTICITY BY A PERTUSSIS TOXIN-INSENSITIVE DEPRESSION OF EVOKED RESPONSES IN THE DENTATE GYRUS.

E. C. Burgard and J. M. Sarvey. Department of Pharmacology, Uniformed Services University of the Health Sciences, Bethesda, MD 20814.

We have previously demonstrated that bath application of 1μM muscarine can facilitate the induction of long-term potentiation (LTP) of evoked responses recorded in the rat dentate gyrus (Burgard and Sarvey, *Neurosci Lett.*, in press). Here we show that a higher concentration (10μM) of muscarine produces a pertussis toxin (PT)-insensitive depression of evoked responses that blocks the induction of isoproterenol-induced long-lasting potentiation (LLP), but does not affect LTP induction.

In the hippocampal slice, stimulation of the medial perforant path evoked a population spike (PS) and a dendritic EPSP recorded extracellularly in the dentate gyrus. A 10min bath application of D,L muscarine·Cl depressed evoked responses with an approximate EC_{50} = 3μM. This depression could be blocked by muscarinic receptor antagonists with a relative potency of 4-DAMP > pirenzepine > AFDX-116, and is therefore probably mediated by M3 or M1 receptors. 10μM Muscarine depressed the PS to 12% of baseline and the EPSP to 81%. *In vivo* pretreatment with PT (1μg PT intradentate 3 days before slice preparation) had little effect on the depression produced by 10μM muscarine (PS: 30% of baseline, EPSP: 74%). This treatment significantly reduced $GABA_B$-mediated responses. In other studies, bath application of isoproterenol (1μM for 20min) produced LLP of the evoked population spike amplitude (191% of baseline) and EPSP slope (170%). 10μM muscarine blocked this induction of LLP (PS: 98% of baseline, EPSP: 97%) but did not affect the induction of LTP (PS: 202% of baseline, EPSP: 134%) induced by high frequency stimulation (100Hz for 2sec). This data suggests that a PT-insensitive muscarinic receptor can depress evoked responses and modulate ß-adrenergic-induced synaptic plasticity in the dentate gyrus. (supported by NIH grant NS23865)

275.1

BEHAVIORAL AND BIOCHEMICAL EFFECTS OF INTRASTRIATAL IMPLANTATION OF HUMAN NEUROBLASTOMA CELLS IN THE RAT. J. L. Cadet, R. Last*, V. Kostic, S. Przedborski, and V. Jackson-Lewis. Columbia University, New York, New York 10032.

Unilateral injections of 6-hydroxydopamine into the rat striatum result in amphetamine-induced rotation. This behavior was associated with an almost complete loss of dopamine (DA) uptake sites in the basal ganglia. Quantitative receptor autoradiography revealed significant increases in striatal DA D2 receptors. In contrast, there were no significant changes in striatal DA D1 receptors. Intrastriatal implantation of human neuroblastoma cells LAN-5 attenuated the circling behavior observed in the operated animals. Transplantation of these cells caused a crescent-like reappearance in dopamine uptake sites on the medial hemisphere of the lesioned striatum without evidence of any survival of the transplanted cells. In addition, there were increases in DA uptake sites in the contralateral substantia nigra and ventral tegmental area of the transplanted animals. Nevertheless, the increases observed in striatal DA D2 receptors were not altered by transplantation. DA D1 receptors were also not affected. These studies show the feasibility of using receptor autoradiography to document, in a quantitative fashion, the recurrence of dopamine terminals in the lesioned basal ganglia. The present results support the possibility that trophic factor-induced regeneration might play an important role in behavioral recovery after transplantation of various tissues in the injured brain.

275.2

TRANSPLANTS OF EMBRYONIC CORTEX OR ASTROCYTES BUT NOT BASIC FGF PROMOTE RECOVERY FROM LONG-TERM SPATIAL LEARNING DEFICITS AFTER SOMATO SENSORY CORTEX ABLATION. J.P. Kesslak, V. Valouskova*, C.W. Cotman. Dept Psychobio, Univ. Calif, Irvine, CA 92717; Inst Physio, Czechoslovak Acad Sci, 142 40 Prague 4-Krc.

Recovery from behavioral deficits induced by neural loss in the CNS may be induced by transplants of neural and nonneural tissue and administration of neurotrophic factors. Somato sensory cortex damage results in long-term deficits on the Morris water maze, a spatial learning task. Embryonic somato sensory cortex transplants have been shown to facilitate recovery from these deficits (Valouskova & Macias-Gonzales, *Soc Neurosci Abst*, 15: 108.2, 1989). Recovery from somato sensory cortex damage was assessed in the present study after transplants of embryonic cortex or cultured astrocytes or after chronic infusion of basic fibroblast growth factor (FGF).

Embryonic neocortex (EC; n = 14) or cultured astrocytes (AS; n = 10) were transplanted into the cavity in the left hemisphere immediately after bilateral aspirative lesion of the somato sensory cortex. Chronic infusion of basic FGF (FGF; n = 7) into the left lateral ventricle was also initiated immediately after cortical ablation. These groups were compared with undamaged control animals (CNT; n = 11) and animals with bilateral ablation and no treatment (LES; n = 9). Rats were tested for three consecutive days on a Morris water maze at 14 days, 2, 4 and 6 months post-lesion. Analysis of variance showed that bilateral ablation of somato sensory cortex produced long-term deficits on the Morris task. Groups EC and AS performed significantly better than did group LES and did not differ from CNT during each test period. Administration of FGF did not significantly improve performance during any of the test sessions. These results can be interpreted to indicate that neural tissue and astrocytes can be beneficial in reducing the behavioral deficits observed after damage to the neocortex.

275.3

RECOVERY OF SPATIAL MEMORY PERFORMANCE IN RATS WITH HIPPOCAMPAL EXCITOTOXIC LESIONS AND FETAL CELL IMPLANTS. Yvonne Wilsdon[1], Hugh A. Tilson[2], and Richard A. King[1] [1] Psych. Dept., Univ. of N.C. Chapel Hill, N.C. 27599 [2] Neurotox. Div., Mail Drop 74B, U.S. Environmental Protection Agency, Research Triangle Park, N. C. 27711.

Young adult male Fischer 344 rats were anesthetized and injected intracerebrally with 10μg. per site of N-methyl-D-aspartate (NMDA) or artificial cerebrospinal fluid (the vehicle) in each of four hippocampal sites, targeting either CA3 pyramidal cell fields or dentate gyrus granule cell fields. Two weeks after original lesion surgery, half of the subjects were injected with E-17/18 fetal hippocampal cell suspensions and the other half received vehicle. Three weeks, six weeks, or four months after the original lesion surgery, rats were trained in a standard Morris water maze place task procedure, with a free swim (probe trial) after seven days of four-trial per day acquisition training.

A statistically significant impairment in memory for place was observed at 3 weeks and at 6 weeks post-lesion in the lesion-only subjects. However, at 4 months post-lesion, lesion-only animals performed at the same level as unlesioned controls. Thus, spontaneous recovery of memory performance occurred within 4 months after the lesion. Lesioned animals which received hippocampal cell injections, and in which Nissl staining indicated that the implanted cells had survived, showed some improvement in memory performance at 3 weeks post lesion. Implant effects at 4 months could not be assessed due to the spontaneous recovery described above.

275.4

CHANGES IN MOTOR COORDINATION AND SPONTANEOUS NOCTURNAL LOCOMOTION AFTER HOMOTOPIC AND HETEROTOPIC GRAFTS INTO THE LESIONED STRIATUM. M. Giordano and P.R. Sanberg. Division of Neuroscience, University of Cincinnati College of Medicine, Cincinnati, OH 45267.

Neural grafts can reverse behavioral, pharmacological and biochemical deficits induced by a variety of lesions. However, the mechanisms underlying this recovery are not clear. Grafts may act by diffusion of trophic factors and neurotransmitters, by rebuilding the damaged neural circuitry, or by providing anatomical support for sprouting axons.

Using the kainic acid (KA) animal model of Huntington's disease, this study examined the effects of post-lesion delay, and homotopic vs heterotopic grafts on behavioral recovery. We previously reported that with regard to drug-induced behaviors, homotopic grafts successfully attenuated KA-induced deficits. On the other hand, heterotopic grafts had inconsistent effects dependent on the length of the post-lesion delay.

We are now presenting data on spontaneous nocturnal locomotion and sensorimotor function. KA induced a temporary deficit in motor coordination which was not present in those animals with homotopic and heterotopic grafts, regardless of post-lesion delay.

These data seem to indicate that homotopic grafts are needed to restore pharmacological integrity to the lesioned striatum, whereas both homotopic and heterotopic grafts have generalized beneficial effects on spontaneous motor behaviors. This hypothesis will be tested against the results of the spontaneous locomotion tests. Supported by NINDS, TSA, HDSA, STRC and Omnitech.

275.5

FETAL TRANSPLANTS SPEED RECOVERY OF LOCOMOTION AFTER SENSORIMOTOR CORTEX REMOVALS IN RATS. J.M. Held. Dept. of Physical Therapy, Univ. of Vermont, Burlington, VT 05405

This study evaluated if fetal sensorimotor (S-M) cortex transplants would reduce the initial deficit or enhance recovery of locomotion in rats which have had S-M cortex removals. 47 mature male Sprague-Dawley rats were preoperatively trained and postoperatively tested on a narrow elevated runway. 14 rats were sham-operated (SHAM), and 33 underwent bilateral S-M cortex removals. 8 days later, one group of 16 lesioned rats received fetal transplants from S-M cortex of 15 day old fetuses (SM15) while the other 17 lesioned (LES) plus the SHAM rats had a sham operation. Both LES and SM15 rats were found to be impaired on the first day of postoperative testing; however, SM15 rats recovered faster than LES rats. Movement analysis revealed that both LES and SM15 rats demonstrated aberrant patterns after recovery. Thus, although fetal transplants speeded behavioral recovery, they did not restore normal movement patterns. HRP tracing suggests some interconnections with host brain.

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275.6

MONOAMINERGIC NEURAL TRANSPLANTS TO THE NEOCORTEX REDUCE IMMOBILITY TIME IN RATS DURING BEHAVIORAL DESPAIR TESTING C.E. Sortwell* and J. Sagen. Dept. Anat. and Cell Biol., Univ. IL at Chicago, Chicago, IL 60612.

According to the classical monoamine theory of depression, depression may be caused by a central imbalance in norepinephrine and serotonin function, and effective antidepressant therapies work by correcting this imbalance. A novel approach towards the restoration of imbalanced functioning in the CNS is the transplantation of pharmacologically relevant tissues into local CNS regions. Previous work in our laboratory has demonstrated that monoaminergic neural transplants into the rat frontal neocortex prevent the development of learned helplessness, a condition that models human depression. Another popular model for depression is the behavioral despair (BD) model, which is widely used for screening potential antidepressant therapies. In order to assess the ability for monoaminergic transplants to reduce BD, either adrenal medullary tissue, pineal gland tissue, a combination of adrenal medullary and pineal tissue, or equal volumes of sciatic nerve were transplanted into the rat frontal neocortex. BD testing began 6-8 weeks following surgery. During a pretest session, rats were forced to swim in a plexiglass swim cylinder for a 15 min period. Twenty-four hours later, the rats were forced to swim again for a 5 min test session during which immobility time was measured. The duration of immobility may indicate a level of despair, and immobility times are reliably reduced by antidepressant therapies. Immobility times were reduced in rats with adrenal medullary grafts, pineal grafts, and a combination of both adrenal and pineal grafts to the frontal neocortex. In contrast, immoblity times were not reduced by control sciatic nerve tissue grafts. This reduced immobility was probably not due to non-specific effects on motor function, since the transplants did not produce alterations in motor activity in an open field test. Morphological studies revealed that the grafted monoaminergic tissues survived well and continued to produce high levels of monoamines. These results provide further evidence to suggest that neural transplants may provide a long-term source of monoamines for the alleviation of depression.

275.7

FUNCTIONAL ENHANCEMENT OF INTRASTRIATAL DOPAMINE CELL GRAFTS BUT NOT CHROMAFFIN CELL GRAFTS BY THE CO-TRANSPLANTATION OF SCIATIC NERVE TISSUE IN 6-HYDROXYDOPAMINE LESIONED RATS. Craig G. van Horne, Ingrid Strömberg, Wayne L. Wittenberg*, John Hudson*, Lars Olson, and Barry Hoffer, Dept. of Pharmacology, Univ. of Colorado Health Sciences Center, Denver, CO 80262, and Dept. of Histology and Neurobiology, Karolinska Institute, Stockholm, Sweden.

Peripheral nerve "bridges" demonstrate the ability to facilitate axonal growth and regeneration of adult and fetal central nervous system tissue. The purpose of this study was to determine if co-grafted peripheral nerve tissue could enhance the ability of fetal dopamine (DA) cell or adrenal medullary cell transplants to reinnervate host striatum that had been denervated unilaterally. Male Fisher-344 rats were unilaterally lesioned with 6-hydroxydopamine to eliminate the nigro-striatal DA pathway. A total of 41 rats demonstrated a pattern of rotation indicative of a greater than 98 % depletion in DA. Rats were either kept as non-grafted controls (n=6), grafted with sciatic nerve (SN) minces (n=6), grafted with fetal ventral mesencephalon (VM) (n=10), co-grafted with VM and SN minces (n=9), grafted with adrenal chromaffin tissue (n=5), or co-grafted with chromaffin tissue and SN minces (n=5). All groups were then tested for changes in apomorphine-induced rotational behavior. The SN control group showed no significant differences in rotation when compared to pre-grafting levels, and to the lesioned non-grafted group. Both the VM grafted group and the VM-SN co-grafted group showed significant ($p<0.01$, one way ANOVA) decreases in rotations begining at 1.5 weeks post-grafting. There was a progressive decrease in rotations up to 12 weeks, the last test point. Interestingly, the co-graft group revealed a significantly greater decrease in rotation ($p<0.05$) than the VM group beginning at 5 weeks and continuing out to the 12 week test point. While the chromaffin grafts and the chromaffin co-grafted animals both showed a slight decrease in rotation at 5 weeks, there were no differences between the two groups. Taken together, these findings suggest that SN tissue enhances the ability of fetal VM grafts but not chromaffin grafts to reinnervate host brain. Supported by (NS09199, AG04418, PMA).

275.8

EFFECTS OF ADRENAL MEDULLA GRAFTS ON PLASMA CATECHOLAMINES AND ROTATIONAL BEHAVIOR.

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NIMH Neuroscience Center at St. Elizabeths, Washington D.C. 20032 and Dep. of Psychol. and Neurosci. Prog., Univ. of Michigan, Ann Arbor, MI 48104

In an animal model of Parkinson's disease adrenal medulla grafts located in the lateral ventricle reduce the behavioral manifestations of nigrostriatal dopamine depletion. The reasons for the functional effects of adrenal medulla grafts are, however, uncertain. We have hypothesized that changes in blood catecholamines and a compromised blood-brain-barrier may mediate behavioral recovery following transplantation of adrenal medulla into the brain.

Male Sprague-Dawley rats received unilateral 6-OHDA lesions of the substantia nigra and either bilateral adrenalectomy (n=50) or a sham operation (n=50). Animals were tested for rotational behavior following 0.05 mg/kg apomorphine S.C. and 0.75 mg/kg amphetamine I.P. and then received intraventricular adrenal medulla (n=25), sciatic nerve (n=15) or sham (n=10) grafts. After transplantation, animals were tested for apomorphine, amphetamine and nicotine-induced rotation. Blood samples were collected before and after transplantation. Catecholamines in the plasma were assayed using HPLC with electrochemical detection. This experiment is currently in progress.

275.9

NORMALIZATION OF CIRCLING INDUCED BY INTRA-NIGRAL MUSCIMOL BY FETAL NIGRAL GRAFTS IN THE STRIATUM. D. Gaudin, J.A. St-Pierre*, L. Tremblay, P.J. Bédard and M. Filion, Lab Neurobiol. Depts. of Anatomy and Physiology Univ. Laval, Québec, (Qc), CANADA G1K 7P4.

In the present study, we have investigated the effects of a fetal nigral graft in the striatum on circling induced by unilateral microinjection of muscimol in the target structure of the striatum, the substantia nigra pars reticulata (SNr). In a group of 28 ovariectomized female rats, 20 received a unilateral nigral lesion with 6-OHDA. The lesioned animals were then tested for circling with amphetamine 5 mg/kg and apomorphine 0.25 mg/kg. The animals which displayed circling with these two drugs were divided into two groups. One group received a graft of 1.5 x 10^6 cells taken from the ventro-mesencephalon of 13-14 day old rat embryos. The other group was not grafted. 4 to 6 months after the graft, all animals received one microinjection of muscimol (25 ng/0.5 µl) (gabaergic agonist) into the SNr using indwelling guide cannulae (20 ga) situated 2 mm above the target site. The injection site was determined by stereotaxic and electrophysiologic techniques. Circling was monitored during ninety minutes after the injection. Unilateral injection of muscimol into the SNr of the intact side of controls, lesioned and grafted animals induced a similar contralateral circling (800 counts/90 min). In the SNr of the lesioned side the contralateral circling was decreased by 80%. Circling after the muscimol injection on the grafted side was similar to the intact side of the three groups. Our results show that a fetal nigral graft implanted into the striatum can normalize the changes in GABAergic sensitivity in the SNr caused by dopaminergic denervation.
Supported by MRC of CANADA

275.10

CHRONIC LEVODOPA TREATMENT IMPAIRS RECOVERY OF ROTATIONAL BEHAVIOR IN DOPAMINE-GRAFTED RATS. D.M. Yurek, K. Steece-Collier, T.J. Collier, and J.R. Sladek, Jr. Dept. of Neurobiology & Anatomy, Univ. of Rochester School of Medicine, Rochester, New York 14642.

The effect of chronic levodopa treatment on the function of embryonic mesencephalic tissue grafts was assessed in rats by monitoring rotational behavior elicited by dopamine (DA) agonists before and after neural grafting. Rats were given unilateral 6-hydroxydopamine (6-OHDA) lesions of the nigrostriatal pathway and baseline measures of rotational behavior induced by D1 receptor stimulation, D2 receptor stimulation, or amphetamine were determined. Subsequently, DA grafts were implanted into the lesioned striatum and chronic regimens of either saline or levodopa [50 mg/kg, i.p., twice daily] began one day after neural grafting and were continued for 7 weeks. Rotational behavior elicited by the D1 agonist, SKF 38393, was completely attenuated throughout the six week period following the commencement of levodopa treatment, regardless of the absence or presence of a DA graft. Conversely, rotational behavior elicited by the D2 agonist, quinpirole, was significantly elevated in ungrafted animals receiving chronic levodopa. Grafted animals receiving chronic levodopa did not show a significant reduction in rotational behavior, whereas grafted animals receiving chronic saline showed a significant 67% reduction in quinpirole-induced rotational behavior. Amphetamine-induced rotational behavior was reduced in both levodopa and saline treated animals, however grafted animals receiving chronic levodopa treatment showed a reduction of rotational behavior that was uncharacteristic and less compensatory than that observed in grafted animals receiving chronic saline treatment. The results of the present study suggest that the impaired recovery in quinpirole- and amphetamine-induced rotational behavior in grafted animals receiving chronic levodopa treatment may be related to (1) impaired graft function, (2) an alteration in pre- and postsynaptic mechanisms in the host DAergic system, or (3) a combined effect of (1) and (2). The morphology of DA grafts will be examined using catecholamine histofluoresecence and tyrosine hydroxylase immunocytochemistry. Supported by the United Parkinson Foundation.

275.11

THE EFFECT OF EMBRYONIC NIGRAL GRAFTS ON STRIATAL DOPAMINE RECEPTORS IN UNILATERALLY LESIONED RATS FOLLOWING CHRONIC LEVODOPA TREATMENT. K. Steece-Collier, D.M. Yurek, T.J. Collier, and J.R. Sladek, Jr. Department of Neurobiology and Anatomy, University of Rochester School of Medicine, Rochester, NY 14642.

We have been interested in what effect chronic treatment with levodopa, a drug which increases striatal dopamine (DA) turnover, may have on the viability and morphological development of embryonic nigral DA neurons grafted into a lesioned caudate. Previously, we reported that twice daily injections of Sinemet (50 mg levodopa: 5 mg carbidopa/ kg) for 6 weeks has a detrimental effect on embryonic nigral DA neurons (*Soc. Neurosci Abstr.*. 15:1354, 1989). While there was no significant difference in number of grafted neurons between control and levodopa treated animals, the grafted neurons in the levodopa treated animals had significantly reduced cell body size, reduced staining for tyrosine hydroxylase in a majority of these neurons, increased infiltration of macrophages within the grafts and less extensive neurite outgrowth. The functional efficacy of grafts in control and levodopa treated animals has been investigated by monitoring rotational behavior in rats with unilateral nigrostriatal DA lesions elicited by DA agonists before and after neural grafting (*Soc. Neurosci. Abstr*, D.M. Yurek *et al.*, 1990). The results from these behavioral studies indicate that chronic levodopa impairs recovery of the rotational behavior in animals recieving grafts. This apparent decreased functional effect as a consequence of chronic levodopa may be related to impaired graft function, an alteration in DA receptor mechanisms or a combination of these. To better elucidate the mechanisms involved in these morphological and behavioral results, we are currently examining the impact of neural grafts on striatal D1 and D2 receptors and DA uptake binding sites in control and levodopa treated animals. Results of these receptor binding studies will be presented and discussed. (Supported by grants from the UPF and the Pew Foundation.)

275.12

PAIN REDUCTION BY THE TRANSPLANTATION OF ADRENAL MEDULLARY EXPLANTS IN THE RAT SPINAL SUBARACHNOID SPACE. H. Wang and J. Sagen. Dept. Anat. and Cell Biol., Univ. Il at Chicago, Chicago, IL 60612.

Previous studies in this laboratory have demonstrated that transplants of adrenal medullary tissue or isolated chromaffin cells can reduce pain sensitivity as assessed by both acute and chronic analgesiometric tests. This analgesia is most likely mediated by the local release of neuroactive substances, including opioid peptides and catecholamines, from the transplanted cells. We have recently found that adrenal medullary tissue can survive and continue to release these agents for at least one month in explant tissue culture. The advantage of maintaining tissue in explant culture is that it appears to allow for recovery from anoxic trauma following dissection. In addition, it may reduce immunologic responses by allowing for the migration of passenger leukocytes out of the tissue. The purpose of the present study was to assess the potential for using adrenal medullary explants as a graft source for the reduction of pain sensitivity. Rat adrenal medullae were maintained in explant culture for 0 - 30 days following dissection from cortical tissue. The explanted tissue was transplanted in the subarachnoid space of host rats following various intervals in culture. Control animals received equal volumes of control explanted striated muscle. Pain sensitivity was assessed using acute analgesiometric tests from 2 - 16 weeks following implantation. Results indicated that adrenal medullary tissues maintained in culture for up to 30 days were able to reduce pain sensitivity when transplanted into the subarachnoid space. Ten to 15 days in culture appeared to be optimal, since the most potent analgesia was observed in animals with explants from this time period. Morphological studies revealed the presence of clusters of healthy looking chromaffin cells in the explants 16 weeks after transplantation into the rat spinal subarachnoid space. Results of this study suggest that adrenal medullary explants may be a potential graft source for the reduction of pain. (Supported by NIH grant NS25054)

275.13

TIME OF IMPLANTATION AFTER SUPRACHIASMATIC NUCLEUS (SCN) LESION AFFECTS THE OUTCOME OF SCN TRANSPLANT. M.A. Vogelbaum and M. Menaker, Department of Biomedical Engineering and Department of Biology, University of Virginia, Charlottesville VA 22901.

We implanted τ_{SS} (period about 20 hours) golden hamster fetal hypothalamic blocks into partially SCN lesioned normal hamster hosts with residual 24 hour rhythmicity. These implants were done either about 30 days after lesioning ("delayed"), or immediately thereafter, to evaluate the effects of recovery from neuronal injury on the physiology of coupling between graft and host.

When transplantation is delayed, both 20 and 24 hour locomotor rhythms are expressed in the host: about 30 days after implantation, a rhythm with period close to 20 hours appears and lasts for 7 to 10 cycles, at which point a 24 hour rhythm takes over. This alternation of rhythms continues with each rhythm typically lasting 5 to 7 cycles, at which point there is a transition to the other rhythm. There is very little temporal overlap of the two rhythms, and there does not appear to be phase shifting of one rhythm by the other.

When transplantation immediately follows the lesion, a single rhythm is expressed with a greater amplitude than in the case of delayed implants. In most cases, the period of the restored rhythm was close to that of the donor SCN, but we do have one case with an intermediate (21.7 hour) period. In no case were two alternating rhythms seen, although in some a beat frequency of about 24 hours is present throughout the activity record.

These results suggest that the stage of recovery from neuronal injury influences the degree of coupling between the SCN implant and the host SCN. We hypothesize that a physical barrier is formed after lesioning due to the development of a glial scar. Perhaps neural integration within the SCN is prevented after a glial scar is allowed to develop. Accordingly, we have examined glial scar formation in both the delayed and immediate implant cases.

275.14

DEVELOPMENT OF THE LIGHT-ACTIVATED PUPILLARY RESPONSE IN RATS, AS MEDIATED BY OPTIC INPUT FROM NORMAL OR TRANSPLANTED RETINAE. R.D. Lund, J.D. Radel and S. Das*. Dept. Neurobiology, Anatomy & Cell Science, Univ. Pittsburgh Sch. Med., Pittsburgh, PA 15261.

We have shown in mature rats that pupilloconstriction can occur in response to illumination of retinae that had previously been transplanted to the rat's midbrain. Because the transplanted retina is developmentally 1 week younger than host retinae at the time of transplantation (E13 vs. P1), we questioned if this developmental handicap would be reflected in a delayed acquisition of the pupillary reflex.

The eyelids of normal rats or rats with transplanted retinae were opened, then one eye or the transplant was illuminated and the pupillary response recorded. We first detected a sluggish pupillary constriction at P7 in normal rats but not until P14-16 if driven through a transplant. The response matured to a brisk, adult-like constriction in normal rats by P12-14, and in host rats by P20-22. All animals were processed for ultrastructural analysis immediately after testing. In both normal and host rats, the initial pupillary response occurs as the retina develops rudimentary outer segments and asymmetric, ribbon-less synaptic contacts in the inner plexiform layer (IPL). Mature responses coincide with the appearence of ribbon synapses in the IPL.

We conclude that the development of light-activated pupilloconstriction is regulated by the developmental stage of the retina rather than by non-retinal elements, and that the timetable of development for transplanted retinae is determined by endogenously rather than by the host. (Supported by NIH grants EY05283, EY05967 & EY05308)

275.15

CORTICAL GRAFTS PRESERVE THALAMIC FUNCTION IN MATURE RATS FOLLOWING LESIONS OF SOMATOSENSORY CORTEX. M. F. Gonzalez, S. Ciricillo*, M. P. Jasper* and F. R. Sharp†. Depts. of Neurology and †Physiology, UCSF, and VA Medical Center, San Francisco, CA 94121.

Previous work has shown that unilateral tactile stimulation of the mystacial vibrissae of normal rats elicits an increase of ^{14}C 2-deoxyglucose (2DG) uptake in the contralateral ventrobasal (VB) thalamic nuclei. In this study we examined this phenomenon in mature rats with primary whisker somatosensory cortex (S1) lesions, or with fetal cortical tissue grafts surviving in injuries of this site. Lesions consisted of removing cortical gray matter from 0 to 3 mm posterior to bregma, and from 3 to 6 mm to the right of the sagittal suture. Cortical transplants were performed by placing slabs of fetal cortical tissue in these cavities one week after injury.

Tactile stimulation of the left whiskers of rats with S1 cortical lesions resulted in an 9.8% inhibition of 2DG uptake in the VB thalamic nucleus, as compared with the unstimulated left side ($N=4$, $t=5.097$, $P<0.015$). The same treatment produced opposite effects in 4 subjects that had large surviving cortical grafts. Their VB thalamic nuclei exhibited a 24% increase in 2DG uptake ($t=11.15$, $P<0.002$). An additional subject with a surviving graft did not exhibit any thalamic activation following stimulation. This data suggests that some cortical transplants may prevent the functional atrophy of host brains' structures caused by cortical injury.

275.16

USE OF c-*FOS* TO DETERMINE THE NEURAL PATHWAY ACTIVATED BY INTRACRANIAL RETINAL TRANSPLANTS IN RATS S.L. Craner, G.E. Hoffman, J.S. Lund, and R.D. Lund. University Pittsburgh School of Medicine, Pittsburgh, PA 15261.

We examine here activation patterns in the host brain after illumination of intracranial retinal transplants, using c-*fos* labelling techniques. Embryonic (E13) retinae were transplanted over the midbrain of postnatal (P1) rat hosts and one host eye removed to enhance the transplant projection. At maturity, the remaining host eye was removed from those animals which had a demonstrable transplant-mediated pupillary reflex. After a suitable recovery period, these rats were anesthetized and the transplanted retinae stimulated with flashes of light (800 msec duration every 3.5 seconds) for one hour. The animals were terminated after a further 30 mins and the brains processed for immunohistochemical labeling of the c-*fos* protein. Suitable control groups of non-transplanted animals provided information regarding stimulus-specific c-*fos* activation following light flashes presented to the eye.

Several visual centers, connected with the stimulated eyes, both *in situ* and transplanted, showed levels of c-*fos* that were higher than in unstimulated controls. Most prominent were the superior colliculus and several divisions of the pretectum. This provides further evidence of stimulus activation of host visual centers by way of a retinal transplant in a manner differing little from normal animals studied after visual stimulation. Supported by NIH grants EY05282, EY05308 & EY06194.

275.17

FETAL CORTICAL GRAFTS USED IN THE REPAIR OF ASPIRATION AND NMDA NEOCORTICAL LESIONS MADE IN ADULT RATS. A SINGLE UNIT STUDY. E.J. Neafsey, T.P. Hogan*, P.L. Shaw*, E.B. Pedersen* and A.J. Castro. Dept. of Cell Biology, Neurobiology and Anatomy, Loyola University Chicago Stritch School of Medicine, Maywood, IL 60153.

Rat fetal (E15) presumptive sensorimotor neocortex was grafted into the adult rat sensorimotor cortical area using two procedures: 1) a tissue BLOCK into an aspiration lesion cavity in the cortex (n=3) or 2) a cell SUSPension injected into a cortical lesion made by multiple intracortical NMDA injections 5 days prior to grafting (n=6). Two to three months later the rats were anesthetized with ketamine HCl (100 mg/kg, IP), and the responses of single units in the transplant to electrical stimulation (0.1 msec pulses) of the thalamus (THAL; current < 500 uAmp) and contralateral and ipsilateral forepaws (CFP, IFP; 1 mAmp) were examined. The table below summarizes our findings.

Graft Type	# of units	Resp THAL	Mean Laten msec (SEM)	Resp CFP	Mean Laten msec (SEM)
BLOCK	10	80%	6.6 (1.1)	40%	21.5 (3.7)
SUSP	20	81%	6.7 (0.9)	60%	15.2 (1.4)

These results are similar to those seen in controls and in block transplants which have been placed in newborn hosts (Neafsey et al., BR 493:33, 1989) and demonstrate that such transplants can receive sensory input from the body. (Support by NIH grant NS13230)

275.18

HYPEREXCITABILITY IN TRANSPLANTED STRIATAL NEURONS: MODULATION BY EXCITATORY AMINO ACIDS. S.M. Sivin, J.P. Walsh, M.S. Levine and N.A. Buchwald. MRRC, UCLA, Los Angeles, CA 90024.

Previous work from our laboratory has characterized the electrophysiological properties of transplanted striatal neurons grafted into the striatum (Synapse, 2:37) or substantia nigra (Soc Neurosci Abs, 15:1353). The grafted cells become hyperexcitable and develop large amplitude, long duration depolarizations along with bursts of action potentials. These depolarizations occur spontaneously or following extracellular stimulation of host tissue and are never observed in host striatal cells. In the present study, we assessed the extent to which hyperexcitability in transplanted striatal cells is due to activation of excitatory amino acid (EAA) receptors. Cell suspensions obtained from 17 day old fetal striata were grafted into both the striatum and substantia nigra of adult rats. One to 4 months after transplantation, the rats were sacrificed, brains removed and 400 μm thick slices were taken through the transplant for intracellular recording. Stimulation of the host tissue resulted in large amplitude (> 20 mV), long duration (> 150 msec) depolarizations accompanied by bursts of action potentials. Responses were similar for cells grafted into the striatum and into the substantia nigra. Bath application of the broad spectrum EAA antagonist kynurenic acid (0.5 mM) abolished almost all post-synaptic activity, as did the specific kainate/quisqualate antagonist CNQX (1-3 μM). The effects of the specific NMDA antagonist AP5 (20 μM) were more variable, ranging from no effect to an 80% reduction in amplitude and duration of the depolarizations. NMDA receptor antagonists like AP5 are usually ineffective in blocking responses in host striatal cells unless the voltage dependency of the receptor is negated by removing Mg^{2+} from the bathing medium. These data suggest that EAA receptors contribute significantly to the hyperexcitability of transplanted striatal cells. Supported by USPHS HD05958.

276.1

INDUCIBILITY OF AN EARLY GROWTH RESPONSE GENE IN SONGBIRD BRAIN: LINKS WITH ADULT NEURAL PLASTICITY

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RNAs for several presumed regulators of gene transcription rise rapidly following stimuli associated with neuronal differentiation and depolarization. To test the possibility that early growth response genes may be involved in regulating plasticity in the adult nervous system, we examined expression of one (the canary homologue of zif-268, egr-1, NGFI-A and Krox-24) in the brain of songbirds following metrazole-induced seizures. The anatomical pattern of activation of the gene (revealed by in situ hybridization) closely coincides with sites in the brain that receive new neurons in adulthood in canaries. Furthermore, substantially lower levels of induction were seen in the stable song control circuit of adult male zebra finches, who are incapable of modifying their song. These results suggest the inducibility of the gene may reflect or predict sites of synaptic plasticity in the adult nervous system.

276.2

A CANARY SONG CENTER-ENRICHED RNA ENCODES THE HOMOLOGUE OF N-CHIMAERIN, A PROTEIN RELATED TO THE BCR ONCOGENE AND PROTEIN KINASE C.

J.M. George and D.F. Clayton. Lab. of Animal Behavior, The Rockefeller Univ., New York, NY 10021

We are characterizing RNAs enriched in the plastic regions of the songbird brain, and describe here a clone we call hvc-2. Localization by in situ hybridization reveals a largely neuronal distribution throughout the neo- and hyperstriatum, with a particularly high level of expression in the plastic song center Area X. The sequence encodes a presumed protein of 299 a.a., the N-terminus of which shares 40% identity with the regulatory domain of protein kinase C, including the zinc finger consensus $C\text{-}X_2\text{-}C\text{-}X_{13}\text{-}C\text{-}X_2\text{-}C\text{-}X_7\text{-}C\text{-}X_7\text{-}C$ believed to mediate phorbol ester binding. The C-terminus is 43% identical to bcr, the product of the breakpoint cluster region gene, involved in the Philadelphia chromosome (Ph^1) translocation. Our clone, hvc-2, shows 97% a.a. identity to n-chimaerin, a recently described human gene with a predominately neuronal distribution and greatest abundance in hippocampus and cerebral cortex (Hall, C. et al. J. Mol. Biol. 211:11, 1990). This high degree of conservation between human and avian species suggests a fundamental role for the gene product, perhaps as a component of signal transduction mechanisms involving phospholipid metabolism.

276.3

B-50/GAP43 GENE EXPRESSION IN THE DEVELOPING AND AGING RAT OLFACTORY SYSTEM.

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The olfactory neuroepithelium exhibits neurogenesis throughout adulthood, and in response to lesions, a phenomenon that distinguishes this neural tissue from the rest of the mammalian nervous system. It has been suggested that denervation and reinnervation of olfactory bulb neurons by primairy olfactory neurons may occur throughout life. Thus, these properties enable the study of neurogenesis and synaptogenesis in the adult animal. In this study we reported the expression of the growth associated protein B-50/GAP43 and its mRNA in the olfactory system during postnatal development and aging. In neonatal rats B-50/GAP43 mRNA was expressed in virtually all primary olfactory neurons and in their target cells in the olfactory bulb, the mitral- and juxtaglomerular cells. In contrast in 6 and 18 month old animals B-50/GAP43 expression was restricted to neurons in the basal region of the neuroepithelium and to some of the target mitral- and juxtaglomerular cells in the olfactory bulb. If the persistance of B-50/GAP43 expression in mitral- and juxtaglomerular cells reflects the continuously changing input from primary olfactory neurons in the olfactory bulb then it implies longterm mitral cell synaptic plasticity in the pyriform cortex.

276.4

MAP-2 IS MORE SUSCEPTIBLE TO CALPAIN HYDROLYSIS THAN IS SPECTRIN.

G.V.W. Johnson, J.M. Litersky and R.S. Jope. Departments of Neurology, Psychiatry and Cell Biology & Anatomy, University of Alabama, Birmingham, Alabama 35294.

In brain, MAP-2 and spectrin are major components of the neuronal cytoskeleton and therefore the processing and degradation of these proteins is likely to play an important role in the modulation of the form and function of neurons. In this study we examined the in vitro degradation of these two proteins by calpain using quantitative immunoblot analysis.

Like tau (BBRC 163:1505, 1989), there are at least two populations of MAP-2 in brain based on calpain-sensitivity, a calpain sensitive form associated with microtubules (2XMT MAP-2) and a calpain-resistant form (total MAP-2) that represents another population of MAP-2. 2XMT MAP-2 is approximately 10 times more sensitive to calpain than total MAP-2. The two brain spectrin isoforms (240 and 240E) also show differential sensitivities to calpain. Brain spectrin (240) is significantly more sensitive to calpain than spectrin (240E), which is degraded at a rate similar to total MAP-2. Calmodulin significantly increases the rate and extent of calpain-induced degradation of both isoforms of brain spectrin, but does not alter the breakdown of either 2XMT or total MAP-2. However even in the presence of calmodulin, both isoforms of brain spectrin are significantly less sensitive to calpain than is 2XMT MAP-2.

Given these results and the similar subcellular localization of MAP-2 and brain spectrin (235/240E) (i.e., in dendrites and postsynaptic densities), it is likely that the calpain-mediated breakdown of MAP-2, as well as spectrin, contributes to neuronal plasticity, such as may occur with long term memory, and calcium-mediated neurodegeneration which may be associated with ischemia or neurodegenerative diseases, such as Alzheimer's disease.

Supported by NIH grants #NS27538 and AG06569 and the Alzheimer's Association/Mary Sue Glover Memorial Pilot Research Grant.

276.5

ACTIVATION OF BRAIN PROTEIN KINASE C SUBTYPES BY OMEGA-3 CIS-FATTY ACIDS.

S.G. Chen, K. Conley* and K. Murakami. Dept. of Biochem. Pharmacol., State Univ. of New York, Buffalo, NY 14260.

Protein kinase C (PKC) plays an important role in the regulation of synaptic plasticity. Cis-fatty acids such as oleate and arachidonate have been shown to strongly activate purified brain PKC in the absence of phospholipids, diacylglycerol, and Ca^{2+} (JBC 261, 15424, 1987). It has been further demonstrated that cis-fatty acids differentially activate brain PKC subtypes (BBRC 145, 797, 1987).

Here we report that PKC subtypes can be activated by omega-3 cis-fatty acids such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) in the absence of phospholipids and diacylglycerol with different sensitivity to Ca^{2+}.

PKC was purified from rat brain by four-step liquid chromatography. Three PKC subtypes (type I, II, and III) were further separated on a hydroxyapatite column. In the presence of Ca^{2+}, EPA and DHA can significantly activate these three PKC subtypes at concentrations well below 100 μM. The concentrations required for half maximal activation (Ka values) for type I, II, and III were 22, 14 and 23 μM for EPA and 21, 17 and 31 μM for DHA, respectively. In the absence of Ca^{2+}, EPA and DHA showed a 3-9 fold lower affinity for type III as compared to the other subtypes.

Since omega-3 cis-fatty acids are enriched in retina and brain, the ability of these cis-fatty acids to activate PKC subtypes suggests their involvement in neuronal processes.

276.6

SIGNAL-SELECTIVE PHOSPHORYLATION OF PROTEIN KINASE C SUBSTRATES IN NEUROBLASTOMA N1E-115.

K. Murakami, S.G. Chen and A. Routtenberg#. Dept. of Biochem. Pharmacol. State Univ. of New York, Buffalo, NY 14260 and #Cresap Neuroscience Lab. Northwestern Univ., Evanston, Illinois 60208.

At least seven subtypes of protein kinase C (PKC) are expressed in the brain (Nature 334, 661, 1988). Our previous studies have shown that cis-fatty acids can activate purified brain PKC independently of Ca^{2+}, diacylglycerol and phospholipids and we proposed that cis-fatty acids liberated by the action of phospholipase A2 may play a second messenger role in the activation of PKC (FEBS Lett. 192, 189, 1985).

In this study, we examined the effects of different PKC activators on protein phosphorylation in a neuroblastoma clone, N1E-115. Endogenous PKC activation by cis-fatty acid and Ca^{2+}/diacylglycerol/phospholipids lead to distinct subsets of PKC substrate phosphorylation in N1E-115 cells. One particular type of PKC substrate, a 40 kDa protein, was selectively phosphorylated by oleic acid in the absence of Ca^{2+}. This phosphorylation was also stimulated by exogenous PKC but suppressed by both H-7, a PKC inhibitor and in PKC-depleted cells by TPA. Ca^{2+}/diacylglycerol/phospholipids did not fully phosphorylate this protein.

Existence of a cis-fatty acid sensitive PKC substrate indicate that the heterogenous PKC subtypes may be selectively activated by different signals and each subtype may phosphorylate specific sets of PKC substrate.

276.7

SYNAPTIC PLASTICITY: MOLECULAR AND CELLULAR APPROACHES TO THE STUDY OF PROTEIN KINASE C (PKC) REGULATION OF PROTEIN F1/GAP-43 PHOSPHORYLATION. P. Meberg*, F-S. Sheu*, X. Xiang, Y.Y. Huang*, P. Colley*, B. Kapella*, E. Valcourt* and A. Routtenberg. Northwestern Univ., Evanston, IL 60208.

In situ hybridization of protein F1 mRNA demonstrates selective regional expression. Brainstem cells containing biogenic amines (e.g., locus coeruleus, dorsal raphe, substantia nigra-pars compacta) show extensive F1 mRNA expression, while other brainstem cells (e.g., red nucleus and pontine nucleus) and cholinergic cells (e.g., medial habenula) show little. The most striking selectivity is seen in adult hippocampus: pyramidal cells are heavily labeled while granule cells are at near background levels as quantified by image analysis (also, Rosenthal et al., EMBO J. 6:3641, 1987). Developmental analysis of F1 mRNA shows peak expression in pyramidal cells at 12 days, and in granule cells at 16 days; pyramidal cell F1 mRNA expression is 4- to 8-fold greater than the granule cells at all ages.

Protein F1 is phosphorylated by PKC, whose subtype distribution, like F1, is regionally selective (Brandt et al., Cell 49:57, 1987). Protein F1 and beta-PKC mRNA are expressed at parallel levels (high-pyramidal; low-granule) in hippocampus, while gamma-PKC is expressed similarly in both cell types. Furthermore, beta-PKC, compared to gamma-PKC, preferentially phosphorylated either purified protein F1 or recombinant F1 made with protein-secreting bacteria.

PKC regulates long-term potentiation (LTP; Routtenberg, Behav. Neural Biol. 44:186, 1985). Use of PKC inhibitors in the synaptic zone suggests PKC regulation of LTP persistence not its initiation. The role of PKC in regulating plasticity in the cell body is currently under study using intracellular injection of PKC inhibitors into CA1 pyramidal cells in the hippocampal slice preparation. A protocol has been implemented to enable recording baseline activity with inhibitor-filled pipettes and ejection either before or after LTP. It is attractive to think that beta-PKC regulates persistence of LTP by phosphorylating presynaptic proteins. (Supported by NIMH 25283 and AFOSR 90-0240).

276.8

TRANSIENT RISE IN IMMEDIATE EARLY GENE PRODUCTS IN HIPPOCAMPAL NEURONS AFTER ELECTROCONVULSIVE TREATMENT. Y. Nakabeppu*, J.M. Baraban, B. Christy*, P.F. Worley. Howard Hughes Medical Institute, Depts. of Molecular Biology and Genetics, and Dept. of Neuroscience, Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

Cell surface receptor stimulation by neurotransmitters or growth factors can elicit a rapid and transient activation of a set of genes, referred to as immediate early genes (IEGs), thought to play a key role in long-term changes in cellular activity. Electroconvulsive treatment produces a robust rise in mRNA levels for several IEGs that code for known or putative transcription regulatory factors in dentate granule cells. Accordingly, we have used this stimulation paradigm to characterize the expression of these gene products in brain neurons.

Immunoblot analysis of hippocampal nuclear extracts with affinity purified polyclonal antisera demonstrates a rise in Zif/268, c-Jun, Jun-B and c-Fos that peaks at 1-2 hrs and returns to basal levels by 4-8 hrs. These IEG products contribute to increased binding activity to DNA consensus sequences previously identified for Zif/268 and Jun-Fos complexes. By contrast, binding activity for the consensus sequence of Spl, a constitutively expressed transcription factor, is unchanged. Immunohistochemical studies with these antisera demonstrate prominent increases in staining in nuclei of dentate granule cells. These results suggest that activation of these genes in brain neurons leads to transient increased expression of their gene products.

276.9

KINDLING INDUCED CHANGES IN CALMODULIN KINASE II IMMUNOREACTIVITY. J.M. Bronstein, D.B. Farber, P.E. Micevych, R. Lasher, and C.G. Wasterlain. Dept. of Neurology, UCLA School of Med., Los Angeles, CA 90024.

The distribution of Type II calmodulin kinase (CaM kinase) immunoreactivity was studied in control and septally kindled rat brains. CaM kinase was concentrated in limbic structures, such as the hippocampus, lateral septum, and amygdala. Within the hippocampus, the molecular layer of the endal limb of the dentate gyrus, the statum radiatum, and lacunosum moleculare of CA1 were the most heavily stained regions. The cerebellum was stained only in the molecular and Purkinje cell layers, and very low amounts of immunoreactive protein were present in the brainstem and white matter. Kindling resulted in a significant decrease in CaM kinase immunoreactivity in CA3 and the dentate of the ventral hippocampus but not in the lateral septum. These data suggest that kindling decreases the number of CaM kinase molecules or alters its antigenic distribution, and provides further evidence that alterations of this enzyme may be important in the kindling phenomenon.

276.10

ENTORHINAL CORTEX LESION INDUCES C-FOS IN THE RAT HIPPOCAMPUS. S.S.Schreiber, G.Tocco, N.J.Laping, J.R.Day, A.A.Blain, C.E.Finch. Andrus Gerontology Center and USC School of Medicine Department of Neurology, University of Southern California, Los Angeles, CA 90033.

The induction of c-fos in the deafferented rat hippocampus was examined by in situ hybridization as part of an ongoing study of molecular events associated with reactive synaptogenesis. Adult male rats were subjected to unilateral electrolytic entorhinal cortex lesion (ECL) under pentobarbital anesthesia. The animals were sacrificed by decapitation either one or four hours after surgery. Unoperated but anesthetized, and sham operated animals served as controls. In situ hybridization was performed on cryostat sections (10um) fixed in 4% paraformaldehyde. A full length c-fos antisense riboprobe labeled with 35S-UTP was hybridized to sections for three hours at 50 C. In intact and sham-operated control animals levels of c-fos expression were unchanged indicative of a low level of basal expression. One and four hours after ECL c-fos expression was increased in the ipsilateral cortex (n=2). However, at four hours increased signal was also detected in both hippocampi (3 out of 4 animals). These results suggest that c-fos induction occurred by way of trans-synaptic activation from the lesioned afferent pathway. Furthermore, the uncharacteristic delay in, and bilaterality of, this response suggests a role for proto-oncogene fos in adaptation to changes in neuronal circuitry. Characterization of the response in terms of time course and cellular localization will be reported. (Supported by NIH Grant NS01337 to SSS; NIA AGO5142; NIA AGO7909)

276.11

RAPID SEPTAL ASTROCYTIC RESPONSE FOLLOWING ENTORHINAL CORTEX LESION. C.Zarow and C.E.Finch. Ethel Percy Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

Astrocytic responses to entorhinal cortex lesion (ECL) by the hippocampus suggest that astrocytes play a role in phagocytosis of debris as well as nutritive support for new sprouts. As part of a study of septal responses to the ECL, changes in glial fibrillary acidic protein (GFAP) message and protein product were examined. Unilateral electrolytic ECL were performed on young male rats, with 2, 6, 10, or 14 d post lesion survival. Unoperated animals served as controls. RNA was extracted from ipsilateral and contralateral hippocampi and combined septa. Labeled cRNA corresponding to a portion of the coding region of mouse GFAP (clone a gift of E. Lazarides) was used as a probe in all studies. Another group of brains was paraffin-embedded, sectioned at 10 μm, and labeled for GFAP message (in situ hybridization (ISH)) and product (immunocytochemistry (ICC)). By Northern blot and RNA solution titration analyses, GFAP mRNA peaked at 2d post lesion in each region and decreased to control levels by 6d in septum and the contralateral hippocampus. In contrast, GFAP mRNA in the ipsilateral hippocampus returned more slowly to baseline, by 14d. GFAP mRNA levels were increased over control by 5-fold in the septum, 3-fold in the contralateral hippocampus and >10-fold in the ipsilateral hippocampus. In the ipsilateral septum, ISH signal was increased at 2d, but increases in protein were not apparent (ICC) until 10d post lesion. These data suggest a rapid and robust astrocytic response in the septum. The 5-fold increase in message is apparently limited to the ipsilateral septum, suggesting a specific interaction between the astrocytes and the neurons which are sprouting. It is an open question whether these astrocytes are responding directly to the ECL induced degeneration or indirectly as a result of septal sprouting. (Supported by NIA AGO7909)

276.12

RECOMBINANT HUMAN NERVE GROWTH FACTOR STIMULATES ACETYLCHOLINE SYNTHESIS IN CHOLINERGIC NEURONS SURVIVING PARTIAL SEPTO-HIPPOCAMPAL LESIONS. P.A. Lapchak, E. O. Junard and F.F. Hefti. Andrus Gerontology Center, U.S.C., Los Angeles, CA, 90089.

In adult rats with lesions of the septo-hippocampal S-H) pathway, nerve growth factor (NGF), given intraventricularly (icv), prevents the loss of cholinergic cell bodies in the septum and increases ChAT activity in the hippocampus. The present study determined whether the NGF-induced increase in ChAT activity results in changes of presynaptic cholinergic function in the hippocampus. Adult rats received partial unilateral transections of the fimbria as described previously (Hefti et al. Brain Res. 293: 305-311, 1984). They were injected icv (during 3 weeks) every second day with 1 μg of rhNGF through chronically implanted cannulas. We then determined whether NGF treatment altered the amount of 3H-acetylcholine(ACh) formed from 3H-choline by hippocampal slices in vitro. 3H-ACh formed by hippocampal slices of cytochrome c (cc)-treated lesioned animals was reduced to 55.4 $\pm$5.7 % of the contralateral intact tissue. Following treatment with NGF, 3H-ACh synthesis was increased to 81.7 $\pm$ 10.9 % of the contralateral intact tissue. NGF did not alter the amount of 3H-ACh formed by the unlesioned contralateral intact tissue. These results are compatible with the idea that NGF treatment stimulates presynaptic cholinergic function following brain injury (Supported by FRSQ, Quebec).

276.13

GM1-ENHANCED RECOVERY OF DRL-20 AND OPEN FIELD ACTIVITY AFTER BILATERAL ENTORHINAL CORTEX LESIONS IN RATS. J. J. Ramirez, B. Fass-Holmes, S. E. Karpiak, C. Tuite*, C. Alexander*, and A. Grewal*. Dept. of Psychology, Davidson College, Davidson, NC 28036, N.Y.S.P.I., Columbia Univ., N.Y.C., NY 10032.

Administration of gangliosides accelerates recovery of function after bilateral entorhinal cortex (EC) lesions on open field activity and learned spatial alternation tasks. We previously demonstrated that a reduction in heterologous sprouting is associated with this enhanced recovery. The present study examined whether gangliosides (GM1) enhance recovery from bilateral EC lesions on a timing task (i.e., differential reinforcement of low-rate responding -- DRL) as well as open field activity. Optical densitometry measurements were taken to assess sprouting by the septo-dentate pathway. Eighteen rats were assigned to sham/GM1, lesion/GM1, or lesion/saline conditions, tested on both tasks preoperatively, and tested postoperatively for 30 days. Rats in the lesion/GM1 group showed enhanced recovery on the DRL-20 and the open field tasks. Optical densitometry analyses revealed a reduction of septal sprouting in the molecular layer of the dentate gyrus for the lesion/GM1 group. GM1 treatment may be facilitating recovery from bilateral EC lesions by reducing the trauma of injury and denervation, reducing heterologous sprouting, or both.

Supported by NC Board of Science and Technology.

276.14

THE EFFECT OF FEMALE CIRCULATING SEX HORMONES ON HIPPOCAMPAL SYMPATHETIC INGROWTH. L. E. Harrell, D. S. Parsons*, A. Peagler* and J. Litersky. Department of Neurology, Veterans Administration and University of Alabama Medical Center, Birmingham, Alabama 35294

Previously, we have demonstrated an interaction between male circulating sex hormones and the behavioral and biochemical effects of peripheral sympathetic ingrowth, which occurs in the hippocampus after medial septal lesions (MSL). In this study we assessed the effect of female circulating sex hormones. Seventeen adult female Sprague-Dawly rats underwent sham ovariectomy (SOx), while 21 were Ox. Animals within each group then underwent 1 of 3 neurosurgical procedures: CON-sham MSL + sham ganglionectomy (Gx); MS-MSL + SGx; MSGx - MSL + Gx. Eight weeks later, animals were sacrificed, the brain dissected into a septal block (to assure appropriate lesion placement) and a hippocampal block, which was dissected into dorsal (D) and ventral (V) regions. Norepinephrine (NE) and choline acetyltranferase (ChAT) were then measured in the hippocampus. MS lesions were found to reduce ChAT activity in both D and V of both SOx and Ox groups when compared to CON ($p<.0001$). This reduction was less in the Ox than SOx group ($p<.05$) in both D and V ($p<.0006$). Analysis of NE revealed that Ox did not alter NE in CON. However, Ox MS animals were found to have significantly greater levels of NE in both D and V ($p<.05$), while Ox MSGx animals were found to have lower levels of NE than SOx MSGx. This suggests that circulating female sex hormones interact with both MS lesions, as measured by ChAT activity, and hippocampal sympathetic ingrowth in a complex fashion. Further studies are ongoing to assess the behavioral effects of female sex hormones in HSI.

276.15

CORTICOSTERONE DECREASES GLIAL FIBRILLARY ACIDIC PROTEIN mRNA AND SULFATED GLYCOPROTEIN-2 mRNA IN THE HIPPOCAMPUS OF ADRENALECTOMIZED AND ENTORHINAL CORTEX LESIONED MALE RATS. N. J. Laping, N. R. Nichols, J. R. Day, and C. E. Finch. Gerontology Center, University of Southern California, Los Angeles, CA 90089.

Glial fibrillary acidic protein (GFAP), a marker of astrocyte activation, and sulfated glycoprotein-2 (SGP-2), a marker of programmed cell death in peripheral tissues, are elevated during neurodegeneration. As shown previously unilateral entorhinal cortex lesion (ECL) increases GFAP and SGP-2 mRNA in the hippocampus (Poirier et al, 1988; Lampert-Etchells, these Proceedings). Moreover, GFAP mRNA is increased in the hippocampus following adrenalectomy (ADX). This study examined the effect of corticosterone (CORT) (200 μg/ml in drinking water) on GFAP and SGP-2 mRNA levels in the hippocampus of ECL rats. CORT decreased GFAP mRNA in ADX rats. The combination of ADX + ECL increased GFAP mRNA additively in the ipsilateral hippocampus over the ADX and intact groups. CORT reduced GFAP mRNA to intact levels in the contralateral hippocampus of ADX+ECL rats and to a lesser degree in the ipsilateral hippocampus. CORT decreased SGP-2 mRNA in the ADX and ADX+ECL group as well. These experiments show that CORT can independently regulate both GFAP and SGP-2 mRNA in ADX and ECL conditions. Also, the ECL-surgery induced surge of CORT probably does not play a major role in the long term regulation of GFAP mRNA. (Supported by: J.D. & C.T. MacArthur, Program in Successful Aging; ONR Grant N00014-85-K-0770; NIA 07909).

276.16

GONADECTOMY ELICITS A SEXUALLY DIMORPHIC PATTERN OF GENE EXPRESSION IN THE RAT HIPPOCAMPUS AFTER ENTORHINAL CORTEX LESION. J.R. Day, N.J. Laping and C.E. Finch. Andrus Gerontology Center, University of Southern California, Los Angeles, CA 90089.

Unilateral lesion of the rat entorhinal cortex (ECL) induces synaptic remodeling in the adult hippocampus. Following deafferentation, dramatic changes in gene expression and morphology occur as the synapses of the perforant pathway are gradually replaced by remaining afferents. The pattern of gene expression induced by this lesion is similar to that seen during aging and in Alzheimer's disease. Specifically, the astrocyte marker glial fibrillary acidic protein (GFAP) and a putative neuronal constituent, sulfated glycoprotein-2 (SGP-2), are elevated in the hippocampus after ECL in rats as they are during neurodegeneration in humans. Northern blot analysis showed SGP-2 and GFAP mRNAs to be elevated 21 days post-castration, and by 2 days post-ECL. SGP-2 and GFAP mRNAs increased 5-fold after castration alone and an additional 3- to 5-fold respectively, in castrate males after ECL. In females, ovariectomy did not increase these mRNAs above those levels induced by ECL in intact females, and ovariectomy alone had no effect. These results indicate that the hormonal control of gene expression, subsequent to injury in the brain of females is different from males. (Supported by: J.D. & C.T. MacArthur Program in Successful Aging; NIA 07909)

276.17

EVIDENCE FOR SYNAPTIC REMODELLING IN HIPPOCAMPAL CA1 FOLLOWING ENTORHINAL CORTEX LESIONS. M.A. King, D.W. Walker, and B.E. Hunter, Gainesville VA Medical Center and Dept. of Neuroscience, University of Florida, Gainesville, FL 32610.

Entorhinal cortex (EC) lesions provoke a well documented reactive synaptogenesis in the dentate gyrus which is reflected by changes in the pattern of acetylcholinesterase (AChE) staining. Since the entorhinal cortex (EC) also projects to regio superior we examined AChE staining patterns in CA1 to determine whether and how synaptic remodelling occurs there. Unilateral electrolytic EC lesions in adult male Long-Evans rats were followed by a 40 d. survival. Digital images of AChE-stained sections from the ipsi- and contralateral dorsal hippocampus were sampled from the alveus to the fissure at lateral (->CA3), central, and medial (subicular) CA1. Intensities at each 1% of normalized stain profiles were analyzed for lesion and CA1 position effects with 2-way ANOVA and Duncan's means tests. CA1 location effects were found mainly in distal strata radiatum and lacunosum-moleculare. Ipsilateral to the lesion significant AChE intensification was observed in distal apical lateral CA1, similar to that seen in the dentate. Central and medial lesion effects were characterized more by an AChE clearing in distal stratum radiatum. Effects in strata pyramidale and oriens were minor. In the context of the meaning of AChE reorganization in the dentate gyrus these data indicate that synaptic reorganization may also occur in CA1 following deafferenting lesions. The data furthermore offer a quantitative description of variations in AChE parallel and transversely perpendicular to the primary dendritic axis of CA1 pyramidal cells.

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276.18

NMDA RECEPTOR ACTIVATION INCREASES AXON TERMINAL EXCITABILITY IN VITRO: A NOVEL FORM OF NEURONAL PLASTICITY. **S. F. Stasheff and W. A. Wilson, Depts. of Pharmacology and Medicine, Duke University and Durham Veterans Administration Medical Centers, Durham, NC.**

We previously reported that kindling-like stimulation increases the occurrence of ectopic action potentials ("baseline spikes" (BLSs)) in CA3 cells of the hippocampal slice, via NMDA receptor activation (Neurosci. Lett. 111:144-50, 1990; Neurosci. Abst. 478.2, 1989). Like known forms of CNS plasticity such as LTP, this increase in excitability is long-lasting and, once established, no longer dependent on NMDA receptor activation. However, the initiation site of these action potentials, and thus of the alteration in excitability, is clearly not somatic. We have now used three approaches which suggest that the increase in BLS firing results from the development of hyperexcitability in CA3 axon terminals.

First, we performed intracellular collision tests. We collided known anti- and orthodromic spikes to find the critical period for collision in each cell. We then tried to collide BLSs with known antidromic spikes. We usually found that they failed to do so, indicating that BLSs are antidromic. Second, we analyzed the waveforms of spikes triggered by various means. We found that antidromic spikes possess an inflection on their rising phase which is not present in orthodromic (synaptic, current-evoked, or spontaneous) spikes. BLSs also exhibit such an inflection, indicating their antidromic origin. Finally, we performed intracellular excitability tests before and after the induction of BLSs, measuring the threshold for antidromic activation at putative terminal and non-terminal sites along the Schaffer collaterals. This threshold decreased only at terminal sites, in parallel with the development of BLSs.

These results suggest the novel idea that NMDA receptors can mediate lasting increases in the excitability of not only dendrites and somata, but also axon terminals of CNS neurons. Such changes may contribute to phenomena such as epileptogenesis and LTP. Supported by NS 17701 and the Veterans Administration.

276.19

VISUALIZATION OF LIVING HIPPOCAMPAL SYNAPSES USING CONFOCAL SCANNING LASER MICROSCOPY. C. L. Keenan, A. C. Nobre, B.J. Claiborne, and T.H. Brown. Dept. of Psychology, Yale Univ., New Haven, CT 06520 and Div. of Life Sciences, Univ. of Texas, San Antonio, TX 78285.

We have been interested in optical methods that offer good spatial and temporal resolution of cellular and subcellular details in brain slices (Keenan et al., Brain Res. Bull., 21:373, 1988; Keenan et al., Soc. Neurosci. Abstr., 15:980,1989). Here we report the successful use of confocal scanning laser microscopy (CSLM) for visualizing the mossy-fiber (mf) synapses of hippocampal region CA3.

Hippocampal slices were placed into a chamber attached to the stage of an inverted microscope, which was equipped with a long-working distance (500 μm), oil-immersion objective (63X, 1.25 NA). The (presynaptic) mf expansions were stained by pressure ejection of DiIC18(3) into either the stratum lucidum, the hilus or the stratum granulosum. The (postsynaptic) thorny excrescences were stained by intracellular injection of lucifer yellow into the CA3 neurons. Serial optical sections of mf expansions and thorny excrescences were then obtained using CSLM. Sections were taken at intervals of 0.1 - 2.0 μm and then reconstructed to yield 3-D images.

Using appropriate scanning times and filtering for the particular dye, we were able to visualize clearly both the mf expansions and the thorny excrescences. High resolution images were obtained in as little as 6 sec, at 1 scan/sec, with relatively low laser power. Sometimes we could see small filamentous extensions of the mf expansions, reminiscent of growth cone filopodia.

We have been particularly interested in possible structural changes that may accompany use-dependent synaptic modifications, such as long-term potentiation (LTP) (reviewed in Brown et al., In: Byrne and Berry, Neural Models of Plasticity, 266, 1989). If there are in fact LTP-associated structural modifications, as suggested by electron microscopic studies, the present methods may enable them to be visualized in real time. (Supported by the AFOSR and NIH)

276.20

2-AMINO-3-PHOSPHONOPROPIONATE (AP3) BLOCKS INDUCTION OF ASSOCIATIVE LONG-TERM DEPRESSION (LTD) IN HIPPOCAMPAL FIELD CA1. S. Chattarji, P.K. Stanton[1] and T.J. Sejnowski. Salk Institute, La Jolla, CA 92037 and [1]Albert Einstein Coll. Med., Bronx, NY 10461.

A test input, which by itself does not elicit long-term potentiation (LTP), exhibits an associative form of LTP when it is activated at the same time as a separate conditioning input. Recently, we have reported an associative long-term depression (LTD) in field CA1 that is produced when a low-frequency test input (TEST) is negatively correlated in time with a high-frequency conditioning input (COND). LTD can also be produced by activating presynaptic terminals while a postsynaptic neuron is hyperpolarized. The present study investigates the effect of 2-amino-3-phosphonopropionate (AP3), a putative antagonist for a metabotropic quisqualate receptor, on the induction of LTD in hippocampal field CA1.

Extra- and intracellular recordings were made in rat hippocampal slices (400μm thick) in an interface chamber at 34°C. The COND stimulus, activating Schaffer collateral/ commissural fibres, was trains of 10 bursts of 5 pulses each (100 Hz burst frequency, 200 msec interburst interval). The TEST stimulus, activating separate subicular inputs, was a 5 Hz train of single shocks. The single shocks of the TEST input were given either superimposed on the middle of each burst of the COND input (*in phase*), or symmetrically between the bursts (*out of phase*).

In control experiments, the TEST stimuli alone induced no change and the COND stimuli alone induced homosynaptic LTP of the COND input without affecting the TEST input. In extracellular experiments, *out of phase* stimulation induced associative LTD at the TEST input (Δepsp slope = -25.1±2.1%, Δpop. spike = -44.2±3.7%, n=17). In contrast, 25μM bath applied AP3 blocked induction of LTD at the TEST input (Δepsp slope = +5.6±2.2%, Δpop. spike = -2.9±1.6%, n=13), without affecting LTP of the COND input. In intracellular experiments, pairing of hyperpolarizing current injection with synaptic stimulation elicited LTD (Δepsp slope = -26.4±4.6%, n=6), which was blocked by AP3 (Δepsp slope = +18.7±14.9%, n=8). Thus, a metabotropic quisqualate receptor may be involved in the induction of LTD.

276.21

EFFECTS OF CARBACHOL AND L-AP4 ON PAIRED-PULSE PLASTICITY IN THE PERFORANT PATHWAYS OF THE DENTATE GYRUS OF THE RAT. J. S. Kahle and C.W. Cotman, Department of Psychobiology, University of California, Irvine, CA 92717.

The pharmacological profile of paired-pulse (PP) plasticity in the dentate gyrus was studied in order to further define the mechanisms underlying these phenomena. Lateral and medial entorhinal layer II cells both terminate on dentate granule cells, but the lateral perforant path exhibits PP potentiation whereas the medial perforant path exhibits PP depression at interstimulus intervals (isi) between 40-800msec. Previously, carbachol (CARB; an acetylcholine agonist) has been shown to preferentially reduce medial perforant path field potentials. Conversely, L-2-amino-4-phosphonobutyric acid (L-AP4; a glutamate analog) has been shown to specifically reduce lateral perforant path field potentials. The effects of CARB and L-AP4 on PP plasticity as a function of isi were compared. Pairs of evoked extracellular field potentials were recorded differentially from the medial and lateral perforant pathways from rat hippocampal slices maintained *in vitro*.

In CARB (20μM), PP depression reversed to PP potentiation (30%) at short isi, was attenuated (50%) at intermediate isi, and not affected at longer isi. Furthermore, in CARB, PP potentiation tended to increase at short isi. In L-AP4 (10μM), PP depression was attenuated (50%) at intermediate isi, whereas PP potentiation increased (240%) at all isi investigated. The effects of CARB and L-AP4 were not due simply to the reduction of the field potential amplitude.

The effects of L-AP4 may be explained in part by the blockade of a late, slow inhibitory post synaptic potential that may be part of feed-forward inhibition onto the dentate granule cells. The effects of both CARB and L-AP4 on PP depression in the medial perforant path appear to be dependent on the pattern of activity of this pathway.

276.22

BETA-ADRENERGIC AGONIST-INDUCED LONG-LASTING SYNAPTIC MODIFICATIONS IN HIPPOCAMPAL DENTATE GYRUS REQUIRE ACTIVATION OF NMDA RECEPTORS, BUT NOT ELECTRICAL ACTIVATION OF AFFERENTS. D.Dahl, Univ Texas-Dallas and J.M.Sarvey, Uniformed Services University, Bethesda, MD.

Norepinephrine (NE) in the presence of an alpha-adrenergic receptor antagonist has differential effects on activation of granule cells by separate components of the perforant path (PP): medial PP responses potentiate (LLP) and lateral PP responses depress (LLD). LLP requires co-activation of the NMDA receptor subtype of glutamate, as D[-]APV and CPP, both antagonists, block its induction. D[-]APV and CPP also show a pathway selectivity limited to medial PP EPSPs.

It is not known whether induction of beta-adrenergic LLP or LLD requires electrical activation of the PP. Furthermore, although D[-]APV and CPP block NE-induced LLP, it is not known whether LLP or LLD induced by the specific beta-adrenergic agonist isoproterenol (ISO) is also blocked by NMDA receptor antagonists.

We have found that electrical stimulation of the PP is not required for LLP or LLD induction and that D[-]APV blocks ISO-induced LLP and LLD, but prior exposure to D[-]APV and ISO prevents induction of LLD, but not LLP, by a subsequent exposure to ISO. (Supported by NS 23865 to JMS.)

mRNA REGULATION: ENDOCRINE CONNECTION

277.1

INDUCTION OF TYROSINE HYDROXYLASE MRNA BY KCl DEPOLARIZATION IS SEX DEPENDENT. D.R. Studelska and K.L. O'Malley. Anatomy & Neurobiology, Washington Univ. Sch. Med., St. Louis, MO 63110.

Increased extracellular K+ has been reported to increase levels of tyrosine hydroxylase (TH) in sympathetic neurons. Here we report that this effect is restricted to males. Superior cervical ganglion (SCG) neurons were dissociated from male or female E21 rat fetuses and cultured on collagen in a medium containing 10% human placental serum, 2% chick embryo extract, and 20 ng/ml nerve growth factor. After 3 to 5 days, the cells were switched to a defined medium (N2) with or without 40 mM KCl. After 6 to 16 days in culture, total RNA was isolated and reverse-transcribed using oligonucleotide primers complementary to TH. Primers complementary to actin mRNA and 18S rRNA were used in parallel reactions as internal controls. The resulting cDNAs were amplified using the polymerase chain reaction. In 3 separate experiments K+ depolarization led to 5-fold increases in TH mRNA from males but not from females when compared with actin transcript levels. 18S rRNA levels were correlated with actin mRNA levels except in male neurons treated with KCl where 18S rRNA also exhibited a 5-fold increase with respect to actin mRNA. Estradiol treatment for 6 to 18 h had no effect on TH message levels in SCG neurons cultured from either sex. Together these results suggest that sex differences in SCG may be modulated transynaptically in vivo.

277.2

ANDROGEN AND ESTROGEN RECEPTOR mRNA EXPRESSION IN THE PREOPTIC AREA OF MALE AND FEMALE RAT BRAINS. J.N. BICKNELL, C.D. REFSDAL*, M.A. Miller and D.M. DORSA. GRECC, VAMC, Seattle, WA 98108.

We have examined sexual dimorphism of expression of androgen receptor (AR) and estrogen receptor (ER) mRNA in the rat brain. The brains of 60 day old male and female Wistar rats were hand dissected, the various regions pooled by sex, and RNA extracted. Total RNA was size fractionated on agarose formaldehyde gels and transferred to Nytran membranes and then hybridized with either an AR or ER randomly primed probe.

In previous studies we found that male rat brain contains two forms of AR mRNA (9.5kb and 11kb transcripts). Both forms were also evident in female brain. In both male and female preoptic area (POA), these two AR messages are present in about equal abundance. This pattern of expression is similar to the hippocampus, but different from the other brain areas which express the smaller message in greater abundance.

Estrogen receptor probe preferentially hybridized to a single band of RNA from the POA of both males and females and was equilvalent in size to that observed when probing RNA from the uterus. ER message levels were elevated in female POA when compared to that of the male. We propose that sex related differences in circulating gonadal steroids underlie sexual dimorphism of steroid receptor gene expression.

We conclude that the two isoforms of AR mRNA are present in the POA of male and female rat brain. In addition, ER mRNA levels may be greater in the POA of females than in males.

Supported by NS20311

277.3

DECLINE OF VASOPRESSIN mRNA AND IMMUNOREACTIVITY IN THE BNST FOLLOWING CASTRATION: A TIME-COURSE STUDY. M.A. Miller, D.M. Dorsa and G.J. DeVries. GRECC, VA Medical Center, Seattle, WA 98108, and Department of Psychology, University of Massachusetts, Amherst, MA 01003.

Vasopressin (VP) neurons in the bed nucleus of the stria terminalis (BNST) of the rat are steroid dependent. Castration of adult male rats makes VP cells virtually undetectable throughout the BNST by both immunocytochemical (ICC) and in situ hybridization (ISH) techniques. In this study, we have compared the decline over time of VP mRNA and VP-immunoreactivity in the BNST following castration. Male Wistar rats (90 d) were sacrificed as sham-operated controls or at 1,3,or 8 wks post-castration. All animals used for ICC and some animals used for ISH were pretreated with colchicine. The peak number of VP mRNA expressing cells sampled unilaterally in 20μm sections decreased significantly ($p \leq 0.01$) by 1 wk post-castration (37±4 vs. 5±3 , mean±SEM). The remaining cells exhibited a reduced number of grains/cell ($p \leq 0.01$). No VP expressing cells were detectable at 3 or 8 wks post-castration. Preliminary evidence suggests that colchicine treatment may significantly reduce grains/cell ($p \leq 0.05$) but not cell number. VP-immunoreactive cells (50μm section) were not decreased at 1 wk post-castration (64±12 vs. 46±9, mean±SEM) but were significantly reduced ($p \leq 0.001$) at 3 and 8 wks post-castration (10±3 and 6±1, mean±SEM, respectively). Fiber density showed a similar rate of decline. These results indicate that decreases in VP mRNA levels occur much more rapidly than changes in peptide content.

277.4

ESTROGEN RECEPTOR mRNA EXPRESSION IN RAT HYPOTHALAMUS AS A FUNCTION OF SEX AND ESTROGEN DOSE. A.H. Lauber, C.V. Mobbs, M. Muramatsu*, and D.W. Pfaff. Laboratory of Neurobiology and Behavior, Rockefeller University, New York, NY 10021.

Recent in situ hybridization experiments confirm our previous findings (Soc. Neurosci Abst, 1989, p. 984; J. Neuroendo, in press) that estrogen down-regulates estrogen receptor (ER) mRNA in female rat mediobasal hypothalamus. Here, we show that ER mRNA level is regulated according to EB dose and genetic sex. Ovariectomized (ovx) rats were treated with 10 ug estradiol benzoate (EB) for 0, 2, 6 or 18 hrs; gonadectomized males (gdz) for 0, 6 or 18 hrs. Tissue sections were hybridized with ^{3}H-single stranded DNA probe prepared from the region of the rat cDNA corresponding to the steroid binding domain. Relative ER mRNA level was assessed by counting the number of grains over cells in ventrolateral-ventromedial nucleus (VLVM), arcuate, dorsomedial nucleus (DM) and amygdala. Values were expressed as mean grains/cell after subtracting background (mean grains/cell in thalamus and cortex of the same brain section). There was no effect of sex or EB on ER message levels in DM or amygdala. EB decreased ER mRNA level in female VLVM by 42% (2 hr) to 55% (18 hr) and in the arcuate by 65% (6 hrs) to 74% (18 hr) as compared to ovx. Notably, gdz males had significantly lower ER mRNA levels than ovx females (51% VLVM; 56% arcuate) and EB failed to down-regulate significantly ER message level in males. In a separate study ovx rats were implanted for 2 wks with silastic capsules containing 0%, .1%, 1%, 10% or 100% estradiol. Estradiol induced a monotonic dose-dependent decrease in ER mRNA levels in females. Message levels declined in VLVM by 28% (1%).to 57% (100%) and in arcuate by 36% (1%) to 62% (100%). The data show regional specificity and sex differences in the relative level and hormonal regulation of ER mRNA expression. Further, ER mRNA down-regulation in females exhibits dose-dependence at a time point following EB treatment which ensures the system is at steady-state.

277.5

CORTICOSTERONE REGULATES CALBINDIN-D_{28K} mRNA AND PROTEIN IN RAT HIPPOCAMPUS. S. Christakos and A.M. Iacopino, UMDNJ-New Jersey Medical School, Newark, NJ 07103.

Corticosterone administration (10 mg/day for 7 days) to intact rats significantly increased levels of the 28,000 M_r calcium binding protein (calbindin) (43%) and calbindin mRNA (125%) in hippocampus. Adenalectomy (ADX) (animals were sacrificed 7 days after ADX) produced a significant decrease in hippocampal calbindin protein (85%) and mRNA (80%) compared to intact controls. Immunocytochemical analysis of tissue sections indicated a marked depletion of calbindin immunoreactivity in CA_1 and the dentate gyrus of the hippocampus 2 weeks after ADX. When ADX rats were treated with corticosterone, calbindin protein and mRNA levels in hippocampus were restored to levels observed in intact controls. No changes in calbindin in kidney, cerebellum, striatum or cerebral cortex were noted in ADX rats or in intact rats treated with corticosterone when compared to controls, indicating the specificity of the effect on calbindin for the hippocampus. Since CA_1 and the dentate gyrus of the hippocampus contain the highest concentration of brain corticosterone type I receptors, our findings suggest glucocorticoid receptor mediated regulation of hippocampal calbindin gene expression. These studies present the first evidence of a hormonal regulator of calbindin gene expression in the brain.

277.6

REGULATION OF VASOPRESSIN EXPRESSION IN CULTURED MAGNOCELLULAR NEURONS BY GLUCOCORTICOIDS Ch. Pilgrim[1], P. Oeding[2]*, H. Schmale[2] and K. Schilling[1] Abt.Anatomie und Zellbiol.[1], Univ.Ulm, D-7900 Ulm, and Inst.f.Zellbiochem.[2], Univ. Hamburg,D-2000 Hamburg, Fed. Rep. Germany.

We used primary diencephalic cultures to investigate why adrenalectomy-induced up-regulation of vasopressin (VP) expression affects only parvocellular but not magnocellular (MC) hypothalamic neurons. In cultures derived from 14 day old fetal rats, which contain MC but no parvocellular VP neurons, selective neutralization of glucocorticoids (GC) in the culture medium by the drug RU 38 486 increased numbers of MC VP cells and levels of VP mRNA 2-3-fold. This effect was specific for VP neurons , as neither the expression of oxytocin nor of general neuronal marker proteins was affected by RU. RU was not mitogenic for VP neurons. RU increased VP expression also in cells that were grown in 14 mM Mg^{++}, showing that the effect was not mediated transsynaptically. These results show that GC can regulate VP expression in MC hypothalamic neurons. The absence of such an effect in vivo may be best explained by an inhibitory synaptic input, which is lacking in vitro. Supported by the DFG (Ri 192/17-6, Schi 271/1-2).

277.7

REGULATION OF POMC mRNA BY DEXAMETHASONE AND 8-Br-cAMP IN ANTERIOR AND INTERMEDIATE LOBES OF THE RAT PITUITARY DURING EARLY POST-NATAL DEVELOPMENT. R.E.M. Scott and J.E.Pintar. Department of Anatomy and Cell Biology, Columbia University P&S, NY, NY. 10032.

POMC RNA synthesis in the adult anterior lobe (AL) is under negative regulation by glucocorticoids. However, glucocorticoids have very little, if any, positive or negative effect in the adult intermediate lobe (IL). The non-responsiveness of the IL to glucocorticoids is thought to result from a lack of functional glucocorticoid receptors in this lobe. Using the reverse haemolytic plaque assay, our laboratory has previously shown that the glucocorticoid analogue dexamethasone (DEX) inhibited CRH stimulated secretion from dispersed melanotrophs at fetal and early post-natal ages but that this inhibition disappeared at p3.

Solution hybridization/RNA protection assays with a POMC cRNA probe were used to measure changes in neurointermediate lobe (NIL) and AL POMC mRNA levels in short term cultures in response to 8-Br-cAMP and DEX at early post-natal ages. In the AL, at p1, 8-Br-cAMP (1mM) increased POMC mRNA levels above control. Incubation with DEX (10^{-6}M) for 1 hour prior to addition of 8-Br-cAMP inhibited the cAMP mediated increase in POMC mRNA, which remained at the level of control. Similar results were observed at p10, where 8-Br-cAMP alone increased POMC mRNA levels while addition of DEX inhibited this increase. In the NIL, at p10, 8-Br-cAMP increased POMC mRNA levels, but DEX inhibition of 8-Br-cAMP mediated increase was not detected. However, at p1, 8-Br-cAMP mediated increase in POMC mRNA was inhibited by pretreatment with DEX and POMC mRNA levels remained at the level of control. These results, along with those reported earlier, indicate that corticosteroids effect both POMC secretion and mRNA levels in the NIL at early post-natal ages but that the responsiveness disappears as the IL undergoes a maturation process that is closely linked with the arrival of the dopaminergic input into the IL at p3.

277.8

COLD INCREASES THYROTROPIN-RELEASING HORMONE mRNA LEVELS IN PARAVENTRICULAR NUCLEUS DESPITE ELEVATED T_3. R.T. Zoeller and H.E. Albers. Dept. Anat/Neurobiol., Univ. Missouri Sch. Med., Columbia, MO and Dept Biol. Georgia State Univ, Atlanta, GA

TRH neurons are found throughout the CNS; however, TRH neurons in PVN control pituitary-thyroid function. TRH mRNA levels in PVN appear linked to neuronal activity: hypothyroidism increases TRH secretion and TRH mRNA levels, and thyroid hormones (T_3) reverse these effects. Cold exposure increases thyroid activity perhaps by activating PVN TRH neurons. If so, TRH mRNA levels should become elevated in PVN after cold. We exposed male Sprague-Dawley rats to 5°C for 6, 24, 30, and 48 hours and measured plasma TSH and T_3 by RIA and TRH mRNA by in situ hybridization (ISH). ISH allowed us to measure single cell levels of TRH mRNA and multiple mRNAs in the same animal. TSH and T_3 were elevated in plasma after 6h of cold, and TRH mRNA was elevated in PVN compared to controls maintained at 25°C. The effect of cold was specific for TRH mRNA in PVN: TRH mRNA levels were not altered in thalamus, and β-actin and oxytocin mRNA levels were not altered in PVN. TSH and T_3 in blood and TRH mRNA in PVN were higher in 25°C controls at 24h than in 25°C controls at 6h, and cold did not produce a further increase. This pattern was repeated at 30h and 48h cold. The 24-h profile of TRH mRNA changes in PVN of resting animals corroborated these findings. Thus, time of day, not duration of cold, was the important variable in observing an effect of cold on TRH expression. We found no evidence that cold activates a subset of TRH neurons separate from those inhibited by T_3. These findings indicate that TRH gene expression in PVN neurons is linked to neuronal activity which is regulated hormonally (T_3) and neurally (cold), and these signals interact within TRH neurons of the PVN during the physiological response to cold.

277.9

EFFECT OF STARVATION ON GROWTH HORMONE mRNA LEVELS IN GENETICALLY OBESE AND LEAN ZUCKER RATS. J. A. Finkelstein and I. Ahmad. Dept. of Anatomy, N.E. Ohio Univ. Coll. Med., Rootstown, OH 44272.

Plasma growth hormone (GH) levels are known to be diminished by both starvation and obesity. In genetically obese Zucker rats, the decrease in plasma GH is associated with a decrease in GH gene expression as measured by the steady state level of GH mRNA. The purpose of the present study was to investigate the effects of starvation on GH mRNA levels in both lean and obese Zucker rats. Groups of 5-month-old male genetically obese (fa/fa) (mean body weight 560 g) and lean (Fa/-) (380 g) rats were studied. Half of the animals of each genotype were deprived of food for 72 hours, and the other half were not. After sacrifice, the pituitaries were removed and processed for determination of GH mRNA levels using the dot blot technique. The largest difference was seen between the two ad libitum groups, with the obese animals having 55% lower level of pituitary GH mRNA. Starvation resulted in similar decreases in GH mRNA levels in the lean group (26%), and the obese group (23%). Therefore, the chronic metabolic abnormalities associated with genetic obesity are more effective in lowering GH mRNA levels than those associated with an acute bout of food deprivation.

277.10

EFFECT OF ANESTHESIA AND ADRENALECTOMY ON HEAT-INDUCED HSP70 mRNA EXPRESSION IN BRAIN REGIONS MEDIATING THE NEUROENDOCRINE STRESS RESPONSE.
M.J. Blake, D.D. Norton* and N.J. Holbrook*. Lab. of Mol. Genet., NIA/ Geront. Res. Cntr., Baltimore, MD 21224

We have recently demonstrated that exposure of animals to increased ambient temperatures induces the expression of the cellular stress protein, HSP70, in several regions of the rat brain known to coordinate the neuroendocrine stress response. Areas showing selective expression included the paraventricular nucleus, dorsomedial hypothalamic nucleus, supraoptic nucleus, dentate gyrus, and medial habenula, as well as choroid plexus, cerebellum and a global expression in the epithelial cells of the cerebral microvasculature. In order to delineate the neural mechanisms responsible for this site-selective HSP70 mRNA expression following heat-stress, adrenalectomized (ADX) and control rats given either saline or pentobarbital injections were exposed to an ambient temperature of 40^{0}C for 90 min and subsequently processed for in situ hybridization. Adrenalectomy did not alter the ability of unanesthetized rats to thermoregulate in response to heat-stress. HSP70 mRNA expression in unanesthetized ADX and Control rats was restricted to the paraventricular nucleus, choroid plexus, cerebellum and microvasculature. Pentobarbital injections resulted in a reduced ability of both ADX and Control rats to thermoregulate as evidenced by a rapid rise in colonic temperature (T_c) that continued throughout the duration of exposure. The increased T_c attained by anesthetized control rats resulted in the addition of the dentate gyrus, medial habenula, and dorsomedial hypothalamus to areas showing heat-induced HSP70 mRNA expression. However in ADX rats these additional areas were not recruited. The blunting of thermoregulatory mechanisms by pentobarbital and the removal of endocrine factors by adrenalectomy greatly affects which brain regions display HSP70 mRNA expression in response to heat-stress.

GENE STRUCTURE AND FUNCTION III

278.1

THE GENOMIC ORGANIZATION OF HUMAN MAO B GENE.
Joseph Grimsby*, Kevin Chen*, Nancy Lan and Jean C. Shih. Div. Biol. Sci., Sch.of Pharm., Univ. of South.California, L.A. Calif. 90033

Monoamine oxidase A and B (MAO A and B) play an important role in oxidative deamination of neuroactive and xenobiotics amines including the parkinsonism-producing neurotoxin MPTP. These two forms of the enzyme are defined by their substrate and inhibitor specificities. The deduced amino acid sequences from the previously cloned human liver MAO A and B cDNAs share 70% identity and they may be derived from separate genes (PNAS, 85, 4934-38, 1988). This report describes the genomic organization of human MAO B gene. Subfragments of human MAO B cDNA were ^{32}P-labeled to screen a partial digested human multiple X chromosome library. The complete MAO B gene, contained in 7 overlapping λ clones, has 15 exons and 14 introns spanning larger than 40 Kb. Positive Hind III or EcoR1 genomic fragments were subcloned into pUC 19 and extensively mapped. Conveniently sized positive fragments ~ 1 Kb were then subcloned into M13 and sequenced. The exon-intron organization, the structural significance of the exon boundaries, and the functions of protein domain encoded by individual exons will be discussed. (Supported by NIMH grants R37 MH39085 (Merit Award), K05 MH00796 (Research Scientist Award), and Welin professorship).

278.2

THE ROLE OF CYSTEINE RESIDUES IN MAO B CATALYTIC ACTIVITY.
H.F. Wu, K.Chen*, N. Lan and J.C. Shih. Divi. Biol. Sci., Sch.of Pharm., Univ. of Southern Calif., L.A., Calif. 90033

We and others have shown previously that sulfhydryl reagents like N-ethylmaleimide, iodoacetamide and 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) inhibited purified bovine MAO B activity. These results suggested that cysteine residues may be important to MAO B catalytic activity. The deduced amino acid sequence from human liver cDNA encoding MAO B showed that there are 9 cysteine residues in MAO B (PNAS,85,4934,1988). We report here the role of each cysteine studied by site-directed mutagenesis. We altered each cysteine by oligonucleotide-directed mutagenesis. Each mutagenic clone was sequenced then cloned into an expression vector, pECE. High-efficiency $CaPO_4$ precipitation transient transfection was performed. The amount of MAO B mutants transfected was determined by Western blotting, and the catalytic activity was determined by radio-assay using ^{14}C-phenylethylamine as substrate. Our results indicate that some mutants exhibited no activity, some with partial activity,and some with full activity when compared with the controls. The role of each cysteine residue in MAO B catalytic acitivity will be discussed (Supported by NIMH grants R37 MH 39085 (merit award) and KO5 MH00796 (research scientist award); and Welin professorship).

278.3

ISOLATION OF SEQUENCES AT OR NEAR THE 5' ENDS OF THE HUMAN MAO A AND B GENES. R. M. Denney, Sanat Dave* and Abha Sharma*. Dept. of Human Biological Chemistry and Genetics, University of Texas Medical Branch, Galveston, TX 77550.

Monoamine oxidases (MAO) A and B oxidize catecholamine and indoleamine neurotransmitters and are encoded by X-linked genes in man. We screened a human X chromosome-enriched, genomic library with synthetic oligonucleotides representing the most 5', untranslated 48 nucleotides of the human MAO A and B cDNAs (Bach et al. PNAS 85:4934, 1988). For comparison, protein coding sequences start at nucleotides 75 and 78 of the MAO A and B cDNAs, respectively. Both the MAO A 5' 48-mer and a cDNA fragment (nucleotides 96-552, protein-coding sequence) hybridize to a .67 kb Hae II-Pst I fragment from the 16 kb putative MAO A clone, which therefore may encode some or all the 5'-untranslated region and the first protein-coding exon of MAO A cDNA. The MAO B 5' 48-mer hybridizes to a small Hae II fragment (ca. 200 bp) within the putative, 15 kb MAO B clone. Proximity of a Hae II site to the 48-mer hybridizing region of the MAO B clone is interesting, since MAO B cDNAs (Bach et al. 1988; our laboratory) have a Hae II site 28 bp downstream from the 48-mer. Sequencing and transfections with appropriate reporter constructs are needed to test whether these putative 5' MAO A and B genomic fragments contain promoters and cis controlling elements involved in cell type-specific expression of MAO A and B. [Supported by NS 19543.]

278.4

ISOLATION AND CHARACTERIZATION OF A PARTIAL cDNA FOR A PLASMA MEMBRANE CALCIUM-ATPASE FROM EXCITABLE TISSUES.
P. Brandt, T.C. Vanaman*, R.L. Neve. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717 and Dept. of Biochemistry, University of Kentucky, Lexington, KY 40536.

Complementary DNA (cDNA) clones for the 3'-terminus of a unique isoform of the plasma membrane Ca^{2+}-ATPase, designated PMCA5, have been isolated from bovine brain and human fetal brain cDNA libraries. The deduced protein sequences contain putative calmodulin-binding and transmembrane domains, as well as a potential recognition site for N-glycosylation. The transmembrane domains were similar in structure to those of the sarcoplasmic reticulum Ca^{2+}-ATPase which are thought to be involved in high affinity Ca^{2+}-binding and transport. Examination of a gene fragment corresponding to this region of human PMCA5 showed that an intron separates the proposed regulatory domain from the calmodulin-binding domain. The mRNA for the PMCA5 isoform was localized in excitable tissues by polymerase chain reaction amplification of cDNA synthesized from tissue-specific RNAs. Further examination of the tissue distribution of other plasma membrane Ca^{2+}-ATPase isoform mRNAs by this method showed that PMCA1 was localized exclusively in tissues of the central nervous system. PMCA2 mRNA was localized in these tissues as well as in liver and skeletal muscle. Analysis of the tissue distribution and developmental expression of additional Ca^{2+}-ATPase isoforms is in progress.

278.5

ISOLATION AND CHARACTERIZATION OF TWO NOVEL PITUITARY cDNAS HOMOLOGOUS TO KEX2 AND FURIN: CANDIDATES ENCODING PROHORMONE PROCESSING PROTEINASES. L. Gaspar, P. Mion*, M. Marcinkiewicz*, M. Mbikay*, M. Chrétien and N.G. Seidah*. J.A. DeSève Labs of Molecular and Biochemical Neuroendocrinology, Clinical Research Institute of Montreal, Montreal, Que. H2W 1R7

In neuroendocrine cells specific converting enzymes are responsible for the processing of prohormones. One of such candidates, the yeast KEX2, has been characterized at the molecular level. More recently, human furin was shown to be a possible mammalian counterpart to KEX2. We applied the technique of PCR to mouse pituitary cDNAs using oligonucleotide primers designed according to the well conserved active sites of subtilisins Ser* and Asn* found in human furin. Two distinct but similar cDNA sequences (mPC1 and mPC2) were isolated and completely characterized from a mouse pituitary cDNA library. The sequences showed ≥ 50% homology to KEX2 and human furin. Northern blot hybridization revealed mRNAs 3.0 and 2.8 kbp in sizes, respectively. By in situ hybridization and Northerns mPC1 was found mostly in pituitary, adrenals, hypothalamus and brain as well as in AtT20 pituitary tumor cell line. mPC2 transcripts are abundant in pituitary, brain, GH3 tumor cells and pancreatic beta-T3 tumor cells but relatively much rarer in AtT20 cells. The hybridization signals were absent from cells which normally do not process polypeptide prohormones.

278.6

PRELIMINARY CHARACTERIZATION OF THE HUMAN THYMOSIN BETA 10 GENE AND ITS EXPRESSION IN THE DEVELOPING HUMAN BRAIN. Michael R. Condon[1*], Thomas W. Lysz[2], Joseph J. Seebode[1*] and Alan K Hall[1*]. *1 Urology Research Laboratory and 2 Department of Surgery, UMDNJ, Newark, N.J., 07103 U.S.A.

Two overlapping cDNA's of the human thymosin beta 10 (B10) gene were used to investigate its genomic structure, and expression in the developing human brain. Restriction mapping, revealed a pattern consistent with multiple genes and/or introns. However, using the gene specific 3' non-translated (NT) region of the human cDNA a single Eco RI fragment of approximately 10 kilobase (Kb) was observed, suggesting the B10 gene may be composed of a single exon. Previous studies by us, have shown the B10 peptide isolated from fetal and adult human brain tissues is subject to developmental regulation. Hybridization analysis of total RNA from human fetal and adult brain tissues with the 3' NT cDNA fragment, detected a mRNA species of approximately 600 nucleotides, with the highest levels of expression occurring in the fetal brain and declining to near undetectable levels in the adult brain tissue. Present studies are aimed at the isolation and identification of the promoter/enhancer region of the human B10 gene. (Supported by NIH/NCI grant CA49422-01 to A.K. Hall)

278.7

STRUCTURAL ORGANIZATION AND SEQUENCE OF THE HUMAN CILIARY NEUROTROPHIC FACTOR GENE. B.Cordell, A.Lam*, F.Fuller, J.Miller*, S.Varon+, and M.Manthorpe+. California Biotechnology Inc. 2450 Bayshore Pkwy, Mountain View, CA 94043; +Dept. Biology, UCSD, La Jolla, CA 92093.

Ciliary Neurotrophic Factor (CNTF) is a potent polypeptide hormone whose actions appear to be restricted to the nervous system where it promotes survival, neurotransmitter synthesis and neurite outgrowth in responsive neuronal populations. Recently cDNAs encoding rat and rabbit CNTF have been described (Stockli, K.A., et al, Nature, 342:920, 1989; Lin, L.F.H., et al, Science, 246:1023, 1989). We have cloned the gene encoding human CNTF which appears to be a unique copy gene with a simple genetic organization - the coding domain is interrupted by only a single intron. The chromosomal localization of the human CNTF gene has also been determined. The CNTF protein has been well conserved in evolution with amino acid sequences of rat and rabbit sciatic nerve CNTFs displaying ~85% homology to the inferred amino acid sequence for human CNTF.

278.8

ISOLATION AND CHARACTERIZATION OF THE MOUSE CORTICOTROPIN-RELEASING HORMONE GENE. A.F. Seasholtz, F. Bourbonais*, T. Saunders*, S. Keller*, and S.A. Camper*. Mental Health Research Institute and Dept. of Human Genetics, University of Michigan, Ann Arbor, MI 48109

The mouse corticotropin-releasing hormone (CRH) gene has been isolated from a mouse Balb/c liver genomic library and characterized by DNA sequence analysis. The gene exhibits a structural organization similar to that of the human, rat and ovine CRH genes. The mouse CRH peptide exhibits 100% conservation to the rat and human CRH peptides at the amino acid level, with only 3 nucleotide changes from the rat sequence in the peptide-encoding region. Additionally, the mouse CRH gene displays extensive nucleic acid homology to the rat CRH gene in the 5' flanking sequence.

The sequence information from the mouse CRH gene is being used to localize transcriptional control elements. Central to these studies is the use of transgenic mice for the identification of tissue-specific DNA elements in the rat CRH gene. The information gained from the mouse CRH gene sequence allows the production of specific probes which can discriminate the endogenous mouse CRH gene and mRNA from the mRNA produced from the injected rat CRH gene or rat CRH-reporter gene constructs.

Supported by ADAMHA Small Grant 1 R03 MH46532 to S.A.C. and A.F.S.

278.9

SEQUENCE AND EXPRESSION OF THE RAT MELANIN CONCENTRATING HORMONE (MCH) GENE. Robert C. Thompson, Sharon Burke*, Huda Akil and Stanley J. Watson. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109-0720

Melanin concentrating hormone (MCH) is a neuropeptide first identified in the salmon where it appears to be involved in the regulation of body color. Additionally, this peptide has been suggested to play a role in the hypothalamic-pituitary axis. The amino acid sequence (Kawauchi, et al. 1983) of this peptide was confirmed through the cloning and sequencing of cDNA clones from this species which contains two MCH encoding mRNAs (Ono, et al. 1988 and Minth, et al. 1989). Recently, the mammalian MCH peptide has been isolated, sequenced (Vaughan, et al. 1989) and the protein precursor deduced from isolated cDNA clones in the rat (Nahon, et al. 1989). This work suggested that in addition to MCH, other peptides could be derived from this protein precursor (NEI, NGE). These same authors have described the anatomical localization of MCH- and NEI-like immunoreactivity in the lateral hypothalamic region (Nahon, et al. 1989). Our laboratory has also been interested in this lateral hypothalamic region and thus MCH-precursor derived neuropeptides. To further our understanding of this neuropeptide and its relationship to both magnocellular components as well as other peptide-regulators of pituitary functions, we isolated rat hypothalamic cDNA clones and used these clones as hybridization probes to isolate the rat MCH gene. We report here the complete nucleotide sequence of the rat MCH gene and examine the central nervous system expression by Northern blot and in situ hybridization analysis. This work supported by NIMH Fellowship MH09904-01 to R.C.T. and NIMH MH422251-04 to S.J.W. and H.A..

278.10

IDENTIFICATION OF AN ANTI-CYTOCHROME P-450$_{LA\omega}$ IMMUNOREACTIVE PROTEIN AND ITS cDNA IN RAT BRAIN. S.A. Signs, M.D. Schechter and J.P. Hardwick*, Depts. of Pharmacology and Biochemistry, Northeastern Ohio Univ. College of Medicine, Rootstown, Ohio 44272

Antibody raised against rat hepatic lauric acid ω-hydroxylase (cytochrome P-450$_{LA\omega}$) was used to screen a λ gtll expression library constructed from Sprague-Dawley rat brain mRNA. Immunoreactive fusion proteins were identified using alkaline phosphatase-conjugated goat anti-rabbit IgG and upon plaque purification, seven recombinant cDNA clones were isolated all of which cross-hybridized with the largest cDNA of the group; a cDNA insert of 3200 basepairs (ω-9). ^{32}P-labeled ω-9 cDNA hybridized with a single mRNA of approximately 4000 bases on a Northern analysis of total RNA isolated from whole rat brain as well as from dissected striatum, hippocampus and cerebellum. Anti-cytochrome P-450$_{LA\omega}$ antibody was also used for Western analysis of rat brain subcellular protein fractions. SDS-PAGE resolved a prominent immunoreactive protein (M_r=55,000)in rat brain synaptosomes, mitochondria, and plasma membranes (less abundant), but absent from microsomes. Synaptosomal preparations from rats treated for 4 days with chlofibric acid (100 mg/kg/day) or ethanol (12g/kg/day) exhibited increased immunoreactivity to anti-cytochrome P-450$_{LA\omega}$. Studies are ongoing to examine the role of this gene and its protein in central nervous system eicosanoid metabolism.

278.11

CLONING AND PARTIAL CHARACTERIZATION OF A PUTATIVE cDNA ENCODING HUMAN BRAIN SEPIAPTERIN REDUCTASE (SR).
R.A. Levine[1,2], J. Solus[3], S. Goustin[3], S. Tait[1*], S.K. Demetriou[1*], B. Citron[4], and S. Kaufman[4]. [1]Laboratory of Molecular Neurobiology, Lafayette Clinic and [2]Department of Psychiatry, Wayne State University, Detroit, MI; [3]Center for Molecular Biology, Wayne State University; and [4]Laboratory of Neurochemistry, National Institutes of Mental Health, Bethesda, MD.

Sepiapterin reductase catalyzes the final reaction in the synthesis of tetrahydrobiopterin (BH_4), the essential cofactor for tyrosine and tryptophan hydroxylase. Cloning of sepiapterin reductase and other BH_4-related genes will assist in studying the coordinate regulation of BH_4 and biogenic amine synthesis. Anti-SR serum was used to isolate a positive clone upon screening a lambda ZAPII rat liver cDNA library. Eco RI digestion of the insert yielded 2 bands (.67 and .48 kb). The P-32 labelled 0.67 kb rat cDNA fragment was used to screen a lambda ZAP human frontal cortex cDNA library. Following screening of 300,000 clones (40% formamide, 42°C), one positive clone was identified on duplicate filters and remained positive upon re-screening. Following plasmid isolation and Eco RI digestion, the excised inserts were 1.67 kb on agarose minigels. A southern blot of insert DNA was then probed with the P-32 labelled .48 kb Eco RI fragment of the rat SR-cDNA, which also hybridized with the excised insert, thus helping validate the authenticity of the putative human SR-cDNA. The size of 1.67 kb for the putative SR-cDNA is larger than the minimum predicted size of the full-length rat SR-cDNA (1.2 kb), based on rat SR-cDNA sequence information and the molecular weight of rat sepiapterin reductase. Thus, we may have obtained the full-length human SR-cDNA. This will be determined through sequencing the insert, which is currently underway.

278.12

ISOLATION OF G-PROTEIN COUPLED RECEPTOR cDNAs FROM LOCUS COERULEUS BY PCR.
J. M. Rimland, E. J. Nestler and R. S. Duman. Laboratory of Molecular Psychiatry, Depts of Psychiatry and Pharmacology, Yale Univ. Sch. Med., New Haven, CT 06508.

We are interested in isolating and characterizing cDNA clones for a number of G-protein coupled receptors enriched in the locus coeruleus (LC), particularly the VIP, CRF, μ-opiate and GABA-B receptors. The approach we used was the *in vitro* amplification, by the polymerase chain reaction (PCR), of cDNA derived from bovine LC mRNA. Two degenerate oligonucleotide primers, containing the consensus nucleotide sequences for conserved amino acids in transmembrane regions III and VI of the G-protein coupled receptor family were used to selectively amplify such receptors. The amplified cDNAs were blunt-end cloned into pBluescript to create a PCR cDNA library. Various clones were screened for inserts using PCR and four clones were chosen for further study. Partial DNA sequencing revealed that all four clones had predicted amino acid sequences with some degree of homology to other members of the G-protein coupled receptor family. PCR clone 3 is 92% similar to the human β_2-adrenergic receptor and thus is considered to be the bovine equivalent of this ß-adrenergic receptor subtype. PCR clones 1, 2, and 9 have 42-65% homology and similar hydrophobicity plots to other members of the G-protein coupled receptor superfamily, making them candidates for novel receptors. We are currently screening a LC cDNA library to obtain the complete coding sequence of each clone. The full length clones will then be expressed so that the nature of their receptor binding and functional properties can be determined. (Supported by USPHS grant MH45481)

278.13

CLONING AND ANALYSIS OF THE PSEUDOGENE FOR HUMAN EPINEPHRINE SYNTHESIZING ENZYME, PHENYLETHANOLAMINE N-METHYLTRANSFERASE(PNMT).
Y.H. Suh, I.S. Park*, W. Choi*, J.I. Woo* and C. W. Park*. Dept. of Pharmacol., Seoul Nat. Univ. College of Med., Seoul 110-460, Korea

A intronless pseudogene for human phenylethanolamine N-methyl transferase(PNMT), terminal enzyme catalysing the final step in the catecholamine biosynthetic pathway, was isolated from a human recombinant DNA library by hybridization with a bovine cDNA probe and characterized in detail. This gene has five distinct sequence characteristics found in processed pseudogene. First, this gene lacks completely the intervening sequences. Second, this gene is truncated at the 5' end peptide encoding region by 433 base pairs. Third, the 502 base pairs of this gene containing poly(A) singnal are completely identical to the 3' half of mRNA encoding region of functional gene. Fourth, this has a poly(A) tail. Fifth, this gene is flanked by direct repeat of 6 base pairs. Here we report the complete sequence of a human pseudogene for phenylethanolamine N-methyltransferase and this is the first report of cloning of pseudogene for catecholamine biosynthetic enzymes.

NEUROGLIA AND MYELIN II

279.1

ELECTROPHYSIOLOGICAL AND NEUROCHEMICAL RESPONSES OF THE FASCIA DENTATA TO MICRODIALYSIS WITH HIGH K^+: ROLE OF GLIA.
J.C.Szerb. Dept.of Physiol.& Biophys., Dalhousie U. Halifax, N.S.B3H 4H7.

Transmitter release is often evoked in vivo by microdialysis with high K^+. To see the effects of high K^+, maximal extracellular EPSP in the fascia dentata due to 0.1 Hz angular bundle stimulation was established in anesthetized rats. The tip of a 3 mm BAS microdialysis probe was lowered 1.5 mm below and 1.5 mm caudal to the recording electrode. Perfusion (3.4 μl/min) with 25 mM KCl reduced glutamine (GLN) release without changing that of glutamate (GLU) or the EPSP. In about half of the experiments with 50 mM KCl several large (40-60 mV) spreading depression-like waves (SDW) appeared while EPSP-s nearly disappeared. GLU release was increased about three fold, whether SDW-s were present or not. 100 mM K^+ regularly induced 6-8 SDW-s during 30 min and about 8 fold increase in GLU release. Perfusion with 20 mM fluoroacetate (FAA), an inhibitor of glial Krebs cycle, reduced the spontaneous release of GLN and GLU and SDW-s appeared even with 25 mM K^+, accompanied by a suppression of EPSP-s. Results suggest increased release of transmitters, evoked by perfusion with 50 or 100 mM KCl, is accompanied by spreading depression and inhibition of electrical activity. The effects of lower conc. of K^+ are limited by the activity of glia. (Supported by the MRC of Canada).

279.2

PURIFICATION OF A MEMBRANE-ASSOCIATED PROTEIN MITOGENIC FOR SCHWANN CELLS.
M. Nordlund, X. Fei, D. Hong and N. Ratner. Department of Anatomy and Cell Biology, Univ. of Cincinnati Col. Med., Cincinnati, OH 45267.

Neuronal membranes contain a heparin binding mitogen for Schwann cells (Ratner, N., PNAS 85:6992-6996). We have partially purified this mitogen from fetal cow brain membranes by heparin affinity chromatography, carboxymethyl ion exchange chromatography and elution from non-denaturing 12% polyacrylamide SDS gels. The mitogen has an apparent molecular weight of 50,000 daltons.

Mitogen eluted from polyacrylamide gels retains its ability to stimulate proliferation of cultured rat Schwann cells, but is not mitogenic for NR6-3T3 cells, which respond to the heparin-binding mitogens acidic and basic FGF. These data confirm that the neuron-derived growth factor (NDGF) is distinct from acidic and basic FGF.

Partial characterization of NDGF has revealed that 1) NDGF activity is abolished following reduction by 2-mercaptoethanol 2) NDGF activity is also extremely sensitive to exposure to pH<6 and >8 3) NDGF activity can be stabilized by low levels of the detergent Tween-20 during purification. These characteristics, together with our ability to purify large quantities of mitogen from bovine CNS, should facilitate purification of NDGF to homogeneity.

Supported by the National Multiple Sclerosis Society.

279.3

MICROGLIA ORIGIN: NEW INSIGHTS WITH VAULT IMMUNOFLUORESCENCE. D. C. Chugani, N. L. Kedersha* and L. H. Rome. * UCLA School of Medicine, Los Angeles, CA 90024.

The developmental appearance of microglia in rat brain has been examined by immunofluorescent localization of vaults, recently described ribonucleoprotein particles (Kedersha and Rome, 1986). In brain, vault antiserum is highly specific for both ameboid and ramified microglia. The developmental profile of vault immunoreactivity (IR) in rat brain slices suggests that microglia enter the brain by 2 routes with different time scales for each. The first migration, which begins before embryonic day 15 and subsides between postnatal days 7 and 14, was identified by vault-IR and Bandeiraea simplicifolia B4-isolectin. Cells appear to enter from blood vessels and display a ramified morphology as soon as they are detected in brain. The second microglial migration occurs in the first postnatal week when ameboid microglia appear in large fiber tracts. Ameboid microglia appear to differentiate into ramified microglia between postnatal days 4 and 14. Vault IR, as a very early microglial marker, provides new insight regarding the much debated origin of the ramified microglia. It is quite clear that ameboid cells are not the sole source of ramified microglia since ramified cells can be detected before the influx of ameboid microglia. Colocalization studies with monocyte/macrophage markers ED1 and OX42 show that both ramified and ameboid microglia originate from monocyte lineage. (Supported by NIH NS15654, GM38097, HD 06576; DOE DE-FC03-87ER60615)

279.4

HIGH MOLECULAR WEIGHT POLYPEPTIDES RELATED TO GLIAL FIBRILLARY ACIDIC PROTEIN (GFAP) ISOLATED FROM MAMMALIAN SPINAL CORD. G. Shaw* and J. Hawkins. Department of Neuroscience, JHM Health Center Box J-244, Gainesville FL 32610, USA.

A group of proteins of apparent molecular weight around 140kDa have been identified in cytoskeletal preparations of spinal cords from several different mammalian species. These proteins are recognized by several antibodies to distinct epitopes on GFAP and by anti-IFA, a pan specific antibody to intermediate filament subunits, but are not reactive with antibodies to neurofilament subunits or vimentin. The proteins coelute with GFAP in ion-exchange chromatography, cosediment with GFAP under polymerization conditions and have an amino acid profile close to that of pure GFAP. They produce very similar chemical digests to that obtained with GFAP. They are unaffected by disulphide reducing agents and are also found in material prepared rapidly in the presence of transglutaminase inhibitors. The simplest explanation for their origin is that they are formed by in vivo covalent multimerization of GFAP protein, possibly under the activity of endogenous transglutaminases.

279.5

DEGRADATION PRODUCTS OF MYELIN PROTEINS IN A CNS SUBCELLULAR FRACTION. H. Persson. Dept. of Anatomy, Section of Neuroanatomy, University of Göteborg. Box 33031, S-400 33 Göteborg, Sweden.

The fraction floating on 0.32 M sucrose when normal mammalian spinal cord homogenate after discontinuous density gradient centrifugation is highly enriched in Marchi-positive material. *In situ* this material is located along paranodal myelin sheath segments. We show here by immunoblotting that a 26 kDal degradation product of the myelin oligodendrocyte glycoprotein (MOG) is present in the Marchi-positive floating fraction which is not found in the myelin fraction. Also a monoclonal antibody, FD1, has been produced which in myelin binds to a protein of a MW of 40-41 kDalton and in the floating fraction to an additional band with a MW of 25 kDal. This protein is absent from other membrane fractions in the CNS. Immunohistochemistry showed that this antibody bound preferrably to paranodal regions of myelin sheaths and cells identified as oligodendrocytes by a monoclonal antibody against galactocerebroside (kindly provided by Dr. Barbara Ranscht). Previous biochemical analyses of the floating fraction have demonstrated a gross composition closely resembling myelin and the presence of degradation products of MAG (myelin-associated glycoprotein) and CNP (2',3' cyclic nucleotide 3'-phosphodiesterase) has been established in earlier studies (Persson, H., and Cornelius, O., Neurochem. Res. 12:1177, 1989). These data in conjunction with metabolic studies showing the specific activity of incorporated amino acids to proceed with time from heavier to lighter myelin subfractions (Smith, M.E., and Benjamins, J.A. Model systems for study of perturbations of myelin metabolism. In Morell, P. (ed.), Myelin, Plenum Press, New York 1984, pp. 441-487) strongly suggest that normally occurring Marchi-positive bodies represents an intermediate stage in myelin catabolism.

279.6

THREE-DIMENSIONAL FINE STRUCTURE OF CYTOSKELETAL-MEMBRANE INTERACTIONS AT THE NODE OF RANVIER. Takao Ichimura & Mark H. Ellisman. Department of Neurosciences, Laboratory for Neurocytol., University of California San Diego, La Jolla, California, 92093

Descriptions of the ultrastructural morphology of the nodal and paranodal regions of the node of Ranvier have been based mainly on images obtained with conventional freeze-fracture EM techniques. It has been established that the myelinating cells form intimate associations with the axon at the paranodal and nodal regions with characteristic intramembrane particles. Some of the particles in the nodal region are thought to be correlates of voltage sensitive Na+ channels while those of the paranodal "glial axonal junction" (GAJ) are suspected correlates for K+ channels. In order to learn more about the structure of PNS and CNS nodes in these regions we have applied two EM imaging techniques, deep-etch rotary shadowing and selective staining. The images obtained have yielded additional information about the 3-D fine structure in three important areas: 1) beneath the nodal membrane where the axoplasmic cytoskeleton associates with the nodal axolemma; 2) extracellular to the nodal region, where structures link the Schwann cell microvilli or astrocyte processes with the nodal axolemma; and 3) at the GAJ where there are characteristic associations observed between axoplasmic intermediate filaments and the paranodal axolemma. Here there are also systematic connections with the myelinating cell via cytoskeletal and membrane specializations within the myelinating glia. These intricate connections, which effectively cross the GAJ, are the most complex and appear to involve many interlinked components. For example, spectrin-like microfilaments, and axoplasmic intermediate filaments appear anchored to the axolemma by filaments of approximately 4nm in diameter. In the most revealing images these filamentous structures are seen to form a continuum connecting paranodal intramembrane particles to cytoskeletons on both sides of the junctional complex. The paranodal loops of the myelinating cell contains bundles of circumferentially oriented filaments. Cross-linking filaments are observed that appear to mediate the anchoring of these glial filaments to the membrane. We suspect this intricate association at the GAJ provides for the transcellular ion exchanges that occur at this site as well as for the stabilization of intercellular attachments.

279.7

ALTERED REGULATION OF TRANSCRIPTION OF MYELIN GENES IN THE SHIVERER MUTANT MOUSE. A. Roach and M. Wiktorowicz, Samuel Lunenfeld Research Institute of Mount Sinai Hospital, Toronto, Canada M5G 1X5.

The *shiverer* mutation consists of a deletion of the 3' end of the myelin basic protein gene, which completely prevents production of mature mRNA and protein, and results in severe dysmyelination and a trembling behaviour. We have used the transcription run-on technique to measure the MBP transcription rate in brains from mice of wild-type and homozygous *shiverer* genotypes at several ages spanning postnatal development. In wild-type brains a major factor in the developmental regulation of MBP expression is the control of transcription rate. In *shiverer* brains, the transcription rates for the undeleted 5' end of the gene follow closely those seen in wild-type animals up to the age at which maximal myelination normally occurs. Total MBP transcripts follow a similar profile but at less than 5% the level seen in wild-type, and, as expected, no mature mRNA is detected. Thus the *shiverer* deletion does not remove information required for efficient, developmentally-regulated transcription, and the low level of MBP transcripts must be a result of their reduced stability. A 2-3 fold higher than normal MBP transcription rate in older *shiverer* animals suggests the possibility of a feedback mechanism which might normally down-regulate gene expression in response to accumulation of mature myelin.

279.8

DISTRIBUTION OF MYELIN PATCHES IN THE MOSAIC OPTIC NERVES OF THE MYELIN DEFICIENT RAT HETEROZYGOTE. I.D. Duncan, J. P. Hammang, K.F. Jackson, D. Marren and C. Iida, School of Veterinary Medicine, University of Wisconsin, Madison, WI.

Previous work has shown that the female carriers of the myelin deficient (md) rat trait show extensive myelin mosaicism in the optic nerve (Duncan et al. J. Neuropath. exp. Neurol. 1988, 47,384). The present study was designed to examine the distribution of myelin patches along the optic tract and nerves from the md rat heterozygotes and relate these findings to the embryological population of the nerve with glia cell precursors.

The optic nerves and tracts from both known and putative md rat heterozygotes were dissected from chiasm to retina after the rats were perfused with aldehyde fixative. Those optic nerves which showed a gross myelination defect, being transparent or containing only "streaks" of myelin, were embedded whole, and skip-serial sections cut at 50-100μ intervals. Examination of these sections showed that patches of myelin were often discontinuous along the course of the nerve. While the majority of nerves showed this deficit along their entire length, 15 nerves had a more severe defect towards the retina. 3-D images of certain areas showed that patches of myelin appeared to arise from a single clone. Mosaicism was also seen in the optic tracts of those rats which had severe optic nerve abnormalities. The spatial distribution of the patches might confirm the longitudinal migration of the O-2A progenitor cell. However, a radial migration from the embryonic optic stalk also cannot be entirely ruled out by these results. (Supported by NIH NS23124 and NMSS RG 179).

279.9

CHARACTERIZATION OF THE MYELIN-LIKE MEMBRANES IN EARTHWORM CNS USING IMMUNOLABELLING TECHNIQUES. BEATRICE CARDONE and BETTY I. ROOTS. Dept. of Zoology, Univ. of Toronto, Toronto, Canada, M5S 1A1.

The ventral nerve cord of the earthworm Lumbricus terrestris contains three dorsal giant axons (2 lateral and 1 medial). These axons are ensheathed by multi-lamellar myelin-like membranes. We are exploring the relationships which exist between these membranes and true myelin. Previous studies in our laboratory have resulted in the isolation and partial characterization of these myelin-like membranes (Pereyra and Roots, Neurochem. Res., 13:893, 1988). In order to localize component proteins of this sheath, we have generated both polyclonal and monoclonal antibodies against two subfractions (F_1 and F_2) of the nerve cord preparation which are enriched in these myelin-like membranes. The clones were screened using Western immunoblotting for cross-reactivity to the protein bands P28, P32, P42 and P80 which are the major protein bands of the subfraction. These antibodies were then used to identify the location of cross-reactivity in frozen tissue sections of earthworm nerve cord using immuno-fluorescence. The localization of the antibody binding to specific membrane proteins was visualized by immunogold electron microscopy. The production of these antibodies will now allow us to investigate the phylogenetic distribution of these myelin-like membrane proteins and the relationship to the evolution of myelin.

279.10

CYTOARCHITECTURE OF SCAR FORMATION FOLLOWING KAINIC ACID INDUCED LESIONS IN RAT HIPPOCAMPUS. CM SEVERIN, CL BOWMAN AND JW SWANN. SCHOOL OF MEDICINE, SUNY-BUFFALO, BUFFALO, NY 14214, AND WADSWORTH CENTER, NYS DEPARTMENT OF HEALTH., ALBANY, NY 12201.

An electron microscopic and cell culture study was performed on kainic acid induced scar formation in the rat hippocampus. Following a unilateral stereotaxic injection of 4 nmols of kainic acid into the hippocampal CA3 region, the animal was allowed to survive from 1 hour to 6 months. In two different series of animals, the resultant scar tissue was then processed for either electron microscopy or cell culture. In the electron microscopic study the animals were sacrificed and perfused with a glutaraldehyde-paraformaldehyde solution. Two cores of tissue were taken from the center of the scar, sectioned and stained. Examination of serially sectioned tissue showed the scar to be composed of 6 cellular elements and numerous myelinated and unmyelinated fibers. Consistently present in the 1 hour to 6 month scars were astrocytes, endothelial cells, microglia, fibrocytes, and oligodendroglia. Neurons were present only in 1 to 3 hour lesions. Long cellular processes containing a dark cytoplasm, numerous vacuoles, and lamellar-like endings were also prominent in the scars. Examination of dissociated scar tissue in cell cultures also verified the presence of astrocytes, microglia- and fibrocyte-like cells. This investigation demonstrates a complex cellular network in the kainic acid induced lesions of the rat hippocampus.

279.11

EFFECTS OF ASTROCYTIC SWELLING ON Ca^{2+} TRANSPORT AND RELEASE OF ^{3}H-D-ASPARTATE. E.R. O'Connor, A. Schneider, and H.K. Kimelberg. Dept. of Pharmacology/Toxicology and Div. Neurosurgery, Albany Medical College, Albany, NY 12208.

Swelling of astrocytes in hypotonic media causes regulatory volume decrease (RVD), membrane potential depolarization, and release of amino-acids. We are examining whether such swelling results in increased Ca^{2+} uptake. Swelling of astrocytes in hypotonic medium (removal of 50 mM NaCl) causes increased influx of $^{45}Ca^{2+}$, which is partially blocked by 1 μM Nimodipine and 1-10 μM Verapamil. This finding suggests that voltage-gated L-channels are being opened, possibly due to the swelling induced depolarization (Kimelberg and O'Connor, Glia 1:219 (1988)). In contrast, gadolinium, up to 10 μM, which has been reported to block stretch-activated non-specific cation channels (SACS), did not inhibit the swelling induced increased uptake of Ca^{2+}. Although cell swelling leads to increased uptake of Ca^{2+}, nominally Ca^{2+} free media (20 μM) appeared to have no effect on RVD or swelling induced ^{3}H-D-aspartate release. Intracellular [Ca^{2+}] will be measured using Fura-2 to determine (i) if Ca^{2+} is released from internal stores during astrocytic swelling and (ii) if events associated with astrocytic swelling are dependent on an increase in cytosolic free Ca^{2+}.
(Supported by grant NS 23750 to H.K.K.).

279.12

VOLTAGE-ACTIVATED K^+ AND CA^{++} CHANNELS IN ACUTELY DISSOCIATED HIPPOCAMPAL ASTROCYTES. S.Duffy, F.W.Tse, D.Hochman, D.D.Fraser and B.A.MacVicar. Neurosci.Res.Group, University of Calgary, Calgary, Alberta T2N4N1.

Astrocytes in primary culture have been shown to express several voltage- and ligand-gated ion channels. We have developed a technique which permits the acute isolation of hippocampal astrocytes from mature brain. Dissociated astrocytes were identified by their morphology and by immunocytochemical staining for glial fibrillary acidic protein. Whole cell voltage clamping of astrocytes revealed two prominent voltage-activated K^+ currents similar to the neuronal delayed rectifier and the A-currents as well as a high threshold Ca^{++} current. Experiments employing the Ca^{++} sensitive dyes, indo-1 or fura-2 to measure intracellular Ca^{++} revealed that these cells have a resting Ca^{++} level of 100-150 nM which increased several fold in response to depolarization. This Ca^{++} transient is dependent on the presence of extracellular Ca^{++} and is attenuated by the Ca^{++} bolcker verapamil (100-200 uM). These results indicate that astrocytes in the hippocampus normally express voltage-activated K^+ and Ca^{++} channels and that the functions of the cells in regulating the extracellular milieu might be dynamically modulated. Supported by the Medical Research Council.

279.13

THE CONCEPT OF THE INTERNAL CORTICAL GLIAL LIMITING ZONE. T.I. Mandybur and S. Guy. Dept. of Pathology and Laboratory Medicine, Univ. of Cincinnati, Cincinnati, OH 46267.

The concept of the cerebral cortical external glial limiting membrane (EGLM) is well established. Herewith we postulate existence of an internal glial limiting zone (IGLZ) of the cortex. GFAP stained sections of normal human (and animal) cerebral cortex with neighboring white matter exhibited a concentration of fibrous astroglial cell bodies (AB) in a zone approximately 1-2 mm underneath the lamina 6, partly involving also the deeper lamina 6.

The density of AB in this zone was approximately up to 10x greater than in the deeper white matter in a given segment of the cortex, the number of AB in this zone was roughly similar to those in the EGLM, although the density here of the AB was of much lesser degree. Curiously, such zone was not observed about the basal ganglia or other deep gray structures. One role of the IGLZ could be in providing a sealer to the cortex from the white matter by producing here a denser astrocytic fiber network.

279.14

α , β AND $5HT_2$ RECEPTORS REGULATE THE UPTAKES OF GLUTAMATE, GABA AND TAURINE IN ASTROCYTES.

Elisabeth Hansson, Institute of Neurobiology, University of Göteborg, P.O.Box 33 031, S-400 33 Göteborg Sweden.

From experiments using dissociated primary astroglial cultures from newborn rat cerebral cortex, stimulation of monoamine receptors (α, β and $5HT_2$) was shown to affect the kinetics of the high affinity uptake of glutamate, GABA and taurine. In the presence of the α_1 agonist phenylephrine, there was an increased active uptake of glutamate, while β-adrenoceptor activation caused a slight inhibition of the glutamate uptake and a stimulation of the GABA and taurine uptakes. $5HT_2$ receptor stimulation caused a slight inhibition of the taurine uptake. The mechanisms behind these effects were also studied. The GABA uptake seems to be regulated by the G-protein / adenylate cyclase complex in the receptor domain. Also the K^+-channel seems to be involved. The taurine uptake seems not to be regulated by the same mechanism as for GABA uptake nor did the glutamate uptake. By the expression of receptors, uptake carriers and interaction between these systems , astrocytes might supervise, control, facilitate and partly regulate synaptic transmission in many synaptic regions.

279.15

INHIBITION OF POTASSIUM UPTAKE BY CALMODULIN ANTAGONISTS: A POSSIBLE ROLE OF ASTROCYTES IN EPILEPTOGENESIS. J.T. Neary, A.S. Bender, L. Baker*, J. Blicharska*, L.O.B. Norenberg* and M.D. Norenberg. Lab. Neuropath., Vet. Admin. Med. Ctr. and Univ. of Miami, Miami, FL 33101.

It has been suggested that a defect in K^+ buffering by astrocytes may contribute to epileptogenesis. In support of this, we previously reported that K^+ uptake was reduced in astrocyte cultures derived from genetically epilepsy prone rats, GEPRs (Soc Neurosci Abst 13:943, 1987). We also found that astrocytes from GEPRs have decreased calcium, calmodulin-dependent protein phosphorylation (Trans Amer Soc Neurochem 20:230, 1989), a mechanism which has also been linked to epileptogenesis. These findings led us to speculate that the reduction in K^+ uptake may be due to the decrease in calcium, calmodulin-dependent protein phosphorylation. To test this hypothesis, we investigated the effect of calmodulin antagonists on K^+ uptake in cultured astrocytes from normal rats. We found that W-7 and W-13 inhibited K^+ uptake (IC_{50}=90 and 200uM, respectively) whereas W-12, which has a very low affinity for calmodulin, was ineffective. Calmidazolium (IC_{50}=25uM) and trifluoperazine (IC_{50}=55uM) also inhibited K^+ uptake. Since the latter drugs can also inhibit protein kinase C (PKC), we tested this possibility by down-regulating PKC with extended phorbol ester treatment. Since K^+ uptake was only minimally diminished, it is unlikely that these drugs are acting via PKC. These findings indicate that calmodulin antagonists potently inhibit K^+ uptake in astrocytes and, together with previous results, suggest that the regulation of K^+ uptake by calmodulin-dependent protein phosphorylation may contribute to epileptogenesis.

279.16

ACTIONS OF EXTRACELLULAR ATP ON ASTROCYTES IN PRIMARY CULTURE: POTENTIAL ROLE IN REACTIVE GLIOSIS. M.D.Norenberg, J.T.Neary, L.Baker, J.Blicharska, and L.O.B.Norenberg*. Lab. of Neuropathol., Vet. Admin. Med. Ctr. and Univ. Miami Sch. of Medicine, Miami, FL 33101.

A number of factors have been implicated in the proliferative and hypertrophic processes which characterize reactive astrocytosis. One such factor we have been exploring is ATP, an agent that is released by injured cells following tissue destruction. Activation of P2 purinergic receptors by ATP leads to increased intracellular levels of calcium (EC_{50}=10uM) (BBRC 157: 1410, 1988) and to the phosphorylation of a protein which co-migrates with glial fibrillary acidic protein (GFAP) (Soc Neurosci Abstr 15: 352, 1989). Since calcium appears to be involved in astroglial stellation (MacVicar BA: Brain Res 420: 175, 1989), and since the degree of GFAP phosphorylation appeared to be affected by ATP, we investigated the possibility that ATP might be a factor in triggering reactive astrocytosis. For this purpose, we used primary astrocyte cultures derived from neonatal rats. Light microscopic studies disclosed marked stellation of astrocytes after 1 hr exposure to extracellular ATP (100 uM). As measured by ELISA, GFAP content increased by 40%. Additionally, [3H]thymidine incorporation was increased 3-fold following treatment with ATP for 3 days, consistent with cellular proliferation. These findings show that extracellular ATP reproduces many of the features associated with reactive gliosis and suggest that extracellular ATP may be involved in the activation of astrocytes following CNS injury.

279.17

THE ROLE OF PHOSPHOINOSITIDE HYDROLYSIS IN VOLUME REGULATION IN ASTROCYTES. A.S.Bender, J.T.Neary, J.Blicharska*, L.O.B.Norenberg* and M.D.Norenberg. Laboratory of Neuropathology, Department of Pathology, University of Miami School of Medicine and Veterans Administration Medical Center, Miami, FL 33101.

Astrocytes, when exposed to hypotonic stress, undergo swelling with subsequent volume decrease, i.e., regulatory volume decrease (RVD). Calcium mobilization plays an important role in this phenomenon. Since phosphoinositide (PI) hydrolysis is thought to be involved in intracellular signaling via mobilization of calcium, we tested the effect of hypotonic stress on the formation of inositol phosphates (IPs) in astrocytes. Astrocytes were prelabelled with [^{3}H]-myo-inositol, cultures were then exposed to hypotonic stress (45 mM Na^+) and IPs were separated from myo-inositol by Dowex anion-exchange chromatography. A transient rise of 70-80% in the content of IPs was found at 0.5-1 min after hypotonic exposure. This was followed by a decline in the formation of IPs which paralleled RVD. We also found that agents which are known to induce receptor-mediated formation of IPs in astrocytes such as endothelin-1 (20 nM), bradykinin (15 uM) and ATP (0.5 mM) were able to accelerate RVD when added to the hypotonic medium. These findings suggest an important role of PI hydrolysis and calcium in the regulation of volume in astrocytes following hypotonic stress. (Supported by M.R.C. of Canada and the Veterans Administration)

279.18

PRESENCE OF THE TYPE II (β) AND III (α) FORMS OF PROTEIN KINASE C IN ASTROCYTE CULTURES PREPARED FROM THE RAT NEOCORTEX. L. Rochat* and P.L. Mobley. Dept. of Pharmacology, Univ. of Texas Hlth. Sci. Cntr., San Antonio, TX 78284.

Previous studies have demonstrated the presence of protein kinase C in cultured astrocytes from rat neocortex. The present studies were conducted to determine which forms of the kinase are present in these cultures. Using monoclonal antibodies for the type I (γ), II (β), and III (α) forms of protein kinase C and immunocytochemical staining techniques, staining was observed with the antibodies for the type II and III forms of the enzyme but not with the antibody for the type I form. To confirm that binding was to protein kinase C, cells were subjected to 1-dimension gel electrophoresis and electroblotted to nitrocellulose. Using antibody overlay and peroxidase staining techniques, the antibody to the type II and III forms of the kinase were found to associate only with an M_R 80,000 band. No staining was observed with the antibody to the type I form even though this antibody reacted with western blots of protein kinase C obtained from adult rat brain. These studies suggest that astrocyte cultures obtained from the neocortex of newborn rats contain only the type II and III forms of protein kinase C. (Supported by N.I.H. grant NS25766.)

279.19

CULTURED TYPE 2 ASTROGLIA EXHIBIT REGIONAL DIFFERENCES IN THEIR EXPRESSION OF NEUROTRANSMITTER RECEPTORS THAT INFLUENCE INTRACELLULAR CALCIUM LEVELS.
K.D.McCarthy and V.Dave*, Department of Pharmacology, The University of North Carolina at Chapel Hill, NC 27599

Recent studies completed in this laboratory indicate that cerebral type 2 astroglia exhibit at least seven different receptor types linked to the mobilization of calcium. Experiments indicate that cerebral type 2 astroglia are heterogeneous with respect to their expression of neuroligand receptors influencing calcium levels. We have assessed the ability of cerebellar and optic nerve type 2 astroglia to respond to neuroligands with elevation of calcium levels in order to further examine the question of pharmacological heterogeneity among type 2 astroglia. Type 2 astroglia were prepared from neonatal cerebral cortex, 4 day old cerebellum, and 14 day old optic nerve segments. The influence of neuroligands on calcium levels was examined using a video-based imaging system and fura-2 loaded cells. All analyzed cells were subsequently identified as type 2 astroglia through their glial fibrillary acidic protein and A2B5 immunoreactivity. A similar percentage of type 2 astroglia isolated from cerebellar and cerebral tissue responded to carbachol (>60%), 2-methyl thio ATP (2mt-ATP; > 60%), serotonin (<15%), and glutamate (<15%). Compared to cerebral type 2 astroglia, a smaller percentage of cerebellar type 2 astroglia responded to norepinephrine, histamine and bradykinin. In striking contrast to the responsiveness of cerebral and cerebellar type 2 astroglia, very few optic nerve type 2 astroglia responded to any of the neuroligands examined. 2mt-ATP appears to be the most effective for increasing calcium in these cells and to date only 5 of 32 optic nerve type 2 astroglia have been observed to respond to this agonist. Our results indicate that type 2 astroglia isolated from any given brain region are pharmacologically heterogeneous and that the set of receptors exhibited by these cells varies between brain regions. (Supported by NS 20212)

279.20

MECHANISM OF REGULATORY VOLUME DECREASE IN HUMAN ASTROCYTES. S. Medrano and E. Gruenstein. Dept. Mol. Gen., Biochem. and Microbiol. Univ. of Cincinnati, Cincinnati, OH 45267.

Swelling of astrocytes occurs after brain injury and stroke. Since these cells constitute 20-25% of human brain volume, their swelling is a major factor in the morbidity and mortality associated with cerebral edema. Here we report the results of studies designed to elucidate the mechanisms of the regulatory volume decrease (RVD) which occurs after astrocytes are swollen by exposure to hypotonic medium. Using UC-11MG cells, a well characterized human, astrocytoma-derived line (Lomneth et. al., Brain Res.486:95, 1989), we observed an increase in membrane permeability to both K^+ and Cl^- during RVD, consistent with a net loss of these ions. In some cell types RVD has been found to occur via activation of a stretch activated, cationic channel (SAC), which causes an influx of Ca^{2+} and membrane depolarization. In UC-11MG cells RVD was blocked by Gd^{3+}, an inhibitor of the SAC. Although increases in intracellular free Ca^{2+} (Ca^{2+}_i) were also observed, they only occurred well after the onset of RVD. Furthermore, the RVD was not affected by blocking the Ca^{2+}_i increase with dm-BAPTA or removal of extracellular Ca^{2+}. These results indicate that the RVD in UC-11MG cells is due to the activation of an SAC, is independent of changes in Ca^{2+}_i, and is therefore probably dependent on membrane depolarization.

279.21

PREFERRED PATHWAYS FOR ASTROCYTE MIGRATION. C. Andersson, M. Tytell, J. K. Brunso-Bechtold. Department of Neurobiology and Anatomy, Wake Forest University, Winston-Salem, NC 27103

A central issue in understanding the migration of transplanted astrocytes is the question of preferred pathways guiding the migration. LM analysis has shown that the major pathways for donor astrocytes migrating into host tissue are on fiber bundles, and along blood vessels and pial surfaces (Goldberg and Bernstein, 1987). However, we have reported that donor astrocyte migration patterns in neonates change dramatically over time. At 1- wk post-injection (PI), donor astrocytes were located in cingulate and parietal cortex only, but by 3- and 5-wk PI, the cells were observed solely in corpus callosum, hippocampus and septal nucleus (Andersson et al, 1989). These results suggest that the pathways utilized for short distance migration may differ from those pathways which guide long distance migration. Therefore, we examined donor astrocyte migration in neonatal rats at 3 developmental stages: post-natal day (PND) 5 (premyelination), PND 20 (early stage myelination), and PND 35 (late stage myelination). A cell suspension of cultured type 1 astrocytes, labeled with either Fast Blue (LM) or 15 nm colloid gold beads (EM) were injected into the right parietal cortex. Preliminary LM findings correlate long distance migration of donor astrocytes primarily with myelinated fiber pathways. EM studies are underway to determine the cellular interactions between the transplanted astrocytes and host oligodendrocytes. Supported in part by NSF grant BNS-8811178 to MT, EY05028 to JB-B.

279.22

HISTAMINE-INDUCED INTRACELLULAR CALCIUM ELEVATION IN TYPE-2 ASTROCYTES. N.Inagaki*, H.Fukui*, S.Ito*#, A.Yamatodani and H.Wada*. Dept. Pharmacol. II, Faculty of Med., Osaka Univ., Osaka 530, and #Osaka Bioscience Institute, Suita 565, Japan.

In the previous study (Soc. Neurosci. Abstr. 15; 235, 1989), we reported that histamine induced intracellular calcium elevation in type-2 astrocytes and the calcium elevations were classified into 4 patterns, i.e.; transient, oscillatory, sustained and biphasic patterns. In this study, we further investigated the histamine-induced intracellular calcium elevation in rat type-2 astrocytes in primary culture using fura 2-based microfluorimetry. The histamine induced calcium elevations were first observed in the processes of type-2 astrocytes and calcium waves propagated to the cell soma within a few seconds. In some cells calcium elevations were seen only in the processes. Some of the calcium elevations in the processes were oscillatory. These observations suggest that the processes are sensitive to histamine signals. The calcium elevations were blocked by H1-antagonists but not by H2- or H3-antagonists and induced by H1-agonists but not by H2- or H3-agonists. Phorbol ester inhibited the calcium elevations but pertusssis toxin and voltage dependent calcium channel blockers had no effects. When extracellular calcium was omitted or La^{3+} was added into extracellular medium, sustained phase of calcium signals disappeared and only transient and oscillatory patterns were observed. this suggests sustained and biphasic patterns are composed of intracellular calcium mobilization and extracellular calcium influx.

IONS CHANNELS: CHLORIDE AND OTHER CHANNELS

280.1

FAST CHLORIDE CHANNELS ARE PRESENT IN MOST DISSOCIATED RAT CEREBRAL CORTEX NEURONS. A. L. Blatz. Dept. of Physiology, UT Southwestern Medical Center, Dallas, TX 75235.

Chloride-selective channels with fast gating kinetics have been described in tissue-cultured rat skeletal muscle (Blatz and Magleby, 1985, Biophys. J. 47:119). Cl channels with essentially identical properties have now been observed in most acutely dissociated rat cerebral cortex neurons. Neurons were obtained with a trypsin dissociation technique. Cell-attached and inside-out configurations of the patch clamp technique were used. Single Cl channel currents were recorded mainly in inside-out patches with the extracellular solution containing 140 mM KCl and the intracellular solution containing 1000 mM KCl. The excised patch temperature was maintained at either 22 or 7 °C. Both solutions contained 1 mM EGTA and 5 mM TES buffer at pH 7.0. Under these conditions fast neuronal Cl channels exhibited the following properties that were very similar to muscle Cl channels: 1) unitary conductance of 140 pS at room temperature and 75 pS at 7 ° C; 2) Cl/K selectivity ratio of about 5-7; 3) dependence on membrane potential with the percent time open decreasing with hyperpolarization; 4) complex gating kinetics with at least two open kinetic states with similar mean lifetimes and at least six closed kinetic states with mean lifetimes ranging from tens of microseconds to several seconds; 5) presence of altered modes of kinetic activity including the "buzz" mode and an inactivated state of very long duration; 6) subconductance states with apparent unitary conductance of 2/3 times the normal level. Fast Cl channels in neurons often do not become active until several seconds to minutes following excision from the soma. Supported by NIH Grant GM39731.

280.2

CHANGES IN GABA SENSITIVITY OF ACUTELY DISSOCIATED LARGE DORSAL ROOT GANGLION (DRG) NEURONS MAINTAINED IN CULTURE D.L. Eng, J.D. Kocsis, J. Caffrey, A.H. Cornell-Bell. Dept. of Neurology, Yale Univ.Sch.Med., West Haven V.A.Med.Cntr., New Haven, CT 06510

DRG neurons from L4 and L5 of adult rats were acutely dissociated and cultured for 1 to 5 days on PORN/laminin-glass coverslips in the absence of neuronotropic growth factors (DMEM:F12 (1:1), + 10% FBS and antibiotics). Large DRG neurons (40 to 60 microns) were impaled under visual control with glass microelectrodes. The cultures were continuously washed with a modified Krebs' solution. Single electrode current and voltage clamp experiments were carried out to characterize action potential, resting potential, input resistance, inward rectification, and conductance and membrane potential changes elicited by GABA or the $GABA_A$ receptor agonist muscimol. These properties were studied over time in order to determine if they could be maintained in the absence of neuronotropic growth factors such as NGF. The mean depolarization elicited by GABA on dissociated DRG neurons tested between 0 and 24 hrs was 16 mV (n=9) which is comparable to responses in intact DRG, declined to 9.4 mV (n=22) for neurons studied between 2 and 5 days. However, neurons, exhibiting reduced GABA responses, maintained similar action and resting potentials, input resistance, and a prominent inward rectification throughout the tested period. These results indicate that there is a selective reduction in GABA receptor function in isolated large DRG neurons maintained in culture. One interpretation of these data is that a trophic factor present in vivo, but absent in the culture media, maintains the expression of DRG GABA receptors. Experiments to test this hypothesis are currently underway. Supported by the Medical Reseach Service of the VA.

280.3

IMMUNOHISTOCHEMICAL AND BIOPHYSICAL CHARACTERIZATION OF ADULT AND NEONATAL RAT DORSAL ROOT GANGLION CELLS IN MONOCULTURE. P. G. Thomas*, J. Caffrey, A.H. Cornell-Bell, J.D. Kocsis and S.G. Waxman. Dept. of Neurology, Yale Univ.Sch.Med., W.Haven V.A. Med.Cntr., New Haven, CT 06510

Enriched monocultures of rat dorsal root ganglion neurons and satellite cells were used to study physiological and immunohistochemical properties of peripheral $GABA_A$ receptors. L4 and L5 ganglia were digested in 1 mg/ml type II collagenase and 20 U/ml papain in HEPES-buffered physiological saline for 40 min; then triturated in Earle's MEM, 5% FBS, 1 mg/ml BSA and 1 mg/ml trypsin inhibitor with a siliconized pipet. Cells diluted into 0.75% BSA/Ca^{++}-free saline were loaded onto a 3-6% continuous BSA gradient using a STA-PUT SP-120 sedimentation chamber at an initial rate of 10 ml/min for 5 min and brought to a final volume of 250 ml at 40 ml/min. 6 ml fractions collected following 40 min sedimentation were centrifuged (225 g x 5 min). Cells were resuspended in DMEM:F12 (1:1) + 5% FBS and plated on polyornithine/laminin-coated coverslips. Neonatal neuronal diameter distributions were multimodal, having four main peaks at 12 μm (15%), 20 μm (51%), 28 μm (28%) and 40 μm (4%) (n=510). Using patch voltage clamp methods, all cells expressed "L-type" Ca^{++} currents; Na^+ and "T-type" Ca^{++} currents were small or undetectable in cells < 20 μm. Using intracellular recording techniques, cells > 20 μm displayed a prominent depolarization and conductance increase to bath-applied GABA. Fixed cells were permeabilized with 0.1% Triton X-100 (2 min) and labelled with rabbit α-GABA (30 min). Cells were incubated in biotinylated secondary (horse α-rabbit IgG) followed by avidin-FITC. Cells having diameters >20 μm preferentially labeled with α-GABA receptor antibodies. These results indicate that rat DRG cells separated by size into monocultures show distinct differences in biophysical and immunohistochemical properties. Supported by Medical Research Service., V.A. Med.Cntr..

280.4

MOLECULAR CLONING OF A NEONATAL GLYCINE RECEPTOR FROM RAT CORTEX E.Barbosa*, S.Logan, and M.Van Der Ent*, Dept of Neurol., Johns Hopkins School of Med., Balto., MD 21205.

Glycine and γ-aminobutyric acid (GABA) are the major inhibitory neurotransmitters in the CNS of the adult rat, with GABA acting predominantly in the cortex, and glycine in the brainstem and spinal cord. cDNA cloning has established the existence of a gene super-family for neurotransmitter gated ion channel receptors. Recent studies have indicated that a developmentally regulated neonatal form of the glycine receptor exists both in the spinal cord and the cortex. By low stringency screening of a Day 1 rat cortex cDNA library using using a redundant oligo to the conserved cystein region from the published adult receptor (Betz et al., *Nature*, July 87), we identified a cDNA whose nucleotide sequence shows 74.6% homology to the adult glycine receptor and 57% identity to the Bovine alpha subunit of GABA. The primary structure of this clone exhibits similarities characteristic of other genes in the super-family: a putative 21 amino acid (21 a.a.) hydrophobic signal sequence, a disulfide bonded extracellular domain with two cysteine residues spaced 14 a.a. apart and the four hydrophobic transmembrane domains. Like GABA alpha subunits, low sequence conservation characterizes the extended putative intracellular loop between the M-3 and M-4 domains. Northern analysis of RNA extracted from rat cortex identified a single 3.8 kb RNA species which is most abundant at Days 1 through 10 and subsequently declines to low but detectable levels at Day 15 into adulthood indicating that it is developmentally regulated. Current studies focus on the expression in Xenopus oocytes in an effort to determine sensitivity of the channel both to glycine and its antagonist strychnine.

280.5

WHOLE-CELL AND SINGLE-CHANNEL ANALYSIS OF SWELLING-INDUCED CHLORIDE CURRENTS IN NORMAL AND CYSTIC FIBROSIS EPITHELIAL CELLS. C. K. Solc and J. J. Wine, *Cystic Fibrosis Research Laboratory, Stanford University, Stanford CA 94305-2130, USA.*

Two recent reports have characterized a swelling-induced Cl^- current at the whole-cell level in normal epithelial cells (Worrell et al., *Am J Physiol* 256:C1111-9, 1989; McCann et al., *J Gen Physiol* 94:1015-36,1989). In the present study, we extend this work by identifying the single channels underlying the current, and investigate if the induction or properties of these channels are altered in cells from CF individuals.

Outwardly-rectifying Cl^- channels in T84, sweat coil, sweat duct and tracheal epithelial cells were induced by exposure of the cells to hypotonic solutions or by perfusion in the whole-cell configuration. There was no apparent difference in the induction, single-channel or whole-cell properties between CF and normal cells. Whole-cell outward current at +100 mV, carried primarily by Cl^- (CsCl in the pipet), was typically 2 to 4 pA/pF in unswollen cells and 10 to 40 pA/pF in swollen cells. The underlying Cl^- channels were mostly open at negative voltages but inactivated above ~+50 mV. The time constant of inactivation ranged from 100 to 600 ms at +100 mV. Whole-cell currents were blocked by bath-applied DPC, NPPB and DNDS. DNDS block was voltage-dependent, with more effective inhibition of outward currents (inward Cl^- movement) than inward currents. Outwardly-rectifying single-channel currents (25-50 pS near 0 mV) with voltage-dependence, kinetics, selectivity, and pharmacology corresponding to the whole-cell current were recorded in cell-attached and outside-out patches. Open probability was not affected by altering the hydrostatic pressure inside the pipet. Mean open durations at voltages above 0 mV were 100 to 400 ms. In all patches studied, the maximum number of channels in the patch was observed immediately after obtaining a tight seal, and the channels typically disappeared with time during the experiment. The conductance, selectivity, and pharmacological properties of these channels are very similar to those of the voltage-induced outwardly-rectifying Cl^- channels thought to underlie the CF defect in airway epithelia, but induction and gating properties are, at least in part, different. (Supported by NIH R01 DK 39659, HL 42368, CFRI, and the Cystic Fibrosis Foundation)

280.6

ION SENSITIVE MICROELECTRODE STUDY OF CHLORIDE BALANCE IN THE SOMATIC MUSCLE BAG CELLS OF ASCARIS SUUM. H. Rheinallt Parri*, M.B.A. Djamgoz*[1], L. Holden-Dye* and R.J. Walker. Dept. of Neurophysiology, University of Southampton, Southampton SO9 3TU, [1]Dept. of Pure and Applied Biology, Imperial College, London SW7 2BB.

Ion sensitive microelectrodes incorporating the exchanger Corning 477913 were used to determine intracellular chloride levels in Ascaris muscle cells. Resting membrane potential was -25 ± 1 mV, free internal chloride concentration was 18 ± 0.5 mM, and E_{Cl} -47.1 ± 0.7 mV (n=49). In other experiments the reversal potential of the GABA event was determined to be -54 ± 1 mV (n=62). Membrane potential decreased by 9 ± 1.7 mV (n=3) per 10 fold change in external chloride. Internal chloride did not fall as much as expected on exposure to low chloride solutions, and indicated an interfering ion concentration of 12.1 ± 2.4 mM.

The apparent high resting conductance to chloride, and displacement of E_m positive to E_{Cl} indicates the presence of an outwardly directed Cl^- pump in these cells.

We are grateful to the SERC for funding.

280.7

c-AMP-DEPENDENT PROTEIN KINASE-MEDIATED ACTIVATION OF CHLORIDE CURRENT BY PGE_1 IN JURKAT T-LYMPHOCYTES. M.A. Schumann, D. Maldonado*, and P. Gardner, Dept. of Medicine, Stanford Univ., Stanford, CA 94305.

The prostaglandins are inflammatory mediators that modulate cell secretion in the immune system. We have used tight seal-whole cell recordings to investigate the effect and mechanism by which Prostaglandin E_1 affects a Cl^- current in Jurkat lymphocytes. Addition of PGE_1 to the bathing solution induced, within .5-1 minutes, a dose-(range 1μM-10μM) and time-dependent increase in current peak amplitude. Diphenylcarboxylamine, DPC, (8×10^{-4}M), a blocker of anion transport added to the bath, diminished the Cl^- current response to PGE_1 (10μM). The mechanism by which PGE_1 induces an increase in Cl^- current was then investigated. IBMX, a phosphodiesterase inhibitor, was found to augment the effect of PGE_1 on the Cl^- current when added to the bath. The peak increase in amplitude attained with PGE_1 without the use of IBMX was 53.0%, SE 11.6, (n=4) after 3 minutes, compared to 101.9%, SE 14.2 (n=4) with IBMX (1×10^{-4}M) after 1 minute. The time constant increased 2-3 times (n=4) with PGE_1 and IBMX. The PGE_1-induced Cl^- current was inhibited with the use of Walsh inhibitor, a competitive inhibitor of cAMP protein kinase, in the pipette. The Cl-current was decreased by 19.7%, SE 5.9 (n=3) with 1μM Walsh inhibitor. The effect of PGE_1 was also inhibited by the peptide, R_p cAMP, (1mM; n=4), a competitive analog of cAMP which antagonizes the activation of cAMP-dependent kinase. The data illustrate the increase in Cl^- current induced by PGE_1 on T lymphocytes and are consistent with an cAMP-dependent phosphorylation mechanism.

280.8

WITHDRAWN

280.9

DEPOLARIZATION PREVENTS MAGAININ CYTOLYTIC ACTIVITY ON TUMOR CELLS. R. A. Cruciani1, O. Colamonicci2*, H.-C. Chen3* and J. L. Barker1, [1]Lab. of Neurophysiology, NINDS, [2]Endocrinology and Reproduction Research Branch, NICHD, NIH Bethesda MD 20892 and [3]Dept. of Medicine, Section of Hematology/Oncology, University of Chicago, IL 60637.

The magainins are a family of antibiotic peptides recently isolated from the skin of the African Clawed frog Xenopus Laevis. Previously we reported the ability of these peptides to form cation-selective channels in artificial lipid bilayers. Magainins exhibit cytolytic activity against human hematopoietic and solid tumors cells studied in vitro. The viability of tumor cells was compromised at peptide concentrations that had little effect on circulating human blood cells (red blood cells, lymphocytes and neutrophils) and neural cells from different stages of embryonic development and differentiation. The IC_{50}'s of magainins G, A and B was 10-20 μg/ml, while magainins 1 and 2 were less effective. To study the effect of magainins on membrane potential we utilized a membrane potential-sensitive dye (oxonol). Changes in fluorescence signals were studied utulizing a fluorescence spectrophotometer. In order to determine the potentiometry of the fluorescence signal we incubated the cells in the presence of 200 nM gramicidin D, a Na^+-K^+ ion selective peptide. Gramicidin increased the fluorescence signal when the cells were incubated in regular saline. When NaCl was replaced by 140 mM NMG-Cl the polarity of the signal was inverted. At low, non-cytolytic concentrations magainin produced fluorescence signals comparable with non-selective cation channels. Remarkably, tumor cells remained vital, excuding trypan blue, when depolarized either by resuspension in 150 mM KCl or by 200 nM gramicidin in physiological saline and then exposed to cytolytic concentrations of the peptide. The selectivity of the ionophoric peptide for tumor cells and the protection afforded by depolarization may be related to physico-chemical differences between tumor and normal cell membranes.

280.10

MUTANT OF ESCHERICHIA COLI WITH ALTERED PRESSURE AND DRUG SENSITIVITY OF THE MECHANOSENSITIVE ION CHANNEL. C. Li, B. Martinac, C. Kung and J. Adler. Departments of Biochemistry and Genetics and Laboratory of Molecular Biology, University of Wisconsin, Madison, WI 53706

We have studied the effect of chlorpromazine (CPZ) on the pressure sensitivity of the mechanosensitive (MS) ion channel of E. coli by use of the patch-clamp recording technique. We have isolated several CPZ-resistant mutants of E. coli. One of the isolates with aberrant morphology has MS channels which differ from wild-type MS channels in two ways: (i) CPZ does not affect the opening probability of the channels as it does in the wild-type strain, and (ii) the MS channels in the mutant have reduced pressure sensitivity as compared with the wild-type channels. We do not know yet what causes the altered properties of the MS channels in the mutant strain. Parallelly to the electrophysiological studies we are characterizing the mutant by genetic approach. The localization of the mutation responsible for the MS channel phenotype in the mutant by gross and fine mapping is in progress. Supported by NIH grant DK39121 and by a grant from the Lucille P. Markey Trust.

281.1

THE MUTATION *SLO* ALTERS MEMBRANE EXCITABILITY AND CA-ACTIVATED K CURRENT IN CULTURED *DROSOPHILA* "GIANT" NEURONS. M. Saito and C.-F. Wu, Dept. of Biology. Univ. of Iowa. Iowa City, IA 52242.

The mutation *slowpoke* (*slo*) in *Drosophila* is known to eliminate a Ca-activated transient K current in larval and adult muscles. Consequently, the Ca action potential in *slo* muscle is prolonged. This phenotype is enhanced when repetitive action potentials are induced with sustained current injection which inactivates A current. It is important to ask whether *slo* also affects the neuronal membrane current and excitability. We have developed a "giant" neuron culture system which allows collection of both current- and voltage-clamp data from the same cells. Embryonic neuroblasts treated with cytochalasin B differentiated without cytokinesis and became multinucleated giant neurons with well-developed neurites. In wild-type giant neurons, four different types of voltage responses were observed under current-clamp: all-or-none, graded multiple-peak, graded single-peak, and "passive" responses. In *slo* neurons, all 4 types of responses were present. However some all-or-none action potentials showed longer duration (10 to 26 ms) than that in wild-type neurons (7 to 15 ms). In addition, *slo* neurons displayed irregular responses which did not fall in any of the above categories, such as variable voltage oscillations or bursts of action potentials riding on slow depolarizing fluctuations. Voltage-clamp studies showed that *slo* eliminates a Ca-activated current in a subset of neurons. However, the defect was not limited to the transient component of Ca-activated current, as observed in muscle, but often affected a sustained component. The differential effects of *slo* in nerve and muscle suggest molecular diversity of Ca-activated K channels.

281.2

MOLECULAR CHARACTERIZATION OF THE SLOWPOKE CODING REGION. G.A. Robertson, N.S. Atkinson and B. Ganetzky, Laboratory of Genetics, University of Wisconsin, Madison,WI 53706.

Calcium-activated potassium channels are key elements in the control of membrane excitability. The mechanisms of gating and modulation of these channels are incompletely understood, however, due to the lack of information regarding their molecular structure. Even less is known about how the expression of these or other ion channels is regulated by the genome. In *Drosophila*, a mutation in the gene *slowpoke* (*slo*[1]) eliminates a specific calcium-activated potassium current in muscle and neurons (Elkins, Ganetzky and Wu, PNAS, 83:8415, 1986). We generated additional mutations to localize the gene cytogenetically and to clone DNA from the region (Atkinson, Robertson and Ganetzky, Soc.Neurosci. Abs.15:541,1989). The immediate goal of this project is to determine whether *slo* encodes a structural component of the calcium-activated potassium channel or a polypeptide otherwise governing its function or expression.

We have identified several related cDNAs within the *slo* region. These cDNAs were isolated on the basis of their homology to genomic DNA that spans two genetic lesions associated with a *slo* phenotype. One cDNA was used to probe Northern blots of wild-type head mRNA and hybridizes to two transcripts of 10.5 and 5.8 kb. These transcripts are not present in head mRNA from flies carrying a *slo* mutation. We have begun sequencing this cDNA and from the deduced amino acid sequence we hope to gain insight into the structure and functional role of the *slo* gene product.

Supported by postdoctoral fellowships from the NIH (GAR) and the Muscular Dystrophy Assn (NSA) and by NIH grant NS-15390 (BG).

281.3

K^+ CHANNEL SUBFAMILIES HIGHLY CONSERVED IN *DROSOPHILA* AND MOUSE. Michael D. Pak*, Manuel Covarrubias*, Ann Ratcliffe*, Alice Butler*, and Lawrence Salkoff. Dept. Anatomy and Neurobiol., Washington Univ. Sch. Med., Box 8108, 660 S. Euclid Avenue, St. Louis, MO 63110.

We show that mouse and *Drosophila Shab* (*mShab* and *fShab*) represent an instance of K^+ channels in distantly related species that are both functionally and structurally conserved; most kinetic, voltage-sensitive, and pharmacological properties are similar for the two channels. The greatest functional difference between the currents is recovery from inactivation which is several times slower in *mShab* than *fShab*. Another interesting difference is the delay in current rise in response to depolarization, which is greater for *mShab* than *fShab*. This difference is interesting because it occurs between two channels which are structurally very similar with mostly conserved differences. *mShab* and *fShab* are, thus, naturally occurring structural variants which may prove valuable in analyzing channel kinetics in the future. In addition to conserved structure, *mShab* has an unusually long nonconserved region at the carboxyl end of the protein. Truncation of 293 residues from the carboxyl end produced no noticeable change in voltage-sensitive, kinetic or pharmacological properties. Thus, the measured functional properties of *mShab* are determined by the remaining 564 residues, most of which are conserved. The properties of *mShab* closely resemble those of a native delayed-rectifier type potassium channel in hippocampal neurons. A *Shal* homolog from mouse brain which has very high homology to *Drosophila Shal* is currently being expressed. Supported by NIH 1 RO1 NS24785-01, a research grant from the Muscular Dystrophy Association, and grant from Monsanto-Searle.

281.4

FUNCTIONAL EXPRESSION OF BOTH TRANSIENT AND DELAYED OUTWARD K^+ CURRENTS IN XENOPUS OOCYTES RESULTING FROM INJECTION OF A CLONED HUMAN K^+ CHANNEL mRNA. R.E. Hice, L. Philipson*, D.J. Nelson*, G. Bell*, K. Schaefer*, and D. Steiner*. Depts. of Medicine, Biochemistry and Molecular Biology, Neurology, and the Howard Hughes Medical Institute, University of Chicago, Chicago, IL 60637.

A cDNA clone encoding a voltage dependent K^+ channel was isolated from a human fetal skeletal muscle cDNA library by screening at low stringency with a related human K^+ channel cDNA probe. The 4kb cDNA contained a 1959-base pair open reading frame. The deduced 653-amino acid protein is 95% identical to RCK4, a previously described rat cortex K^+ channel clone (Stühmer *et al., EMBO 8*:3235,1989). The two proteins are highly conserved, with most of the amino acid substitutions occurring in the N-terminal domain. Nine of the 22 substitutions (plus one deletion) are conservative. A 2.3kb segment of the cDNA containing the open reading frame was subcloned into the sP64T vector for production of *in vitro* transcribed RNA using SP6RNA polymerase. After microinjection of pgm amounts of sRNA into *Xenopus* oocytes, large outward K^+ currents (>10μA) could be observed by 12 hours. Outward current during depolarizing steps from holding potentials more negative than -40 mV was composed of both transient and delayed outward K^+ conductances. The delayed K^+ current could be observed in isolation by either holding at -40mV or more positive or by adding 10mM 4-AP to the external solution. The I_A is activated by -40mV with a time to peak of ca. 7 msec at 22°C. Inactivation of the I_A is largely complete at the end of 200 msec depolarizations. The delayed outward component was present even when total outward current was kept below 3μA and was present in sRNA produced from multiple independently subcloned plasmids. The presence of two distinct kinetic components in the expressed outward current indicates that variability in K^+ channels could be due to differential oligomeric interactions or post-translational modifications. Supported by NIH GM36823 (DJN).

281.5

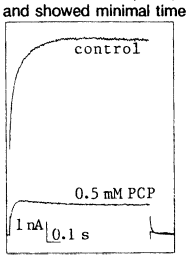
ESTABLISHMENT OF TRANSFORMED FIBROBLASTS WITH POTASSIUM CHANNEL GENES, NGK1 AND NGK2. T. Kawamura *#, S. Yokoyama*, I. Yamashita*# and H. Higashida. Departments of Biophysics and *Neurosurgery, Kanazawa Univ. Sch. of Med., Kanazawa 920, Japan.

Two types of potassium channels expressed in NG108-15 mouse neuroblastoma x rat glioma hybrid cells have been identified by molecular cloning technique (Yokoyama et al. FEBS Lett., 259: 37, 1989). NGK1 is identical with a rat brain potassium channel BK2, and NGK2 is structually related to the Drosophila Shaw gene. Both channel proteins expressed in Xenopus oocytes have properties of delayed rectifiers. We have established transformed B82 mouse fibroblast cells which stably express voltage-dependent potassium channels, NGK1 and NGK2 gene products, respectively. Each of the NGK1 and NGK2 cDNAs was ligated to the mammalian expression vector, pKNHneo, containing the SV40 early promotors and an aminoglycoside-3'-phosphotransferase gene. These recombinant pKNH-NGK1 or pKNH-NGK2 plasmids were independently introduced into B82 cells by a cationic liposome-mediated transfection method and the cell lines were selected by geneticin. Northern blot analysis of total RNA from two types of isolated clones probed with the entire coding region of NGK1 or NGK2, respectively, identified proper transcripts. Electrophysiological studies in the two types of clones by whole cell voltage clamp and single channel recording technique revealed the appearance of voltage-dependent potassium channels with difference in kinetics.

281.6

POTASSIUM CURRENTS WITH DELAYED RECTIFYING PROPERTIES IN FIBROBLAST CELLS STABLY TRANSFECTED WITH A POTASSIUM CHANNEL GENE. T.R. Werkman[1], T. Kawamura*[2], S. Yokoyama*[2], H. Higashida[2] and M.A. Rogawski[1]. [1]Medical Neurology Branch, NINDS, NIH, Bethesda, MD 20892, and [2]Kanazawa University School of Medicine, Kanazawa 920, Japan.

The pharmacological properties of a voltage-dependent K^+ channel were studied in a fibroblast cell line (CL1023) stably transfected with a potassium channel gene (NGK1) from the NG108-15 neuroblastoma-glioma hybrid cell line (Yokoyama *et al., FEBS Lett.*, **259**: 37, 1989; Kawamura *et al., this meeting*). NGK1 is identical with the rat brain K^+ channel protein BK2. Upon step depolarization to potentials more positive than -20 mV we recorded a large (up to several nanoamperes) outwardly directed current that slowly rose to a plateau and showed minimal time dependent inactivation. Tail current analysis revealed the outward current to be carried by K^+ ions. The K^+ current was relatively insensitive to tetraethylammonium (100 mM: ~ 50% block) but was more sensitive to 4-aminopyridine (1 mM: ~ 60% block; 3 mM: ~ 80% block) and the dissociative anesthetic phencyclidine (PCP) (50 μM: ~ 50% block; 500 μM: ~ 90% block; figure: depolarizing steps from -60 to +60 mV). In view of the recently described heterogeneity of voltage-dependent K^+ channels, non-neuronal cells expressing a single K^+ channel subtype provide a useful system to characterize the unique pharmacological properties of the various molecular species.



281.7

CLONING, EXPRESSION, AND MODULATION BY SEROTONIN OF A MOUSE BRAIN POTASSIUM CHANNEL.
J.H.Hoger*, A.E.Walter*, D.Vance*, Lei Yu[1], C. Labarca, H.A.Lester, and N.Davidson. Divs. of Biology and of Chemistry. Caltech, Pasadena CA 91125, and [1]Dept. Medical Genetics, Indiana School of Medicine, Indianapolis, IN 46202.

Modulation of ion channels by neurotransmitter and hormone receptors is a common mechanism for the regulation of cellular excitability. We have used Xenopus oocytes to study the modulation of a cloned ion channel by a cloned receptor. A mouse brain potassium (K) channel was cloned (MBK1a) and expressed in Xenopus oocytes. The K current expressed in the oocytes displayed characteristics similar to delayed rectifier type K currents. This current underwent slow inactivation (τ=5-7s) and was blocked by external tetraethyammonium ($IC_{50\%}$=0.4 mM). The K channel was co-expressed with a mouse brain serotonin receptor ($5HT_{1C}$) in Xenopus oocytes. Activation of the $5HT_{1C}$ receptor by 0.1μM serotonin resulted in suppression of 56% of the K current amplitude over a period of 20 min. In contrast, other cloned K or Na ion channels were not suppressed by the $5HT_{1C}$ receptor in oocytes. The K current amplitude could also be suppressed by intracellular injections of GTP-γ-S or Ca. The suppression of the K current was blocked by EGTA, confirming that the suppression requires an increase in intracellular Ca. We have found that the suppression is sensitive to the calmodulin antagonists W-7 and trifluoperizine. In addition, experiments indicated that protein kinases of type A, type C or calmodulin kinase II were not involved in the suppression of I_K. Support: GM-29836 and 10991.

281.8

SHAW, A K^+ CHANNEL FROM *DROSOPHILA*, HAS UNUSUALLY LOW VOLTAGE SENSITIVITY AND POOR K^+ SELECTIVITY. K. Baker, and L. Salkoff. Dept. Anatomy and Neurobiology, Washington U. Sch. of Med., Box 8108, 660 S. Euclid Ave. St. Louis, MO. 63110.

The *Shaw* gene from *Drosophila* (Butler et al. Science 234) expresses a voltage-gated potassium current in *Xenopus* oocytes (Wei et al. Science in press). The putative gating charge region (S4) is novel because it only has 4 positive charges in an uninterrupted +00 format. In addition, 2 negative charges are found in positions occupied by positive charges in other K^+ channels. Thus, *Shaw* may have a lower equivalent gating charge than other cloned K^+ channels. This predicts that the probability of being open will change gradually over a broad voltage range. A clear 'threshold' for activation is therefore not expected. Experimental observations support these predictions. Active current is observed at -100 mV and conductance continues to increase beyond +50 mV. Thus, *Shaw* activates over an extremely broad voltage range (-100 mV to +50 mV) and shows no clear 'threshold'. Another anomalous property of *Shaw* is poor K^+ selectivity compared with other cloned K^+ channels. *Shaw*'s sodium to potassium permeability ratio (P_{Na+}/P_{K+}) is over 2 times larger than other cloned K^+ channels. We are presently characterizing a site-directed mutant that alters the selectivity of *Shaw*. Although no *in vivo* role has been ascribed to *Shaw* it will likely be unique due to its unusual properties as compared to other K^+ channels. Supported by NIH 1 RO1 NS24785-01, and research grants from the MDA and Monsanto-Searle.

281.9

SITE-DIRECTED MUTAGENESIS OF *SHAKER* POTASSIUM CHANNELS. G. A. Lopez*, Y.N. Jan & L.Y. Jan. Howard Hughes Medical Institute and Departments of Physiology and Biochemistry, UCSF, San Francisco, CA. 94143

The recent cloning of several potassium channel genes has made it possible to carry out structure-function studies. The approach taken in this study is to examine the potential function served by those hydrophobic residues that are highly conserved. A comparison of the primary amino acid sequences of several potassium, sodium, and calcium channels reveals dramatic conservation in the S4 segment which is the hypothesized voltage sensor. The S4 segment consists of a repeated triplet containing a positively charged amino acid, usually an arginine, and two hydrophobic amino acids. It has been shown previously that charge neutralizations in S4 affect voltage dependence. We now show that conservative hydrophobic substitutions in S4 also give a similar phenotype in voltage dependence. Using site-directed mutagenesis, we have substituted each of several hydrophobic amino acids in various transmembrane segments with hydrophobic residues. Mutated channels were expressed in *Xenopus* oocytes and analyzed using two electrode voltage clamp. We have found that mutating certain hydrophobic residues in the S4 segment altered the position of the activation curve but not its slope. No effect was seen in channel kinetics or potassium selectivity. These results reveal that the S4 sequence as a structural entity is important in the voltage-dependent channel gating.

281.10

CLONING OF RAT BRAIN POTASSIUM CHANNELS: A NEW MEMBER OF THE RCK FAMILY AND REPRESENTATIVES OF TWO NOVEL FAMILIES. J.A. Drewe, G.E. Kirscht, S. Verma, A.M.J. VanDongen, A.M. Brown and R.H. Joho, Dept. of Molecular Physiology and Biophysics and †Dept. of Anesthesiology, Baylor College of Medicine, Houston, TX 77030.

Voltage-activated potassium (K^+) channels in rat brain are represented by three families, *rck*, *drk* and *ngk*. We report here the cloning of a novel K^+ channel (*rck6*) whose amino acid homology suggests membership in the *rck* family, and the isolation of two additional putative K^+ channel clones. *rck6* was isolated by hybridization to an oligonucleotide corresponding to seven amino acids (NEYFFDR) located in the amino terminus within all three families. The characteristics of the expressed K^+ currents is presented at this meeting (Kirsch *et al.*). Two additional clones were obtained via low-stringency cross-hybridization screening, utilizing nick-translated *drk1*. They have been sequenced in their entirety, with single open reading frames of 547 and 505 amino acids. Sequence homology within the six transmembrane core region suggests that the clones correspond to K^+ channels which would represent new families. However, no expression of K^+ currents was observed in *Xenopus* oocytes injected with cRNA from the latter two clones. Supported by the NIH, the Advanced Technology Program of the State of Texas and the American Heart Association (Texas Affiliate).

281.11

COMPARATIVE PHARMACOLOGY OF CLONED RAT BRAIN DELAYED RECTIFIER POTASSIUM CHANNELS. M. Taglialatela, J.A. Drewe, S. Verma, A.M. Brown, R.H. Joho and G. E. Kirscht. †Dept. of Anesthesiology and Dept. of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.

We have begun a comparative analysis of the pharmacological properties of rat brain K^+ channels from the *drk*, *ngk* and *rck* families, expressed in *Xenopus* oocytes. Our results show that *drk1* and an *ngk2*-like clone are the most pharmacologically inert; they are blocked by 4-aminopyridine (4-AP) and tetraethylammonium (TEA) at millimolar concentrations, but are insensitive to peptide toxins at micromolar concentrations. By contrast *rck6* (Drewe *et al.*, this meeting) and an *rck1*-like clone were sensitive to dendrotoxin, noxiustoxin and mast cell degranulating peptide at nanomolar concentrations. The *rck* clones also showed a voltage- and frequency-dependent response to 1 mM 4-AP which resembles that observed in the squid axon and frog node of Ranvier delayed rectifiers; repetitive depolarizing test pulses to +40 mV at a frequency 1 Hz resulted in a progressive relief of block. These results provide further support for the idea that *rck* is a mammalian delayed rectifier homolog. Supported by the NIH, the Advanced Technology Program of the State of Texas and the American Heart Association (Texas Affiliate).

281.12

EXPRESSION AND CHARACTERIZATION OF A NOVEL MEMBER OF THE RCK FAMILY OF RAT BRAIN POTASSIUM CHANNELS. G.E. Kirscht, J.A. Drewe, S. Verma, A.M. Brown and R.H. Joho. †Dept. of Anesthesiology and Dept. of Molecular Physiology and Biophysics, Baylor College of Medicine, Houston, TX 77030.

We have cloned from rat brain a novel voltage-activated K^+ channel (*rck6*; Drewe, *et al.*; this meeting) belonging to the *rck* family. RNA transcripts of *rck6* injected into *Xenopus* oocytes expressed delayed rectifier K^+ channels. Test pulses to +50 mV evoked K^+ current (2-6 μA) which did not inactivate in 5 s. The conductance-voltage relationship followed a Boltzmann distribution with midpoint and slope factors, -12.3 and 11.9 mV, respectively. Single channel conductance in frog Ringers was 8.2 pS. Current was blocked by 4-aminopyridine, tetraethylammonium, dendrotoxin, noxiustoxin, and mast cell degranulating peptide; but not by charybdotoxin ([CTX]< 200nM). A short amino terminus (175 residues), similar to that of other non-inactivating *rck*s, may be responsible for the lack of inactivation in *rck6*. CTX resistance may reflect differences in the extracellular domains; *rck6* has longer S1-S2 and S3-S4 linkers than the other *rck*s. Supported by the NIH, the Advanced Technology Program of the State of Texas and the American Heart Association (Texas Affiliate).

281.13

POTASSIUM CHANNEL GENE STRUCTURE AND EXPRESSION AND AUDITORY FUNCTION IN THE DEAF WADDLER MOUSE. L.A. Adams*, P.Weisleder, N. Copeland, N. Jenkins, L. Lock, E. W Rubel, and B.L.Tempel. Depts. of Pharmacology, Medicine, and the Hearing Devel. Labs., Univ. of WA, 98195; GRECC, Seattle Veterans Admin. Med. Center, Seattle, WA, 98108; and Natl. Cancer Inst., Frederick, MD, 21701.

Potassium channels are a diverse class of membrane proteins important in controlling the activity of excitable cells. We are studying one such potassium channel gene (MK1) in the deaf waddler (dfw) mouse, a mutant strain in which the homozygotes (dfw/dfw) exhibit a wobbly gait when walking, and appear to be deaf. Brainstem electrodes were used to record auditory evoked responses to tone bursts ranging in frequency from 0.5-16 kHz, which were presented in a free field. Heterozygotes (dfw/+)had thresholds comparable to those reported in the literature for normal mice. In dfw/dfw, on the other hand, we were unable to evoke replicable responses at any frequency, even when stimuli exceeded 100 dB SPL. The confidence interval for the chromosomal locus of MK1 and the dfw mutation overlap on mouse chromosome 6. Northern blot analysis of MK1 expression in brain tissue reveals a 30-40% decrease in MK1 mRNA levels in dfw/dfw compared to their heterozygote (dfw/+) littermates. In situ hybridization studies in normal mouse brain demonstrate that MK1 is expressed in brainstem auditory and motor relay nuclei; an examination of the expression of MK1 in the dfw/dfw brain is presently underway. Genomic DNA from dfw/dfw is being examined by direct nucleotide sequencing and by Southern blot analysis of restriction fragment length polymorphisms. Supported by NIH NS 27201 and DC 00395.

281.14

SENSITIVITY TO BLOCKING AGENTS OF CLONED POTASSIUM CHANNELS EXPRESSED IN XENOPUS OOCYTES. M.P. Kavanaugh, P.B. Osborne, M.J. Christie, A.E. Busch, R.P. Hartshorne, R.A. North and J.P. Adelman. Vollum Institute, Oregon Health Sci. Univ. Portland, OR 97201

Clones were expressed in Xenopus oocytes and membrane currents were evoked (-80 to 0 mV) by depolarizing pulses. RBK1 currents were inhibited in a dose-dependent manner by four fractions of dendrotoxin (DTX) and by the related toxin I which are derived from the venom African mamba. The order of potency was δ-DTX < toxin I α-DTX = γ-DTX < β-DTX (respectively IC_{50} values in nM: < 0.03, 0.1 $\pm$ 0.02, 0.3 $\pm$ 0.02, 0.3 $\pm$ 0.3 and 5.1 $\pm$ 3). The interaction of TEA with the channel was also studied. RBK1 and the structurally homologous clones RBK2 and RGK5 have widely different sensitivities to TEA (respective K_D's: 0.3 mM, > 100 mM and 10 mM). Chimeras were constructed utilizing a conserved restriction site (MscI) immediately N-terminal to the S4 putative transmembrane segment. The TEA sensitivity of the expressed channels could be correlated with the source of the C-terminal region beginning at S4. Chimeras with the RBK1 sequence in this region had K_d's for TEA between 0.2 and 0.5 mM, regardless of the origin of the remaining portion of the molecule. Likewise chimeras that had RBK2 or RGK5 sequence in this region had respective K_d's of > 100 mM and in the 10-50 mM range. These data suggest that the putative extracellular loop between S5 and S6 is a critical determinant of TEA sensitivity.

281.15

REGULATION OF THE BRAIN K_{v1} POTASSIUM CHANNEL GENE IN PITUITARY CELLS. L. Hemmick, E.S. Levitan, D. Saal, J. Marshall, N. Birnberg, and L.K. Kaczmarek. Yale Univ. Sch. Med., New Haven, CT 06510.

The rat brain gene K_{v1} encodes a delayed rectifier K^+ channel when expressed in frog oocytes (Swanson et al., 1990). Here we report that Northern blots indicate that a 3.5 kb polyA+ K_{v1}-like transcript is expressed in native anterior pituitary and rat GH pituitary tumor cells. No transcript was detectable in AtT-20, PC12 or NG108 cells, suggesting that expression is cell type specific. A GH cell cDNA library was screened and a clone was isolated. Preliminary DNA sequence and RNAse protection data indicate that the GH cell gene is identical to K_{v1}. Dexamethasone increased the K_{v1} transcript level ~2 fold. Thus, corticosteroids may regulate secretion, in part, by altering ion channel gene expression. Treatment of GH cells with secretagogues (TRH, TPA, cAMP analogs, Bay K, high K^+) failed to alter K_{v1} mRNA levels. Interestingly, GH cells do not have a simple delayed rectifier. Rather, their K^+ channels either inactivate or are Ca^{2+} sensitive. We hope to use hybrid arrest to identify the component of current that is carried by the K_{v1} channel protein and to determine its role in GH cells.

281.16

IDENTIFICATION OF A DELAYED RECTIFIER K^+ CHANNEL IN RABBIT SCIATIC NERVE BY cDNA CLONING TECHNIQUE. J-H Hsu*, G.K. Wang, and S-Y Wang. Dept. of Anesthesia, Harvard Med. Sch., Boston, MA 02115 and Dept. of Biology, SUNY at Albany, Albany, NY 12203.

Neuronal-like delayed rectifier K^+ channels are present in dissociated Schwann cells from rabbit sciatic nerve (Chiu, J. Physiol. 396:173). It was suggested that these channels are synthesized in vivo in the sciatic nerve. We performed Northern blot analysis to demonstrate that mRNA transcripts homologous to the cDNA clone of RCK1 (delayed rectifier K channels) were indeed present in adult rabbit sciatic nerve. The hybridization signal of these mRNA transcripts was comparable with its brain counterpart in its mobility (~8 Kb) and intensity. cDNA cloning and sequencing further indicate that both the rabbit brain and rabbit sciatic nerve express identical delayed rectifier K^+ channels. The sequence analysis also shows that the coding region of the RCK1 gene is highly conserved (over 90%) among rat, mouse, and rabbit. We conclude that rabbit sciatic nerve expresses neuronal K^+ channels in vivo. The origin of these gene transcripts in sciatic nerve is most likely from Schwann cells. (Supported by NIH GM39939.)

281.17

A POTASSIUM CHANNEL HOMOLOGOUS TO SHAB IS PRESENT IN THE *APLYSIA* NERVOUS SYSTEM. E. A. Quattrocki, R. J. Knox, J. Marshall*, and L. K. Kaczmarek Dept. of Pharmacology, Yale University School of Medicine, New Haven, CT 06510.

Potassium channels, by their diversity and ability to undergo modulation underlie many of the complex changes in electrical excitability occurring in neurons. The modulation of K^+ currents has been extensively studied in *Aplysia californica*; yet, at the molecular level much remains unknown. In an effort to study the molecular basis for modulation of K^+ currents we have isolated putative K^+ channel clones from the *Aplysia* nervous system. Screening head ganglia libraries with a rat brain K^+ channel, Kv1, as a probe, yielded two cDNAs of approximately 400 and 700 bases that exhibit a high degree of similarity to other K^+ channels. These cDNAs code for a single peptide sequence that spans from the S4 region of a K^+ channel out towards the 3' end, including two other potential membrane spanning regions, S5 and S6. Comparisons with other known K^+ channels show that this clone is most similar, approximately 80%, to the *Drosophila* K^+ channel Shab; the membrane spanning regions being the most similar. Characteristic to Shab and Shaw, but unlike Kv1 or the other Shaker-like channels, this *Aplysia* clone has only five positively charged amino acids instead of seven in the S4 region, the presumed voltage sensor. Efforts now focus on obtaining the full length clone for expression studies and on investigating this putative channel's role in intact cells using antisense hybrid arrest techniques.

281.18

CLONING AND EXPRESSION OF K_V4,A RAT BRAIN DELAYED RECTIFIER K^+ CHANNEL CDNA. C.J.Luneau*,S.A.Buhrow*,J.Marshall*,J.Antanavage*,E.Levitan, J.Smith*,R.Swanson,K.Folander,C.Bennett*,C.Oliva*,L.Kaczmarek, R.B.Stein*,and J.B.Williams. Depts. of Pharmacology and Biological Chemistry, Merck, Sharp & Dohme Res. Labs, West Point, PA 19486 and Dept. of Pharmacology, Yale U. School of Med., New Haven, CT 06510

K_V4, a cDNA clone which encodes a K^+ channel, was isolated from a rat brain library with a probe derived from K^+ channel clone K_V1 (Swanson,R., Neuron, 4, 1990). The clone spans 4 kb and contains an ORF which encodes a protein of 585 amino acids (M_r = 65,353). Isolation of several genomic clones spanning regions of the cDNA clone indicates that the coding sequence of K_V4 is interrupted by introns. K_V4 possesses 6-7 transmembrane regions and is very similar to K^+ channel clone NGK2 (Yokoyama, S., et al., FEBS Lett, 259: 37, 1989) but diverges significantly at the C-terminus. The protein is encoded by a 4.5 kb mRNA which Northern analysis has revealed to be present only in brain; no hybridizing species were evident on Northern blots prepared from heart, spleen, kidney, skeletal muscle or lung mRNA. The abundance of the mRNA increases in the rat over the course of development from neonate to adult. In vitro transcribed K_V4 RNA, when injected into Xenopus oocytes, elicits a voltage-activated current which displays delayed rectifier characteristics.

281.19

WITHDRAWN

281.20

CLONING OF POTASSIUM CHANNEL cDNAS FROM RAT LEUKOCYTES AND HUMAN T CELLS K. Folander, C.S. Lin, J.S. Smith, R. Swanson, and R.B. Stein Departments of Pharmacology and Immunology Research, Merck Sharp and Dohme Research Labs

A cDNA encoding a potassium channel was cloned from rat peripheral white blood cell mRNA using the polymerase chain reaction. The DNA sequence of the clone is identical to K_V3, a delayed rectifier K^+ channel previously cloned from rat brain (Swanson *et al.*, Neuron, 1990). Injection of RNA transcripts of the clone into *Xenopus* oocytes resulted in the expression of K^+ currents with properties intermediate to those of the *n* and *n'* type K^+ currents previously characterized in mouse T cells. Thus, the rat channel (like the *n'* channel) is half activated by depolarization to -10 mV, is blocked by charybdotoxin (IC_{50}= 1 nM), and is relatively insensitive to block by TEA (IC_{50} >40mM). The current, however, does display some use-dependent inactivation upon repetitive depolarization, as does the *n* type current.

Human T lymphocytes were purified from peripheral blood by density gradient centrifugation, sheep erythrocyte rosetting, nylon-wool column purification, and plastic adherence. These T cell preparations were >98% $OKT3^+$ and contained both $CD4^+$ and $CD8^+$ populations as assessed by flow-cytometry analysis. An ~1100 bp cDNA was cloned from these T cells and encodes a partial protein with >90% amino acid identity to rat K_V3. The region of the protein that has been cloned consists of 364 amino acids that extend from upstream of the first hydrophobic domain to within the carboxy terminal region of the channel. Work is now in progress to clone cDNAs to complete the entire protein coding sequence.

281.21

LOCALIZATION AND TOPOLOGY OF K^+-CONDUCTING MEMBRANE PROTEIN I_{SK} IN RAT KIDNEY CELLS. T. Sugimoto, T. Ueyama, T. Houtani*, M. Ikeda*, H. Ohkubo+*, T. Takumi+*, Y. Tanabe+* and S. Nakanishi+*. Dept. of Anatomy, Kansai Medical Univ., Moriguchi, Osaka 570 and +Inst. Immunology, Fac. Med., Kyoto Univ., Kyoto 606 Japan.

A novel rat membrane protein that induces selective permeation of K^+ ions by membrane polarization was previously identified (Science 242: 1042-1045, 1988). This protein termed I_{SK} consists of 130 amino acids with a single putative membrane domain. We raised antisera against several oligopeptides representing parts of the I_{SK} protein. Tissue sections obtained from the perfusion-fixed rat kidney were immunostained for I_{SK} protein using different antibodies which recognized aminoterminal (N-I) and carboxyterminal (C-I) peptide fragments. Both antibodies recognized the apical membrane portion of epithelial cells in the proximal tubule as reported recently (J. Membrane Biol. 113: 39-47, 1990). At the ultrastructural level, the immunolabel was densely and uninterruptedly distributed over the apical membrane of the epithelial cells and of the microvilli. N-I immunoreactive product was also present in the surface coat portions; C-I immunoreactive product was localized to the membrane and the contiguous submembranous zone of the cytoplasm. Basolateral cell surfaces were virtually free from the immunolabel. The results indicate that I_{SK} protein in renal tubular cells is situated at the apical membrane with its aminoterminal portion directed toward the tubular lumen, and support our previous conclusion that I_{SK} protein plays an important role in K^+ permeation from the epithelial cells to the lumen.

SYNAPTIC STRUCTURE AND FUNCTION II

282.1

A COMPARISON OF KINETIC MODELS OF THE NEUROMUSCULAR JUNCTION USING MONTE CARLO SIMULATION. T.M. Bartol Jr.*, E.E. Salpeter[1]*, M.M. Salpeter. Section of Neurobiology and Behavior, and [1]Laboratory of Nuclear Studies Cornell University, Ithaca, NY 14853.

Previous studies have modelled the kinetics of the nicotinic acetylcholine receptor (AChR) at the neuromuscular junction (NMJ) using simultaneous differential equations. For case in solving these equations, the full kinetic scheme,

$$A+R \underset{k_{-1}}{\overset{2k_{+1}}{\rightleftharpoons}} A+AR \underset{2k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} A_2R \underset{\alpha}{\overset{\beta}{\rightleftharpoons}} A_2R^*$$

can be replaced by an approximate simplified scheme,

$$A+R \underset{k_{-1}}{\overset{2k_{+1}}{\rightleftharpoons}} A+AR \underset{2k_{-2}(1-g)}{\overset{k'_{+2}}{\rightleftharpoons}} A_2R',$$

(Adams in *Myasthenia Gravis*, Albuquerque & Eldefrawi eds., Chapman & Hall publ., 1983, p. 131). This scheme uses a reduced forward binding rate constant $k'_{+2} = k_{+2}(\alpha+\beta)/(\alpha+\beta+2k_{-2})$ and a factor $(1-g) = \alpha/(\alpha+\beta+2k_{-2})$, which effectively gives the fraction of doubly bound receptors which are in the closed state and from which the actual unbinding occurs. If unbinding is slow compared to isomerization, $2k_{-2} << (\alpha+\beta)$, there is no doubt about the validity of the simplified scheme; for such cases k'_{+2} reduces to k_{+2} and (1-g) reduces to $\alpha/(\alpha+\beta)$. If unbinding is fast, on the other hand, it is not obvious how well the simplified scheme would mimic the full scheme. We have developed a Monte Carlo method for modelling the NMJ, which follows the fate of each individual ACh molecule in the cleft. This method allows an accurate evaluation of each of the two kinetic schemes. We have carried out such calculations for a number of cases, ranging from slow to fast unbinding. We have established that, for both conditions, the shape (and amplitude) of a miniature endplate current described by the simplified scheme does indeed approximate that given by the full scheme. This research was conducted using the Cornell National Supercomputer Facility, a resource of the Center for Theory and Simulation in Science and Engineering and was funded by NIH grant NS09315.

282.2

FREEZE-FRACTURE ULTRASTRUCTURE OF THE FROG NEUROMUSCULAR JUNCTION ACTIVE ZONE REVEALS NO MARKED PROXIMAL-DISTAL GRADIENT IN SYNAPTIC STRUCTURE. **P. A. Pawson and A. D. Grinnell, Jerry Lewis Ctr, UCLA Sch. Med., Los Angeles, CA, 90024.**

To resolve disagreements in the literature, we have looked again for structural correlates of the commonly observed decrease in release efficacy from the distal regions of frog motor nerve terminals. Since the relevant synaptic structure is believed to be the pre-synaptic active zone (AZ), we have examined quantitatively the proximal-distal distribution of AZ structure, using a freeze-fracture technique that produces replicas of large fractions of terminals, including the region of nerve entry, so that we know the proximal/distal position of each branch. From 18 endplates we obtained fractures of 66 branches, 28 of which exceeded 20 micrometers in length. We selected for analysis the nine branches that measured > 50 micrometers (from 8 endplates). Only one of these branches showed a marked distal decrease in AZ length/unit length of terminal, while several had short regions (5-10 micrometers), either proximal or distal, that exhibited amounts of AZ that were substantially greater or smaller that the average value for that terminal branch. In general, however, these branches did not display a significant proximal-distal gradient in AZ length/micrometer terminal length. **(Supported by NIH,NSF,MDA).**

282.3

REGULATION OF SINGLE QUANTAL CURRENT AT THE SNAKE NEUROMUSCULAR JUNCTION. R.S. Wilkinson and J.J. Stevermer*. Department of Cell Biology and Physiology, Washington University School of Medicine, St. Louis, MO 63110.

The strength, or efficacy, of a synapse depends not only on the number of transmitter quanta released (quantal content) but also on the postsynaptic current produced by each quantum. Thus one potential mechanism for regulation of synaptic strength is regulation of quantal current. To test this possibility we compared spontaneous miniature endplate currents (mepc's) among fibers of different type (faster twitch, F; slower twitch, S; and tonic, T) and input resistance (R_{in}) in the thin transversus abdominis muscle of the garter snake.

Muscle fibers were identified by anatomical criteria as F, S, or T and impaled near their endplates with 2 microelectrodes. R_{in} was determined from the slope of current-voltage plots near resting potential. Amplitudes of mepc's were either calculated by dividing miniature endplate potential amplitudes by R_{in} or recorded directly by voltage clamping the endplate region of fibers (-100 mV). Mean mepc amplitudes (average of 100-200 events/fiber) ranged from 0.5-5 nA. The mean amplitudes varied inversely with R_{in} (range, 0.3-4 MΩ) such that larger fibers (smaller R_{in}) received larger mepc's. The slope of this relation was steeper for F fibers; their mepc's were generally larger than those of S or T fibers having similar resistance. These results suggest that single quantal current is regulated at the snake motor endplate, and are consistent with the theoretical needs of larger and faster cells for more synaptic current. Supported by NIH grant NS24752 and the MDA.

282.4

EVOKED MINIATURE SYNAPTIC CURRENTS IN THIN HIPPOCAMPAL SLICES. G.B. Richerson and C.F. Stevens. Dept. of Neurology, Yale University School of Medicine, New Haven, CT 06510, and The Salk Institute, Howard Hughes Medical Inst., La Jolla, CA 92037.

The characteristics of evoked miniature synaptic currents were studied using whole cell patch clamping from thin hippocampal slices. 100 uM rat hippocampal slices were prepared using a vibratome. Neurons in the CA1 region were visualized using Nomarski optics on an inverted microscope, and the cell membrane was cleaned with a fire-polished patch electrode (2-3 Mohms). The same electrode was then used to voltage clamp neurons in the whole cell patch clamp mode. Miniature excitatory postsynaptic currents (mepscs) were induced by a puff of hypertonic sucrose onto the dendritic field of neurons. These mepscs had a reversal potential near 0 mV, and were blocked by Kynurenic acid (1 mM), a glutamate receptor antagonist. Movement of the sucrose pipette by 20-30 uM resulted in loss of the mepsc response, suggesting that mepscs were only induced in a localized region. The mepscs had a large variability in amplitude which did not appear to be related to a difference in electrotonic decay, since the amplitude was not inversely proportional to the decay time constant. This data suggests that quantal release may not be uniform in size in central neurons. Supported by the Howard Hughes Medical Institute and grant NS12961 from the National Institutes of Health.

282.5

CABLE ANALYSIS OF RAT HIPPOCAMPAL NEURONS IN CULTURE: A NOVEL APPROACH. J. M. Bekkers* and C. F. Stevens. The Salk Institute, Howard Hughes Medical Institute, La Jolla, CA 92037.

Groups of neurons in cell culture provide a useful model system for the study of synaptic currents. Our objective was to characterize the cable properties of the dendrites of such neurons in order to estimate the fidelity with which currents originating in distal synapses are recorded at the soma. Hippocampal neurons, 2-3 weeks post-plating, were whole-cell voltage clamped at the soma via a patch electrode containing Lucifer Yellow. Bath solution made hypertonic by the addition of sucrose was applied to localized lengths of dendrite, eliciting miniature excitatory postsynaptic currents (mepscs) at the point of application. As solution was applied more distally, the mean mepsc measured at the soma became slower and smaller, compatible with increasing cable distortion. For example, in one dendrite with diameter ~0.8 um the mean mepsc amplitude and decay time constant (at a holding potential of -60 mV) were 67 pA and 1.2 ms when solution was applied at the soma, 38 pA and 2.6 ms at 70 um, and 21 pA and 3.5 ms at 120 um. Averaging across 6 cells, the mean mepsc amplitude declined with distance x (in um) approximately as exp(-x/170), after normalizing all dendritic diameters to 1 um. In contrast, charge transfer to the soma (which reflects the DC length constant) was much less attenuated.

Neurons were afterward fixed and their dendritic arbors accurately measured under a fluorescence microscope. Assuming homogeneity of membrane and mepsc properties, we are currently modeling neurons as a passive network in which a known current (the mean mepsc elicited at the soma) is injected at known points on the dendritic tree. Comparison of the calculated and observed cable filtering of the mepscs will allow estimation of passive membrane properties. A conclusion is that significant cable attenuation of the amplitudes of distally originating fast synaptic currents occurs in our system.

282.6

ELEMENTARY EXCITATORY SYNAPTIC CURRENTS IN DENTATE GRANULE AND HIPPOCAMPAL PYRAMIDAL CELLS.
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Institute of Neurophysiology, Karl Johans gt. 47, 0162 Oslo, Norway.

Excitatory postsynaptic currents (EPSCs) were recorded at the soma of dentate granule cells and CA1 pyramidal cells in slices (19-24°C, 10μM bicucculine) using a whole-cell patch-clamp technique (Edwards et al., 1989). EPSCs from three different conditions were compared: (1) spontaneous EPSCs, probably due to asynchronous discharge in afferent fibers, since they were blocked by 1μM TTX; (2) EPSCs elicited by ejection of ruthenium red (RR) from a pipette; these were TTX-resistant and are probably caused by spike-independent release of transmitter quanta; and (3) EPSCs elicited by electrical stimulation of a small number of afferent fibers. In some cells there was a clear amplitude step between the failures and the smallest EPSCs, suggesting that these represent input from a single afferent fiber.

The three groups of EPSCs showed no obvious differences in either amplitude (0-20pA, typically 4pA), rise-time (RT, 4-12ms) or half-decay time (HDT, 7-30ms). This supports the idea that the smallest stimulated EPSCs were due to input from a single fiber. Furthermore, if RR gives release of single transmitter quanta, the spontaneous and stimulated EPSC which are equally small, may each be due to a single quantum. This supports the idea that central excitatory boutons contribute only one or a few quanta per impulse. The scatter in amplitude, RT and HDT was remarkably wide for all three groups of EPSCs. Still, in a given cell RT varied less for electrically stimulated EPSCs than for spontaneous or RR elicited EPSCs, as expected if the former is generated in a restricted part of the dendritic tree. However, for a given RT the large amplitude variation suggests that the variability in amplitude is not only due to electrotonic attenuation. There may be genuine differences in the elementary EPSC sizes, perhaps reflecting different functional states of the synapses.

282.7

RECURRENT EXCITATORY SYNAPSES (AUTAPSES) IN CULTURED RAT HIPPOCAMPAL NEURONS.
G.D. Clark[1], L.L. Thio[2], D.B. Clifford[3] and C.F. Zorumski[2]. Dept. of [1]Neurology, LSU School of Medicine, New Orleans, LA 70112 & Depts. of [3]Neurology, [2]Psychiatry, [2]Anatomy & Neurobiology, Washington University School of Medicine, St. Louis, MO 63110.

Observations of depolarizations following action potentials have been made in the rat hippocampal slice preparation. Some of these have been speculated to result from the release of transmitter from an autapse, a recurrent process forming a synapse on or near the neuron cell body. We have observed early (< 5ms) depolarizations following action potentials in cultured rat hippocampal neurons studied in synaptically connected pairs. Presynaptic neurons manifesting an afterdepolarization always produced an excitatory (probably glutamate mediated) postsynaptic event (N=16). The latencies of the after action potential events were such that at most one synapse could be interposed (range 3.5-5 ms). 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (.5 μM) reversibly diminishes the size of afterdepolarizations by 24 ± 11%, N = 3 suggesting that these are synaptically mediated events. Wheat germ agglutinin, a potent recruiter of desensitized glutamate postsynaptic receptors, augments both the postsynaptic excitatory events and the afterdepolarizations (increase of 20 ± 4%, N=5). Zinc ions potentiate only postsynaptic events not the afterdepolarizations. These results provide evidence for the existence in cell culture of autapses. Autapses, because of their proximal location, could serve as important feed-forward potentiators of central nervous system synaptic transmission.

282.8

cAMP-DEPENDENT PROTEIN KINASE INJECTIONS ENHANCE ELECTROTONIC AND CHEMICAL EXCITATORY RESPONSES AT MIXED SYNAPSES ON THE MAUTHNER CELL. Alberto Pereda, Angus C. Nairn, Paul Greengard and Donald S. Faber. Dept. of Physiology, SUNY-Buffalo, Buffalo NY 14214 and The Rockefeller Institute, New York NY 10021.

It was previously shown that intracellular injection of cAMP in the goldfish Mauthner cell lateral dendrite (Wolszon and Faber, 1989) enhanced both the electrical and the chemical glutamatergic components of the mixed EPSPs evoked by stimulation of posterior 8th nerve. Due to the presence of gap junctions in these terminals and the low molecular weight of cAMP, this effect may have occurred pre- or post-synaptically.

In order to localize their origin, the catalytic subunit of cAMP-dependent Protein Kinase (bovine heart), which has a molecular weight of 40.000, was injected into Mauthner cell lateral dendrites (n=5). Injection produced a sustained increased in both excitatory responses, which on the average were: 20.9% for the electrical and 19.8% for the chemical components. Resting potential remained unchanged during the experiments, and antidromic spike height , a measure of the Mauthner cell input resistance, showed no significant changes. Control injections using vehicle solution (n=3) or heat inactivated kinase (n=2) produced a decrease in both components of the EPSP.

The data suggest that the cAMP effect is, at least partially, of post-synaptic origin, and imply that phosphorylation of gap junctions and the glutamate receptor may be involved.

282.9

HEMI-GAP JUNCTIONS IN SOLITARY HORIZONTAL CELLS.

S. H. DeVries* & E. A. Schwartz. Committee on Neurobiology, The University of Chicago, Chicago, IL 60637.

We have studied gap junctions that form between isolated pairs of catfish horizontal cells (DeVries & Schwartz, *J. Physiol.* **414**: 351-375, 1989). Junctional conductance can be decreased by 50% within seconds after exposure to 10 nM dopamine. The intracellular pathway involves a rise in cAMP concentration and activation of a cAMP-dependent kinase. Two additional 2nd messengers, cGMP and protons, independently decrease junctional conductance. We now describe a membrane current of solitary horizontal cells which is modulated by dopamine thru a cAMP-dependent kinase. This current is also independently modulated by cGMP and protons. When measured in the same cell pair, cAMP, cGMP, and protons reduce membrane current and junctional conductance with similar rates of onset and recovery, and with the same relative magnitude of effect. The current-voltage properties of both the dopamine-sensitive membrane current and the junctional conductance were determined. The current is fully activated at depolarized voltages and undergoes a slow voltage-dependent inactivation during steps to hyperpolarized voltages. The junctional conductance displays voltage-dependent behavior consistent with the end-to-end anastomosis of two "hemi-junctions". We conclude that horizontal cells express hemi-junctions and that these hemi-junctions can join with those in apposed cells to form a full gap junction. The voltage-dependent properties of the whole gap junction follow directly from those of the hemi-junction.

282.10

ELECTRONIC COMMUNICATION BETWEEN GLOMUS CELLS OF RAT CAROTID BODY. L. Monti Bloch, V. Abudara* and C. Eyzaguirre. Depts. Physiol. Facult. Med., Montevideo, Uruguay and Univ. Utah Sch. Med., Salt Lake City, UT, USA

The carotid body is an arterial chemoreceptor whose glomus cells synthesize, store and release dopamine, ACh and peptides when stimulated by hypoxia, hypercapnia or acidity. Many glomus cells are electrically, and bidirectionally, coupled at rest (L. Monti Bloch and V. Abudara, 1988). We now report on the effects of hypercapnia, hypoxia, lactate and dopamine on intercellular coupling. Carotid bodies, excised from 50 g anesthetized rats were superfused with oxygenated physiological saline, pH 7.4 at 30°C. Two adjacent glomus cells were simultaneously impaled under visual control with 3 M KCl-filled micropipettes (80-100 MΩ). Their membrane potential was -30 to -50 mV. Current pulses (0.1-1.0 nA) were delivered through the recording electrode to measure input resistance (R_o). The coupling resistance (R_c) and coupling coefficient (K_C) between cells were calculated. At rest R_o = 69.2 ± 16, R_c = 17.5 ± 4.7 MΩ and K_C = 0.54 (n=22). Applications of 100% CO_2 depolarized the cells and R_o increased. R_c augmented to 37 ± 3.7 MΩ and K_C decreased to 0.16. Applications of 100% N_2 (hypoxia) had similar effects but changes had longer latencies. Lactic acid (10^{-6} M) increased R_o and cells depolarized for 5-10 min. R_c increased to 61.8 ± 5.3 MΩ and K_C decreased to 0.08. Finally, dopamine 1 μM also uncoupled the cells while producing 2-5 mV depolarization. Thus, natural stimulation and dopamine uncouple glomus cells. Results suggest that, as in other organs, secretory activity of glomus cells is accompanied by uncoupling, and that dopamine plays an important role. Supported in part by NS grants 05666 and 07938.

282.11

EXCITATORY AND INHIBITORY SYNAPTIC TRANSMISSIONS ON CANINE INTRACARDIAC GANGLION CELLS. X. Xi, W.C. Randall and R.D. Wurster. Physiol. Dept., Loyola Univ. Med. Ctr., Maywood, IL 60153.

Vagal control of the sinoatrial node in dogs is mediated by intracardiac ganglia located in the pulmonary vein fat pad of right atrium. The synaptic transmission properties of mammalian intracardiac ganglia have not been previously investigated. Thus, canine intracardiac ganglia were intracellularly studied *in vitro*. Presynaptic fibers were activated by stimulating interganglionic nerves using a bipolar suction electrode. In each ganglion cell, a single stimulation (0.1 ms) evoked one or more fast excitatory postsynaptic potentials (f-EPSPs) which normally reached threshold and initiated an action potential. The f-EPSPs of one ganglion cell may have different time delays and thus, summate into different patterns. These f-EPSPs were blocked by hexamethonium (10^{-4} M) and low Ca^{++}, high Mg^{++} Krebs solution but not by atropine (10^{-6} M). Train stimulation (20 Hz, 0.2 - 0.9 S) evoked a slow hyperpolarizing inhibitory postsynaptic potential (s-IPSP) on 43 of 60 neurons. These s-IPSPs lasted for a few seconds with varying amplitude, depending on the frequency, duration and voltage of stimulation. Slow IPSPs were not evoked by repetitive action potential activation via intrasomal current injection. Atropine (10^{-6} M, n=7) and low Ca^{++}, high Mg^{++} Krebs solution (n=11) blocked the s-IPSPs, but hexamethonium (10^{-4} M, n=5), propranolol (10^{-5} M, n=3) or phentolamine (10^{-5} M, n=4) had no effect. Little or no conductance changes accompanied these s-IPSPs. These data suggest that: 1). Canine intracardiac ganglion cells receive multiple synpases. 2). Synaptic excitation and inhibition involve nicotinic and muscarinic receptors, respectively. (Supported by HL 27595)

CALCIUM CHANNELS II

283.1

CALCIUM CURRENT IN AXOTOMIZED X-ORGAN CRAYFISH NEURONS. García-Colunga, J.*, García, U.*, Aréchiga, H. and Valdiosera, R.* Dept. of Physiol., Biophys. and Neurosci. CINVESTAV-IPN Ap. Postal 14-740, México, D. F. 07000.

Calcium current (Ica) was recorded with whole cell clamp in ligatured x-organ somata perfused with: NMGCl 154, $CaCl_2$ 20, CsCl 30, TEACl 20 and HEPES 10; the pipette solution contained: CsCl 214, $CaCl_2$ 2.86, EGTA 10, MgATP 2 and HEPES 10 (mM). Ica activates at -30 mV with a sigmoidal time course and increases gradually with depolarization reaching a maximum peak value of 1.17 ± 0.47 nA (n = 11) at 30 mV in 3.8 ms declining exponentially afterwards. Tail currents could be fitted with a single exponential (τ = 300 μs). Changing the holding potential between -90 and -50 mV had no effect on Ica. In the presence of Ba^{2+} the current did not decline indicating calcium dependent inactivation. This possibility was further explored with double pulse experiments. Ica during the test pulse depends on calcium entry during the conditioning pulse. This phenomenon did not occur when Ba^{2+} was substituted for Ca^{2+}. The permeability sequence for divalent cations was: $Ba^{2+} \geq Sr^{2+} > Ca^{2+} > Mg^{2+}$, Ni^{2+}, Co^{2+} and Mn^{+} when the external calcium was substituted equimolarly for each cation. The blocking sequence was: $Zn^{2+} > Cd^{2+} > Ni^{2+} > Co^{2+} > Mg^{2+}$ when 2 mM of each ion was added to the external solution. Nifedipine (100 μM) had no effect on Ica.

283.2

ION CHANNELS IN *APLYSIA* SMOOTH MUSCLE: CURRENTS ACTIVATED BY VOLTAGE AND ACETYLCHOLINE (ACh). **J.L. Ram and L.-X. Liu, Physiology, Wayne State U., Detroit, MI 48201**

Buccal muscles of *Aplysia* are smooth muscles used in voluntary feeding movements. The objective of this study was to determine whether voltage-dependent calcium channels (VDCaCh) mediate the contractile response to the physiological activator, ACh. Buccal muscle I5 was dissociated with collagenase (2-4 hr at 30 °C). With K in the patch electrode (whole cell recording) and the fiber in normal medium (SW), depolarization elicited outward current. With TEA and 4-AP SW outside and Cs as the major pipet ion, depolarization elicited inward current. Inward current was blocked completely in 0 Ca SW and La^{+++} SW and reduced 80-90% in 1 μM nifedipine SW, suggesting this current to be VDCa. Lowest voltage to elicit inward current averaged -43 mV and peak current (mean=2.6 nA) was elicited by -2 mV. ACh elicited inward current at -80 mV which reversed (in most cells) above -35 mV. The I-V curve for ACh-elicited current had two slopes, changing to a shallower angle just above or below reversal. In intact muscles, ACh depolarized muscle fibers to no greater than -35 mV and the contractile response could be blocked 50% by nifedipine. A role of VDCaCh in ACh-elicited contraction is supported by a) the voltage sensitivity of VDCaCh (within range of ACh depolarization) and b) a drug that blocks VDCaCh reduces the contractile response. Supported by MDA and NIH RR 08167.

283.3

VOLTAGE-SENSITIVE CALCIUM CURRENTS IN IDENTIFIED NEURONS OF A JELLYFISH. J. Przysiezniak and A. N. Spencer. Dept. of Zool., Univ. of Alberta, Edmonton, AB, CANADA, T6G 2E9 and Bamfield Marine Station, Bamfield, BC, CANADA, V0R 1B0.

Calcium currents from a primitive invertebrate, the jellyfish *Polyorchis penicillatus*, were studied using the tight-seal, whole-cell, voltage-clamp technique, applied to identified, isolated 'swim' motor neurons (SMNs).

Depolarizing voltage steps elicited a rapidly-activating (T_{peak} 3-10ms), rapidly-inactivating (τ_{Inact} ~25ms) calcium current. This fast I_{Ca} was blocked by 0.1-0.5mM cadmium applied externally, which also 'shaved off' the plateau phase of action potentials recorded in current-clamp. The fast I_{Ca} first activated around -20mV, reached a maximum about +10mV, and reversed around +50 to +60mV. This I_{Ca} was half-inactivated at -18mV.

Inactivation was sometimes incomplete at the end of 1 sec. depolarizing pulses, suggesting the presence of a slow, non-inactivating I_{Ca}. In some of the cells studied, the I-V curve exhibited a shoulder at ~+50mV, also suggesting the presence of a second I_{Ca} with a maximum at very positive voltages.

Pharmacological characterization of these two currents will allow us to distinguish what their roles are in synaptic transmission and/or spike propagation.

283.4

WHOLE-CELL CALCIUM CURRENTS IN ACUTELY DISSOCIATED MEDIAL SEPTUM/DIAGONAL BAND NEURONS. W.H.Griffith, L. Taylor and M.J.Davis*. Depts. of Medical Pharmacol. & Toxicol. and Medical Physiol. College of Medicine, Texas A&M University, College Station, TX 77843.

We have identified both low-voltage activated (LVA) and high-voltage activated (HVA) calcium (Ca^{2+}) currents in acutely dissociated neurons from adult guinea pig. Whole-cell patch clamp procedures were used with trypsin (65,000 units/treatment) dissociated cells. Barium (Ba^{2+}) (2 mM) was used as the charge carrier and both Na^+ and K^+-free solutions were used to reduce interfering conductances. An ATP-regenerating system (4 mM MgATP, 0.1 mM GTP, 5 mM creatine phosphate and 20 units/ml creatine phosphokinase) enabled currents to be recorded for up to 2 hrs. All drugs were applied by bath perfusion. From a V_h of -100 mV, LVA currents could be induced by step depolarizations positive to -75 mV and showed marked voltage-dependent inactivation at -80 mV. These transient currents were reduced by nickel (20 - 100 μM) and cadmium (10 - 500 μM). A non-inactivating HVA current began positive to -50 mV and was sensitive to nifedipine (5 - 10 μM) as well as the divalent blockers.

Selective expression of these Ca^{2+} currents could be observed in different cell types within the basal forebrain, suggesting that LVA and HVA calcium currents play a critical role in the firing pattern of these cells. (Supported AG07805, NS22456, HL-38104, AHA-881161).

283.5

LOW THRESHOLD TRANSIENT Ca^{++} CURRENTS IN ACUTELY DISSOCIATED HIPPOCAMPAL LACUNOSUM-MOLECULARE INTERNEURONS. D.D.Fraser and B.A.MacVicar. Neurosci.Res.Group, Univ. of Calgary, Calgary, Alberta T2N4N1.

Intracellular recordings from lacunosum-moleculare (LM) interneurons suggested that these cells might have low-threshold Ca^{++} spikes (Lacaille and Schwartzkroin, 1988). To determine the types of ion currents present in these cells we have developed a method to acutely isolate these cells from hippocampal slices. Following incubation of slices in trypsin-hyaluronidase, the LM was dissected free and this area was mechanically triturated. Cell morphology of dissociated LM interneurons was distinct from that of CA1 pyramidal neurons. TTX-insensitive low threshold transient inward currents were observed during every stable whole cell voltage clamp. They were similar to T-type Ca^{++} currents described in other cells in that they were blocked by Ni^{++} (200 uM) but not by low concentrations of Cd^{++} (200 uM). The current was inactivated when holding potential was -60 mV. At a holding potential of -100 mV the current was observed to activate at -60 mV and had a peak of 400 pA at -30 mV. Low threshold Ca^{++} currents could be important in generating rhythmic activity in LM interneurons and possibly the hippocampus as in the thalamus and the inferior olive. Supported by MRC (Canada).

283.6

ASYMMETRICAL REGIONAL DISTRIBUTION OF TETRODOTOXINE-SENSITIVE CALCIUM-CONDUCTING CHANNELS IN THE RAT HIPPOCAMPUS. N. Akaike and K. Takahashi*. Dept. of Neurophysiology, Tohoku Univ. Sch. of Med., Sendai 980, JAPAN

Experiments were performed on the pyramidal neurons freshly isolated from rat hippocampal CA1 region. Voltage-dependent Ca^{2+} currents (I_{Ca}s) in the neurons can be classified into T-, N-, and L-types on the basis of their current kinetics, voltage-dependence, and pharmacological sensitivities. We found an additional tetrodotoxin (TTX)-sensitive transient Ca^{2+} current (termed 'TTX-I_{Ca}') through the Na^+ channel in the dissociated pyramidal neurons. The TTX-I_{Ca} was not affected by Ca^{2+} channel blockers but was sensitive to Na^+ channel blockers and scorpion toxin. The TTX-I_{Ca} developed linearly with increasing extracellular Ca^{2+} concentration, and the selectivity was $Ca^{2+} >> Sr^{2+} > Ba^{2+}$. We first report here an asymmetrical regional distribution of the TTX-I_{Ca} and T-type I_{Ca} in the CA1 region: the former is observed dominantly at the dorsal portion while the latter at the ventral portion.

283.7

UNITARY CALCIUM CURRENTS IN ADULT RAT HIPPOCAMPAL NEURONS. Vazquez, M.*, Rodriguez, V.*, Larrazolo, A.*, Guevara, R. and Garcia, D. Depto. de Fisiologia, Fac. de Medicina, UNAM. Apdo. Post. 70250, Mexico 04510, D.F. Mexico.

It is known that an increase in the strenght of synaptic transmission is produced when repetitive stimulation is delivered to excitatory pathways in the hippocampus (J. Physiol. 232: 331-356, 1973). Thus it has been proposed that some amino acids produce synaptic responses in these excitatory relays (Nature 297: 496-498, 1982). Even calcium influx has been involved in this neuronal hyperexcitability (J. Neurochem. 28: 63-70, 1977). The aim of these experiments was to investigate the unitary calcium current activity in control and in glutamate conditions. Method: proteolytic treatment of hippocampal slices; granule cells in cell-attached configuration; solutions formulated to block all potassium and sodium currents, bath (mM): 125 Cs^+; patch pipette: 10 Ba^{2+}, pH= 7.35. Current records were filtered a 10 KHz and sampled at 5 KHz. Results: in a preliminar approach it can be observed that glutamate (1 mM) increases the single calcium channel activity (see below).

283.8

PROPERTIES OF TWO LONG-LASTING CALCIUM CURRENTS IN NON-DISSOCIATED, ADULT HIPPOCAMPAL CA1 PYRAMIDAL CELLS. L.W. Campbell, Q. Thibault*, S-Y Hao*, P.W. Landfield. Department of Physiology & Pharmacology., Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

It is clear that there are multiple calcium currents in peripheral and central nervous system neurons (Llinas and Yarom, 1981; Fox *et al.*, 1985; Tsien, 1987; Miller, 1987). Recent studies in dissociated hippocampal neurons (Gray and Johnston, 1987; Madison *et al.*, 1987; Mogul & Fox, 1989; O'Dell and Alger, 1989) have also found rapidly inactivating (T,N) currents and a slowly inactivating, long-lasting (L) Ca current. Most studies of multiple Ca currents have used dissociated-cell/patch-clamp methods. In the present studies, conducted with intracellular single electrode voltage-clamp in non-dissociated adult rat hippocampal slice neurons, we also found evidence of at least 3 types of Ca currents.

However, in contrast to previous studies, we find 2, rather than 1, long-lasting Ca currents. The 3 types observed, in order of activation ranges, are as follows:

1. "R" current ("resistant"); low voltage activated (-55 to -50 mV, holding potential of -80 mV); low amplitude; highly resistant to voltage or Ca inactivation, and to dihydropyridines (DHP) or ω-Conotoxin (CgTx).
2. "N" current (similar to the peripheral N, but may have T-like components); medium-range activation (approximately -45 to -40 mV, holding at -80 mV), relatively rapid inactivation (~ 100 ms time constant), and sensitivity to CgTx and Cd.
3. "L" current (many properties similar to L-type); medium-range activation (-40 to -35 mV), slow inactivation (> 1000 ms) and sensitivity to DHPs, CgTx and Cd. This current is also charaterized by unusually long tail currents. Several factors suggest that the tail is not entirely due to space-clamp problems: the tail is extremely sensitive to changes in holding potential, and is present in somata after severing apical dendrites.

283.9

EXPRESSION OF CALCIUM CONDUCTANCES IN PURKINJE NEURONS IN CULTURE. D.L. Gruol and C.R. Deal*, Dept. of Neuropharm., Res. Inst. Scripps Clinic, La Jolla, CA 92037.

During maturation, cerebellar Purkinje neurons (PNs) acquire the ability to generate patterns of high frequency spike activity. We are investigating the ionic basis for this developmental change using current and voltage clamp techniques. In intracellular recordings from immature PNs at 7 days *in vitro* (DIV), small depolarizations evoked repetitive, fast TTX (1 μM) sensitive Na+ spikes. Strong depolarizations evoked fast Na+ spikes and slow Cd^{2+} (30 μM) sensitive Ca^{2+} spikes. At 14-16 DIV, when PNs have well developed dendritic structure and exhibit high frequency firing patterns, small and large depolarizations elicited an initial Cd^{2+} sensitive burst event followed by repetitive TTX sensitive Na+ spikes. A similar burst event was evoked at the termination of a hyperpolarizing current pulse. In voltage clamp recordings from the older PNs, in the presence of Na+ and K+ channel blockers, depolarization from a holding potential of -90 mV evoked a low threshold transient Ca^{2+} current and a high threshold Ca^{2+} current with a transient and a sustained component. The transient currents were not evident when the potential was held at -40 mV. At 7 DIV, Ca^{2+} currents were small but appeared to include both transient and sustained components. These date indicate that the contribution of Ca^{2+} currents to PN activity increases with development. Supported by NS21777.

283.10

DEVELOPMENT OF CALCIUM CURRENTS IN CULTURED RAT CEREBELLAR GRANULE CELLS. C. Marchetti, C. Carignani* and M. Robello*, Istituto di Cibernetica e Biofisica, CNR, Genova, Italy.

We have characterized a voltage-dependent calcium current in cerebellar granule cells from 8-day-old rats. Currents were measured with the patch-clamp technique in an external bath containing barium or calcium (20 mM) and TEACl, and with cesium in the pipette to minimize the other ionic currents. During the first 3 days in vitro (DIV) only 16% of granule cells displayed barium currents with amplitude ≥ 50 pA, whereas from 5 to 13 DIV all the cells had a current ≥100 pA. This current activated around -25 mV, reached a peak between +10 and +20 mV and reversed around +70 mV. The dehydropyridine BayK 8644 (0.1 - 5 μM) produced: (i) an increase in the current amplitude, (ii) a shift of its activation in the hyperpolarizing direction and (iii) a slowing down of deactivation kinetics. The effects were dose-dependent and independent of DIV. The barium current was also reversibly depressed by 100 μM Baclofen (28±7% inhibition in 9 cells, from 5 to 13 DIV). These results suggest that cerebellar granule cells possess a high-voltage-activated calcium current which develops during the first 4 DIV and can be modulated by GABAb agonists.

283.11

HIGH- AND LOW-THRESHOLD CALCIUM CURRENTS IN RAT SENSORIMOTOR CORTICAL NEURONS. R.J. Sayer, P. C. Schwindt, and W. E. Crill, Dept. of Physiology & Biophysics, Univ. of Washington Sch. of Med., Seattle, WA 98195.

The Ca^{2+} currents of rat neocortical neurons were investigated by voltage clamping acutely isolated cells. Slices of sensorimotor cortex from rats 14-28 days old were treated with papain for 1.5 h and then mechanically dissociated to produce single cells. Whole-cell recordings of Ca^{2+} currents were made at 20-23°C. High-threshold (HT) and low-threshold (LT) components of the current were observed in all neurons. The HT current was evoked by commands positive to -40 mV, was maximal at about -10 mV and was slowly inactivating. The LT current was only seen when holding potentials were more negative than -70 mV. It was activated by commands positive to -65, and was maximal at about -30mV. The LT current was transient, with a time constant for inactivation of approximately 70 ms at -30 mV. After 800 ms hyperpolarizations to -100 mV, which removed inactivation from the LT current, the ratio of maximal LT to maximal HT amplitude averaged 0.33. In comparison to the LT current, the HT current was more sensitive to block by Cd^{2+} and less sensitive to block by Ni^{2+}. Racemic BAY K 8644 (1μM) enhanced the HT current at potentials between -40 mV and -10 mV, but had no effect on the LT current. Nimodipine (10 μM, by puffer pipette) reversibly suppressed both currents by about 60%. ω-Conotoxin (16 μM, by puffer pipette) reduced the HT currents by a mean of 23%, with little recovery on washing, but did not affect the LT current. Supported by NINDS grant NS16792.

283.12

CALCIUM CURRENTS IN NEURONS ISOLATED FROM HUMAN NEOCORTEX. W. E. Crill, R.J. Sayer and P. C. Schwindt, Dept. of Physiology & Biophysics, Univ. of Washington Sch. of Med., Seattle, WA 98195.

Ca^{2+} entry plays a key role in the control of neuronal excitability, so it is important to characterize the Ca^{2+} currents in cells from regions prone to epileptic activity. Neocortical tissue was obtained from adult patients during resection of epileptic foci from the temporal lobe (either side). We used samples from areas which needed to be removed but which were considered to be relatively more "normal" than the epileptic foci. The tissue was sliced, treated with papain for 1.5 h and then mechanically dissociated to produce single cells. Ca^{2+} currents were recorded under whole cell voltage clamp at 20-23°C. Two components of current were identified by their thresholds for activation: (a) a high-threshold (HT) current evoked by commands positive to -40 mV, which was slowly inactivating, and (b) a low-threshold (LT) current activated positive to -60 mV which was rapidly inactivating. The LT current was only seen with holding potentials more negative than -70 mV, and its peak amplitude was always much smaller than the HT current. The HT current was more sensitive than the LT current to block by Cd^{2+}, while the reverse was true for Ni^{2+}. Racemic BAY K 8644 (1 μM) increased the HT current most strongly for command potentials between -40 mV and -10 mV, but had no effect on the LT current. Nifedipine (10 μM, by puffer pipette) reversibly reduced the maximal HT current by approximately 60%. These results indicate some similarities between the Ca^{2+} currents of human neocortical neurons and those of other mammals. Supported by NINDS grants NS16792 & NS20482.

283.13

CALCIUM CHANNELS PARALLEL SODIUM CHANNEL MATURATION IN MOUSE NEURODEVELOPMENT B.B.Grover, *S.M.Saderup & M.J. Litzinger Depts. of Pediatrics, Biology, Neurology & Physiology, Univ. of Utah, SLC,UT. & Albert Eistein College of Medicine, N.Y.,N.Y.*

Recent studies on the Na^+/Ca^{++} exchange mechanism have suggested the importance of the Na^+ & Ca^{++} ion concentration gradient to be in place for neurodevelopment to occur (Mills et.al.,1989). The maturation of these ion channels is crucial to the establishment of this gradient. H^3- TTX (Na^+channel probe) showed a two fold increase in binding between days 10 & 15 in mouse neurodevelopment (Hafemann & Unsworth, 1972). Erman et.al.,1987, reported a sudden increase in Nitrendipine binding in mouse brain between days 7 & 21.

The present study seeks to establish more closely the parallel maturation of Ca^{++} channels during mouse neurodevelopment. Nimodipine (L type Ca^{++}channel)binding site density increases between days 10 & 15. A 50% increase in N type Ca^{++} channels (with w-conotoxin) was demonstrated between days 11 & 14. This time period corresponds to a "critical period" noted by Himwich, 1962, which signaled the activation of electrocortical maturation. These binding studies show the parallel maturation of Na^+ & Ca^{++} channels during this period of rapid mouse neurodevelopment and suggests the need for the availability of these ion channels for the maturation of the Na^+/Ca^{++} exchange system. This work was sponsored by NICHD K08 00886-01.

283.14

DIFFERENTIAL MODULATION OF CALCIUM CHANNELS BY GUANINE NUCLEOTIDE ANALOG IN HUMAN NEUROBLASTOMA CELLS. E. Reuveny and T. Narahashi. Dept. of Pharmacol., Northwestern Univ. Med. School, Chicago, IL 60611.

Guanine nucleotide-binding proteins (G-proteins) couple the membrane receptors for neurotransmitters to a variety of enzymes and ion channels. The L-type calcium channels are modulated by leucine enkephalin via δ receptors, whereas the N-type calcium channels are modulated by norepinephrine via α_2-adrenergic receptors. G-proteins are believed to be involved in these two systems. We have previously shown that human neuroblastoma cells (SH-SY-5Y) express N- and L-like calcium channels (Reuveny and Narahashi, Biophys. J. 57 520a, 1990). We now report the effect of direct activation of G-proteins on calcium channels in these cells. N-like calcium channel currents were reduced by the internal perfusion of cells with the non-hydrolyzable analog of GTP, GTPγS (100 μM), while L-like currents were not reduced. Interestingly, the activation kinetics of L-like calcium channel currents were slowed. In contrast, internal perfusion of cells with GTP up to 5 mM was not effective in reducing either the N- or L-like calcium channel currents. The effects of GTPγS on the N- and L-like calcium channel currents were attenuated by preincubation of cells with pertussis toxin (100 ng/ml) for 18 hr. These results suggest that the N-like calcium channels in SH-SY-5Y cells are tonically suppressed by G-proteins. Supported by NIH grant NS14144.

283.15

INTERACTION OF "L"-TYPE CALCIUM CHANNELS AND PROTEIN KINASE C IN MODULATING [^{3}H]-5HT RELEASE FROM RAT SPINAL CORD SYNAPTOSOMES. V.C. Gandhi and D.J. Jones. Depts. Anesth. & Pharm., Univ. Texas Hlth. Sci. Ctr., San Antonio, TX 78284-7838

Previous studies demonstrated that protein kinase C (PKC) modulates the release of monoamines from central neural tissues. Since we have shown previously that Ca^{++} channel agonists modulate release under similar conditions, the present studies investigated the role of PKC in the Ca^{++}-dependent release of 5-HT from spinal cord synaptosomes along with the possible involvement of dihydropyridine (DHP)-sensitive "L" Ca^{++} channels in regulating this release.

DHP agonists such as Bay K 8644 maximally enhance K^+-stimulated release at a K^+ concentration of 15 mM. Similar to these results, the PKC activators phorbol 12-myristate 13-acetate (PMA) and 1,2-oleoyl acetylglycerol (OAG) also demonstrated peak enhancement of [^{3}H]-5HT release at 15 mM K^+ when tested over a range of 5-60 mM K^+. This increase in [^{3}H]-5HT release with submaximal depolarization of the synaptosomes was dependent on the concentration of PMA or OAG and was not evident when inactive phorbol esters were added to the perfusion medium. Facilitation of [^{3}H]-5HT release by both PMA and OAG was dependent on the concentration of Ca^{++} in the superfusion media. The effects of PMA and Bay K 8644 were additive when added together at submaximal concentrations. Nimodipine, an "L" Ca^{++} channel antagonist, while having no independent effect on K^+-induced [^{3}H]-5HT release, abolished the Bay K 8644 as well as PMA-induced enhancement. In addition the PKC inhibitor polymyxin B blocked both PMA- and OAG- enhanced release, as well as Bay K 8644 enhanced release in the presence of 15 mM K^+. These results demonstrate that PKC-dependent mechanisms are involved in the release of [^{3}H]-5HT in rat spinal cord synaptosomes. Moreover, these studies also indicate a possible linkage between PKC-dependent release of [^{3}H]-5HT and "L" type voltage-sensitive Ca^{++} channels. Supported by NSF BNS-8820008.

283.16

D_1 RECEPTORS ACTING VIA A cAMP/ PROTEIN KINASE A PATHWAY ACTIVATE "FACILITATION" Ca^{2+} CHANNELS IN CHROMAFFIN CELLS
C.R. Artalejo*, M.A. Ariano, R.L. Perlman, & A.P. Fox*, The University of Chicago, & The University of Vermont.

Bovine chromaffin cells exhibited two components of whole-cell Ca^{2+} current. The "standard" Ca^{2+} current was activated by brief depolarizations. The standard Ca^{2+} current was carried by a dihydropyridine insensitive 14 pS channel. The other component, called "facilitation", was activated by prolonged pre-depolarizations to very positive potentials (Fenwick et. al., 1982; Hoshi & Smith, 1987), or by repetitive small depolarizations in the physiological range. Facilitation Ca^{2+} currents were carried by a dihydropyridine sensitive 27 pS Ca^{2+} channel that was relatively inactive in unstimulated cells. Dopamine (100 nM) or apomorphine (10 nM) activated facilitation Ca^{2+} channels in the absence of pre-depolarizations or repetitive activity. This response could be mimicked by the specfic D_1 dopamine receptor agonist SKF 38393. The effect of SKF 38393 could be blocked by the specific D_1 dopamine antagonist SCH-23390. The D_1 effect was mimmicked by 8-4-Chlorphenylthio-cAMP and inhibited by a specific protein kinase A inhibitor peptide (PKI). D_2 dopamine agonists like quinpirole had no efffect on whole-cell current at concentrations up to 100 μM. Previous studies reported the existence of D_2 but not D_1 receptors in chromaffin cells. Using fluorescence microscopy the rhodamine conjugate of the 4'-amino derivative of the D_1 antagonist SCH 23390 (Ariano et. al., 1989) was found to bind to almost all of the cells in culture. This binding was displaced by 10 μM SCH 23390 but not by the 5-HT antagonist ketanserine, identifying specific D_1 receptors in bovine chromaffin cells. Dopamine release from chromaffin cells may provide a positive feedback signal capable of regulating secretion.

283.17

NOREPINEPHRINE DECREASES THE CALCIUM CURRENT OF ADULT RAT SYMPATHETIC NEURONS VIA A G-PROTEIN. Geoffrey G. Schofield, Department of Physiology, Tulane University Medical School, New Orleans, LA 70112.

Norepinephrine decreases the Ca^{2+} current of acutely isolated adult rat superior cervical ganglion (SCG) neurons via an α_2-adrenoceptor (Schofield, *Eur. J. Pharmacol.* in press). In dorsal root ganglion neurons the norepinephrine-induced inhibition of the Ca^{2+} current is transduced via a guanine nucleotide binding protein (G-protein) (Holtz, et al. *Nature* 319: 670, 1986). Experiments were performed to investigate if a G-protein is involved in the norepinephrine-induced inhibition of Ca^{2+} currents in acutely isolated adult rat SCG neurons. Ca^{2+} currents were recorded using the whole-cell patch-clamp technique in solutions designed to isolate Ca^{2+} currents. Inclusion of 500 μM GTP-γ-S in the patch-pipette mimicked the effect of norepinephrine, decreasing the Ca^{2+} current amplitude as well as slowing the rising phase of the current. In GTP-γ-S dialysed neurons norepinephrine did not produce further inhibition of the Ca^{2+} current. Incubation of SCG neurons with pertussis toxin (200 ng/ml) eliminated the norepinephrine-induced inhibition of the Ca^{2+} current. On the other hand, intracellular application of 500 μM c-AMP and 500 μM IBMX had no effect on the norepinephrine-induced inhibition of the Ca^{2+} current. These results suggest that the norepinephrine-induced decrease of the Ca^{2+} current of adult rat SCG neurons is transduced by a pertussis toxin sensitive G-protein independently of c-AMP. Supported by the Pharmaceutical Manufacturers Association Foundation and American Heart Association, Louisiana Affiliate.

283.18

FREQUENCY-DEPENDENCE OF AFTERHYPERPOLARIZATION IN BULLFROG SYMPATHETIC NEURONES. B.S. Jassar* and P.A. Smith. Dept. of Pharmacology, Univ. Alberta, Edmonton, Canada, T6G 2H7.

The action potential afterhyperpolarization (ahp) in bullfrog sympathetic neurones involves a voltage-insensitive $G_{K,Ca}$ termed I_{AHP} (Pennefather et al., PNAS. 82, 3040, 1985). When neurones were stimulated once every 2s, the duration of the ahp (recorded with intracellular microelectrodes and measured at 50% amplitude) was 70.1±3.8% (n=34) of that seen when neurones were stimulated once every 90s. This slow frequency-dependence was partly attributable to frequency-dependence of Ca^{2+} influx as demonstrated by a change in duration of 'Ca^{2+} spikes' (recorded in 100mM TEA/2uM TTX) or by a change in the amplitude of the peak Ca^{2+} current studied by whole-cell patch-clamp recording (100ms voltage command from -55 to 0mV; Cs^+ replacing K^+ inside, 105mM TEA outside). The frequency-dependence of the ahp was enhanced by 5mM caffeine and reduced by 5μM ryanodine suggesting that Ca^{2+}-induced Ca^{2+} release may also be significant under physiological conditions. Changes in ahp duration reflect an intrinsic mechanism which enhances ganglionic transmission during high frequency activation, yet depresses transmission during low frequency activation. Supported by MRC/NSERC/AHFMR.

283.19

A HIGH-THRESHOLD, DIHYDROPYRIDINE-RESISTANT CA^{2+} CURRENT IN N1E-115 NEUROBLASTOMA CELLS. Andrew G. Knapp, Lee Margolin* and Deborah Daly*. Cambridge NeuroScience, Inc., Cambridge, MA 02139.

We have confirmed that N1E-115 neuroblastoma cells induced to differentiate with 2% DMSO express two kinetically distinct components of calcium current (Narahashi et al. J. Physiol. 383, 1987): a low-threshold current that requires negative holding potentials, activates at potentials positive to -60 mV and inactivates completely within 200 msec; and a high-threshold current that is insensitive to holding potential, activates at potentials positive to -20 mV, and shows little inactivation over hundreds of msec. In common with classical L-type currents, the high-threshold component conducts Ba^{2+} more effectively than Ca^{2+}, and is blocked by relatively low concentrations of Cd^{2+}; however, it is unaffected by dihydropyridine antagonists (nimodipine and/or nitrendipine) in concentrations as high as 10 μM. Antagonists at 100 μM block both components of the calcium current. These results suggest that N1E-115 cells may be a useful model for central neuronal high-threshold Ca^{2+} channels, a subset of which displays a similar insensitivity to dihydropyridines (e.g., Sah et al. Soc. Neurosci. Abstr. 15, 1989).

284.1

ROLE OF NMDA RECEPTORS IN DOPAMINE D1-, BUT NOT D2-, MEDIATED CHANGES IN STRIATAL NEUROTENSIN. N.A. Singh, K.M. Merchant, L.G. Bush, J.W. Gibb and G.R. Hanson. Dept. Pharmacol. and Toxicol., Univ. of Utah, Salt Lake City, UT 84112.

Previous studies have demonstrated that methamphetamine-induced increases in striatal neurotensin (NT) levels are completely blocked by dopamine D1 receptor antagonists as well as noncompetitive N-methyl-D-aspartate (NMDA) antagonists. To elucidate further these D1- and glutamate-mediated NT changes, we coadministered selective dopaminergic drugs with the noncompetitive NMDA antagonist, MK801. Animals were given a combination of MK801 (1 mg/kg) 15 min prior to SKF 38393 (20mg/kg; a D1 agonist) for 3 doses at 6-hr intervals and sacrificed 18 hr after the last dose. MK801 alone did not alter striatal NT levels but completely blocked the increases caused by SKF 38393. The possibility of interaction between D2 and NMDA receptors was also tested by combining MK801 with treatments of quinpirole (selective D2 agonist) and sulpiride (selective D2 antagonist) which cause significant decreases and increases, respectively, in striatal NT levels. Pretreatment with MK801 did not alter the changes in NT levels mediated by either the selective D2 agonist or antagonist. These results suggest that NMDA-glutamate systems play a role in the regulation of striatal NT systems mediated by D1 receptors but not those associated with D2 receptors. (Supported by USPHS grants DA 00869 and DA 04222).

284.2

INCREASE IN STRIATAL DOPAMINE RELEASE FOLLOWING LOCAL PERFUSION OF THE NMDA RECEPTOR ANTAGONIST 2-AMINO-5-PHOSPHONOPENTANOIC ACID. R.J. Gruen, R.H. Roth, B.S. Bunney and B. Moghaddam. Depts. Psych. & Pharm., Yale Univ., New Haven, CT 06510 and Dept. Psychol., New York Univ., New York, NY 10003.

It has been suggested that excitatory amino acids tonically enhance striatal dopamine (DA) release by acting on the N-methyl-D-aspartate (NMDA) receptor. *In vivo* microdialysis techniques were used to examine the effect of local application of the NMDA receptor antagonist 2-amino-5-phosphonopentanoic acid (AP5) on DA release in the striatum of chloral hydrate anesthetized adult rats. AP5 was perfused for 15 minutes at a rate of 2 µl/minute through concentric microdialysis probes. Perfusion of 1 µM and 100 µM AP5 was without a significant effect on extracellular DA while 1 mM AP5 significantly enhanced DA release. Our findings do not support the idea that excitatory amino acids have a tonic facilitatory effect on DA release which is mediated through the NMDA receptor. The enhancement of DA release following 1 mM AP5 may be due to Ca^{++} channel agonist or partial NMDA agonist properties of that drug at higher concentrations. An alternative explantation for the observed increase in DA is that excitatory amino acids have tonic facilitatory effects on neuromodulators which inhibit DA release. Supported by MH28849 and MH14092.

284.3

PARTIAL CHARACTERIZATION OF KAINIC ACID-INDUCED STRIATAL DOPAMINE RELEASE USING IN VIVO MICRODIALYSIS. D.P. Carrozza*, T.N. Ferraro, G.T. Golden, P.F. Reyes and T.A. Hare. Departments of Pharmacology & Neurology, Thomas Jefferson University, Phila., PA 19107.

Three major subtypes of EAA receptors have been described which preferentially recognize the ligands N-methyl-D-aspartate, quisqualate and kainate (KA). The aim of this study was to partially characterize interactions between striatal EAA receptors and dopamine (DA) release using in vivo microdialysis. Male Sprague-Dawley rats underwent surgery while anesthetized with ketamine (100 mg/kg), acepromazine (10 mg/kg) and pentobarbital (10 mg/kg) for placement of bilateral guideshafts at coordinates designed to allow striatal microperfusion. After a 24-48 hour recovery period, animals were lightly anesthetized for insertion of a removable, concentric microdialysis probe and perfused with modified Ringer's solution at 2.75 µL/min. Samples were collected at 15-minute intervals and analyzed using HPLC with EC detection. After baseline DA release was established, an isosmotic solution of 100 mM KCl in Ringer's was perfused for 10 minutes to standardize DA release. All subsequent pharmacological manipulations were compared to KCl-induced release and expressed as a percent value. In one group of animals, KA (12.5 mM in Ringer's) was administered via the microdialysis probe in 2, 3, 5 or 10-minute pulses resulting in release of DA which was 15.7±3.9%, 30.3±11.3%, 67.5±15.0% and 92.9±19.8% of KCl-induced DA release, respectively (n=6 for each dose). In other rats, cadmium chloride (0.6 mM in Ringer's) was perfused for 30-45 minutes prior to a 10-minute KA pulse. Results showed a significant reduction of KA-induced DA release after cadmium treatment when compared to control values (from 92.9±19.8% to 45.5±10.8%, $p<0.0005$, n=6). The effect of i.p. administration of anesthesia on KA-induced DA release was also investigated. Microdialysis probes were inserted in freely-moving rats followed by establishment of baseline and standardization with 100 mM KCl. After a 3-minute KA pulse, animals were anesthetized with ketamine, acepromazine and pentobarbital as described above. After 30 minutes, animals were given another 3-minute KA pulse. Results showed a significant inhibition of KA-induced DA release when compared to control values (from 25.0±4.1% to 9.5±3.4%, $p<0.02$, n=4). These findings show that KA-induced DA release is dose-dependent, inhibited by certain anesthetics, and at least partially dependent upon activation of calcium channels.

284.4

NMDA RECEPTORS MODULATE STRIATAL DOPAMINE RELEASE AND METABOLISM IN VIVO: A MICRODIALYSIS STUDY. James P. Bennett, Jr. (1,2,3) and Catherine A. Leslie (2) Departments of Neurology (1), Behavioral Medicine and Psychiatry (2) and Pharmacology (3) University of Virginia School of Medicine, Charlottesville, VA 22908.

Studies in striatal slices suggest that excitatory amino acids (EAAs), principally glutamate (GLU), are the neurotransmitters of the massive corticostriatal/accumbens projections, and that GLU modulates dopamine (DA) release. We have examined the influence of the EAA agonist N-methyl-d-aspartic acid (NMDA) on levels of striatal extracellular fluid (ecf) DA and metabolites and amino acids in vivo using the technique of intracerebral microdialysis combined with simultaneous animal activity monitoring.

Guide cannulas were placed 48 hours prior the insertion of 3 mm length dialysis probes (Carnegie Medicin) into the dorsolateral striata of awake, freely moving animals. Probes were perfused with Mg+2-free artificial csf at 1 ul/min and dialysates were collected in 30 minute fractions while activity was continuously monitored (Columbus Instruments). After two hours of stable baseline, perfusate was switched to Mg+2-free csf with 10 mM NMDA. Intense stereotypic behavior followed, ecf DA increased 370% from baseline control levels by the third fraction after beginning NMDA infusion and then decreased to 138% of baseline two hours later. Ecf DOPAC, HVA and GLU fell to 49%, 39% and 30%, respectively, of baseline. The specificity of these effects was tested by systemic treatment with the NMDA cation channel antagonist MK801. MK801 (2 mg/kg i.p.) injected 30 minutes prior to NMDA infusion blocked the stereotypic behavior and increase in ecf DA. NMDA-induced fall in ecf DOPAC and HVA was also blocked by MK801. MK801 alone did not alter basal ecf DA levels but increased ecf DOPAC and HVA 20-30%. MK801 had no effect on the NMDA-induced decline in striatal ecf glutamate.

NMDA receptor subtype activation increases DA release and decreases apparent DA turnover in the rat striatum in vivo. MK801-induced increase of DA metabolites suggests that there is some NMDA receptor-mediated tonic suppression of nerve terminal DA synthesis. Failure of MK801 to block the decrease in ecf glutamate produced by NMDA infusion suggests that this effect is not mediated through the NMDA-type EAA receptor. (Supported by NIMH-PSA to C.A.L. and NINDS-RCDA, NS26581 and the American Parkinson Disease Association to J.P.B.)

284.5

N-METHYL-D-ASPARTATE STIMULATED DOPAMINE RELEASE FROM RAT STRIATAL SLICES IS GREATLY ENHANCED BY ALUMINUM FLUORIDE. John J. Woodward. Dept. of Pharmacology and Toxicology, Medical College of Virginia, Richmond, VA 23298.

N-methyl-D-aspartate (NMDA) stimulated the release of endogenous dopamine from striatal slices prepared from adult Sprague-Dawley rats. The EC_{50} for NMDA was approximately 140 uM. Release was inhibited by the specific glycine antagonist 7-Cl-kynurenic acid, magnesium, and the competitive NMDA antagonist, AP-5 indicating that release was due to activation of the NMDA receptor-coupled ionophore. Sodium fluoride (NaF; 5 mM) significantly potentiated the NMDA stimulated release of dopamine. This effect was further increased when a mixture of NaF and aluminum chloride ($AlCl_3$) was added to the slices prior to NMDA stimulation. NaF (2.5 mM) and $AlCl_3$ (5 uM) doubled the NMDA stimulated release over that of the control containing no added fluoride. A seven-fold increase in NMDA stimulated dopamine release was obtained with 5 mM NaF and 10 uM $AlCl_3$. The NaF/$AlCl_3$ mixture had no effect on the non-stimulated basal release of dopamine. No increases in NMDA stimulated release were observed when NaF was replaced with NaCl. Similarly, $AlCl_3$ alone had no effect on NMDA stimulated release. The fluoride induced increase in NMDA stimulated dopamine release was totally blocked by 7-Cl-kynurenic acid or magnesium and was markedly reduced by 1 uM tetrodotoxin. Striatal slices depolarized with KCl also released dopamine and this release was similarly potentiated by the fluoride mxiture. However, KCl-stimulated dopamine release from striatal synaptosomes was not potentiated by concentrations of fluoride that greatly increased release from striatal slices. These results suggest that aluminum fluoride may enhance depolarization-induced dopamine release from striatal slices through activation of a GTP binding protein. Supported by NIAAA AA08089 and a grant from the Alcoholic Beverage Medical Research Foundation.

284.6

THE EFFECT OF SELECTIVE AGONISTS AND ANTAGONISTS OF EXCITATORY AMINOACID RECEPTORS ON DOPAMINERGIC AND CHOLINERGIC NEUROTRANSMISSION IN AWAKE RATS: POSSIBLE CLINICAL IMPLICATIONS. A. Imperato, L.H. Jensen§, L. Alivernini*, M.G. Scrocco*, S. Bacchi* and L. Angelucci. Farmacologia Medica 2a, Università "La Sapienza", Roma, Italia, and § Neurosearch, K-2600 Glostrup, Denmark.

Perfusion with the selective agonists kainate (KA) and quisqualate (QA) enhaced the release of dopamine (DA) in both caudate and accumbens nuclei inducing behavioural activation; both effects were prevented by perfusion with the QA-KA antagonist DNQX. On the contrary, N-methyl-D-aspartate (NMDA) did not affect the release of DA in these areas, while was very potent in stimulating acetylcholine (ACh) release in the hippocampus. The selective NMDA antagonist, CPPene, decreased cholinergic transmission in the hippocampus. Moreover, perfusion of the nucleus accumbens and striatum with CPPene and MK801 enhanced DA release, also inducing a strong behavioural activation. This study suggests that the DA system is under an excitatory control through KA and QA receptors, while not through the NMDA ones, and that the QA-KA antagonists might be used to modulate DA release in case of its hyperactivity. On the contrary, the ability of NMDA antagonists to enhance DA release suggests that the blockade of these receptors could indirectly releave DAergic neurons from an inhibitory control, and that these compounds might have a therapeutical efficacy in syndromes linked to DAergic deficits. Moreover, the hippocampal cholinergic system is under a strong NMDA control, and the ability of NMDA antagonists to reduce the release of ACh indicates the in vivo biochemical basis of their learning and memory impairing effects.

284.7

MG^{2+}, MK-801, AND GLYCINE DIFFERENTIALLY MODULATE NMDA-EVOKED RELEASE OF ADENOSINE AND [^{3}H]NORADRENALINE FROM RAT NEOCORTEX K. Hoehn, C.G. Craig and T.D. White, Dalhousie University, Halifax, Nova Scotia, Canada, B3H 4H7

Adenosine is an important inhibitory neuromodulator in the cortex. NMDA is more potent at releasing adenosine (EC_{50}=10μM) than [^{3}H]NA (EC_{50}=330μM) from superfused rat cortical slices. Block of NMDA-evoked adenosine release by each of 3 uncompetitive antagonists, that act at different sites on the NMDA receptor-ion channel complex, can be overcome by high NMDA concentrations. Thus 1.2mM Mg^{2+} (channel antagonist) blocks 10 and 20μM NMDA-evoked adenosine release but does not block 500μM NMDA-evoked adenosine release; release of [^{3}H]NA evoked by 500μM NMDA is blocked by 1.2mM Mg^{2+}. With 500μM NMDA, release of both [^{3}H]NA and adenosine are blocked by 3μM MK-801 (channel antagonist); however, 0.3μM MK-801 blocks [^{3}H]NA release without affecting adenosine release. The glycine site antagonist, 7-chloro-kynurenate (100μM) blocks adenosine release evoked by 20μM NMDA but does not block release evoked by 500μM NMDA; release of [^{3}H]NA evoked by 500μM NMDA is blocked by 100μM 7-chlorokynurenate. These results suggest that maximal adenosine release requires activation of only a small fraction of available NMDA receptors (i.e. there are spare receptors for NMDA-evoked adenosine release). The adenosine released at low levels of NMDA receptor activation could provide an inhibitory threshold against further NMDA-mediated neurotransmission in the cortex. (Supported by the MRC of Canada).

284.8

EXCITATORY AMINO ACIDS AUGMENT MET-ENKEPHALIN RELEASE FROM SLICES OF THE RAT STRIATUM AND GLOBUS PALLIDUS. K. Jhamandas and B.B. Ruzicka. Department of Pharmacology and Toxicology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

To determine if a functional interaction exists between excitatory amino acids (EAA's) and opioid-containing neurons, the effects of four EAA's - L-glutamate (GLU), N-methyl-D-aspartate (NMDA), kainate (KA) and quisqualate (QUIS) - on the release of endogenous met-enkephalin (ME) from slices of the rat striatum or globus pallidus were examined. GLU and NMDA (1-5 mM), in the absence of Mg^{2+}, increased the ME release from both striatal and pallidal slices in a concentration related manner. A higher concentration (10 mM) of the two EAA's produced smaller increases in the ME release. The NMDA (5 mM)-induced elevations in striatal and pallidal ME release were attenuated by Mg^{2+} (1.2 mM) and the NMDA receptor antagonists, TCP (10 μM) and CPP (5 μM). KA (1-10 mM) also stimulated ME release from both the striatum and globus pallidus. The striatal release response was concentration-dependent, and was greater than that observed in the globus pallidus. In the striatum, the KA (5 mM)-evoked ME release was Ca^{2+}-dependent and was partially reduced by tetrodotoxin (0.3 μM), Mg^{2+} (1.2 mM) and CPP (5 μM), suggesting that a component of the KA action involved the activation of NMDA receptors. QUIS (5-10 mM) only weakly stimulated the release of ME from both the striatum and globus pallidus. These results show that activation of EAA receptors increase the release of ME from rat striatum and globus pallidus *in vitro*.
(Supported by the Medical Research Council of Canada)

284.9

EFFECTS OF GLYCINE AND Mg^{+2} ON QUINOLINATE-INDUCED ENDOGENOUS AMINO ACID RELEASE FROM CULTURED CEREBELLAR NEURONS. M.A. Barry, F. Stastny and G.R. Dutton, Department of Pharmacology, University of Iowa, College of Medicine, Iowa City, IA 52242.

Quinolinic acid [QUIN], a metabolite of tryptophan, mimics the neurotoxic action of N-methyl D-aspartate [NMDA] resulting in selective necrosis of granule cells in the rat cerebellum. Thus, we studied amino acid release from cultured cerebellar granule neurons produced by QUIN [1, 5, 10 mM]. Cells were stimulated with QUIN in the presence and absence of Mg^{+2} [1 mM] and amino acid release was measured using HPLC. In the presence of Mg^{+2}, QUIN increased the release of aspartate, glutamate, alanine, GABA, serine and adenosine in a dose dependent manner. Taurine release increased only in response to 10 mM QUIN. Removal of Mg^{+2} increased basal release levels of only serine and GABA. Stimulation with QUIN under these conditions further increased the release of adenosine, serine, taurine and GABA in a dose dependent manner. In the presence of Mg^{+2}, glycine [1 μM] potentiated the effect of 10 mM QUIN alone on GABA and adenosine release. On the other hand, in the absence of Mg^{+2}, glycine potentiated the effect of QUIN on GABA and taurine release. Glycine alone had no effect in either the presence or absence of Mg^{+2}. These results suggest that the QUIN-evoked release of taurine and GABA may be modulated through the glycine site on the NMDA receptor.

(This work was supported by NS 20632; MAB is with the Neuroscience Program.)

284.10

Changes in somatostatin (SRIF) in rat brain following quisqualic acid (QUIS) administration. M.Sadamatsu*§, A.Masui*, T.Itoshima*, H.Kanai*, M.Akaike*§§, T.Higuchi§§§, N.Kato and Y.Aoki*§
§ Setagawa Hosp.,Otsu 520-21, Dept.of Psychiat., Shiga Univ.Med.Sci.,Otsu 520-21, §§ Hoechst Jap. Ltd.,Pharma Res.Labs.,Kawagoe 350, §§§ Dept. of Psychiat.,Gumma Univ.Med.Sch.,Maebashi371, Japan.

We found the treatment with glutamate to neonatal rats resulted in an elevation in immunoreactive(IR)-SRIF in the limbic structures after matured. It was reported that lesions by quinolinic acid, a NMDA-receptor agonist, spares SRIF neurons. To clarify the role of excitatory amino acid receptor subtypes on SRIF neurons, changes of IR-SRIF in the rat brain were examined following intraventricular administration with QUIS (2, 10ug; 1h to 14 days after the treatment) and compared with those following kainate treatment. QUIS(10 ug) induced a reduction in IR-SRIF in the hippocampus, amygdala, hypothalamus and piriform/entorhinal cortex. This was most evident and long lasting in the hippocampus. Preliminary data exhibited the kainate treatment without effect on brain IR-SRIF levels. The effect of Joro-spider toxin, a putative QUIS-receptor antagonist, on SRIF is currently under study, but it appears the toxin fails to inhibit QUIS-induced changes in IR-SRIF.

284.11

3 H-D-ASPARTIC ACID RELEASE IN CORTEX AND HIPPOCAMPUS OF ADULT AND AGED FISCHER 344 RATS. M.J. MELDRUM, P. GLENTON*, R. DAWSON, DEPT. OF PHARMACODYNAMICS, COLL. OF PHARMACY, UNIV. OF FLORIDA, GAINESVILLE, FLORIDA. 32610.

Endogenous excitatory amino acid induced excitotoxicity has been implicated in several neurodegenerative diseases. Age-related increases in glutamate release may contribute to the neuronal loss attributed to the "normal" aging process. The present study used basal and stimulated 3H-D-Aspartic acid (3H-D-ASP) release as a marker for glutamatergic activity in adult (6 month) and aged (28-30 month) male Fischer 344 rats. KCL (40,56 mM) and electrical induced stimulation of 3H-D-ASP release was measured in superfusion studies in cortical (frontal and temporal) and hippocampal slices. KCL (56 mM) induced 3H-D-ASP release was elevated in hippocampus but not in frontal cortex of aged Fischer 344 rats compared to adult animals. Frequency dependent (2,5,10,15,20 Hz) 3H-D-ASP release was elevated at 10 Hz in hippocampus and 15 Hz in temporal cortex in aged vs. adult animals. In comparison to 3H-norepinephrine (3H-NE) release measured in the same brain areas, 3H-D-ASP release required much higher levels of stimulation to induce release. Omega-Conotoxin GVIA (5 x10^{-9} M, 30 min) failed to decrease 3H-D-ASP release in both adult and aged animals in either brain area but decreased 3H-NE release > 70 %. These data suggest that 3H-D-ASP release is altered in temporal cortex and hippocampus but not frontal cortex of aged Fischer 344 rats. The data also suggest differences in the glutaminergic neuronal release system when compared to catecholamines. (Supported by American Federation for Aging Research)

284.12

A CONFORMATIONALLY DEFINED INHIBITOR OF SYNAPTOSOMAL L-GLUTAMATE UPTAKE R.J. Bridges, M.A. Anderson*, T.N. Blakely*, E.R. Whittemore, C.W. Cotman and A.R. Chamberlin*, Depts. of Neurology, Psychobiology, and Chemistry, University of California, Irvine, CA 92717.

L-Glutamate binds to several different proteins during the course of excitatory synaptic transmission (e.g., transmitter receptors, transport systems). *In vivo*, endogenous L-glutamate binds to all of these sites, while *in vitro*, analogues of L-glutamate that mimic specific conformations exhibit selective affinities and can, thus, differentiate among the binding sites. In the present study we have identified a conformationally restrained analogue of L-glutamate (L-*trans*-2,4-pyrrolidine dicarboxylate; L-*t*-2,4-PDC) that potently and selectively blocks the transport of ^{3}H-D-aspartate through the sodium-dependent uptake system in rat cortical synaptosomes. When included in the uptake assay at an equal concentration with ^{3}H-D-aspartate (50μM), the novel analogue reduced the rate of uptake by about 50%. In contrast to its ability to block transport, L-*t*-2,4-PDC was ineffective at inhibiting the binding of ^{3}H-KA, ^{3}H-AMPA and ^{3}H-L-glutamate to kainate, AMPA, and NMDA receptors, respectively. The D-*trans*, L-*cis*, and D-*cis* isomers of 2,4-PDC did not inhibit the synaptosomal uptake of the ^{3}H-D-aspartate. These results suggest that L-*t*-2,4-PDC embodies specific structural/conformational characteristics necessary for the binding to the sodium-dependent uptake system that are distinct from those required for binding to the, KA, AMPA, and NMDA receptors.

284.13

EFFECT OF SYSTEMIC KAINATE ADMINISTRATION ON THE PRODUCTION OF KYNURENIC ACID IN THE RAT BRAIN IN VITRO AND IN VIVO. W.A. Turski, H.-Q. Wu and R. Schwarcz. Maryland Psychiatric Research Center, Baltimore, MD 21228.

Kynurenic acid (KYNA) possesses anticonvulsant properties in animal models of epilepsy. Conversely, KYNA function may be compromised by convulsant agents. We therefore examined the effect of kainate (KA; 10 mg/kg, s.c.) on KYNA production from its bioprecursor L-kynurenine (KYN) in vitro and in vivo.

Slices from piriform cortex, hippocampus and striatum were obtained at various timepoints following KA administration and incubated with 50 μM KYN for 2 h. No changes in extracellular KYNA were observed in slices obtained during status epilepticus. After one month, KYNA production was increased in the piriform cortex (+370%) and the hippocampus (+218%) but not in the striatum. Subsequently, microdialysis (perfusion with 200 μM KYN) experiments were performed in the piriform cortex of unanesthetized rats. KA, administered after attainment of steady-state KYNA levels, did not change extracellular KYNA levels during an additional 4 hours (i.e. during status epilepticus). One month after KA, KYNA production from KYN was doubled as compared to controls. These data demonstrate 1) qualitative resemblence of in vitro and in vivo assessment of KYNA function; 2) a lack of KYNA involvement in KA-induced seizures and 3) an increase in KYNA function in chronically KA-lesioned brain areas. (Supported by grant NS 16102).

284.14

CARDIOVASCULAR RESPONSES TO COMPETITIVE AND NON-COMPETITIVE NMDA ANTAGONISTS. L.A. Martin*, M.E. Abreu and J.W. Ferkany. Nova Pharmaceutical Corporation, Baltimore, MD 21224

Several lines of evidence suggest that excitatory amino acid (EAA) antagonists and in particular antagonists of the NMDA receptor are effective in attenuating neuronal damage following ischemic or hypoxic insult to brain. Included in this would be hypoxia or ischemia associated with myocardial arrest. For this reason, EAA antagonists are of considerable interest as novel therapeutics.

In the current study competitive and noncompetitive NMDA antagonists were evaluated for effects on cardiovascular function following intravenous administration in urethane-anesthetized rats. Cumulative doses of the compounds were administered at 10 minute intervals and arterial pressure (MAP) and heart rate (HR) were monitored. The noncompetitive antagonist (+) MK-801 produced dose-dependent transient decreases in MAP with a maximal hypotensive effect (50% reduction in MAP) observed at 0.5 mg/kg. Similar MAP decreases were observed with (-) MK-801 although 5-10-fold higher doses were required. The competitive NMDA antagonists, CPP and CGS 19755, elevated MAP 15-20% above baseline at the lowest doses (5 and 0.5 mg/kg, respectively) while hypotension was evident at higher doses (40-50 mg/kg). Interestingly, NPC 12626 (competitive NMDA antagonist) had virtually no effect on arterial pressure, producing only minor decreases at the highest doses tested (250 mg/kg). Heart rate was not affected with any compounds tested.

These results suggested that NPC 12626 elicits fewer cardiovascular effects than other NMDA antagonists when evaluated in anesthetized animals. Since other pharmacological actions of NPC 12626 occur at doses similar to CPP and CGS 19755, NPC 12626 may represent a physiologically safer alternative in the treatment of ischemic insult.

284.15

AMAA - A POTENT AND SELECTIVE EXCITATORY AMINO ACID AGONIST AT NMDA RECEPTORS.
U. Madsen*, J.W. Ferkany#, B. Ebert* and P. Krogsgaard-Larsen, Department of Organic Chemistry, The Royal Danish School of Pharmacy, DK-2100 Copenhagen, Denmark, #Nova Pharmaceutical Corporation, Baltimore, Maryland, USA.

Much pharmacological and therapeutic interest is focused on receptors for excitatory amino acids (EEAs). The NMDA subtype of EAA receptors has been characterized in some detail, especially due to the availability of a number of competitive as well as non-competitive antagonists. However, very few potent and selective NMDA agonists have been described. In binding studies and neuropharmacological experiments in different rat cortical tissue preparations (RS)-2-amino-2-(3-hydroxy-5-methylisoxazol-4-yl)acetic acid (AMAA) has been shown to be a very potent NMDA agonist, slightly more active than NMDA, with no significant activity at other EAA receptor subtypes. In contrast to NMDA, AMAA is also a very potent neurotoxin slightly more toxic than kainic acid and considerably more toxic than quinolinic acid. Two series of analogues of AMAA and AMPA (a selective AMPA agonist) have been characterized biochemically and pharmacologically. Distinct differences in the structural requirements for activation of NMDA and AMPA receptors have been revealed. The pharmacological and toxicological profiles of AMAA and the bicyclic analogue, 4-HPCA, as well as quinolinic acid are consistent with heterogeneity of the NMDA receptors.

ACETYLCHOLINE—RECEPTORS: NICOTINIC II

285.1

EXPRESSION OF NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR GENES IN CELLS OF THE SH-SY5Y HUMAN NEUROBLASTOMA. S. Norman, L. Lucero* and R.J. Lukas. Division of Neurobiol., Barrow Neurol. Institute, Phoenix, AZ 85013.

SH-SY5Y is a human neuroblastoma clonal cell line of presumed neural crest origin (Ross et al. *JNCI* 71:741-747, 1983). These cells express functional ganglia-type nicotinic acetylcholine receptors (nAChR) that mediate nicotinic agonist-stimulated $^{86}Rb^{+}$ efflux sensitive to blockade by mecamylamine but not by alpha-bungarotoxin (Lukas, *Soc. Neurosci. Abst.* 15:497, 1989). SH-SY5Y cells also express neuronal nicotinic alpha-bungarotoxin binding sites (nBgtS), which bind radiolabeled toxin with high affinity and appear to have pharmacological features and subunit profiles similar to those of nAChR, but do not appear to function as nicotinic ligand-gated ion channels.

Northern blot analysis of SH-SY5Y cell RNA, using rat neuronal nAChR subunit cDNA as probes (Deneris et al. *Clin. Chem.* 35:731-737, 1989), demonstrates that these cells express transcripts homologous to neuronal nAChR alpha3 and beta2 subunit genes but distinct from those identified on Northern blot analysis of rat PC12 cell RNA (see Boulter et al. *J. Biol. Chem.* 265:4472-4482, 1990). These results suggest that human alpha3-like and beta2-like subunits may be part of a human ganglia-type nAChR. Rat neuronal probes are being used to screen an SH-SY5Y cell cDNA library for clones corresponding to the human alpha3-like and beta2-like transcripts.

285.2

AMPLIFICATION OF GENOMIC SEQUENCES IDENTIFIES A NEW GENE, ALPHA 6, IN THE NICOTINIC ACETYLCHOLINE RECEPTOR GENE FAMILY. E. Lamar, K. Miller*, and J. Patrick. Div. of Neuroscience, Baylor College of Medicine, Houston TX 77030.

It is well established that the subunits of nicotinic acetylcholine receptors (nACHR) are members of a gene family. Five members of the family have been classified as alpha (α) subunits on the basis of contiguous cysteines at positions 192 and 193 (α 1-5); four of these (α 1, 2, 3, and 4) participate in the formation of functional nACHRs in vitro.

We used the polymerase chain reaction technique with highly degenerate primers to amplify rat genomic DNA. Oligonucleotides made to conserved regions of nACHR subunits in the extracellular and membrane-spanning I domains amplified a 321 base-pair open reading frame between amino acids 134 and 241 (corresponding to Torpedo α 1 numbering scheme) that was 76% homologous on the amino acid level to the previously described α 3 subunit. The sequence contained cysteines at positions 142, 192, and 193; these amino acids are conserved in all nicotinic α subunits characterized to date. In addition, the sequence contains a tyrosine residue at position 190, a feature common to all functional nicotinic α subunits. Therefore, we identify our sequence as part of a gene encoding a new member of the nACHR-related gene family, α 6.

Alpha 6 mRNA appears to be more abundant in early developmental stages than in adult rat: Northern blot analysis of brain mRNA showed strong hybridization of α 6 riboprobes to a dominant 3 kilobase band in day 11 and day 22 rats, and only faint hybridization to adult mRNA.

285.3

DEVELOPMENTALLY REGULATED AND BUNGAROTOXIN SENSITIVE AVIAN NEURONAL nAChR SUBUNIT.

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We have isolated from a chicken brain cDNA library a clone defining the novel neuronal nAChR subunit alpha7. The cDNA, 2500 bp in length, contains an ORF encoding a mature protein of 479 residues preceded by a signal peptide and showing homology throughout with all other alpha and non-alpha nAChR subunits. Its amino-terminal sequence closely matches the published amino-terminal 24 residues of one of the subunits of the chick optic lobe alpha-bungarotoxin binding protein. Northern blot analysis and in situ hybridizations demonstrate that alpha7 transcripts transiently accumulate in the optic tectum between E5 and E16, at the time when tectal neurons form their connections with retina and other regions of the brain. Bilateral eye removal at E2 does not prevent this accumulation. The alpha7 cDNA was subcloned into an expression plasmid and injected into Xenopus oocyte nuclei, either alone or in combination with other neuronal and muscle nAChR subunit cDNAs. ACh sensitivity of the injected oocytes was then examined in voltage clamp. The only tested combination of subunits yielding ACh-induced currents was alpha7/beta/gamma/delta and these currents were blocked by alpha-bungarotoxin. We conclude that alpha7 is probably one of the subunits of a developmentally regulated, bungarotoxin binding protein and that these proteins may function as ACh gated ion channels.

285.4

AFFINITY CHROMATOGRAPHY OF A NICOTINIC ACETYLCHOLINE RECEPTOR FROM RAT BRAIN.

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An acetylcholine affinity resin, prepared by reacting bromoacetylcholine bromide with Affi-Gel 401 (Bio-Rad), is commonly used for the purification of nicotinic acetylcholine receptor (NACHR) from electric tissue. We report the use of this affinity gel for the isolation of NACHR from rat brain. Triton-solubilized extract of a membrane fraction from rat brains was incubated with the affinity gel. After extensive washing of the gel, the receptor was specifically eluted with nicotine. Typically, the affinity resin removed 75-90% of [^{3}H]l-nicotine binding sites from solution, and 10-20% of the bound sites were recovered from the resin. Polyacrylamide gel electrophoresis yielded two protein bands, of apparent molecular weights 80,000 and 49,000, only in the specifically eluted fractions. Minor contaminating bands in these fractions were identically present in the previous wash fraction (which lacked nicotine-binding activity), and thus are not part of the receptor. Preliminary results indicate a dissociation constant for l-nicotine of ~8nM both in the original Triton extract and in the specifically eluted material.

Our results provide independent confirmation of the subunit size and composition reported for rat brain NACHR isolated by immunoaffinity methods (Whiting, P., and Lindstrom J., *Proc. natl. Acad. Sci. USA*, 84:595, 1987). Our technique should prove applicable to the study of NACHR from brains of other species.

Supported by the Smokeless Tobacco Research Council.

285.5

LOCALIZATION OF HUMAN NEURONAL NICOTINIC RECEPTOR SUBUNITS.

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Neuronal nicotinic receptors (nAchRs), expressed in rodents and chicken, are multimeric proteins constituted of two different subunits, alpha and beta. Although nAchRs appear to be involved in human degenerative disorders, no information was available on their primary structure and localization in the human nervous system. We have cloned from a human IMR 32 neuroblastoma cell line cDNA library two neuronal alpha subunits, alpha 3 and alpha 5, and a beta subunit which are highly homologous to the rat nicotinic subunits. The anatomical localization of the human alpha 3 subunit was studied using in situ hybridization histochemistry. Both sense and antisense ^{35}S radiolabeled riboprobes were transcribed from an insert subcloned in a pGEM 4 Z vector. Sections (10 um) of autoptic human brain, monkey brain (Cynomolgus), rat brain, containing the medial habenula and the hippocampal formation and sections of human sympathetic lumbar ganglia were hybridized with the radiolabeled probes. A strong specific signal was observed in the human sympatethic lumbar ganglia were the alpha 3 mRNA is expressed in abundance in most of the neurons, but up to now no positive hybridization signal was observed in the other examined tissues. A moderate cross hybridization in the rat brain was noticed in the medial habenula. On the basis of our data we can conclude that a human alpha 3 subunit of the neuronal nicotinic receptor is strongly expressed in human simpathetic ganglia.

285.6

MOLECULAR CLONING OF A NEURONAL NICOTINIC ACETYLCHOLINE RECEPTOR FROM DROSOPHILA.

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The central nervous system of Drosophila contains an α-bungarotoxin binding site with the properties expected of a nicotinic acetylcholine receptor. The receptor was purified 6000-fold by affinity chromatography. Sodium dodecyl sulfate polyacrylamide gel electrophoresis revealed two subunits with apparent molecular weights of 42,000 and 57,000. Polypeptides of identical size were also identified by in situ photoaffinity labeling of the membrane fraction. From sedimentation analysis in H_2O and D_2O a molecular weight of 270000 was determined. The individual polypeptides were blotted on a nitrocellulose membrane, digested in situ with trypsin and separated by microbore HPLC. Isolated peptides were subjected to amino acid sequence analysis. Mixed oligo-nucleotide probes derived from the amino acid sequence were constructed and used for screening of a cDNA library in either SSPE or tetramethylammonium chloride. Several cDNA clones were isolated corresponding to the peptide sequences indicating receptor heterogeneity.

285.7

PHARMACOLOGICAL CHARACTERISATION OF AN ACETYLCHOLINE RECEPTOR ON THE SOMATIC MUSCLE CELL OF ASCARIS SUUM.

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Acetylcholine is the excitatory neurotransmitter in the parasitic nematode, Ascaris suum. We have extensively characterised this cholinoceptor using conventional electrophysiological recording techniques. The agonist profile indicates a nicotinic ganglionic type of receptor; hydroxyphenylpropyltrimethylammonium (HPPT) and dimethylphenylpiperazinium (DMPP) are potent agonists, bethanechol and methacholine are ineffective up to 1 mM. Although mecamylamine is a potent antagonist, indicating a ganglionic-type receptor, hexamethonium is weak. α-Bungarotoxin partially blocks at 1 μM, curare, atropine and pancuronium all block in the low micromolar range.

The results indicate the receptor is nicotinic and resembles the frog ganglionic receptor.

Further experiments including expression of the receptor-channel protein in Xenopus oocytes are being carried out to study receptor characterisation, subunit structure and structure-function relationships.

We are grateful to the SERC and JEA for funding.

285.8

EXPRESSION OF mRNAs IN HUMAN THYMUS CODING FOR THE α3 SUBUNIT OF A NEURONAL ACETYLCHOLINE RECEPTOR.

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Myasthenia gravis (MG) is an autoimmune neuromuscular disease in which the breakdown of tolerance comprises a number of muscle antigens, the best characterized is the nicotinic acetylcholine receptor (AcChR). The association of thymic abnormalities with MG, and the expression in thymus of material that binds α bungarotoxin and/or shows immunological cross-reactivity with antibodies raised against the neuromuscular AcChR led to propose that thymic AcChRs or AcChR-like material may be involved in the triggering and/or maintenance of this autoimmune condition.

To investigate the nature of the AcChR expressed in thymus we searched for cDNA clones present in cDNA human thymic libraries that would hybridize to cDNA probes whose nucleotide sequences encode for the subunits of the neuromuscular AcChR. This experimental approach resulted in the isolation of clones from a human thymus library that code for the α3 subunit of a neuronal acetylcholine receptor (AcChR). The clones hybridize to a major 3.0 Kb mRNA thymic species and 4 minor ones of approximately 2.3, 4.0, 5.0 and 6.5 Kb, but they do not hybridize to human muscle mRNA. In contrast a cDNA probe that encodes for the α subunit of the neuromuscular AcChR hybridizes to a 2.3 Kb mRNA expressed in muscle, but that is not evident in thymus.

Reported findings suggest thymic expression of nicotinic neuronal AcChR could be responsible for cholinergic immunoreactivity found in thymic (epithelial) cells. These thymic clones will be valuable in defining the cholinergic thymic make-up and putative role that thymic AcChR may have in triggering and/or maintenance of an anti-AcChR response in MG.

285.9

INTERACTION OF THYMOPOIETIN WITH NICOTINIC RECEPTORS IN C2 MUSCLE CELLS IN CULTURE. M. Greenbaum*, T. Audhya*, G. Goldstein and M. Quik. Dept. Pharmacol., McGill Univ., Montreal, Canada and Immunobiol. Res. Inst., Annandale, NJ, USA.

Thymopoietin is a thymus derived polypeptide which potently binds to the nicotinic α-bungarotoxin (α-BGT) receptor population in neuronal tissues and electroplax. Current work in our laboratory also indicates that thymopoietin interacts at the nicotinic receptor at the neuromuscular junction. The present experiments were done to determine whether muscle nicotinic receptors could be regulated by thymopoietin using a muscle cell line (C2 cells in culture). Initial studies showed that thymopoietin potently inhibited [^{125}I]α-BGT binding to C2 muscle cells in culture with an IC50 of 2 nM; this was similar to the IC50 for α-BGT, while nicotinic receptor ligands were effective in the uM range. Exposure of the cells in culture to various concentrations of thymopoietin (followed by extensive washing of the cells to remove unbound polypeptide) resulted in a marked reduction in the binding of [^{125}I]α-BGT to the cells, with an IC50 of approximately 3 nM; the decrease in binding was observed as early as 4 hr after exposure to thymopoietin. Thus the present results show that the polypeptide thymopoietin can potently interact at and regulate nicotinic receptors in muscle cells culture.

285.10

THYMOPOIETIN-INDUCED PROCESS FORMATION IN PC12 CELLS IN CULTURE IS DISTINCT FROM THAT INDUCED BY NGF. J. Philie*, S. Geertsen, T. Audhya*, G. Goldstein and M. Quik. Dept. Pharmacol., McGill Univ., Montreal, Canada and Immunobiol. Res. Inst., Annandale, NJ, USA.

Previous studies had shown that thymopoietin (TPO), a thymic polypeptide, potently decreased [^{125}I]α-bungarotoxin (BGT) binding to a nicotinic receptor population in PC12 cells. In addition, TPO resulted in process formation in the cells in culture. NGF also results in neurite extension in PC12 cells. The present experiments were done to determine the relationship between the NGF and TPO induced process outgrowth. A Mab to NGF, which completely prevented the NGF induced changes in PC12 cell morphology, did not alter the neurite extension which occurred in the presence of TPO. Experiments were also done in which the cells in culture were exposed to both NGF and TPO; the process formation which occurred in the presence of the two polypeptide was greater than additive. Exposure of the cells in culture to α-BGT resulted in process formation which was not altered by the NGF Mab; the effect of both NGF and α-BGT was also more than additive. These results suggest that the TPO induced process extension is distinct from that induced by NGF, and furthermore, that the two polypeptides potentiate each others action to initiate process outgrowth.

285.11

DEFINITION OF T CELL EPITOPES IN RECOMBINANT HUMAN ACETYLCHOLINE RECEPTOR α SUBUNIT SEGMENT 1-210. M. Hayashi*, D.J. McCormick*, S. Talib*, T. Okarma*, V.A. Lennon. Mayo Clinic, Rochester, MN 55905 and Applied ImmuneSciences, Inc., Menlo Park, CA 94025.

The pentameric complex of human skeletal muscle's nicotinic ACh receptor (AChR) is the target for pathogenic antibodies in myasthenia gravis. Potential T cell regulatory segments in the α subunit's N terminal (extracellular) domain were investigated by testing in vivo delayed hypersensitivity and in vitro lymphocyte responses to synthetic peptides in rats immunized with a recombinant human AChR α subunit protein comprising residues 1-210.

Of 27 peptides (each 16 residues, overlapping its neighbor by 8 residues), 4 were stimulatory for immune T cells: 9-24 and 41-56 identified two previously unknown T cell stimulatory regions; 121-136 and 129-144 helped define at least two T cell epitopes (provisionally 125-131 and 132-144) in the previously identified myasthenogenic region 125-147 (Ann NY Acad Sci 505:439, 1987; J Immunol 139:2615, 1987).

Recombinant and synthetic autoantigens offer tools for investigation and potential therapy of myasthenia gravis. **Supported in part by NIH NS15057/NS24694 and the Wm.K. Warren Foundation.**

285.12

THE DISTRIBUTION OF α-BUNGAROTOXIN BINDING PROTEIN IN THE CHICK RETINA: AN IMMUNOHISTOCHEMICAL STUDY. K.T. Keyser, #R. Schoepfer,*#W.G. Conroy, *@P.J. Whiting, *#M. Gore, *A. Prechtl-Brzozowska, H.J. Karten and #J. Lindstrom. Department of Neurosciences, UCSD, La Jolla, CA 92093, #Receptor Biology Lab, The Salk Institute, La Jolla, CA 92138 and @Merck, Sharp and Dohme Laboratories, Essex, England.

Alpha bungarotoxin (α Bgt) binds to proteins in the CNS which are related to, but distinct from, acetylcholine receptors in muscle and brain. Their function is unknown. Antibodies that recognize the ligand binding subunit of two subtypes of the α Bgt binding protein (α BgtBP) have been prepared to purified α Bgt and to bacterially expressed peptides from cDNAs. The antibodies were used to investigate the distribution of α BgtBP in chick retina.

Neurons in the inner nuclear layer (INL) and ganglion cell layer (GCL) exhibited α BgtBP immunoreactivity. Stained medium to large cells were found in the inner tiers of cells in the INL while a population of smaller stained cells was restricted to a region closer to the outer plexiform layer (ONL). The dendrites of both populations of cells could be traced to the inner plexiform layer (IPL) where they contributed to a complex pattern of stratified processes. The dendrites of some of the labeled cells in the outer INL projected to the OPL. Many cells of various sizes were labeled in the GCL. The dendrites of these cells also contributed to the complex pattern of fibers in the IPL. Although the function of these proteins is undetermined, their distribution in the retina suggests that they may be important for retinal function. Supported by EY07845 (KTK), EY06890 (HJK) and grants from NIH, MDA & CTR (JL).

285.13

DIFFERENCES BETWEEN THE ADULT AND EMBRYONIC γ-SUBUNIT OF TORPEDO ACETYLCHOLINE RECEPTOR. M.C. Souroujon, S. Carmon*, A. Safran* and S. Fuchs. The Open University of Israel and Department of Chemical Immunology, The Weizmann Institute of Science, Rehovot 76100, Israel.

During development in some mammalian species, the embryonic γ-subunit of acetylcholine receptor (AChR) is replaced by an adult ε-subunit. It is of interest to find out whether Torpedo AChR also displays two alternative forms of the γ-subunit. The known Torpedo γ-subunit possesses a putative phosphorylation site that is present in all known ε-subunits, but is absent in their respective γ-subunits. Antibodies against a synthetic peptide corresponding to residues 346-359 of the Torpedo AChR γ-subunit, and containing this phosphorylation site, react strongly with adult and hardly with embryonic Torpedo γ-subunit. The differential reactivity of the anti-γ peptide antibodies does not reflect differences in the state of phosphorylation. Also, the γ-subunit of the adult Torpedo AChR is phosphorylated by exogenous cAMP-dependent protein kinase to a much higher extent than its embryonic counterpart. Thus it appears that the known Torpedo γ-subunit represents an adult form of this subunit and is different, at least in the region of the phosphorylation site, from the crossreactive embryonic form. It should still be tested whether the embryonic and adult forms of the Torpedo AChR γ-subunit are coded by two different genes.

285.14

EFFECTS OF BARBITURATES ON CHOLINERGIC BINDING TO NEURONAL NICOTINIC RECEPTORS. B.A. Dodson and L.M. Braswell*. Dept. of Anesthesia, UCSF, San Francisco, CA 94143

This study examines the effect of secobarbital, amobarbital, pentobarbital, methohexital and thiopental on high affinity postsynaptic, nicotinic, cholinergic binding to synaptosomes prepared from freshly dissected rat cerebral cortex. The binding of ^{3}H-acetylcholine (^{3}H-ACh, 10nM) to neuronal nicotinic acetylcholine receptors (nAChR) (200μl of 5% brain homogenate ≈ 50-60 ACh binding sites/mg protein) with and without barbiturate (10^{-6}-10^{-2} M) was determined by filtration assay (Schwartz, RD, Molec Pharm 22:56, 1982). Experiments were performed in triplicate at 4°C in TRIS buffer containing atropine to block muscarinic binding. Binding in the presence of 0.1mM carbachol was defined as nonspecific. Secobarbital produced the greatest effect of the barbiturates examined, decreasing ^{3}H-ACh binding to 60% of control values. Amobarbital and pentobarbital were less efficacious with maximum decreases of approximately 20%. Methohexital and thiopental had minimal effects on ^{3}H-ACh binding. These results differ from those obtain in previous studies using *Torpedo* nAChR; in which amobarbital and pentobarbital decreased binding by 75% and 60%, respectively (Dodson, BA, Molec Pharm 32:119, 1987), and secobarbital, methohexital and thiopental increased ^{3}H-ACh binding (Dodson, BA, J. Neurosci 14:914, 1988). These findings suggest that barbiturates may have different mechanism(s) of action for different nicotinic receptor subtypes. (Supported by NIH Grant GM35997)

285.15

[^{3}H]CYTISINE BINDING TO NICOTINIC CHOLINERGIC RECEPTORS IN BRAIN. L.A. Pabreza*, S.R. Wheeler* and K.J. Kellar. Department of Pharmacology, Georgetown Univ. Medical Center, Washington, DC 20007.

Cytisine, a ganglionic type nicotinic agonist, has high affinity for neuronal nicotinic receptors in competition studies against other [^{3}H]agonists. We have characterized the binding of [^{3}H]cytisine to rat brain homogenates. [^{3}H]Cytisine was labeled to a specific activity of 12.4 Ci/mmol by Dr. E. Do (NEN Dupont). Brain homogenates were incubated with 0.2-12 nM [^{3}H]cytisine at 2°C for 75 min in the absence or presence of 10 μM nicotine to measure total and nonspecific binding, respectively. Specific binding represented 50-80% of total binding at all concentrations of [^{3}H]cytisine tested. The K_d of [^{3}H]cytisine for its binding sites is approximately 0.7 nM, which represents an affinity 5-15 times higher than that of other available [^{3}H]nicotinic ligands. The density of sites labeled in various areas of brain is similar to that found using other labeled nicotinic agonists. Drug competition studies indicate that the binding site has a nicotinic cholinergic pharmacology. Thus nicotine, acetylcholine (in the presence of a cholinesterase inhibitor) and carbachol compete with high affinity for [^{3}H]cytisine binding sites. Among antagonists tested, only dihydro-ß-erythroidine (DßE) has high affinity for the site (IC_{50} = 100 nM). Saturation studies of [^{3}H]cytisine binding in the presence of DßE indicated that this antagonist binds in a competitive manner. Mecamylamine, though of much lower affinity, also appeared to bind competitively. If confirmed, this would be in contrast to mecamylamine's noncompetitive binding to sites labeled by [^{3}H]n-methylcarbamylcholine. These results indicate that [^{3}H]cytisine will be a useful ligand for further studies of nicotinic cholinergic receptors. (Supported by DA 06486)

285.16

NICOTINIC CHOLINERGIC RECEPTOR BINDING USING ACETYLCHOLINE RECEPTOR IMMOBILIZED ON A FIBER OPTIC SENSOR. R. G Thompson, D. E. Menking*, K. Rogers*, J. J. Valdes and M. E. Eldefrawi*. Dept. of Army, Chem. Res. Dev. & Engr. Ctr., Biotechnology Div., Aberdeen Proving Ground, MD 21010-5423 and Dept of Pharmacol. & Exptl. Therapeutics, Univ. of MD Sch. of Med., Baltimore, MD 21201

A fiber optic-based biosensor is described in which the nicotinic acetylcholine receptor from Torpedo electric organ is noncovalently immobilized on quartz rods and ligand occupancy of the receptor binding site is measured using fluorescein isothiocyanate (FITC) conjugates of the quasi-irreversible receptor probe, α-bungarotoxin, or the the slowly reversible α-najatoxin probe. Competetion curves for binding of the FITC-toxin probes to the immobilized receptor indicated that the IC_{50} values for agonists such as nicotine and carbamylcholine were higher than those previously reported from radioisotopic measurements in membrane preparation. IC_{50} values for antagonists such as d-tubocurarine, however, were similar to those reported using radioisotope probes. The fiber optic sensor's lower sensitivity for detecting agonist interactions with the receptor may be due to several factors: 1) agonist-induced desensitization of the receptor, 2) greater sensitivity of the agonist binding site to steric hindrance imposed by receptor immobilization, and/or 3) differences in the lipid environment of the receptor in the different binding protocols.

285.17

USE OF MECAMYLAMINE (MEC) AND LOBELINE (LOB) TO ESTIMATE NONSPECIFIC BINDING OF RADIOLABELED NICOTINE (NIC) *IN VIVO* A.S. Kimes, D.F. Wong, and E.D. London. NIDA Addiction Res. Ctr. and Johns Hopkins Medical Inst., Baltimore, MD.

Toxicity of nic has hampered previous attempts to define nonspecific binding of [^{3}H]nic to nicotinic acetylcholine receptors (nAchrs) *in vivo* using unlabeled nic as the competing ligand (Broussolle, E.P. et al., Life Sci., 44:1123, 1989).

In this report, we describe alternative approaches to estimate nonspecific binding of [^{3}H]nic *in vivo* either by blocking toxicity of nic or by using other competing drugs. Mec antagonizes the behavioral and cerebral metabolic effects of nic (London, E.D. et al., J. Neurosci., 8:3920, 1988) as well as nic-induced toxicity, despite weak activity in competing for nic binding sites *in vitro* (e.g., Takayama, H. et al., JPET, 253:1083, 1989). We observed that mec (5 mg/kg) slightly facilitates the entry of [^{3}H]nic into the brain, but does not affect nonspecific binding estimated by nic (5 mg/kg) pretreatment. In the presence of mec, 10 mg/kg unlabeled nic is no more effective at inhibiting binding of [^{3}H]nic than 5 mg/kg, confirming that this dose saturates nic binding sites in mouse brain.

We also used lob (s.c., 0.3 to 30 mg/kg as base), a nAchr ligand with similar affinity to nic *in vitro* (Abood, L.G. et al., Pharmacol. Biochem. & Behav., 30:403, 1988) to inhibit specific [^{3}H]nic binding *in vivo*. High doses of lob ($\leq$ 30 mg/kg) can be given without toxicity. We found that 3 mg/kg lob was sufficient to define nonspecific binding of [^{3}H]nic, suggesting that lob may offer a viable approach to define nonspecific binding of radiolabeled nic to nAchrs.

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285.18

BRAIN NICOTINIC RECEPTORS ISOLATED BY A MONOSPECIFIC ANTIBODY AGAINST A SYNTHETIC ALPHA-3 RECEPTOR ANALOGUE COMPARED TO AN ANTI-NICOTINE IDIOTYPIC ANTIBODY. T.C. Madhok, S.G. Matta, A. Hong,* R.J. Biercke,* J.J. Langone,* and B.M. Sharp*. Endocrine Neuroscience Lab., Department of Medicine, Hennepin County Medical Center and University of Minnesota, Minneapolis. MN 55415 and Department of Medicine and Biochemistry, Baylor College of Medicine, Houston, TX 77030.

This study aimed at characterizing nicotinic cholinergic receptor (nChR) proteins purified from rat brain by immunoaffinity chromatography using the anti-alpha-S_3 antibody (*BBRC* 165: 151-157, 1989) and the 422 F11 anti-idiotypic antibody (*BBRC* 162: 1085-1092, 1989). We have purified to homogeneity the anti-alpha-S_3 antibody from rabbit sera using $(NH_4)_2SO_4$ precipitation, DEAE chromatography and affinity chromatography with thyroglobulin - Sepharose 4B and S_3-peptide-Sepharose 4B. The anti-alpha-S_3-Sepharose 4B was shown to deplete ^{3}H-nicotine binding sites from rat brain membranes. SDS-PAGE of nChR isolated from the 422 F11 column showed a major component (silver stained) with a MW of 45-47K (both by nicotine and citrate, pH 3, elutions) and minor components with MWs of 53K and 63K. nChR isolated from the anti-alpha-S_3 column showed a major protein with MW of 48.5K and a minor protein with a MW of 45.5K. These results show the similarity in molecular size among receptor proteins isolated by these two antibodies, and the usefulness of the anti-alpha-S_3 antibody in isolating nChRs proteins from rat brain. (Supported by DA-04446 and DA-03977)

PEPTIDES—RECEPTORS: ANGIOTENSIN, ENDOTHELIN

286.1

SOLUBILIZATION AND CHARACTERIZATION OF ANGIOTENSIN II RECEPTORS FROM MURINE NEUROBLASTOMA N1E-115 CELLS. I.R. Siemens, H.J. Adler, L.P. Reagan, R. Mir and S.J. Fluharty. Depts. of Animal Biology and Pharmacology, and Institute of Neurological Sciences, University of Pennsylvania, Phila., PA 19104.

The murine neuroblastoma N1E-115 cell line possesses membrane associated receptors for the neuroactive peptide angiotenisn II (AngII). In an attempt to solubilize these receptors we treated N1E-115 membranes with several detergents which have been used successfully to solubilize AngII receptors (AngII-Rs) from peripheral target tissues. Sodium cholate (0.5%) and CHAPS (1%) were effective in solubilizing binding sites for ^{125}I-AngII or the high affinity antagonist ^{125}I-Sarc^1,Ile^8 AngII (SARILE), while digitonin (1%), Triton X-100 (0.5%) and NP-40 (1%) appeared to impair the ability of the solubilized protein to bind the ligand. Further analysis with CHAPS indicated that the solubilized binding sites were specific for AngII-related peptides, although their affinity was less than that observed in intact N1E-115 membranes. Covalent cross-linking of ^{125}I-AngII to solubilized N1E-115 membranes with disuccinimidyl suberate (DSS) followed by size exclusion chromatography or SDS-PAGE identifed a specific binding protein of M_r 73 kDa. Similarly, affinity chromatography using AngII as the ligand also revealed a 73 kDa binding protein on SDS-PAGE as well as a larger protein of M_r 112 kDa under non-reducing conditions, whereas only lower molecular weight binding sites were observed in the presence of reducing agents. Collectively, these results suggest that CHAPS can be used to solubilize and characterize AngII-Rs from a neuron-like cell line. Supported by NS 23986 and MH 43787.

286.2

CROSS-LINKING OF ^{125}I-ANGIOTENSIN II TO SPECIFIC RECEPTORS ON DIFFERENTIATED NG108-15 CELLS. M. D. Carrithers, K. A. Koide, V. K. Raman, S. Masuda and J. A. Weyhenmeyer. Neuroscience Program and College of Medicine, University of Illinois, Urbana, IL 61801.

We previously demonstrated that differentiated NG108-15 cells express a high affinity angiotensin (ANG) receptor that is not detectable on undifferentiated cells, has specificity for ANG II and ANG III, and mediates phosphatidylinositol breakdown (Carrithers et al., BBRC, 167: 1200, 1990). A low affinity site that binds ANG II but not ANG III is present on both differentiated and undifferentiated cells. In the present study, we used the homobifunctional crosslinker BIS (sulfosuccinimidyl) suberate (BS^3) to analyze these binding sites. Membranes from NG108-15 cells were incubated with 1 nM ^{125}I-ANG II in PBS, pelleted, and resuspended in BS^3 (0.5, 1.0, and 5.0 mM). PAGE analysis (7.5% resolving gel) revealed a single major band of ~ 78 kD in membranes prepared from differentiated cells. 1 µM of unlabeled ANG II or ANG III blocked labeling of this site. The estimated K_i (~ 3 nM) for ANG III was similar in differentiated cells as determined by gel densitometric analysis and pharmacological assay. No bands were specifically labeled in undifferentiated cells. These results suggest that the 78 kD band represents a component of the high affinity receptor expressed in differentiated NG108-15 cells.

Supported by NSF BNS 17117 and NIH SITG GM07143.

286.3

USE OF NON-PEPTIDE ANGIOTENSIN II RECEPTOR ANTAGONISTS TO DEMONSTRATE AII RECEPTOR SUBTYPES IN BRAIN. J.K. Wamsley, W.F. Herblin[1], M. Hunt. Neuropsychiatric Res. Inst., Fargo, ND 58103; [1]E.I. du Pont de Nemours and Co., Medical Products Department, Wilmington, DE 19880.

Subtypes of angiotensin II (AII) receptors have been described in the adrenal gland and some vascular tissues, but not in brain. We used the subtype specific AII-type 1 antagonist DUP 753 and the type 2 specific antagonist PD123177 (EXP658) to displace [^{125}I]AII binding in brain and demonstrate the presence of these subtypes.

DUP 753 effectively inhibited [^{125}I]AII binding in specific brain regions of the rat as follows: subfornical organ (SFO) 94%, median eminence (ME) 90%, anterior hypothalamic area (AHy) 60%; habenula (Hb) 48%, superior colliculus (SC) 43%, dorsal nucleus of the medial geniculate body (MGD) 60%. In contrast, PD123177 displaced [^{125}I]AII specific binding quite differently: SFO 18%, ME 0%, AHy 0%, Hb 22%, SC 0%, MGD 58%.

These data clearly demonstrate the presence of both AII receptor subtypes in the mammalian brain. Previous investigations have shown AII receptors to be concentrated in brain regions known to be involved in blood pressure regulation and thirst. These are precisely the areas where AII-type 1 receptors predominate as determined by the selective displacement of [^{125}I]AII by DUP 753. These results imply a central role for this potent non-peptide antihypertensive agent.

286.4

ANGIOTENSIN II DEPOLARIZES SUPRAOPTIC NEURONES RECORDED IN RAT HYPOTHALAMIC EXPLANTS. M.I. Phillips, C.R. Yang, C.W. Bourque and L.P. Renaud. *Dept. of Physiology, University of Florida, Gainsville, FL32610; Centre for Research in Neuroscience, Montreal General Hospital & McGill University, Montreal, Canada, H3G 1A4.*

Angiotensin II (AII) induces firing in rat magnocellular neurosecretory neurones (MNCs) in the supraoptic nucleus (SON), and releases vasopressin following its central administration in vivo via an unknown mechanism. We have used intracellular recording techniques to investigate the cellular mechanisms of AII actions in a superfused rat hypothalamic explant preparation in vitro.

Superfused application of Ile^5-AII and Val^5-AII (0.5-10μM) induced a slow onset (1-2.5min before peak response) and prolonged membrane depolarization (4-12mV for 5-15mins) in 16 of 20 MNCs. This depolarization was accompanied by a mean increase of 20±5.3% in input conductance and persisted when synaptic transmission was blocked in the absence of [Ca^{++}]o (with 6mM Mg^{++} or Mn^{++}). At equimolar concentrations, Val^5-AII was 2-3 times more potent than Ile^5-AII. Extrapolation of the steady-state voltage-current plot for the AII response (from -50 to -110mV) revealed reversal potentials (V_R) between -20 and -38mV. Bath application of a non-peptide AII antagonist, DuP753 (1-5μM), had no effect on resting membrane potential or glutamate-induced depolarization but appeared to block the AII-induced depolarization (n=4 cells). These results indicate that AII acts directly on MNCs to induce a specific receptor-mediated depolarization via activation of a non-selective cationic conductance. (Supported by FRSQ, FCAR & MRC).

286.5

DEVELOPMENTAL REGULATION OF MEMBRANOUS AND CYTOSOLIC ANGIOTENSIN II RECEPTORS IN RAT BRAIN AND ADRENAL. S.Y. Chow, A.N. Epstein and S.J. Fluharty. Depts. of Animal Biology and Psychology, and Institute of Neurological Sciences, University of Pennsylvania, Phila., PA 19104.

The actions of angiotenisn II (AngII) are mediated by receptors (AngII-Rs) located within its target tissues. AngII binding proteins, which may function as receptors, are present in the plasma membrane and the cytoplasm. In this study, we have examined the relationship between these membranous and cytosolic AngII-Rs during development in the rat using the high affinity antagonist ^{125}I-Sarc^1,Ile^8-AngII (SARILE). In the adrenal, SARILE labelled a homogenous population of membranous AngII-Rs, the density of which was highest at 3 days post-partum (B_{max} = 880 fmols/mg prot.). During the next two weeks, however, membranous binding activity rapidly declined and adult levels were less than 40% of those observed at day 3. Cytosolic binding activity exhibited the reverse relation, that is, binding activity was lowest at day 3 and increased to maximal levels of 1530 fmols/mg prot in adulthood. A similar pattern was observed in the hindbrain, although the changes were more gradual. More specifically, membranous binding was highest at day 3 (B_{max} = 29 fmols/mg prot) and thereafter declined to adult levels which were only 30% of the neonatal values. Cytosolic binding, on the other hand, increased over the same time period from 104 to 140 fmols/mg prot. Collectively, these results suggest that there is a reciprocal relationship between membranous and cytosolic AngII-Rs during development in the rat. It remains to be determined if the decline in membranous AngII-Rs, and corresponding increase in binding activity within the cytoplasm, is the result of receptor internalization. Supported by NS 23986, MH 43787, MH 17168, and NS 03469.

286.6

IMMUNOPRECIPITATION OF ANGIOTENSIN II RECEPTORS BY AN ANTI-PHOSPHOLIPASE C POLYCLONAL ANTIBODY IN MURINE NEUROBLASTOMA N1E-115 CELLS. S.J. Mah, R. Mir, R.O. Davies, J.R. Williamson, and S.J. Fluharty. Depts. of Animal Biology and Biochemistry, and Institute of Neurological Sciences, University of Pennsylvania, Phila., PA 19104.

Angiotensin II (AngII) stimulates phosphoinositide hydrolysis in a variety of cells through an association between AngII receptors (AngII-Rs), GTP-binding proteins, and phosphoinositide specific phospholipase Cs (PI-PLCs). In order to examine the relationships between AngII-Rs and these other integral membrane proteins, we have solubilzed membranes from differentiated N1E-115 cells using CHAPS (1%). CHAPS effectively solubilized approximately 40% of membranous binding sites for ^{125}I-AngII, and resulted in a 2.9-fold enrichment of specific PI-PLC activity. Moroever, Western blot analysis using polyclonal antibodies aganist one form of PI-PLC, PLC 60 purified from guinea pig uteri by Bennett and Crooke (J. Biol. Chem. **262**:13789-13797, 1987), identified the presence of an immunoreactive protein with an M_r of 60 kDa. Further analysis indicated that the anti-PLC 60 antisera percipitated a 60 kDa protein in solubilized membranes from cells labelled with [^{35}S]-methionine, which immunoblot analysis indicated comigrated with PLC 60. Finally, anti-PLC 60 antisera also immunoprecipitated AngII-Rs, prelabelled with the agonist ^{125}I-AngII. Successful precipitation of binding activity appeared dependent on prior agonist occupancy of the receptor insofar as the antisera did not precipitate receptors labelled with the antagonist ^{125}I-Sarc^1,Ile^8-AngII. Collectively, these results suggest that detergent solubilized N1E-115 membranes can be used to study the factors regulating the coupling amongest AngII-Rs and PI-PLC 60 within neuronal cells. Supported by NS 23986 and MH 43787.

286.7

CYTOSOLIC BINDING PROTEIN FOR ANGIOTENISN II: RELATION TO MEMBRANOUS RECEPTORS IN MURINE NEUROBLASTOMA N1E-115 CELLS. K.M. Moody, K. Addya, S.Y. Chow, M.A. Ravi Kiron, R.L. Soffer and S.J. Fluharty. Depts. of Animal Biology and Psychology, and Institute of Neurological Sciences, University of Pennsylvania, Phila., PA 19104 and Depts. of Biochemistry and Medicine, Cornell University Medical College, New York, NY 10021.

In addition to membranous angiotensin II receptors (AngII-Rs), many cells appear to possess a cytosolic binding protein which may also function as a AngII-R. In the present study we have characterized this putative cytosolic AngII-R in N1E-115 cells. Binding of the agonist ^{125}I-AngII or the antagonist ^{125}I-Sarc^1,Ile^8-AngII (SARILE) in cytosolic fractions required the organomercurial PCMS, EDTA and CHAPS (0.4%). Cytosolic binding was also specific for AngII-related peptides exhibiting somewhat greater affinity for AngIII than AngII. Covalent cross-linking of ^{125}I-AngII to N1E-115 cytosol with disuccunimidyl suberate (DSS) followed by SDS-PAGE indicated that binding occurred to a single protein of M_r 75 kDa. In addition, polyclonal antisera developed aganist the rabbit liver protein immunoreacted with the N1E-115 protein. Interestingly, as the density of membranous AngII-Rs increased during in vitro differentiation, binding activity in the cytosol decreased. Moreover, PCMS increased binding in differentiated membranes solubilized with CHAPS (1.0%), and most significantly, polyclonal antisera aganist the liver cytosolic protein immunoprecipitated approximately 40% of solubilized AngII-Rs from differentiated, but not undifferentiated membranes. Collectively, these results demonstrate that N1E-115 cells contain a putative cytosolic AngII-R, and suggest that this protein may be recrutied to the membrane during in vitro differentiation. Supported by NS 23986 and MH 43787.

286.8

ENDOTHELIN BINDING IN NORMOTENSIVE AND SPONTANEOUSLY HYPERTENSIVE RAT BRAIN. J.L. Banasik*, H. Hosick*, J.W. Wright and J.W. Harding. Dept. of VCAPP, Washington State Univ., Pullman, WA 99164-6520.

Endothelins (ET), a newly discovered family of three peptides (ET1, ET2, ET3), have 21 amino acids and two disulfide bonds. ET binding and biological activity have been reported in a variety of tissues and organ systems including vascular and nonvascular smooth muscle, fibroblasts, adrenal, cardiac and brain tissues. The endothelins appear to be involved in hemodynamic regulation, having potent vasoconstrictor properties and dose-related effects on blood pressure. Little is known about the role of endothelin in brain, although its function as a neurotransmitter has been postulated. Our studies have aimed to characterize binding properties of the various endothelins in the brain of normotensive (WKY) and spontaneously hypertensive (SHR) rats. ^{125}IET was prepared using the enzymobead lactoperoxidase method and purified with reverse-phase HPLC. Membrane fractions were prepared from homogenates of various brain structures including cerebellum, hypothalamus and brainstem. Our results indicate a wide distribution of ET1 binding in rat brain, brainstem and cerebellum having the highest binding while the cortex demonstrated little binding. Competition experiments demonstrated an IC_{50} value of between .1 and .5 nm for all three endothelins in the cerebellum, hypothalamus and brainstem of WKY rats. Saturation experiments indicate the presence of two receptor binding sites with different affinities. Site one has an apparent K_d of $2x10^{-11}$ M while site two has an apparent K_d of $8.5x10^{-10}$ M. Initial comparisons between SHR and WKY rats suggest differences in the ratio of these two sites.

286.9

ONTOGENY OF ENDOTHELIN-1 RECEPTORS IN THE SPINAL CORD. L. Weston*, M. Connolly*, P. Sweetnam. NovaScreen®, Nova Pharmaceutical Corp., Baltimore, MD 21224

Endothelin-1 (ET-1) is a 21 amino acid vasoactive peptide isolated from endothelial cells. Receptors which mediate the action of ET-1 have a wide tissue distribution, e.g. CNS. ET-1 has been isolated and ET-1-like immunoreactivity has been demonstrated in motorneurons and fibers of the dorsal horn. Physiologically ET-1 has been shown to depolarize ventral roots in newborn rat spinal cord. We have demonstrated the ontogeny of ET-1 receptors in the rat spinal cord by receptor binding assay. ET-1 receptors appear as early as embryonic day 14 and have binding characteristics similar to those found in the adult spinal cord, e.g., K_i = 3.5 nM.

It has been reported that intrathecal injection of ET-1 results in severe spinal dysfunction, possibly the result of motorneuron degeneration. ET-1's ability to induce degeneration was examined by monitoring choline acetyl transferase (ChAT) activity in dissociated spinal cord neurons maintained in culture. Using a number of different ET-1 treatment paradigms we were unable to detect a significant alteration in ChAT activity from that of control levels. This suggests ET-1 binding to neuronal elements may not directly result in the motorneuron degeneration reported *In Vivo*.

286.10

DOWN REGULATION OF ENDOTHELIN RECEPTORS IN GERBILS WITH TRANSIENT FOREBRAIN ISCHEMIA. P. Nambi*, M. Pullen*, C.F. Sauermelch*, G. Feuerstein, and R.N. Willette, Dept. of Pharmacology, SmithKline Beecham Pharmaceuticals, Philadelphia, PA.

Endothelin-1 (ET-1) is a vasoactive 21 amino acid peptide which appears to be part of a small family of endothelin isopeptides. In addition to its vascular effects, ET also elicits direct neuronal effects, causing depolarization in the ventral root of the spinal cord and increased phosphoinositol turnover in cerebellar granular cells. However, correlative evidence for a role of ET-1 in the pathophysiology of stroke is lacking. We studied the effects of transient forebrain ischemia (10 min) on ET receptors in ischemic and non-ischemic regions of gerbil brain. Hippocampi and brainstems were removed from sham and ischemic animals at 15 min, 60 min, 6 hr, and 72 hr intervals and assayed for ET-1 binding. There was no significant change in the receptors in brainstem between the two groups. In contrast, the ET-1 receptors in the hippocampus were downregulated by 8% (p=.01) at 15 min, 15% (p=.002) at 60 min and 12% (p=.05) at 6 hr. At 72 hr there was no significant change. The changes in ET-1 binding did not correlate with histopathologic changes in the hippocampus, but paralled the onset of neurologic deficits and recovery in the ischemic group. In conclusion, the release of ET-1 may contribute to the neurologic deficit and the down regulation of ET-1 receptors associated with transient forebrain ischemia.

286.11

IDENTIFICATION OF ENDOTHELIN RECEPTORS ON CULTURED CEREBELLAR NEURONS. P.G. Lysko, G. Feuerstein, M. Pullen* and P. Nambi*. Dept. of Pharmacology, SmithKline Beecham, King of Prussia, PA 19406.

The endothelin family of 21-amino acid peptides represents the most potent vasoconstrictors known. Three isoforms (ET-1, ET-2, ET-3) have been identified, and are found widely distributed in mammalian tissue, including the brain. The non-vascular distribution pattern of endothelin in brain and the high densities of endothelin binding sites in the granule cell layer of the cerebellum prompted our investigation of primary cultured rat cerebellar granule cells for the presence of endothelin receptors. Endothelin binding assays were performed on 8-day old neurons growing in 24-well culture dishes. Iodinated ET was incubated with cells in buffer at 4C; 1 μM unlabeled ET was used to define nonspecific binding. ET-1 binding was saturable (K_D= 88 pM), time-dependent (equilibrium at 2-3 hr), and specific (90% of total binding). Analysis of binding data revealed a B_{max} of 180 fmol/mg protein, corresponding to about 7400 receptors/cell. ET-3 binding to these cells was also detected, and demonstrated a K_D of 340 pM and a B_{max} of 4000 receptors/cell. Thus, cerebellar granule cells may be a useful model system to explore the function of the endothelin neuropeptides, which have been ascribed various roles as neuromodulators, such as overseeing CNS regulation of cardiovascular function.

286.12

ENDOTHELIN RECEPTORS: EXPRESSION IN XENOPUS OOCYTES. S. Shimada and G.R. Uhl. Lab. of Mol. Neurobiol., NIDA/ARC and Depts. of Neurol. & Nsci., Johns Hopkins Sch. of Med., Bx 5180, Baltimore, MD 21224.

Receptors for the endothelin peptides have been identified based on physiologic actions, ligand binding, and changes in phosphatidyl inositol metabolites. The Xenopus oocyte system frequently allows detection of receptors that can couple to changes in PI turnover. We have thus injected Xenopus oocytes with poly A^+ RNA isolated from brain and peripheral regions and sought responses to application of endothelin, sarafotoxin, and related peptides.

Oocytes injected with 30 ng of poly A^+ RNA prepared from brain and cerebellum gave responses to endothelin peptides (usually endothelin 1, 10^{-7}M) that were present in oocytes tested in 5 separate experiments. These responses ranged from 10 to 50 nA under voltage clamp conditions (-60 to -70 mV holding potential). The responses reversed at -10 mV, consistent with the reversal potential for chloride. Responses desensitized to a marked degree; the second application of endothelin produced almost no response, and this effect persisted for more than 30 min. Endothelin 3 and sarafotoxin showed similar responses.

These results suggest that endothelin receptors can be expressed in the Xenopus oocyte, and provide a approach to sib-selection cloning of this neuropeptide receptor.

MECHANISMS IN TRANSPORTER PHYSIOLOGY

287.1

STUDIES ON [^{3}H]VESAMICOL BINDING IN ISOLATED RAT BRAIN SYNAPTIC VESICLES. J.R.Haigh and S.M.Parsons. Dept. of Chemistry, University of California, Santa Barbara CA 93106

We have investigated the binding of [^{3}H]vesamicol [(-)-(trans)-2-(4-phenylpiperidino)cyclohexanol] to isolated rat brain synaptic vesicles, and crude rat brain membrane fractions. [^{3}H]vesamicol binds specifically and saturably with a k_d of 100nM and B_{max} of 3.2pmol/mg of protein. Under pseudo-first order conditions, the association rate constant for [^{3}H]vesamicol is 0.15 and 0.23 min^{-1} at 27nM and 260nM [^{3}H]vesamicol, respectively. Bound [^{3}H] vesamicol can be displaced by non-radioactive vesamicol, with a dissociation rate constant of 0.13 min^{-1}, a value similar to that found in cholinergic synaptic vesicles from Torpedo electric organ and slide-mounted sections of rat forebrain (Bahr and Parsons.,PNAS, 83:2267, 1986; Marien, et al., PNAS, 84:876). A structure-activity study using several vesamicol and acetylcholine analogues showed that both classes of drugs bind to rat brain synaptic vesicles and inhibit [^{3}H]vesamicol binding. In common with Torpedo vesicles, derivatives of isonipecotic acid were the most potent, inhibiting competitively, and exhibiting a 1000-fold increase in potency compared to acetylcholine. However, most analogues of vesamicol tested were between 3 and 20-fold less potent at inhibiting [^{3}H]vesamicol binding in brain, compared to Torpedo vesicles, although acetylcholine analogues displayed almost equal potency in both membrane systems.

287.2

ISOLATION, CHARACTERIZATION AND PRODUCTION OF ANTIBODIES TO A SOLUBLE VESAMICOL BINDING PROTEIN FROM TORPEDO. Stanley M. Parsons and Barry W. Hicks. Dept. of Chemistry and Neuroscience Research Institute, Univ. of CA, Santa Barbara, 93106

Vesamicol is a potent inhibitor of active transport of ACh into synaptic vesicles. Over 50 publications describe the action of the drug on a variety of preparations. It is presumed that vesamicol exerts its effects by binding to a site on the cytoplasmic side of a transmembrane receptor in cholinergic synaptic vesicles. This receptor has been partially purified and characterized. We report here the isolation of a second, lower affinity (K_d~1uM), vesamicol binding protein. Isolation was accomplished using soft gel and HPLC chromatography. The pure protein elutes at about 450KDal in non-denaturing size exclusion chromatography, yet shows only two bands of about 30KDal and 24KDal in SDS PAGE. The polyclonal antibodies raised to the 30KDal protein showed cross reactivity with the 24KDal protein and to proteins at the same molecular weights in mammalian brain and various tissues from Torpedo. The antisera did not however show any cross reactivity with highly purified synaptic vesicles. This protein differs from the vesicular receptor in its pharmacology, sensitivity to amino acid modification reagents and its pH binding maximum. The physiological role of this protein is currently unknown, but investigators working with vesamicol should be aware that higher concentrations may produce secondary effects.

287.3

PARTIAL PURIFICATION OF THE [^{3}H]RESERPINE BINDING SITE ON THE CATECHOLAMINE TRANSPORTER PRESENT IN THE CHROMAFFIN GRANULE MEMBRANES ISOLATED FROM BOVINE ADRENAL GLANDS. J.D. Deupree, and R. Zielinski*. Department of Pharmacology, University of Nebraska Medical Center, Omaha, NE 68198.

Purification of the catecholamine transporter present in chromaffin granule membranes isolated from adrenal glands has been difficult due to lack of a functional transport assay. [^{3}H]Reserpine, bound to the catecholamine site on the transporter, does not dissociate in the presence of 1 % CHAPS but does dissociate in the presence of 1% SDS. By prelabeling the transporter with [^{3}H]reserpine, we have been able to follow the elution of the transporter under different chromatographic conditions. Over 50% of the [^{3}H]reserpine, still bound to protein, was solubilized using either 1 % deoxycholate or 1 % CHAPS. Chromatography of the [^{3}H]reserpine binding site, solubilized with 1 % CHAPS, on a Superose 6 column resulted in the elution of three major protein peaks. The tritium eluted with the first major protein peak with an estimated molecular weight of 400,000. SDS polyacrylamide gel electrophoresis of the this peak indicated three major peptide bands with molecular weights of 70,000, 40,000 and 20,000. It is not known which of these peptides is associated with the transporter since [^{3}H]reserpine dissociates in SDS. These results indicate that it may be possible to purify the catecholamine transporter by first prelabeling it with [^{3}H]reserpine. This work was supported by NSF grant (BNS-8800887) and NIH grant (NS15187).

287.4

FOURPHIT BINDS IRREVERSIBLY TO THE STIMULANT RECOGNITION SITE ON THE DOPAMINE TRANSPORTER. M.M.Schweri, A.Thurkauf*, and K.C.Rice*. Mercer Univ. Sch. Med., Macon, GA 31207, Neurogen Corp., Branford, CT 06405, and NIDDK, Bethesda, MD 20892.

The effect of the phencyclidine derivative Fourphit (4-isothiocyanato-1-[1-phenylcyclohexyl]piperidine) on the binding of [^{3}H]methylphenidate ([^{3}H]MP) to the stimulant recognition site in the P_2 fraction of rat striatal tissue was studied. Fourphit inhibited [^{3}H]MP binding (determined by a modification of a previously described method [Schweri et al, Life Sci. 45:1689 (1989)]) with an IC_{50} of 7.1 ± 2.1 μM. The reaction proceeded rapidly at 0°C, and the resulting inhibition of [^{3}H]MP binding persisted after three washes. Scatchard analysis of [^{3}H]MP binding in tissue reacted with 29 μM Fourphit and then washed three times showed a significant decrease in the B_{max} (controls, 8.3 ± 0.8 pmols/mg protein; Fourphit, 4.7 ± 0.3 pmols/mg protein), but not the K_D (controls, 97.3 ± 33.3 nM; Fourphit, 99.8 ± 25.1 nM). Preincubation of tissue with saturating amounts of MP afforded little or no protection from inactivation by Fourphit. Equivalent off-rates were observed, however, whether high concentrations of methylphenidate or Fourphit were used to initiate the dissociation of [^{3}H]MP binding. Because Fourphit appears to bind rapidly and irreversibly directly to the [^{3}H]MP binding site, it may prove to be useful as an affinity label for the dopamine transport complex.

287.5

NICOTINIC-RECEPTOR MEDIATED CATECHOLAMINE SECRETION FROM SINGLE ADRENAL MEDULLARY CHROMAFFIN CELLS: CHEMICAL EVIDENCE FOR EXOCITOSIS. D. J. Leszczyszyn, J. A. Jankowski*, O. H. Viveros, E. J. Diliberto, Jr.*, J. A. Near, and R. M. Wightman. The Wellcome Research Laboratory, Division of Medicinal Biochemistry, Research Triangle Park, NC 27709, Medical Sciences Program, Indiana Univ., Bloomington, IN 47405 and Dept. of Chemistry, Univ. Of North Carolina, Chapel Hill, NC 27599-3290.

Secretion from individual cultured adrenal medullary cells has been measured following local application of nicotine by a micropipette. Secretion is detected with a polymer-coated microelectrode placed adjacent to the cells, and voltammetry demonstrates that the detected substances are catecholamines. Nicotine-induced secretion is sensitive to Ca^{2+} channel blockers and is characterized by a large increase in chemical noise. With uncoated electrodes, the electrochemical spikes can be temporally resolved into the apparent secretion of discrete packets of attomole quantities of easily oxidized molecules. These data are consistent with a chemical measurement of exocytosis.

287.6

INVOLVEMENT OF GTP-BINDING PROTEINS IN THE MECHANISM FOR EXOCYTOSIS IN ADRENAL CHROMAFFIN CELLS. K.Kumakura, M.O.-Imaizumi* and N.Kawae*. Life Science Institute, Sophia Univ, Tokyo, Japan 102.

We studied effects of pertussis toxin (PTX) and GTP-γ-S on the secretory function of cultured adrenal chromaffin cells. In the digitonin-permeabilized cells pretreated with PTX, marked increase of Ca^{2+}-dependent release of catecholamine due to an increase of the affinity for Ca^{2+} was observed. Exposure of permeabilized cells to GTP-γ-S inhibited the Ca^{2+}-dependent exocytosis by reducing the affinity for Ca^{2+}. On the other hand, GTP-γ-S by itself evoked catecholamine release from the permeabilized cells in the absence of Ca^{2+}. This Ca^{2+}-independent action of GTP-γ-S was blocked by pretreatment with IAP. IAP-pretreatment of the cells blocked secretagogue action of mastoparan which is also Ca^{2+}-independent. These results suggest that PTX-sensitive GTP-binding proteins are involved in the mechanism for exocytosis in two defferent ways. One controles the Ca^{2+}-triggered process by reducing the affinity for Ca^{2+}. The other one directly coupled with the exocytosis mechanism following the Ca^{2+}-triggered process.

287.7

CNS LOCALIZATION OF A NOVEL TRANSPORTER-LIKE PROTEIN USING ANTIPEPTIDE ANTIBODIES AND NORTHERN BLOT ANALYSIS. J.A. Gingrich*, P.H. Andersen†, T.N. Joergensen¶*, B.S. Guldhammer§, S. El Mestikawy, R.T. Fremeau Jr., M. Tiberi, M.G. Caron, Dept. of Cell Biology, Duke Univ. Med. Ctr, Durham, NC 27710 and Depts. of Biochem. Pharm†, Biolabs¶, and Pathology§, Novo/Nordisk Industries, Copenhagen, Denmark.

We have recently identified an 85 kDa protein which copurifies with the D1 dopamine receptor through four chromatographic steps (biospecific affinity, anion exchange, lectin, and size exclusion). This protein was separated from the D1 receptor by preparative SDS-PAGE, electroeluted, and cleaved with cyanogen bromide. Peptides were isolated by reverse phase HPLC and sequenced. An oligonucleotide derived from the sequence of one of these peptides was synthesized, labelled, and used to screen a bovine brain cDNA library. A clone was isolated which contained three of the isolated peptides within a single open reading frame of 742 amino acids (predicted MW 82.5 kDa). The sequence and predicted structure of this protein are similar to those of several known transport proteins such as the mammalian glucose transporters and the bacterial transporters for arabinose, citrate, xylose, and tetracyline. The mRNA for this protein has a message size of ~4.4-5.5 kb (depending on species) and is localized primarily to the brain. No signal was detected in peripheral tissues such as liver, kidney, heart, lung, spleen, and skeletal muscle. To futher characterize the regional distribution of this transporter-like protein, peptides were synthesized, coupled to a hapten, and used as an immunogen in rabbits and mice. The resulting antibodies recognize a band of ~80-90 kDa on western blot analysis of crude bovine and rat striatal membranes. The labelling of this band is blocked by the antigenic peptide. Western blot analysis of several dissected brain regions suggests that this protein has a wide distribution which includes striatum, cortex, hippocampus, brainstem, and cerebellum. These antibodies should prove to be invaluable tools in elucidating the subcellular distribution and function of this protein.

287.8

EXPRESSION OF SEROTONIN TRANSPORTER mRNA IN *XENOPUS* OOCYTES R. J. Haber* and D. Goldman Laboratory of Clinical Studies, National Institute on Alcohol Abuse and Alcoholism, NIH, Bethesda, Md. 20892

Serotonin (5HT) is removed from the synapse and its action terminated by the 5HT transporter.

We have taken an initial step towards cloning the serotonin transporter by obtaining its expression in *Xenopus* oocytes. We find that mouse brainstem is a suitable source of serotonin transporter mRNA and that synthesis of 5HT transporter occurs steadily over a 3 day period post injection. The ability of mRNA-injected cells to take up ^{3}H-5HT is enhanced by imposition of a sodium gradient, with a high sodium concentration outside the cell. Oocytes injected with mouse brainstem mRNA exhibited a 10-fold increase in uptake of ^{3}H-5HT compared to sham-injected cells. Uptake of ^{3}H-5HT was inhibited by 18 nM citalopram, a specific inhibitor of the serotonin transporter.

287.9

STUDIES ON BINDING SITES FOR DOPAMINE UPTAKE INHIBITORS IN PRIMARY CULTURES OF VENTRAL MESENCEPHALIC NEURONS. J. Hanbauer, M.G. Grilli and A.G. Wright, Jr*. Lab. of Chemical Pharmacology, NHLBI, Bethesda, MD 20892.

The expression of dopamine (DA) transporter and tyrosine hydroxylase immunoreactivity in primary cultures of ventral mesencephalic neurons is developmentally linked to axonal outgrowth. We have used primary cultures of ventral mesencephalic neurons to study the properties of binding sites for cocaine and mazindol. [^{3}H]cocaine or [^{3}H]mazindol binding sites were detectable in intact neurons attached to culture dishes containing Krebs-Ringer-Henseleit (KRH) buffer but were not detectable in washed membrane preparations of neurons cultured for 12 days. [^{3}H]cocaine did not bind to poly-D-lysine coated culture dishes themselves. The specific binding of [^{3}H]cocaine at 4°C was similar to that at 24°C or 37°C indicating that [^{3}H]cocaine was not taken up by an active transport system. [^{3}H]cocaine binding was not altered by four consecutive washes of the cells, ruling out ion trapping of [^{3}H]cocaine. Thus, it is concluded that [^{3}H]cocaine may be sequestered by cytosolic compartments or organelles that were not preserved in membrane preparations. Substitution of Na^+ in KRH buffer by choline chloride or sucrose completely impaired [^{3}H]DA uptake, but failed to alter [^{3}H]cocaine binding, suggesting that in intact cells, unlike in membrane preparations of adult rats, [^{3}H]cocaine binding does not require Na^+. In mesencephalic neurons [^{3}H]DA uptake and [^{3}H]cocaine binding sites were expressed after 1 day in culture and continued to increase proportionally. These data suggest that [^{3}H]cocaine binding sites may be functionally linked to the DA transporter.

LOCALIZATION OF NEUROTRANSMITTER RECEPTORS I

288.1

[^{3}H]BENACTYZINE BINDING SITES IN RAT BRAIN - IN VIVO AUTORADIOGRAPHY STUDY. T. Kadar*, R. Davidovici*., S. Chapman*, and G. Amitai. Dept. of Pharmacology, Isr. Inst. for Biol. Research, Ness Ziona 70450, Israel.

Since the anticholinergic compound benactyzine (BNZ) may induce central adverse effects we have studied its distribution in rat brain in vivo. [^{3}H]-BNZ (4.2 mCi/mmole, 0.5 mg/kg was administered to rats i.m. with or without pretreatment by atropine (1 mg/kg, i.m). Animals were sacrificed 15 min following administration and whole heads were immediately frozen. 40 mμ frozen sections were cut in a coronal plane, freeze dried and attached to [^{3}H] Ultrofilm. The autoradiographic distribution of [^{3}H]BNZ revealed moderate labeling in grey matter areas. Radioactivity was spread homogeneously in cortex, striatum, hippocampus, thalamus, cerebellum and hind brain. However, concentrated labeling was noted in the pyramidal layer of the hippocampus. A decrease in whole brain labeling was observed after pretreatment with atropine. The labeling was spread in a diffused manner (e.g. cortex and thalamus) in contrast to a more localized distribution of either [^{3}H]nicotine or [^{3}H]QNB obtained from in vitro autoradiography. [^{3}H]BNZ labeling appeared in both muscarinic and nicotinic sites. These results conform with our previous data that BNZ inhibits the physiological response of peripheral muscarinic and nicotinic receptors. Supported by DAMD17-84-C-4016.

288.2

CARBON-11 LABELED 5-(N-METHYLAMINO)BENZOVESAMICOL, A MARKER OF PRESYNAPTIC CHOLINERGIC NEURONS. M.R. Kilbourn, Y-W. Jung*, M.S. Haka*, D. Gildersleeve*, F. Buck*, K.A. Frey, and D.M. Wieland*. Div. Nuclear Medicine, Univ. Michigan Med. Sch., Ann Arbor, MI 48109

Radiotracers for in vivo study of presynaptic cortical cholinergic neurons using PET would be of great value in the diagnosis and characterization of Alzheimer's disease and related dementias. We have prepared high specific activity carbon-11 (β+, t1/2 = 20 min) labeled 5-(N-methylamino)benzovesamicol ([11C]MABV) as a ligand for binding to acetylcholine storage vesicles. Mouse brain distribution at 45 min after i.v. injection shows regional selectivity (striatum>cortex>hippocampus> hypothalamus>cerebellum: STR/CER = 8.1 ± 0.44) and stereoselectivity [(-)MABV > (±)MABV > (+)MABV]. Specific binding can be blocked by pretreatment with cold (-)vesa-micol. PET tissue time-activity curves in monkeys (bolus intracarotid inj) were fitted to a three compartment, four parameter model. Data showed high brain extraction (65%) and similar nonspecific binding levels in cortex and striatum. Kinetic estimates of specific binding are 2- to 3-fold higher in striatum than cortex, consistent with in vitro binding estimates. (-)-[11C]MABV shows regional selectivity, stereoselectivity, pharmacological specificity, and in vivo pharmaco-kinetics which support its further development as a presynaptic marker for study of the cholinergic system using PET.

288.3

LOCALIZATION OF MUSCARINIC CHOLINERGIC RECEPTORS ON SINGLE NEURONS IN RAT HIPPOCAMPAL CULTURES. J.M.R. Harrison*, C.A. Levesque, G.A. Banker, G.F. Wooten, and O. Steward. Depts. of Neuroscience and Neurology, Univ. of Virginia, Charlottesville, VA 22908.

Although the distribution of muscarinic receptors in the rodent brain has been extensively investigated, little is known about the subcellular localization and regulation of these receptors. Hippocampal neurons maintained in culture develop elaborate axons and dendrites, allowing the subcellular distribution of receptor to be assessed directly. In the present study, we used [^{3}H]-quinuclidinyl benzilate ([^{3}H]-QNB) in conjunction with autoradiography to investigate the distribution of muscarinic receptors on cultured neurons. Living neurons were exposed to [^{3}H]QNB and then fixed with 1% glutaraldehyde. The neurons were then prepared for autoradiography or scintillation counting. Kinetic studies showed that the binding of [^{3}H]QNB reached steady state levels within 15 minutes at 37°C. Saturation of binding sites occurred at 0.5 nM [3H]-QNB, a concentration similar to that obtained in sections and homogenates. Autoradiographic analysis revealed [^{3}H]QNB binding sites on both cell bodies and dendrites of cultured neurons, but little binding to glia. The amount of nonspecific binding (in the presence of 1 μM atropine) was always less than 10% of the total binding. Pharmacological competition curves suggest that the binding was specific for muscarinic cholinergic sites. Competition with carbachol (an M2 selective agonist) and with pirenzepine (an M1 selective antagonist) indicated that the receptors are predominately of the M1 subtype. These results are consistent with data obtained in sections. Therefore hippocampal pyramidal cells in culture express cholinergic receptors pharmacologically indistinguishable from those expressed in situ, providing an opportunity to study their regulation at a subcellular level. Supported by NS12333 to O.S. J.H. and C.L. were the recipients of postdoctoral fellowships from NIH (5T32HD07192 and 5T32NS07199, respectively).

288.4

BINDING TO PRE- AND POST-SYNAPTIC CHOLINERGIC SITES IN THE STRIATUM AFTER A VOLKENSIN INJECTION TO THE SUBSTANTIA NIGRA. M.B.Harrison, R.G.Wiley#, and G.F.Wooten. Univ. of Virginia, Charlottesville, VA 22908 and #Vanderbilt University, Nashville TN 27232.

Following injection of the retrogradely transported neurotoxin, volkensin, into the SN, both D1 and D2 binding in the striatum show a time-dependent decrease. This effect is seen as early as 5 days, and the decrease is much greater for D1 than D2 at all time points, suggesting a selective localization of D1 receptors on striatonigral neurons. To further investigate the effects of the toxin, we examined binding in the striatum of [^{3}H]-hemicholinium ([^{3}H]-HC), a marker for presynaptic Ach uptake sites and [^{3}H]-QNB, which labels muscarinic cholinergic receptors. Eight rats received 2-2.7 ngs. of volkensin by microinjection into the left SN. After survivals of 5 (n=4) or 21 to 37 (n=4) days, brains were processed for autoradiography with adjacent pairs of sections examined for binding of [^{3}H]-HC and [^{3}H]-QNB. Specific binding in the striatum ipsi- and contralateral to the lesion was compared. At 5 days, [^{3}H]-HC binding on the lesioned side was 97% of that on the intact side (57.7 fmol/mg ± 4.4 vs. 61±7.8 (mean±S.E.); n.s.); [^{3}H]-QNB binding was 99% (383.2±17.4 vs. 388.3±30.9; n.s.). At the later time points, 16 % of [^{3}H]-HC binding sites remained on the lesioned side (7.3±1.4 fmol/mg vs. 46.3±8.1*) with 45% of [^{3}H]-QNB binding remaining (183.2±17.6 vs. 408.1±18.3**). The absence of a significant effect at 5 days suggests that neither [^{3}H]-HC nor [^{3}H]-QNB binding sites are located on striatonigral neurons and that the neurons on which they are located are not subject to the immediate primary neurotoxic effects of volkensin. The nature of its secondary effects is unknown but these results suggest that reuptake sites located on axon terminals are more affected than post-synaptic receptor binding sites on the soma and dendrites. *=p<.05,**=p<.01

288.5

IMMUNOCYTOCHEMICAL MAPPING OF M2 MUSCARINIC RECEPTOR SUBTYPE IN OLFACTORY BULB. M.I. Fonseca*, J.S. Aguilar*, A.F. Skorupa and W.L. Klein. Institute for Neuroscience. Northwestern University. Evanston, IL 60208.

Polyclonal antibodies that recognize the M2 subtype of muscarinic receptors (mAchR) have been raised and used to map the cellular distribution of this subtype in rat olfactory bulb. The antibodies were obtained by injecting BALB/C mice with a BSA conjugated synthetic peptide whose sequence corresponded to the cytoplasmic loop of porcine cardiac M2 subtype. The antiserum recognized the synthetic peptide in ELISA assays and stained a band corresponding to the peak fraction of radiolabeled heart mAchR in Western immunoblots. Immunostaining of rat olfactory bulb sections showed that the antibodies labelled cell bodies and multiple dendritic processes. The patterns of labelling were homogeneous in some cells and punctate in others. M2 positive cells throughout the bulb were mainly present in three layers, representing small fractions of the cells in each region: glomeruli, 6% ; external plexiform layer, 16%; granule layer, 3%. The results show that the antibodies raised against specific sequences of different receptor subtypes can be used to localize the subtypes in situ, and that the M2 subtype within the olfactory bulb is broadly distributed. The mapping of M2-positive cells in olfactory bulb is of clinical interest because loss of M2 subtype and degeneration of olfactory system are observed in Alzheimer's disease.

288.6

IN VITRO AUTORADIOGRAPHIC BINDING AND *IN SITU* HYBRIDIZATION OF MUSCARINIC CHOLINERGIC RECEPTORS IN RHESUS MONKEY THALAMUS. M.V. Wagster, D.M. Weiner*, A.I. Levey, M.R. Brann, L.C. Cork and D.L. Price. Neuropathology Laboratory, The Johns Hopkins University School of Medicine, Baltimore, MD 21205, and Laboratory of Molecular Biology, NINDS, Bethesda, MD 20892.

Muscarinic cholinergic receptor binding site distribution was mapped in monkey thalamus and compared with muscarinic receptor gene expression. In rat brain, the pharmacology of M1 receptor binding appears to correspond to m1, m3, m4, and m5 receptor mRNAs. In rat thalamus, high levels of m2 and m3 mRNAs are expressed, whereas very low levels of m1 and m4 mRNA expression are evident. Based on these previous findings, adjacent sections of thalamus from four rhesus monkeys 9 years (n = 2) and 25 years (n = 2) of age were examined for M1 muscarinic receptor binding sites using [^{3}H] pirenzepine (PZ) and for expression of m2, m3, and m5 receptor mRNAs using a mixture of three 48-base oligodeoxynucleotide probes. Moderate levels of [^{3}H] PZ binding were observed in laterodorsal nucleus, medial dorsal nucleus, and basal ventral medial nucleus of thalamus. Preliminary observations indicate that receptor binding distribution and distribution of receptor mRNAs examined differ somewhat in anatomical localization. Studies are in progress to clarify these distributions.

288.7

MEASUREMENT OF BENZODIAZEPINE RECEPTOR BINDING IN VIVO WITH N-ω-[F-18]FLUOROETHYL FLUMAZENIL AND POSITRON EMISSION TOMOGRAPHY. S.M. Moerlein and J.S. Perlmutter. Mallinckrodt Institute of Radiology and Department of Neurology and Neurological Surgery, Washington University School of Medicine, St. Louis, MO 63110.

Flumazenil (Ro 15-1788) is a selective benzodiazepine (BZP) antagonist that is useful for studies of central BZP receptors in vivo and ex vivo. We have evaluated an F-18 ($t_{1/2}$ = 110 min) labeled analogue of this ligand, N-ω-[F-18]-fluoroethyl flumazenil (FEF), for measurement in vivo of BZP receptor binding kinetics with positron emission tomography (PET). [F-18]FEF was produced via a two-step synthetic sequence that involved the reaction of bis-tosyl ethane with no-carrier-added [F-18]fluoride preceding N-alkylation of the nor-methyl precursor Ro 15-5528. Radiotracer purification was achieved using normal phase HPLC to yield [F-18]FEF in 25-30% radiochemical yield and specific activity > 1000 Ci/mmol within an overall preparation time of 110 min. Separate PET studies were performed on the same baboon, with and without co-injection of unlabeled flumazenil 0.55 mg/kg. Regional cerebral blood flow (CBF) and blood volume (CBV) were measured using [O-15]water and [O-15]carbon monoxide. After [F-18]FEF injection, sequential PET scans were obtained for 3 hours in intervals ranging from 1 minute to 10 minutes. The F-18 labeled ligand selectively localized in vivo within regions rich in BZP receptors (at 38 min: temporal cortex (T)/ cerebellum (C)/white matter (W) = 690/508/443 cps/ml/mCi). Co-injection of unlabeled flumazenil dramatically reduced brain uptake of [F-18]FEF and completely abolished the selective accumulation of the ligand in BZP receptor-rich tissues (at 38 min: T/C/W = 249/323/229 cps/ml/mCi). Non protein-bound [F-18]FEF in blood as determined with micropartition (Centrifree) techniques was 56.15 ± 1.45%. These data indicate the utility of [F-18]FEF for the noninvasive study of central BZP receptor pharmacology in vivo with PET.

288.8

AUTORADIOGRAPHIC LOCALIZATION OF BENZODIAZEPINE RECEPTORS LABELED WITH [^{3}H]ALPRAZOLAM: COMPARISON WITH [^{3}H]DIAZEPAM. L.L. Longlet, M.E. Alburges, M. Hunt, D.R. Mahan[1] and J.K. Wamsley. Neuropsychiatric Research Institute, Fargo, ND 58103; [1]The UpJohn Company, Kalamazoo, MI 49001.

The characterization and localization of sites labeled by [^{3}H]alprazolam has been compared to [^{3}H]diazepam in tissue slices. Both ligands label a finite receptor population and the binding is readily reversible and highly specific. Scatchard analyses indicate a K_d of 7.5 nM and a B_{max} of 197 fmoles/mg tissue for [^{3}H]diazepam, and 4.0 nM and 129 fmoles for [^{3}H]alprazolam. Competition studies indicate a similar profile for the two compounds. [^{3}H]diazepam was then used to label tissue sections in the presence of increasing concentrations of alprazolam, and [^{3}H]alprazolam was used in the presence of increasing concentrations of diazepam. The radioactive ligands overlap in their labeling distribution, but have unique sites as well. Alprazolam uniformally displaced those sites labeled by [^{3}H]diazepam except for areas where the binding was presumably represented by peripheral BZ sites (i.e. the choroid plexus and ependymal lining of the ventricles). Diazepam showed a similar ability to interact with the sites occupied by [^{3}H]alprazolam. These data indicate that, for the most part, the two BZ compounds are labeling similar sites in the brain with comparable binding characteristics.

288.9

***IN VIVO* BENZODIAZEPINE RECEPTOR BINDING IN HUMAN BRAIN: COMPARTMENTAL ANALYSIS OF [11C]FLUMAZENIL DISTRIBUTION AS DETERMINED BY POSITRON EMISSION TOMOGRAPHY.** K.A. Frey, V. Holthoff*, R.A. Koeppe*, D. Jewett*, T.J. Mangner*, and D.E. Kuhl Division of Nuclear Medicine, The University of Michigan, Ann Arbor, MI 48109.

Alterations in the number or function of benzodiazepine binding sites have been suggested in a variety of neurologic and psychiatric disorders. Methods for measurement of *in vivo* radioligand binding with the use of positron emission tomography may allow direct hypothesis testing in clinical populations. We have developed a tracer kinetic model for the compartmental analysis of [11C]flumazenil (FMZ) distribution and binding, which allows estimation of both tracer delivery and uptake in brain as well as local high-affinity binding. Six normal volunteers underwent imaging of [11C]FMZ distribution following intravenous administration. A series of dynamic PET images was obtained over 90 min with the use of a Siemens 931/08-12 scanner. Arterial blood samples were obtained during the imaging period, plasma was chromatographed to exclude labeled metabolites and the [11C]FMZ time course determined. Compartmental analysis of the brain and plasma tracer time courses was conducted on a pixel-by-pixel basis, resulting in maps of both tracer uptake (transport) and distribution volume (proportional to receptor concentration). Estimates of receptor density varied over a 5-fold range (mean±S.D.): pons - 1.0±.1; cerebellum - 2.8±.4; thalamus - 2.9±.4; caudate - 2.3±.2; putamen - 3.1±.4; frontal cortex - 4.8±.6; occipital cortex - 5.4±.7. The present method thus provides reproducible relative receptor density estimates which are in good agreement with results of *in vitro* binding studies. In addition, pixel-by-pixel maps of relative receptor density allow rapid visual inspection of the entire brain, which may prove invaluable in clinical research applications.

288.10

PENTOBARBITAL (PB) ALTERS THE BINDING PARAMETERS OF RAT BRAIN BENZODIAZEPINE RECEPTOR (BZR) IN A REGIONALLY SPECIFIC MANNER. B.X. Carlson and H.A. Baghdoyan. Department of Anesthesia, Penn State Univ., College of Medicine, Hershey, PA 17033.

In cortical homogenates barbiturate-induced enhancement of benzodiazepine binding is due to an increase in BZR affinity. With in vitro receptor autoradiography we have shown regional differences in PB-induced [^{3}H]flunitrazepam (FLU) binding in intact rat brain (Carlson et al, FASEB J. 4:A1006, 1990). The present study is testing the hypotheses that the BZR will exhibit regional differences in K_D and B_{max}, and in PB-induced enhancement of FLU binding. Saturation analysis of FLU binding in 6 regions (cortex, diencephalon, midbrain, pons, medulla, cerebellum) was carried out using fresh brain. Both K_D and B_{max} showed statistically significant differences between brain regions. The highest affinity (K_D=1.07nM) and lowest B_{max} (0.52pmol/mg protein) were observed in the medulla. The lowest affinity (K_D=2.01nM) and highest B_{max} (1.98pmol/mg protein) occurred in the cerebral cortex. PB increased the affinity of BZR for FLU in all brain regions with the greatest increase occurring in the medulla. There were no PB-induced changes in B_{max}. These data show, for the first time, that PB alters binding parameters for BZR in a regionally specific manner.

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288.11

EFFECT OF PARA-CHLOROAMPHETAMINE (PCA) ON ^{3}H-CYANO-IMIPRAMINE (^{3}H-CN-IMI) BINDING TO SEROTONIN UPTAKE SITES

J. Hensler, R. Ferry*, G.B. Kovachich and A. Frazer, Depts. of Pharmacol. & Psychiatry, Univ. of Pa. & Vet. Affairs Med. Ctr., Phila. PA 19104.

There are two distinct serotonin (5-HT) raphe-forebrain projections preferentially innervating different forebrain areas. Treatment of rats with PCA lesions fine 5-HT axons arising from the dorsal raphe (DRN) while sparing beaded 5-HT axons from the median raphe (MRN); 5-HT cell bodies are spared (Exp.Neurol.102:23,1988). It also appears that different regions of brain receive variable innervation from the DRN or MRN. To obtain more quantitative information on the degree of innervation of terminal field areas from the DRN, we have examined by quantitative autoradiography the binding of ^{3}H-CN-IMI to 5-HT uptake sites in rats treated with PCA. Fourteen days after PCA treatment (6mg/kg, i.p., for 2 days) the binding of ^{3}H-CN-IMI in the DRN and MRN was unchanged and 5-HT cell bodies as visualized by 5-HT immunohistochemistry appeared morphologically unaffected. The extent of loss of ^{3}H-CN-IMI binding in different areas was variable: 62±7% in the piriform cortex; 47±3% in the parietal cortex; 40±6%in the frontal cortex; 51±3% in the CA_1 and 38±3% in the $CA_{2,3}$ region of the hippocampus; 53±3% in the basolateral amygdala. PCA treatment resulted in a loss of fine 5-HT axons as visualized by 5-HT immunohistochemistry and the apparent sparing of beaded 5-HT axons. Beaded axons do contain ^{3}H-CN-IMI binding sites as treatment of rats with 5,7-dihydroxytryptamine results in extensive loss of ^{3}H-CN-IMI binding (>85%) and all types of 5-HT axons in terminal field areas. The differences in the extent of reduction in ^{3}H-CN-IMI binding in these forebrain areas after PCA treatment may reflect the degree of 5-HT innervation from the DRN. **(Supported by research funds from the Dept. of Vet. Affairs and USPH grant MH09834).**

288.12

HIGH RESOLUTION AUTORADIOGRAPHIC COMPARISON OF ^{3}H-IMIPRAMINE BINDING IN HUMAN AND RAT HIPPOCAMPUS G.E.Duncan, K.Y.Little*, G.R. Breese, and W.E. Stumpf Dept. of Cell Biology and Anatomy, UNC, Chapel Hill, NC 27599

Within the hippocampal formation we have found substantial species differences between rats and humans in the topographic distribution of binding sites for ^{3}H-imipramine. In the rat, the highest densities of binding sites were found in the dentate molecular layer, CA-3 stratum oriens and CA-3 stratum radiatum. Very low densities of binding sites were observed throughout the pyramidal cell layer in the rat hippocampus. In contrast, in the human hippocampus, the highest densities of binding sites for ^{3}H-imipramine were found in the CA-3 and CA-4 pyramidal cell fields and low densities of binding sites were detected in the CA-3 stratum radiatum. The results raise the possibility that functional consequences to pharmacological actions of imipramine may differ in the rat and human hippocampus. Supported by USPS grants MH-33127, HD-03110, and MH-39144.

288.13

MELATONIN BINDING SITES IN SYRIAN HAMSTER MELANOMA CELLS. D.S. Pickering, L.P. Niles and S.-W. Ying*. Dept. of Biomedical Sciences, Division of Neuroscience, McMaster University, Hamilton, Ontario, Canada L8N 3Z5

In addition to a picomolar melatonin receptor, a nanomolar-affinity site has been observed in various hamster central and peripheral tissues. In order to further characterise this site, we desired to find a continuous cell line expressing this site. The Syrian hamster derived melanoma cell line RPMI 1846 was found to contain a binding site which preliminary pharmacological studies suggest resembles the central nanomolar-affinity binding site: (IC_{50}'s) iodomelatonin, 4.6 nM; 6-chloromelatonin, 25 nM; N-acetylserotonin, 67 nM; melatonin, 155 nM. Scatchard analysis of saturation binding of 2-[^{125}I]iodomelatonin indicates a K_d of 5.1 ± 1.9 nM at 0°C. Measurements of adenylate cyclase activity in semi-purified RPMI 1846 plasma membranes revealed a 5-fold stimulation by 10 μM forskolin, however, neither melatonin nor iodomelatonin significantly affected the basal or forskolin-stimulated cyclase activity at concentrations from 10^{-10} to 10^{-5} M. The nanomolar-affinity melatonin binding site therefore does not seem to be coupled to adenylate cyclase. This is in keeping with the lack of inhibition by guanine nucleotides reported for this site in hamster brain membranes. Studies are underway to explore possible coupling mechanisms of this site. (This work was funded by the OMHF and MRC. D.S.P. is supported by the MRC.)

288.14

MELATONIN BINDING IN CHAPS-SOLUBILISED FRACTIONS FROM CHICK BRAIN. S.-W. Ying*, L.P. Niles and D.S. Pickering. Dept. of Biomedical Sciences, Division of Neuroscience, McMaster University, Hamilton, Ontario,Canada L8N 3Z5

Binding sites for [^{125}I]melatonin ([^{125}I]MEL) were solubilised from chick brains using 5 mM CHAPS (3-[(3-chol-amidopropyl)-dimethylammonio]-1-propane sulfonate). Binding of [^{125}I]MEL to solubilised fractions at 0-4°C was rapid, reversible, saturable and of high affinity. Scatchard analyses revealed a single site with K_d= 328 ± 22 pM and B_{max}= 36.2 ± 2.0 fmol/mg protein (solubilised); K_d= 302 ± 26 pM and B_{max}= 49.5 ± 6.6 fmol/mg protein (crude membranes). Inhibition studies for both preparations yielded the following order of potency: iodomelatonin > melatonin > 6-chloromelatonin >> N-acetylserotonin > serotonin. GTPγS and GTP inhibited binding of [^{125}I]MEL to both preparations by 50-60%. Functional studies indicated that melatonin did not alter basal nor forskolin-stimulated adenylate cyclase activity in chick brain membranes.

These findings demonstrate: (1) that the pharmacological properties and guanine nucleotide sensitivity of [^{125}I]MEL binding sites remain intact following CHAPS solubilisation; (2) the putative melatonin receptor sites in chick brain do not appear to be coupled to adenylate cyclase.

(This work was funded by the MRC and OMHF.)

288.15

FUNCTIONAL MELATONIN RECEPTORS IN ARTERIES INVOLVED IN THERMOREGULATION. M. Viswanathan, J.T. Laitinen and J.M. Saavedra, Sec. on Pharmacology, Lab. of Clin. Sci., NIMH, Bethesda, Maryland 20892.

Melatonin receptors were localized and characterized in the vasculature of the rat, using the melatonin analogue 2-[^{125}I]-iodomelatonin, and quantitative in vitro autoradiography. The expression of these receptors was restricted to the caudal artery and to the arteries which form the circle of Willis at the base of the brain. Specific binding in these arteries was localized exclusively in the smooth muscle layer. 2-[^{125}I]-Iodomelatonin binding in the arteries was stable, saturable, and reversible. Saturation studies revealed that the binding represented a single class of high affinity binding sites with a dissociation constant (K_d) of 33.5 pM in the anterior cerebral artery and 104.9 pM in the caudal artery. The binding capacities (B_{max}) in these arteries were 19.3 and 14.6 fmol/mg protein, respectively. The arterial binding sites had high specificity for melatonin (Iodomelatonin > Melatonin > N-acetylserotonin > prazosin > 8-OH-2(di-n-propylamino) tetraline > > > 5-HT). Norepinephrine-induced contraction of the caudal artery in vitro was significantly prolonged and potentiated by melatonin in a concentration dependent manner, suggesting that these arterial binding sites are functional melatonin receptors. Neither primary steps in smooth muscle contraction (phosphoinositide hydrolysis) nor relaxation (adenylate cyclase activation) were affected by melatonin.

Melatonin, through its action on the tone of these arteries, may cause circulatory adjustments in these arteries which are believed to be involved in thermoregulation.

289.1

LOCALIZATION BY IN SITU HYBRIDIZATION OF GABA$_A$ RECEPTOR ALPHA$_1$ AND GAMMA$_2$ SUBUNITS IN LONG-SLEEP AND SHORT-SLEEP MOUSE BRAINS. N.R. Zahniser, P. Curella*, D. Wilson-Shaw*, C.L. Miller*, R.L. Klein*, W.J. Keir* and J.M. Sikela. Dept. Pharmacology, Univ. Colorado Hlth. Sci. Ctr., Denver, CO 80262.

The genetically selected long-sleep (LS) and short-sleep (SS) mouse lines provide a model for studying mechanisms underlying differences in initial sensitivity to ethanol. Differential sensitivity in GABAergic function is one such mechanism that has been identified. Our goal was to determine whether differences in the subunits composing the GABA$_A$ receptor in the two lines of mice could contribute to the difference in GABAergic function. Northern analyses of inbred LS and SS brain mRNA, utilizing probes derived from mouse α_1 and γ_2 GABA$_A$ receptor subunit cDNAs, revealed no differences between LS and SS mice for either probe. For *in situ* hybridization, the autoradiograms were analyzed with an image analysis system. In every case, the ^{35}S-sense probes showed weak, relatively uniform hybridization. In contrast, in adjacent brain sections the patterns of hybridization for the ^{35}S-antisense probes were region-specific. For example, specific hybridization for the α_1-probe was highest in the inferior colliculus, cerebellum and cerebral cortex whereas that for the γ_2-probe was highest in the cell layers of the dentate gyrus and hippocampus, olfactory bulb and cerebral cortex. While α_1 and γ_2 subunit probes gave differing patterns of hybridization, regional differences between LS and SS mice were not observed for either subunit probe. Our results do not rule out the possibility that expression of these subunits or that other subunits making up the GABA$_A$ receptor may differ between LS and SS mice, but they do suggest that qualitative differences in mRNA for the α_1 and γ_2 subunits cannot explain functional differences between LS and SS mice. Supported by USPHS AA 03527, AA 06399 and GM 07635.

289.2

ADRENALECTOMY INCREASES BICUCULLINE-INDUCED SEIZURE SUSCEPTIBILITY IN LONG-SLEEP AND SHORT-SLEEP MICE. J.M. Wehner, B.J. Bowers, T.Z. Bosy*. Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

Long-sleep (LS) and short-sleep (SS) mice differ in seizure susceptibilty to GABAergic agents, differ in some properties of the GABA$_A$ receptor complex, and in responsiveness to stimulation of the hypothalamic-pituitary-adrenal axis (HPA). Therefore, the effects of adrenalectomy (ADX) was examine in these mice. Within the sham-operated group, SS mice were more susceptible to seizure onset than LS mice. Susceptibility to bicuculline (BIC)-induced seizure onset and tonus was increased in both lines at 7 days after ADX. However, after ADX latencies did not differ between the two lines. Replacement with 10% corticosterone (CCS) pellets in ADX-animals resulted in a return to sham-operated control latencies. In SS mice, dexamethasone (10%) pellets and cholesterol pellets were as effective as CCS replacement. In LS mice, dexamethasone was only effective at a low BIC dose. These results suggest that seizure thresholds may be regulated, at least in part, by the HPA axis and that the effects of ADX on GABA-related seizures may be influenced by genotype such that genetic differences in GABA$_A$ receptor function and in the HPA of LS and SS mice may be responsible for their differential susceptibility to BIC-induced seizures. Supported by AA-03527, AA-07567, and MH-16880.

289.3

THE EFFECTS OF STEROIDS ON GABA$_A$ RECEPTOR FUNCTION IN LS AND SS MICE. B.J. Bowers and J.M. Wehner. Institute for Behavioral Genetics, University of Colorado, Boulder, CO 80309.

Recent studies have shown that steroids are widely distributed in brain and are capable of producing a variety of neurological and behavioral effects. The GABA$_A$ receptor complex has been implicated as a primary site of action. Excitatory and inhibitory modulation of GABA-related responses may be dependent on structural characteristics of the steroid. To determine whether differential responses may also be due to genetic factors the effects of two structurally different steroids, tetra-hydrodeoxcorticosterone (THDOC) and pregnenalone-sulfate (PS), and the steroid anesthetic, alphaxalone (ALPH), on sleep-time and GABA$_A$ receptor function were examined in LS and SS mice. ALPH produced longer sleep-times in LS mice; however, THDOC-induced sleep-times did not differ between the lines. Enhancement of receptor inhibition, measured by [^{3}H]-flunitrazepam (FNZ) binding and $^{36}Cl^-$ uptake, was demonstrated by both ALPH and THDOC with LS\SS differences observed only for ALPH-enhanced [^{3}H]-FNZ binding. The effects of PS suggested a disinhibition of GABA function which did not differ between LS and SS mice. These differences among the steroids on GABA-related responses suggest different sites or mechanisms of action which appear to be due to specific characteristics of the compound. Supported by AA-03527, AA-07567 and MH-16880.

289.4

DEHYDROEPIANDROSTERONE SULFATE, A NEUROSTEROID, BINDS TO RAT BRAIN MEMBRANES AND MODULATES THE FUNCTION OF GABA-A RECEPTORS. S. Demirgören, M.D. Majewska, and E.D. London, NIDA Addiction Research Center, Baltimore, MD 21224

Steroids, such as dehydroepiandrosterone, pregnenolone and their sulfated metabolites (called neurosteroids), are synthesized in the CNS primarily by the oligodendroglia (Hu, Z.Y. et al., Proc. Natl. Acad. Sci. USA 84:8215, 1987). Pregnenolone sulfate (PS) is an allosteric antagonist of the GABA-A receptor (Majewska, M.D. et al., Brain Res. 404:355, 1987). As dehydroepiandrosterone sulfate (DHEAS) inhibits the binding of PS to brain membranes (Demirgören, S. et al., Soc. Neurosci. Abst., 15:994, 1989), it appears that DHEAS may also interact with GABA-A receptors. In the present study, we have examined the binding of [^{3}H]DHEAS to rat brain membranes, and the interaction of DHEAS with GABA-A receptors. Binding of [^{3}H]DHEAS is saturable and protein dependent. The optimal pH for binding is 6.4, and equilibrium is reached after 1.5 hr. At 25°C, the specific binding of 2 nM [^{3}H]DHEAS is 70-80% of the total binding, but at 4°C it is only 50%. The binding is sensitive to heat denaturation as well as to treatment of membranes with phospholipase A_2 and protease. Scatchard analysis of equilibrium binding indicates that [^{3}H]DHEAS binds to two populations of sites: Kd_1= 2.9 ± 0.8 μM and Kd_2 = 554 ± 98 μM (N=6). The binding is inhibited by barbiturates and PS. DHEAS also interferes with barbiturate-induced enhancement of benzodiazepine binding, shifting the barbiturate dose-response curve to the right. The data indicate that DHEAS is a negative modulator of the GABA-A receptor complex.

289.5

STRESS-INDUCED ELEVATION OF GABA$_A$ RECEPTOR-ACTIVE 3α-HYDROXYSTEROIDS IN THE RAT BRAIN. R. H. Purdy[1]*, A. L. Morrow[2], P. H. Moore, Jr.[1]*, S. M. Paul[2], [1]SW Found., San Antonio, TX 78284 and [2]NSB, NIMH, Bethesda MD 20892.

The endogenous anxiolytic steroids, 3α, 21-dihydroxy-5α-pregnan-20-one (allotetrahydrodeoxycorticosterone, THDOC), a metabolite of deoxycorticosterone and 3α-hydroxy-5α-pregnan-20-one (allopregnanolone, AP), a metabolite of progesterone, are amongst the most potent known ligands of the GABA$_A$-receptor complex in the CNS. These steroids potentiate GABA$_A$ receptor-mediated chloride ion conductance in neurons, and have anxiolytic, sedative, and analgesic effects in vivo. We have developed specific radioimmunoassays to measure >25 pg amounts of THDOC and AP in extracts of plasma and brain tissue after purification of the steroids by HPLC. Control levels in plasma (ng/ml) and cerebral cortex (ng/g) of adult male Sprague-Dawley rats were 0.19 and 0.15 for THDOC and 0.06 and 0.39 for AP. Within 5 min after exposure to ambient temperature swim stress there was a 7-20 fold increase in circulating THDOC and AP respectively, and a concomitant 4-7 fold increase of THDOC and AP in cerebral cortex. These increases in plasma and brain were maintained for 1 hr following stress, and then declined to baseline levels by 2 hr. These stress-induced increases did not occur in adrenalectomized animals. We conclude that THDOC and AP are modulators of central GABA$_A$-receptors which may play an important role in the response of the CNS to stress.

289.6

TISSUE-SPECIFIC MODULATION BY STEROIDS OF 35S-TBPS BINDING ON THE GABA-A RECEPTOR. D.M. Turner, D.W.Sapp, U. Witte*, N. Kokka*, and R.W.Olsen. Dept. of Pharmacology, UCLA, Los Angeles, CA 90024

Steroids have been shown to enhance [^{3}H] muscimol and [^{3}H]flunitrazepam binding, and inhibit [^{35}S]TBPS binding to the GABA receptor as well as enhancing both pentobarbital and muscimol-stimulated chloride flux through the GABA-R channel. Steroids inhibit [^{35}S]TBPS binding when present in high concentrations or when GABA is present; we now present evidence that low concentrations of some steroids enhance binding in certain brain regions. [^{35}S]TBPS binding was measured in rat brain sections by autoradiography and in homogenates. Alphaxalone (1 μM) enhanced TBPS binding in inferior colliculus > cerebral cortex = hippocampus > cerebellar molecular layer with little change in the cerebellar granule cell layer. 5α-Androstan-3α,17β-diol (10 μM) enhanced TBPS binding in cerebral cortex deep layers > inferior colliculus = hippocampus > cerebellar molecular layer, while binding in the cerebellar granule cell layer and superficial cerebral cortex were inhibited. 5α-Pregnan-3α-hydroxy-20-one showed little or no effect on TBPS binding at 1 μM, but at 10 μM inhibited binding in all tissues assayed. Thus, steroids differentially modulate the GABA receptor in a subtype-specific manner.

289.7

EFFECTS OF GONADAL STEROID HORMONES ON BENZODIAZEPINE RESPONSES AND RECEPTORS. M.A. Wilson, R.Biscardi* and K.J.Long*. Dept. Pharmacology, Univ. South Carolina Sch. of Medicine, Columbia, SC 29208.

Steroid hormone derivatives have been shown to interact with the GABA/benzodiazepine receptor complex and to modify GABAergic responses. Since benzodiazepines (BZs) similarly modulate GABA responses, we examined if changes in the hormonal milieu modify responses to acute and chronic BZ exposure. Bicuculline seizure thresholds were determined in groups of male, intact female and ovariectomized (OVX) rats following ACUTE diazepam (DZ) treatment or CHRONIC exposure (3 wk) to DZ-filled silastic capsules. Both basal seizure thresholds and anticonvulsant effects of acute DZ treatment (0.5 mg/kg,iv) were similar in these hormonally-distinct groups. Binding studies of several brain areas indicated benzodiazepine receptor levels were comparable in males, females and OVX rats and did not fluctuate over the estrous cycle. Seizure threshold values and BZ binding parameters failed to correlate with circulating levels of estrogen or progesterone. As seen previously in male rats, chronic exposure to DZ resulted in tolerance to the anticonvulsant effects of DZ (Gallager et al.,Brain Res. 342:26,1985). Early results suggest intact females, but not OVX rats, also develop tolerance to DZ's anticonvulsant effects after chronic DZ exposure. All groups had similar brain DZ levels following acute and chronic BZ treatments. Support: PHS R29 DA05932-01, PHS RR05815,& USC RPS Grants.

289.8

GONADAL STATUS IN MALE RATS AFFECTS FUNCTION OF THE BENZODIAZEPINE/GABA RECEPTOR COMPLEX IN REPONSE TO ENVIRONMENTAL CHALLENGE. Renee Primus and Carol K. Kellogg. Dept. of Psychology, University of Rochester, Rochester, N.Y. 14620.

Function at the benzodiazepine (BZD)/GABA receptor chloride channel complex during social interaction (SI) in a familiar vs. an unfamiliar environment was evaluated in young adult male rats as a function of pubertal gonadal status. Male rats were either castrated or left intact at 19 days of age and then subjected to the SI test of anxiety at 60 days. Chloride (Cl-) enhancement of Flunitrazepam (Flu) binding and GABA-mediated Cl- transport were both measured immediately following the test.

Chloride enhancement of Flu binding was facilitated to a greater extent in intact rats exposed to the unfamiliar environment than in intact rats exposed to the familiar environment. There was no differential effect of the two environments on GABA-mediated Cl- transport, but a decrease in the EC50 for GABA stimulation of Cl- uptake was evident in rats exposed to the SI test (regardless of the environment) when compared to naive intact male rats. Cl- enhancement of 3H-Flu binding in juvenile castrated rats was also differentially affected by the two environments, but now facilitation of Flu binding by Cl- was greater in the familiar environment than in the unfamiliar environment. GABA-mediated Cl- transport in juvenile castrated rats, however, was unaltered during environment-related SI and was comparable to that measured in naive intact male rats.

The findings suggest that function at the BZD/GABA receptor complex in intact male rats is differentially affected by various aspects of the SI test. In addition, the finding that juvenile castration alters functional changes at the BZD/GABA receptor complex in response to challenge suggests a role for gonadal function during development in the expression of an organism's response to challenge. Sponsored by Grants MH31850 and MH00651.

289.9

OVARIAN ENDOCRINE STATUS INFLUENCES BEHAVIOR IN THE ELEVATED PLUS-MAZE AS WELL AS THE ANXIOLYTIC EFFECT OF DIAZEPAM. Daniel Bitran and Carol K. Kellogg. University of Rochester, Department of Psychology, River Campus, Rochester, NY 14627.

There are well documented barbiturate-like effects of the reduced metabolite of progesterone (P), 5α[β]-pregnane,3α-ol,20-one (tetrahydroprogesterone - THP), both physiologically and neurochemically, at the level of the GABA/BDZ receptor complex. Based on findings from *in vitro* studies, one would predict that anxiolytic effects should be most apparent at times of high circulating progesterone, i.e., estrous and pregnancy.

The following experiments were aimed at documenting the relationship between ovarian endocrine status and activity in the elevated plus-maze - a behavior that is sensitive to the anxiolytic effects of BDZs. Female Long-Evans rats (200-300 g) were either ovariectomized (OVx) or left intact and cycling. Other females were mated and tested at gestational day 19 or postparturient days 2 and 7. Diazepam (DZ) was administered IP for 6 consecutive days, in order to produce tolerance to its sedative effects. Thirty minutes after the last injection, locomotor activity was monitored for 5 min in a novel arena, followed by a 5 min test in the plus-maze.

Spontaneous behavior in the plus-maze was not different between OVx and estrous females. However, the anxiolytic effect of DZ (1 mg/kg) was dependent on gonadal status. In intact cycling females, DZ significantly increased the number of open arm entries and the time spent in the open arms. There was no response to DZ in OVx rats, but a DZ response was observed in OVx females given a hormonal regimen that results in estrus behavior (estradiol + P). Behavior in the plus-maze also varied as a function of pregnancy and parturition. Postparturient females spent significantly more time on the open arms than did virgin females, whereas the behavior of pregnant and virgin females did not differ. In addition, DZ at 1.0 or 2.5 mg/kg, had no effect on the plus-maze behavior of pregnant females. Together, these data indicate that ovarian hormones are permissive to the anxiolytic effect of BDZs, and, in contrast to predictions from *in vitro* studies, the postpartum period is accompanied by a reduction in anxiety-related behavior. MH31850 and MH00651.

289.10

DEFEAT STRESS IS ASSOCIATED WITH INCREASED GABA$_A$ RECEPTOR SUBUNIT mRNAs. I. Kang, M.L. Thompson, J. Heller*, L.G. Miller. Div. of Clinical Pharmacology, Depts. of Psychiatry and Pharmacology, Tufts-New England Medical Ctr., Boston, MA 02111.

Stress has been shown to affect a number of neurotransmitter systems. The defeat stress paradigm is a model of social stress involving defeat of an intruder by a resident male. Defeat stress leads to transient increases in binding at the benzodiazepine site on the GABA$_A$ receptor; other stress paradigms augment GABA$_A$ receptor function. To assess effects of defeat stress on GABA$_A$ receptor mRNAs, we evaluated mRNAs for the alpha1 and gamma2 subunits in cortex using Northern hybridization in sham, defeated, and resident mice. Control hybridization was determined in each case for GAPDH mRNA. For the alpha1 subunit, mRNA was unchanged from controls immediately after stress. However, at 4 hrs after stress, mRNA was increased by appoximately 20%; at 8 hrs, by 60%; at 24 hrs by 100%; and at 72 hrs, by 120%. mRNA was unchanged in sham mice up to 72 hrs. mRNA was also unchanged in dominant mice at 0 and 24 hrs after stress. Results for the gamma2 subunit were similar; mRNA was increased by 20% at 4 hrs; 50% at 8 hrs; 50% at 24 hrs; and 120% at 72 hrs. No alterations were observed in mRNA of sham mice up to 72 hrs, or resident mice at 0 and 24 hrs. GAPDH mRNA was not affected in any group. Defeat stress is associated with a persistent increase in GABA$_A$ receptor subunit alpha1 and gamma2 mRNAs, which appears to be specific for the defeated animal.

289.11

EFFECT OF ACUTE ADMINISTRATION OF BENZODIAZEPINES (BZD) ON GABA$_A$ RECEPTOR α SUBUNIT mRNAs IN RAT CEREBRAL CORTEX. P. Damschroder-Williams*, P. Montpied, A. L. Morrow, S. M. Paul. Section on Molecular Pharmacology, Clinical Neuroscience Branch, NIMH, Bethesda, MD 20892

It is generally acknowledged that many of the pharmacological actions of BZDs are mediated via an augmentation of GABAergic neurotransmission. In order to investigate the effect of BZDs on the expression of GABA$_A$ receptor mRNA expression, rats were administrated a single acute dose of the short-acting BZD, diazepam (10 mg/kg) or the long-acting BZD, flurazepam (100 mg/kg). Total RNA was extracted from the cerebral cortices of individual animals and a subunit mRNAs were quantified by Northern blot analysis using a human ^{32}P-labelled GABA$_A$ receptor α subunit cRNA probe (J. Neurochem. 51, 1651, 1988). An internal standard (600bp sense β-actin fragment) was added to each tissue sample to control for identical RNA recoveries. Three hours after diazepam or vehicle administration, a significant reduction in GABA$_A$ (α_1 subunit) mRNA was observed in rat cerebral cortex (n=4, -55%, $p<0.05$) from diazepam-treated rats compared to controls. This effect was not observed 24 hours after BZD administration. In contrast, flurazepam administration resulted in a similar reduction at 3 hrs (n=4, -50%, $p<0.05$), that persisted for at least 24 hours (n=3, -50%, $p<0.05$). No significant alteration in the glutamic acid decarboxylase mRNA levels was observed following acute BZD adminstration. These data suggest that acute administration of BZDs alters the level of GABA$_A$ receptor (α_1 subunit) mRNAs in rat cerebral cortex. This reduction in mRNA levels may represent BZD-induced alterations in GABA$_A$ receptor mRNA turnover, gene expression or the diurnal cycle of GABA$_A$ receptor mRNA levels.

289.12

GABA INDUCES A RECEPTOR-MEDIATED DOWN-REGULATION OF GABA$_A$ BENZODIAZEPINE RECEPTOR α SUBUNIT MESSENGER RNAS P. Montpied[1], E. I. Ginns[1], B. M. Martin[1], D. Roca[2], D. H. Farb[2], and S. M. Paul[1]. [1]NSB, NIMH, Bethesda, MD 20892 and [2]Dept. of Anatomy and Cell Biology, SUNY Health Sci. Ctr at Brooklyn, Brooklyn NY 11203.

γ-Aminobutyric acid (GABA), the major inhibitory neurotransmitter in brain, is known to interact with a subclass of GABA receptors that activate a ligand-gated chloride ion channel. Chronic exposure of cultured embryonic chick neurons to physiological concentrations of GABA results in a time-dependent down-regulation of these GABA$_A$ receptors. To delineate the cellular mechanism(s) responsible for agonist induced down-regulation of GABA$_A$ receptors we quantified the levels of GABA$_A$ receptor α subunit messenger RNAs, which encode the subunit(s) containing agonist recognition site(s); and observed a marked reduction in α subunit transcripts following exposure of embryonic chick neurons to GABA. Both the down-regulation of GABA$_A$ receptors and α subunit mRNAs induced by GABA are completely antagonized by the specific GABA$_A$ receptor antagonist SR-95531. These data demonstrate the presence of an agonist-induced receptor-mediated mechanism for regulating the expression of receptor subunit-encoding mRNAs that may be involved in the development of tolerance to the pharmacological actions of drugs known to act via GABA$_A$ receptors.

289.13

PRENATAL EXPOSURE TO DIAZEPAM SELECTIVELY INCREASES SEIZURE SENSITIVITY ONLY TO DRUGS ACTING AT THE GABA/BDZ CHLORIDE CHANNEL RECEPTOR COMPLEX. Carol K. Kellogg and Daniel Bitran. Univ. of Rochester, Dept. of Psychology, Rochester, NY 14627

We reported that adult male rats prenatally exposed to diazepam (DZ) exhibited a decreased threshold to bicuculline-induced seizures and, in parallel studies, a corresponding increased sensitivity to the *in vitro* antagonistic effects of bicuculline on GABA-stimulated chloride influx (Bitran et al., Soc Neurosci Abst 15, 1989). We now report that prenatal exposure to DZ results in an enhanced sensitivity to chemoconvulsants that show pharmacological specificity to the GABA/BDZ receptor.

Pregnant Long-Evans rats were injected SC with vehicle or DZ (1.0 or 2.5 mg/kg) from gestational days 14 to 20. Adult male offspring (400-500 g) were anesthetized (12 mg/kg Ketamine, 10 mg/kg Xylazine) and the jugular vein cannulated. Threshold dosages to facial clonus, myoclonic jerk, forelimb clonus, and extensor tonus were determined during the intravenous infusion (2 ml/min) of the following chemoconvulsants: bicuculline methiodide, a GABAa antagonist (BMI, 0.05 mg/ml); DMCM (0.5 mg/ml), a benzodiazepine inverse agonist; picrotoxin, a GABA-gated chloride channel blocker (PIX, 1 mg/ml); and non-GABAergic convulsants pentylenetetrazol (PTZ, 10 mg/ml), caffeine (CAFF, 20 mg/ml), and strychnine (STRY, 0.1 mg/ml).

Prenatal treatment with DZ decreased the threshold for BMI- and DMCM-induced facial clonus and myoclonic jerk by 35-40% and 20-40%, respectively. Threshold dosages of BMI and DMCM to forelimb clonus and extensor tonus were not affected by prenatal DZ treatment. A 25 to 40% reduction in the thresholds to PIX-induced facial clonus, myoclonic jerk, and forelimb clonus was observed with prenatal DZ treatment. There were no differences in the seizure threshold dosages of PTZ, CAFF, and STRY in adult males prenatally exposed to DZ. These results corroborate and extend previous findings that exposure to diazepam *in utero* induces long-lasting changes in the seizure threshold to chemoconvulsant agents, but only those that act at the GABA/BDZ chloride channel receptor complex. Supported by grant MH31850.

289.14

INTERNALIZATION OF GABA/BENZODIAZEPINE RECEPTORS IN CHICK CORTICAL NEURONS. M.H. Jalilian Tehrani and E.M. Barnes, Jr. Baylor Col. of Med., Houston, TX 77030.

Previous studies have shown that chronic exposure of chick cortical neurons to GABA or benzodiazepine agonists leads to a reduction in the density of GABA/benzodiazepine receptors. In order to study the role of receptor internalization in this process, we have utilized N-(4-sulfophenyl)thiocarbamoyl-1012S [SPTC-1012S] as an impermeant displacer of [^{3}H]flunitrazepam binding to intact neurons. A 24-hr treatment of the cells with 200 µM GABA increased the fraction of intracellular receptors, defined by SPTC-1012S insensitive [^{3}H]flu binding, from 8.0 ± 2.0% to 30.4 ± 2.5% of the cellular total. A similar treatment with 100 nM clonazepam raised the proportion of internal receptors to 19.2 ± 2.3%. The overall level of [^{3}H]flu binding declined by 20% and 12%, respectively, during the exposure to GABA or clonazepam. After a 24-hr treatment of neurons with 10 mM NH_4Cl or 50 µM chloroquine, the fraction of internal receptors was increased to 26.6 ± 3.9% and 14.5 ± 3.3%, respectively, compared to untreated controls (7.0 ± 1.5%). Both compounds produced a decline in the total receptor pool (by 32% and 23%, respectively). The data suggest that both GABA and benzodiazepine agonists accelerate the sequestration of receptors. Permeant amines, however, are more likely to interfere with the recycling of internalized receptors to the neuronal surface. (Supported by NIH grants DK 17436 and NS 11535).

289.15

LOCALIZATION OF BENZODIAZEPINE RECEPTORS: COMPARISON OF IN VIVO SPECT IMAGING, EX VIVO AUTORADIOGRAPHY, AND IN VITRO RECEPTOR AUTORADIOGRAPHY.
E. Sybirska, M. Al-Tikriti, E. W. Johnson, S. Zoghbi and R. B. Innis. Dept. Psychiatry, VA Medical Center and Yale University, West Haven, CT 06516.

To assess the quantitative accuracy of neuroreceptor imaging with SPECT (Single Photon Emission Computed Tomography), we have compared SPECT images of the distribution of benzodiazepine (BZ) receptors in monkey brain with immediate postmortem analyses of the tissue.

Vervet and rhesus monkeys were injected with the BZ antagonist radioligand ^{123}I-Ro16-0154. After SPECT scanning, the animal was killed and the brain sectioned in transaxial planes corresponding to the multiple levels of the SPECT images. Ex vivo autoradiography was used to quantitate the distribution of total ^{123}I radioactivity in 30µm tissue sections. After decay of the ^{123}I radionuclide ($T_{1/2}$ = 13 hr), standard in vitro receptor autoradiography with ^{125}I-Ro16-0154 was used to quantify the distribution of BZ receptors.

SPECT scans showed excellent regional correlations with the actual distribution of tissue radioactivity determined with ex vivo autoradiography. In addition, tissue punches confirmed the concentration of radioactivity in gray matter areas, with Brodmann's area 17 having the highest ratio of gray to white matter of 50:1.

Comparison of ex vivo with in vitro results generally showed an excellent correlation, which confirmed that the distribution of ^{123}I radioactivity corresponds to the actual distribution of BZ receptors. However, some limited brain areas (like cerebellar cortical layers) showed moderate discrepancies between ex vivo and in vitro results. The causes of these relatively small differences could be due to metabolites of the radioligand, the effect of endogenous modulators, or regionally differential washout of the in vivo radioligand.

289.16

QUANTITATIVE SPECT IMAGING OF THE BENZODIAZEPINE RECEPTOR IN NON-HUMAN PRIMATE BRAIN. R.B. Innis, S.W. Woods, S. Zoghbi, E.W. Johnson, M. Al-Tikriti, E. Sybirska, J. Seibyl, R. Malison, P.B. Hoffer, D.S. Charney, and G.R. Heninger. Dept. Psychiatry, VA Medical Center and Yale University, West Haven, CT 06516.

Ro16-0154 is a benzodiazepine (BZ) receptor antagonist analog of the better known agent Ro15-1788. ^{125}I-Ro16-0154 binding to brain tissue homogenates has high affinity (K_I = 0.5 nM at 37°C) and high ratios of specific:non-specific binding (50:1). We have examined the high energy gamma-emitting ^{123}I-Ro16-0154 as an in vivo BZ receptor probe with SPECT (Single Photon Emission Computed Tomography).

In a series of 20 studies, female baboons were injected with 1-18 mCi ^{123}I-Ro16-0154 and scanned for 2-6 hours in the Strichman 810X Brain Imager. Radiolabel uptake was concentrated in cortical areas with highest density in occipital cortex. Maximum uptake was reached within 30-90 min and relatively stable for the following 180 min. Whole body scans showed brain uptake of 11-12% of injected dose at 60-120 min and clearance of greater than 96% of total body radioactivity via urine in 24 hours. Approximately 90% of brain radioactivity could be displaced by Ro15-1788 (0.1 mg/kg i.v.) within 30-45 min, and, thus, appeared to be associated with the BZ receptor. Displacement studies were performed with stepwise increasing i.v. doses of three agonists (diazepam, clonazepam, alprazolam) and two antagonists (Ro15-1788 and "cold" Ro16-0154). The in vitro receptor affinities (K_I) were strongly correlated with the in vivo displacing potencies (ED_{50}) for these five agents (r=.94 on log-log plot). Ex vivo autoradiographic analysis of monkey brain tissue sections obtained approximately 90 min post injection of ^{123}I-Ro16-0154 demonstrated a distribution of radioactivity which exactly mirrored the distribution of BZ binding sites determined with standard in vitro receptor autoradiographic methods.

289.17

IN VIVO CEREBRAL DISTRIBUTION OF THE PUTATIVE BENZODIAZEPINE RECEPTOR LIGAND I-123 Ro 16-0154 IN MAN. S. W. Woods, S. S. Zoghbi*, A. W. Goddard, J. P. Seibyl, I. G. Zubal*, R. M. Baldwin*, D. S. Charney, G. R. Heninger, P. B. Hoffer*, R. B. Innis. Dept. of Psychiatry, Yale U. Sch. of Med., New Haven, CT 06508.

The present study aims to evaluate the cerebral distribution in humans of I-123 Ro 16-0154, a putative benzodiazepine receptor (BZR) ligand for use with single photon emission computed tomography (SPECT). METHOD: Healthy subjects undergo intravenous injection of 5 mCi I-123 Ro 16-0154, followed by serial single slice SPECT acquisitions and repeated whole body scans. RESULTS: In the single subject studied to date, activity peaked in striatum and white matter (WM) at the first measurement 16 min p.i., and in other regions at 40-60 min. p.i. Activity declined after peak at approximately 35%/hr. in striatum and thalamus and approximately 20%/hr. in other regions. 7.3% of the injected dose was present in brain at 3 hrs p.i. The regional distribution of activity was occipital > frontal > temporal = parietal = cerebellum > thalamus > striatum > white matter. The occipital:WM ratio was 10:1 at 90 min p.i. DISCUSSION: The high brain uptake and relatively slow washout suggest that displacement studies are feasible using this ligand. The regional radioactivity pattern is consistent with a BZR distribution but volume averaging effects and more subjects must be investigated.

289.18

BENZODIAZEPINE RECEPTOR ACTIVE COMPOUNDS FROM PLANT MATERIAL. M.R.Witt* and M.Nielsen. Research Laboratories, St. Hans Hospital, Roskilde, Denmark.

Amentoflavone, a biflavonoid, was purified from a plant extract and shown to have high affinity to benzodiazepine receptors in-vitro (Nielsen, M. et al. Biochem. Pharmacol., 37:385, 1988). An estimation of structure/activity on 13 flavone derivatives (obtained from H. Geiger, Stuttgart, Germany) showed that e.g. methylation of hydroxylgroups in amentoflavone decreased the affinity for the benzodiazepine receptor.

Recently we have analysed a series of African medicinal plants. After extraction of plant material and screening for brain receptor interactions using high affinity ligand binding in-vitro, receptor active fractions were purified using HPLC. We have isolated Oxayohimbin-16-carboxylic acid-didehydro-19-methyl, methyl ester (Mayumbin; IC50 = 0,8 µM on inhibition of 3H-diazepam binding) and 2,6 (3,4 methyl-enedioxyphenyl) = -3,7 dioxabicyclo [3.3.0] octane (Sesamin; IC50 = 6 µM for inhibition of 3H-diazepam binding). Sesamin has been reported to have calming and axiety reducing effects in humans and animals (Benecke H.P. and Sherwood B.E, U.S. patent 4,427,694, 1984).

290.1

DISTRIBUTION OF SEROTONERGIC BINDING SITES IN CAT SPINAL CORD. L.M. Pubols, L. Kane* and S. Dawson*. R.S. Dow Neurological Sciences Institute, Good Samaritan Hospital and Medical Center, Portland, OR 97209

The spatial distribution of serotonin (5HT) binding sites in the lumbar spinal cord grey matter of the cat was studied autoradiographically, using 1-2 nM [^{3}H]5HT, with and without 10 μM unlabelled 5HT, to determine non-specific and total binding, respectively. Binding was quantified, using the video counting mode of a Bioquant image analysis system to calculate the percent of total area occupied by silver grains over spinal cord laminae II, III-IV, V-VI, VII, IX, and X. Significant levels of specific binding were found in all of these regions. Specific binding as a percent of total binding was highest in laminae II (84%) and X (72%), and lowest in lamina VII (51%). By analysis of variance, the effect of laminar location on the degree of binding was highly significant for specific binding, but insignificant for non-specific binding, indicating that laminar differences in specific binding were not due to differential quenching of radioactivity.

In contrast to an earlier study (Seybold, 1985), which described dense labelling of serotonin binding sites by [^{3}H]LSD in laminae II and X of the cat, and a lack of labelling in the ventral horn, the present study reveals serotonin binding sites throughout the dorsal and ventral horns in this species. (Support: NIH NS19523)

290.2

LOCATION OF SEROTONIN RECEPTORS MEDIATING RENIN RESPONSE TO DOI. P.A. Rittenhouse, E.A. Bakkum*, K. Kunimoto*, J. Yracheta*, L.D. Van de Kar. Loyola Univ. Chicago, Stritch Sch. Medicine, Dept. Pharmacology, Maywood, IL, 60153.

The serotonin (5-$HT_{2/1C}$) agonist DOI increases plasma renin activity (PRA), in conscious rats, via activation of 5-HT_2 receptors (Rittenhouse et al., The Pharmacologist, 32 1990). The aim of this study was to differentiate central from peripheral receptors which mediate this response. We injected DOI (0, 0.5, 1.0, 10.0 mg/kg, ip) to rats pre-treated with saline or 0.1 mg/kg xylamidine, a peripheral 5-HT_2 antagonist. DOI dose-dependently increased PRA. The dose response curve was shifted to the right in the xylamidine pretreated rats, suggesting a role for peripheral 5-HT_2 receptors in the effect of DOI. Subsequently, we injected DOI into the lateral cerebral ventricles (ICV), using doses lower than the peripherally effective doses: 0, 1, 10, 100, and 200 μg/kg. Only 200 μg/kg DOI significantly elevated PRA. To determine if blood pressure (BP) changes were responsible for DOI's effects, we measured BP through implanted femoral arterial catheters. Injection of DOI ICV at 0, 10.0 and 200 μg/kg produced a dose-dependent rise in BP of 3, 7, and 36 mm Hg respectively, within 5 minutes post-injection. In contrast, intra-arterial injection of 200 μg/kg DOI caused a slower rise (15-30 minutes post-injection) of 20 mm Hg in BP. These data suggest that DOI's elevation of PRA and BP may have both peripheral and central components. Supported by NIDA DA04865.

290.3

IDENTIFICATION OF SPINAL 5-HT_{1C} BINDING SITES. R.Pluchino,* M.R. Pranzatelli, and S. DiMauro. Neurology, Pediatrics, Columbia University, NY

We identified recognition sites in rat spinal cord with the properties of 5-HT_{1C} receptors. The sites were labelled using (^{3}H)mesulergine (10uM 5-HT displacer) or (^{3}H)5-HT (5-HT displacer, pindolol blocker). Bmax values (pmol/g) were 3.8 ± 0.3 and 4.3 ± 0.5, respectively (n=4-7).The corresponding percent maximum specific binding was 70 and 38. K_ds (nM) were 1.8 ± 0.1 and 4.7 ± 0.6, respectively. Inclusion of 20 nM spiperone to block potential 5-HT_2 sites reduced the Bmax for binding sites labelled by (^{3}H)-mesulergine 32% without changing k_d or n_H. However, drug affinities were similar in the presence or absence of spiperone, and the order of drug potencies was consistent with drug affinities for the 5-HT_{1C} receptor: 5-HT, mesulergine, mianserin> Ru24969, ketanserin, DOI> spiperone > 8-OH-DPAT, pindolol. Using either (^{3}H)DOB (5-HT displacer, cinanserin blocker) or (^{3}H)mianserin (5-HT displacer, spiperone blocker), no specific binding attributable to 5-HT_{1C} sites was obtained. These data suggest that a substantial population of spinal 5-HT_1 receptors are of the 5-HT_{1C} subtype and that (^{3}H)mesulergine and (^{3}H)5-HT are useful radioligands for spinal 5-HT_{1C} sites. (Supported by NIH grant 1-K08-NS01158 (CIDA), the Myoclonus Research Fund, the United Cerebral Palsy Education and Research Fund (R381-88), and the William Randolph Hearst Foundation)

290.4

AUTORADIOGRAPHY OF 5-HT_{1A} SITES IN RATS WITH NEONATAL 5,7-DHT LESIONS. M.M. Durkin,* M. R. Pranzatelli, A.I. Barkai,* B.S. McEwen, and T.A. Pedley. Neurology, Pediatrics, & Psychiatry, Columbia Univ.; and Laboratory of Neuroendocrinology, Rockefeller Univ., New York, NY.

We recently reported that rats with brainstem hyperinnervation showed altered behavioral responses to 5-HT agonists after neonatal 5,7-dihydroxytryptamine (5,7-DHT) lesions made by intraperitoneal (ip) compared to intracisternal (ic) injection (Dev Brain Res 50: 89, 1989). To investigate the role of 5-HT_{1A} receptors in these differences, we labelled 5-HT_{1A} sites autoradiographically with 2 nM [^{3}H]DPAT (10 μM 5-HT as displacer) 4 months after ip or ic 5,7-DHT or saline (n=16). The regional distribution of 5-HT_{1A} sites conformed to previous reports of highest receptor densities in hippocampus (CA_1, dentate gyrus), septal nuclei, dorsal and median raphe, mammillary body, and certain cortical regions (cingulum, claustrum). 5-HT_{1A} sites were significantly decreased (-87%) only in the dorsal raphe ($p<0.0004$, ANOVA), and only after ic-made 5,7-DHT lesions. No reductions were found after lesions made by ip injection compared to controls. Further studies will be required to determine if dorsal raphe 5-HT_{1A} sites recovered or were never reduced. 5-HT_{1A} receptor density in the dorsal raphe may contribute to the different behavioral consequences of the route of neonatal 5,7-DHT injection. (Supported by NIH grant 1-KO8-NS01158 (CIDA), the Myoclonus Research Fund, the United Cerebral Palsy Education and Research Foundation (R381-88), and the William Randolph

290.5

A NEW SELECTIVE RADIOIODINATED PROBE FOR LABELING SEROTONIN 1B AND SEROTONIN 1D RECEPTORS. LIGAND-BINDING STUDIES ON THE CENTRAL NERVOUS SYSTEM OF RAT AND GUINEA PIG USING QUANTITATIVE AUTORADIOGRAPHY. P. Boulenguez*[1], J. Chauveau*[2], L. Segu*[1], A. Morel*[2], J. Lanoir[1] and M. Delaage*[2]. [1]CNRS, Lab. de Neurobiologie E6, BP 71, 13402 Marseille Cedex 09; [2]Immunotech, Luminy, Case 915, 13288 Marseille Cedex 09, France.

The serotonin-O-glycyl-tyrosinamide (S-CM-GTNH_2) was radioiodinated on the tyrosine residue. The binding of 0.02 nM S-CM-G[^{125}I]TNH_2 on rat and guinea pig brain sections was reversible and saturable (K_d = 1.3 and 6.4 nM, respectively). The anatomical distribution of the labeling was heterogeneous and corresponded exactly to those of serotonin-1B sites (5-HT_{1B}) in the rat, or 5-HT_{1D} sites in the guinea pig. Hippocampus (5-HT_{1A}) and choroid plexus (5-HT_{1C}) are not labeled. The binding of 0.02 nM S-CM-G[^{125}I]TNH_2 in substantia nigra is inhibited with low concentrations of 5-HT, S-CM-GTNH_2 and CGS-12066B (IC_{50} = 1.7, 3.9 and 79.5 nM respectively in the rat and 1.1, 18.0, 25.4 nM in the guinea pig). Other compounds which selectively bind to 5-HT_2, 5-HT_3, dopaminergic and noradrenergic receptors did not inhibit the radioligand binding at 10^{-5}M in the anatomical structures which contain these receptors. S-CM-G[^{125}I]TNH_2 is therefore a new, powerful probe for 5-HT_{1B} binding sites which does not interact with β-adrenergic receptors. Furthermore, this is the first radioiodinated probe for 5-HT_{1D} binding sites and it could be useful for studying this subtype in human brain. Since the binding of S-CM-G[^{125}I]TNH_2 had a low dissociation kinetic constant and was immobilized with chemical fixatives, we are now studying the subcellular distribution of 5-HT_{1B} and 5-HT_{1D} sites.

290.6

LOCALIZATION OF 5-HT_{1a} RECEPTOR mRNA IN RAT BRAIN. **D. Brousseau, R. Artymyshyn, and P. McGonigle, Department of Pharmacology, University of Pennsylvania, Phila, PA. 19104.**

Using the nucleotide sequence for the human 5-HT_{1a} receptor, a unique 183 base segment of the rat 5-HT_{1a} receptor gene was amplified from a rat genomic library by using PCR technology and subsequently ligated into the transcription vector PGEM-3Z. Seven clones were selected for dot-blot screening against a ^{32}P-labelled probe which was directed against the middle 36 bases of the 183 base 5-HT_{1a} receptor segment. DNA sequencing of a positively labelled clone determined that it contained the desired gene product. Nucleic acid sequence homology between the rat and human 5-HT_{1a} receptor was determined to be approximately 70% in this region. Similar amounts of homology have been observed within discrete regions of other mammalian G-protein linked receptors, particularly around the amino and carboxy termini. Identification of the appropriate clone permitted the synthesis of ^{35}S-CTP labelled anti-sense probe to cellular mRNA for the 5-HT_{1a} receptor. *In situ* hybridization to normal rat brain tissue sections has demonstrated an appropriate and selective regional mRNA distribution for the 5-HT_{1a} receptor as compared with the known distribution of the receptor itself using the radiolabelled ligand 8-OH-DPAT. In particular, regions which were labelled most heavily included the CA fields of the hippocampus as well as the dentate gyrus, the entorhinal cortex, the raphe nuclei, and the anterior olfactory nuclei. This contrasted with a low level of hybridization in the thalamus and basal ganglia and an intermediate level in the frontal cortex. In many areas, including the olfactory nuclei and entorhinal cortex, there was an apparent mismatch between the mRNA and 8-OH-DPAT binding such that mRNA fields surrounded fields of ligand binding.
(Supported by USPHS MH 43821 and GM-07170)

291.1

GABA-A RECEPTORS ON DORSAL COLUMN AXONS IN NEONATAL RAT SPINAL CORD. K. Sakatani, M. Chesler, Z. Hassan* W. Young. Dept. of Neurosurgery, NYU Medical Center, 550 First Ave. New York, NY, 10016

GABA has a depolarizing action on peripheral myelinated sensory axons and their dorsal horn terminals. We sought to determine whether these receptors continue along ascending dorsal column axons, using isolated segments of thoracic dorsal column from 11-17 day-old neonatal rats. Segments were excised from isolated spinal cords under chilled (4-10° C), oxygenated Ringer, placed in a recording chamber, then superfused with room temperature Ringer for 1-2 hours prior to recording. Bipolar platinum electrodes were used to deliver constant current, submaximal stimuli (100 msec, 2 mA, 0.2 Hz). The elicited compound action potential (CAP), was recorded with a glass micropipette (1 M NaCl, 1-2 MΩ) placed 0.5-2mm from the stimulating electrode. And the end of experiments, the isolated tissue was examined histologically to exclude possible contamination of gray matter.

GABA (10^{-4}-10^{-3} M) reversibly depressed the CAP amplitude (10^{-3} M: 56±7% of control; mean±SD, n=5), and prolonged the latency (119±8%). The GABA-A receptor agonist isoguvacine (10^{-4}-10^{-3} M) caused a similar reduction in amplitude (10^{-4} M: 57±10%: n=3) and increase in latency (111±4%). However, at lower concentrations (10^{-5} M), GABA and isoguvacine increased the CAP amplitude (118±5%, n=3) and decreased the latency (92±9%). By contrast, the GABA-B agonist baclofen (10^{-4} M) had no detectable effect on the CAP (n=2).

The results of this study indicate that in spinal cord, GABA-A receptors are present not only on the terminals of primary afferents, but can also be found on ascending dorsal column axons. Whether GABA has similar effects on conduction in the adult spinal cord remains to be determined. While the role of these receptors in the function of neonatal axons is not apparent, their presence suggests that axonal conduction could be subject to modulation by an extra-synaptic mechanism.

291.2

GABAERGIC TERMINALS ON DENDRITES AND DENDRITIC SPINES IN THE PROJECTION ZONE OF THE PERFORANT PATH OF THE DENTATE MOLECULAR LAYER. E. Fifkova, H. Eason* and P. Schaner*. Department of Psychology, University of Colorado, Boulder, CO 80309.

We have studied GABAergic innervation in the dentate molecular layer of 8 mice using postembedding immunogold electron microscopy. In the perforant path (PP) projection zone of the dentate molecular layer (DML), we have regularly observed labeled axon terminals synapsing on dendritic shafts in the form of symmetrical contacts. The dendrites receiving these synapses were GABA negative. In addition, we found dendritic spines contacted by GABA antibody labeled axon terminals. In most instances observed, the labeled axon terminal made a symmetrical contact on the spine stalk, while an unlabeled asymmetrical terminal contacted the spine head. The symmetrical contacts sometimes could be seen extending onto the spine head. GABA positive unmyelinated axons were seen making "*en passant*" synaptic contacts.

One possible source of the GABAergic terminals is the entorhinal cortex (EC). A small population of GABAergic sparsely spinous horizontal-bipolar cells and multipolar cells, located among the projection neurons of layers II and III of the EC, were also shown to terminate in the PP projection zone of DML (Germroth et al., Brain Res., 494, 187, 1989). In addition, strong dendritic inhibition has been observed in the dentate fascia following PP stimulation which appears to be GABA-mediated, as it is alleviated by a GABA antagonist picrotoxin (Wigström and Gustafsson, Brain Res., 295, 153, 1983). It is a feedforward type inhibition of dendrites as it occurs in the absence of granule cell firing. The observed GABAergic terminals synapsing on dendrites and dendritic spines in the PP projection zone could underlie this inhibition. This is the first time that a morphological substrate of a monosynaptic inhibition of dentate granule cell dendrites and spines is described. The authors acknowledge the help of Dr. M. Morales with the immunogold technique. Supported by AAA #AA06196 and MH #41834.

291.3

EVIDENCE FOR $GABA_A$ RECEPTOR SUBUNIT HETEROGENEITY IN THE RAT PITUITARY ANTERIOR AND INTERMEDIATE LOBES. J. Berman*, D.Pritchett, G. Kuchel and J. L. Roberts Fishberg Center For Neurobiology, Mt. Sinai School of Medicine, NY, NY 10029 and Department of Pharmacology, U. of Penn, Phila., PA 19104 .

The heterogeneity of $GABA_A$ receptor ß subunits is believed in most instances to confer distinct pharmacological and regulatory properties upon GABA receptors in a cell and tissue specific manner. Studies utilizing *in situ* hybridization have shown that more than one subunit subtype may be localized to a single specified cell population (Ymer et al., *EMBO,* 8: 1665). Here, we present results implying expression of all known $GABA_A$ receptor ß subunit genes in lactotrophs and melanotrophs. Using the polymerase chain reaction, and for ß1 and ß3 subunits, ribonuclease protection assays, we have demonstrated the presence of ß1, ß2 and ß3 subunit mRNA in both the anterior and intermediate lobes of the rat pituitary. The ß1 subunit is less abundant than ß3 in both tissues, and both messages are substantially more abundant in the anterior pituitary. This finding may reflect the high proportion of anterior pituitary cells which are $GABA_A$ receptor bearing lactotrophs. The anterior and intermediate lobes of the rat pituitary are not known to contain any $GABA_A$ receptor bearing cells other than lactotrophs and melanotrophs respectively. Thus the observation of multiple $GABA_A$ receptor subtypes in these tissues suggests that either multiple receptor types coexist in single cells or that microheterogeneity with respect to receptor composition exists in these cell populations.

291.4

MOLECULAR IDENTITY OF BRAIN GLUTAMIC ACID DECARBOXYLASE (GAD) FORMS IN RAT OVIDUCT AND TESTIS. N.J.K.Tillakaratne, M.G.Erlander, K.F.Greif, G.D. Frantz*, and A.J.Tobin, Dept. of Biology, UCLA, Los Angeles, CA 90024.

The mammalian brain has two forms of GAD, GAD_{65} and GAD_{67}, encoded by two different genes. GAD_{65} and GAD_{67} differ in size, affinity for the cofactor pyridoxal phosphate (PLP), and sub-cellular localization. We examined the distribution of the two forms in non-neural tissues of the rat by immunoblotting, Northern blotting, and GAD activity measurements. GAD_{65} is present in brain and oviduct. GAD_{65} cDNA hybridizes to a 5.7 kb RNA in both brain and oviduct, where it is present in mucosal epithelial cells. The RNA of kidney, liver, pancreas, adrenal gland, uterus, or ovary contains no GAD_{65} sequences. In contrast, GAD_{67} cDNA hybridizes to multiple RNA species in the testis. In the prepubertal testis, a 1.9 kb RNA predominates, and its expression coincides with the temporal course of Sertoli cell development. In the adult testis, the predominant form of GAD_{67} RNA is 2.3 kb long, and its appearance coincides with postmeiotic spermatogenesis. In situ hybridization demonstrates that the presence of GAD_{67} RNA in adult Sertoli cells, spermatids, and spermatozoa. The expression of both GAD forms in non-neural cells suggests that GABA may serve functional roles other than as a neurotransmitter. (Supported by NS22256 to AJT.)

291.5

DIFFERENTIAL DISTRIBUTION OF THE mRNAs ENCODING TWO FORMS OF GLUTAMIC ACID DECARBOXYLASES IN THE RAT BRAIN: INSIGHTS ON THEIR FUNCTIONS. S. Feldblum, N.J.K. Tillakaratne, M.G. Erlander, and A.J. Tobin. Department of Biology, UCLA, Los Angeles, CA 90024.

In the mammalian brain, GABA synthesis depends on two forms of glutamic acid decarboxylase (GAD) which differ in size, subcellular location and affinity for the cofactor pyridoxal-5'-phosphate. These two enzymes, GAD_{65} and GAD_{67}, are encoded by two mRNAs that are the product of two genes. To investigate the possible functional significance of two GADs in different types of neurons we have compared the distribution of the two GAD mRNAs throughout the rat brain using Northern analysis and in situ hybridization. Both forms of GAD were present in all the examined regions except for the triangular nucleus of the septum and the globus pallidus, where only GAD_{67} mRNA was detected. With several exception, GAD_{67} mRNA was present at higher levels than GAD_{65} mRNA throughout the brain. In the olfactory bulb, globus pallidus, and visual and auditory systems GAD_{67} mRNA was more abundant in neurons with no or short axons and restricted dendritic arborization, while GAD_{65} predominated in cells with a more complex spiny dendritic field. We suggest the involvement of the two forms of GAD in different types of synapses, possibly allowing different means of modulating the synaptic GABA. Supported by NS22256 to AJT.

291.6

REGIONAL COMPARISON OF BINDING OF DIFFERENT LIGANDS TO THE $GABA_A$ RECEPTOR. A. M. Mans, K. M. Kukulka* and R. A. Hawkins, Dept. of Physiology and Biophysics, The Chicago Medical School, North Chicago, IL.

The $GABA_A$ receptor mediates inhibitory GABA neurotransmission throughout the brain. It contains a chloride ionophore and three allosterically interacting binding sites : a GABA-, benzodiazepine- and chloride-channel site. To learn more about the regional distribution of this receptor and its subtypes in brain, we measured the binding of four different ligands to rat brain sections in vitro using quantitative autoradiography, with computer analysis and three-dimensional reconstruction. The ligands used were: ^{3}H-muscimol (GABA site), ^{3}H-flunitrazepam (benzodiazepine site), ^{3}H-flumazenil (antagonist at the benzodiazepine site), and ^{35}S-TBPS (chloride-channel site). Nonspecific binding was determined in the presence of excess unlabeled competing ligand in each case. Major differences were found between flunitrazepam and muscimol binding reflecting the different distribution of the low- and high-affinity receptor subtypes respectively. Muscimol binding was generally lower except in the cerebellum, and completely absent in the brainstem structures. The pattern of flumazenil binding was similar to that of flunitrazepam with some subtle differences, e.g., in the superior colliculus and red nucleus. Binding of TBPS was more related to that of flunitrazepam than muscimol; however, significant differences were found, e.g., in the cortex, globus pallidus, thalamic nuclei and inferior colliculus. The results are consistent with the presence of heterogeneous populations of the $GABA_A$ receptor in the brain. Supported by NIH grant NS 16389.

291.7

WITHDRAWAL FROM CHRONIC COCAINE DECREASED DOPAMINE TRANSPORTER SITES IN THE RAT NUCLEUS ACCUMBENS (NAc).
L.G. Sharpe, N.S. Pilotte, W.M. Mitchell*, E.B. De Souza and E.M. Dax*. NIDA, Addiction Res. Ctr. Baltimore, MD 21224.

Cocaine increases the synaptic level of dopamine (DA) by inhibiting DA uptake at terminal transporter sites. This study was designed to assess whether DA-uptake sites changed after chronic cocaine or after cocaine withdrawal. Rats were infused i.v. with saline or 1 mg/kg cocaine every 12 min for 2 hr over 10 days and then killed within 15 min of or 10 days after (withdrawal) the last infusion. Brains were sectioned (10 um) and processed for DMI-insensitive [^{3}H]mazindol binding. Total [^{3}H]mazindol binding in saline control animals was highest in caudate putamen and NAc (250 to 540 fmol/mg protein Eg), moderate in ventral tegmentum, ventral pallidum, and medial prefrontal cortex, and least in substantia nigra, pars reticulata. Although the DMI-insensitive [^{3}H]mazindol binding to DA uptake sites was not changed by chronic cocaine in any of the sites, 10-days of withdrawal from cocaine significantly reduced the density of binding when compared with controls only in the NAc (82%). The reduction of DA uptake sites during cocaine withdrawal may be involved in the development of sensitization to the pharmacologic effects of cocaine.

291.8

MIDBRAIN DOPAMINERGIC NEURONS (NUCLEI A8, A9 & A10) IN THE RAT: 3-DIMENSIONAL RECONSTRUCTION.
K.F. Manaye, M. Sadeq and D.C. German. Depts. of Psychiat. and Physiol., UT Southwestern Med. Cntr., Dallas, TX 75235.

The midbrain dopaminergic (DA) neurons reside within the retrorubral area, substantia nigra, and ventral tegmental area (nuclei A8, A9 and A10 respectively). The present experiment sought to quantify the number of DA neurons within each of the three subnuclei, and map their 3-dimensional distribution within the midbrain. Three male albino rats (175-225 g) were perfuse fixed with 10% neutral buffered formalin, and the brains were blocked in a standard coronal plane. 30 um thick sections were cut on a freezing microtome. Every 3th or 4th section, from rostral to caudal within the midbrain (2.75 mm), was stained with an antibody against tyrosine hydroxylase (1:4000; Eugene Tech), and adjacent sections were stained with cresyl violet. The distribution of cells was bilaterally symmetrical, and on one side of the brain there was an estimated total of 12,430 +/- 694 (mean +/- SD) A8/A9 cells, and 10,771 +/- 847 A10 cells (numbers corrected for split cell counting error). Information about the number and distribution of midbrain DA neurons will be used in conjunction with future studies to further characterize these cells in terms of their co-transmitters and afferent and efferent projections.
Research supported by NIDA (DA-05314).

291.9

CALCIUM-BINDING PROTEIN IMMUNOREACTIVITY IN THE HUMAN MIDBRAIN: RELATIONSHIP TO DOPAMINERGIC NEURONS.
D.C. German, K.F. Manaye, J. Brown* and C.R. Gerfen. Depts. of Psychiatry and Physiology, UT Southwestern Med. Cntr., Dallas, TX 75235; Lab of Cell Biology, NIMH, Bethesda, MD 20892.

In the rat, some dopaminergic (DA) neurons in the retrorubral area (nucleus A8), substantia nigra (nucleus A9), and ventral tegmental area (nucleus A10) contain a 28 kDa calcium-binding protein (calbindin D28k or CaBP) (Gerfen et al., PNAS 82:8780-8784, 1985). The present experiment sought to determine whether there is a comparable distribution of CaBP-containing neurons within the human midbrain DA neurons. Alternate 50 um thick sections were stained with an antibody to CaBP (1:2000) or tyrosine hydroxylase (TH, 1:2000). CaBP-containing cells were observed in: the region surrounding the red nucleus (in the dorsal portion of nucleus A9, along the midline and dorsal and lateral to the red nucleus); nucleus paranigralis; and nucleus parabrachialis pigmentosus. In many cells both CaBP and neuromelanin pigment were present, providing direct evidence of CaBP within DA neurons. The distribution of CaBP/TH-containing cells in the human brain is similar to the distribution of DA neurons which remain in post-mortem parkinsonian brains (German et al. Ann. Neurol. 26:507-514, 1989).
Supported by AG-08013 and Dallas Area Parkinsonism Society.

291.10

THE DISTRIBUTION OF DOPAMINE D1 AND D2 RECEPTOR mRNA'S IN RAT BRAIN.
D. M. Weiner, H. B. Niznik, R. K. Sunahara, B. F. O'Dowd, and M. R. Brann, LMB/NINDS, HHMI/NIH Research Scholars Program, Bethesda MD 20892 and Dept. of Pharmacology, and Addiction Research Foundation, Univ. of Toronto.

Based on the sequence of a dopamine D1 receptor (Sunahara et al, submitted), we synthesized three 48 base oligodeoxynucleotide probes to measure the pattern of mRNA expression in the rat CNS by in situ hybridization histochemistry. Three similar probes to a dopamine D2 receptor have been previously described (Weiner et al, *FEBS LETT*, **253**, 207). Based on several criteria, all of the probes selectively hybridized to their respective mRNA's on both blots and tissue sections. For both the D1 and D2 mRNA's, the strongest signal was observed in the striatum, including the caudate/putamen, the nucleus accumbens, and the olfactory tubercle. Within the caudate-putamen, approximately 50% of the medium sized neurons express each of the mRNA's. Both receptors are also expressed in the frontal and entorhinal cortex, habenula, and distinct nuclei of the amygdala and hypothalamus. For the D2 receptor only, hybridization was seen in the septum, superior colliculus, and substantia nigra, pars compacta. These results are consistent with both the known distribution of dopamine receptor binding sites as determined by receptor autoradiography, and the dopaminergic innervation of the rat brain.

291.11

DIFFERENTIAL EFFECTS OF D2 ANTAGONISTS ON SPONTANEOUS ELECTRICAL ACTIVITY OF SUBSTANTIA NIGRA AND VENTRAL TEGMENTUM.
K.A.Bonnet, X.-K.Gao* and A.J.Friedhoff, Millhauser Labs., New York Univ.School of Medicine, New York, NY 10016

The differential effects of D2 antagonists on rat brain functional activity has been reported to show selective effects on substantia nigra (SN) and ventral tegmentum (VTM). We have studied the relative interaction between ipsilateral VTM, SN, amygdala (AMY), frontal cortex (FC), caudate nucleus (CN) and nucleus accumbens (ACC). Animals were then studied with at least one session each with baseline and (2mg/kg,i.p.) haloperidol (HAL) and separately with baseline and (5 mg/kg) clozapine (CLO) at 15 minute intervals after injection of drug. Each record was analyzed for coherence within wavebands from 1.0 to 30 Hz. With CLO, there is a time-dependent increase in the influence of SN on AMY activity, in the 4-10 Hz wave band, and a decrease in the 12-16 Hz waveband. With CLO there, is an increase in VTM coherence with the AMY and SN and ACC in the 1-8 Hz frequency bands, but a decrease in the same frequency bands in the frontal cortex. With HAL there is a time-dependent increase in the influence of SN activity in the 4-8 Hz frequency bands upon CN and AMY, and a significant decrease in the 8-16 Hz activity that is shared by the SN and the ACC. With HAL, there was no change in the relationship between SN and VTM unlike CLO. We conclude that these differential effects of CLO and HAL will provide more direct information about the pharmacological effects of receptor pharmacology at mesencephalic and diencephalic cell groups and their influence on the functional and adaptive activity in distal, more rostral areas receiving input from those sites.

291.12

IMPROVED D-2 DOPAMINE (DA) RECEPTOR AUTORADIOGRAPHY USING 3H-YM-09151-2, A POTENT BENZAMIDE.
B.L. Waszczak and R.F. Cox. Pharmacol. Sect., Northeastern Univ., Boston, MA.

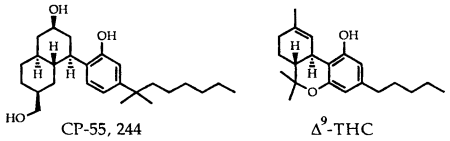
Dopamine D-2 autoradiography is usually performed with 3H-spiperone. A major disadvantage of spiperone is its affinity at α-1, 5-HT2 and spirodecanone sites. 3H-raclopride has better D-2 selectivity but possesses relatively low affinity. A 3H-form of YM-09151-2 (YM), a potent and selective D-2 antagonist, has recently become available and has been used to label D-2 sites in striatal homogenates. We have developed an autoradiographic assay using 3H-YM in order to permit measurement of losses of DA D-2 receptors after treatments with dopamine receptor alkylators (see Cox, Martin and Waszczak, this meeting). Slide-mounted slices of rat brain (12 u) were incubated with 3H-YM at room temperature in a buffer of 25 mM Tris, 100 mM NaCl, 1 mM MgCl2, 1 uM pargyline and 0.001% ascorbate, pH 7.5. Sections were exposed to Hyperfilm for 3 to 4 weeks and regional film densities were analyzed using a computer-assisted imaging system. Association studies indicated that binding equilibrium occurred within 2 to 3 hours. Saturation studies showed a mean Kd of 85 ± 10 pM with regional Bmax values of 0.77 ± 0.04, 0.37 ± 0.04, and 0.49 ± 0.03 pmol/mg protein in striatum, nucleus accumbens and olfactory tubercle, respectively. Non-specific binding, defined in the presence of 50 uM dopamine, was 10-40% of specific binding over a wide range of 3H-YM concentrations (1-1000 pM). Antagonist competition curves fit one-site models and displayed the expected order of potency for D-2 receptors, i.e. (-) sulpiride > mianserin > SCH 23390. Competition by the agonist dopamine resulted in an expected shallow displacement curve best described by a two-site fit. The very high affinity and selectivity of 3H-YM for D-2 sites combine to make this ligand a superior choice for autoradiographic assessment of dopamine D-2 receptor binding.
(Supported by NS 23541.)

291.13

TOTAL SYNTHESIS AND BIOLOGICAL STUDIES OF POTENTIAL AFFINITY LIGANDS FOR THE CANNABINOID RECEPTOR.

S. Richardson,° S. Mirsadeghi,°* A. Lynn,†* G. Wilken,§* M. R. Johnson,Σ * L. S. Melvin,¥* M. Herkenham,† A. Howlett,§ K. C. Rice° °Laboratory of Medicinal Chemistry, NIDDK, Bethesda, MD 20892; †Unit on Functional Neuroanatomy, NIMH Bethesda, MD 20892; §Department of Pharmacology, St. Louis University School of Medicine, St. Louis, MO 63104; ¥Pfizer Central Research, Groton, CT 06340; ΣGlaxo, Inc., Research Triangle Park, NC 27709.

We have prepared a number of non-classical cannabinoids, using the highly potent compound CP-55, 244 as a template for our studies. These compounds bind to the cannabinoid receptor with varying affinities in the nM range. The chemical syntheses of our analogs, binding results, and modulatory effects on adenylate cyclase will be presented, along with our progress to date relating the absolute configuration of CP-55, 244 to that of Δ^9-THC.



CP-55, 244 Δ^9-THC

291.14

AMINOALKYLINDOLE BINDING DATA SUGGESTS A NEW APPROACH FOR STUDYING CANNABINOID ACTIVITY

J. E. Kuster, D. Haycock, A.C. Howlett, B.Subramaniam, S. J. Ward, Dept. Neurosci. Sterling Research Group, Rensselaer, NY; Dept. Pharmacol. St. Louis Univ. Sch. Med., St. Louis, MO.

Aminoalkylindole (AAI) compounds are potent antinoceptive agents. Characterization of AAI binding in rat cerebellar membranes using [3H]-WIN 55,212-2 has revealed a single class of sites (Kd = 2 nM; Bmax = 1.2 pmoles/mg protein) which are saturable, heat-labile, reversible and stereospecific. Of >60 reference compounds tested, only AAIs (Ki 1 nM-3 uM) and cannabinoids compete for this site. Inhibition of binding by AAIs and cannabinoids (1) is competitive (2)correlates with ability to inhibit electrically-induced contractions in mouse vas deferens (MVD) (r=0.98, P<0.001) and (3) correlates with binding affinity in a CP55-940 cannabinoid binding assay (r=0.93). Some AAI analogs produce concentration-dependent rightward shifts in the concentration-effect curves of 55212-2 and THC (pA2 6.5) in the MVD. These data suggest that AAIs & cannabinoids appear to share a common site of action and that AAIs may provide a route to the synthesis of cannabinoid antagonists.

291.15

CHARACTERIZATION AND LOCALIZATION OF AMINOALKYLINDOLES (AAI) IN RAT BRAIN. E.M. Jansen, S.J. Ward, D.Haycock and V.S. Seybold. Dept. of Cell Biol. & Neuroanat., Grad. Prog. in Neurosci., Univ. of Minnesota, Minneapolis, MN 55455; Sterling Research Group, Rensselaer, NY 12144.

Aminoalkylindoles (AAI) have analgesic activity in rodents and humans. Using [^{3}H]WIN55212-2, AAIs have been shown to bind selectively to cannabinoid receptors in studies of homogenates of rat brain. The localization of cannabinoid receptors in mammalian brain has recently been reported by Herkenham et al., (PNAS 87:1932,1990) using a tritiated analogue of Δ^9-THC (CP55,940 Pfizer). The goal of our study was to determine the regional distribution of [^{3}H]WIN55212-2 binding in rat brain and to compare specific binding determined by an unlabeled AAI analog with that of Δ^9-THC analogue desacetyllevonantrodol. Ligand binding with [^{3}H]WIN55212-2 (specific activity 59 Ci/mmole) was done in 20mM Hepes with 0.5% BSA. Tissue was incubated with ligand for 90 min at 30°C; four 10 min washes gave optimum specific binding. Biochemical studies using fresh frozen rat cerebellum tissue sections indicated that [^{3}H]WIN55212-2 had a K_d of approximately 1nM. Competition studies showed that CP55940 and desacetyl-levonantrodol had K_i's of approximately 1 nM. Autoradiographic studies of [^{3}H]WIN55212-2 (1nM) binding to coronal sections of rat brain showed very high amounts of specific binding in substantia nigra and the internal capsule. Moderate levels of binding were observed in cerebellum (molecular layer), basal ganglia, hippocampus, cingulum and layer VI of the cerebral cortex. There was no apparent difference in the regional distribution of [^{3}H]WIN55212-2 specific binding as determined by competition with an unlabeled AAI analog (1μM) or CP55,940 (1μM). These studies confirm the localization of cannabinoid binding sites in rat brain by a compound structurally unrelated to Δ^9-THC. Studies funded by Sterling Research Group.

291.16

THE ONTOGENY OF ADENOSINE A1 RECEPTORS IN RAT BRAIN USING RECEPTOR AUTORADIOGRAPHY. B.A. Etzel and R. Guillet. Dept. of Psychology, Univ. of Rochester, Rochester, NY 14627.

Previous research using brain homogenate assays with rats 14-90 days of age has shown that specific binding to adenosine A1 receptors (A1R) increases as a function of age in cerebellum (with adult levels evident at 42 days) and hippocampus (adult levels at 21 days), but not in cortex, brain stem, or hypothalamus (adult levels at approximately 14-21 days for these three areas). Autoradiography was performed to further specify the regional ontogeny of A1R in the above areas as well as others.

Frozen brain sections from rats 14, 18, 21, 31, and 42 days of age (corresponding to ages at which homogenate assays were done previously) were treated with adenosine deaminase and incubated with 3H-cyclohexyladenosine (3H-CHA) in the presence or absence of L-phenylisopropyladenosine. Sections were examined for specific and nonspecific 3H-CHA localization after a 4-week exposure period.

3H-CHA binding was confined primarily to the hippocampus (CA1, CA2, and CA3) and the thalamus at the youngest age studied (14d). With advancing age, binding to the molecular layer of the cerebellum became more apparent and binding appeared to become more localized to the CA1 region of the hippocampus and less to the CA2 and CA3 regions. Thalamic staining was relatively unchanged with age. Little staining of brain stem or hypothalamus was evident at any age. Cortical staining was nonhomogeneous; its regional ontogeny is currently being examined in more detail. In addition, quantitative analysis of 3H-CHA binding is underway.

Thus, adenosine A1 receptor autoradiography corroborated initial findings using the tissue homogenate assay. It also allowed more specific localization of adenosine A1 receptors in the developing rat as a function of age.

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291.17

HIGH AFFINITY HISTAMINE BINDING IN THE MAMMALIAN BRAIN.

Paul Cumming, Chris Shaw[1] and Steven R. Vincent, Kinsmen Laboratory of Neurological Research, Dept. of Psychiatry, & [1]Dept. of Ophthalmology, University of British Columbia, 2255 Wesbrook Mall, Vancouver, B.C. Canada V6T 1W5.

In addition to the two classical histamine receptor types, a pharmacologically distinct receptor, H_3, has recently been characterized. H_3 was initially described as an autoreceptor, because specific agonists and antagonists altered the synthesis and release of histamine in slices. In the present study, the binding of the H_3 agonist [^{3}H]-N-methylhistamine was characterized in 25 μm cryostat sections from rat forebrain: The K_d was 2 nM and the B_{max} was 25 fmole/section. [^{3}H]-Histamine binding under similar conditions yielded a K_d of 8 nM and a B_{max} of 22 fmole/section. Displacement studies with thioperamide, burimamide, R(-)α-methyl-histamine, and impromidine indicated that the two ligands bound to an identical site, the high affinity histamine site, which had the pharmacological profile of the H_3 receptor.

Autoradiographic studies using [^{3}H]-N-methylhistamine were done in rat, cat and monkey. Specific binding was inhomogeneous and did not match patterns of histamine innervation. Highest binding was observed in the basal ganglia. Unilateral striatal quinolinic acid lesions destroyed histamine binding in the striatum and substantia nigra pars reticulata, but 6-OHDA lesions to the MFB were without effect on striatal H_3 binding. Thus, in addition to their proposed role as autoreceptors, H_3 receptors may be responsible for specific postsynaptic actions of histamine in the basal ganglia.

291.18

LOCALIZING BETA ADRENERGIC RECEPTORS AND THEIR MESSENGER RNA'S IN THE HUMAN EYE

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Several beta adrenergic agents have been clinically exploited for their pressure lowering effects in the treatment of glaucoma. In spite of their wide use, their targets and mechanisms of action in the eye remain unclear. Using in situ hybridization and in vitro autoradiography, sections of entire human eyes were examined for the distribution of beta adrenergic receptors and the mRNA's encoding them. The beta 2 adrenergic receptor had several specific loci of concentration in the anterior segment, including the epithelial and endothelial layers of the cornea, the trabecular meshwork, the ciliary body, and the lens. Both the retina and the retinal pigment epithelium were clearly identified in the posterior segment of the eye. Beta 1 receptors were less inhomogeneously distributed than were the beta 2 receptors, and overall, were of lower density. Quantitative analysis of the distribution of each of these receptors was performed using computerized densitometry. The maps of beta 1 and beta 2 receptors obtained using receptor autoradiography, and of their corresponding messenger RNA's obtained with in situ hybridization, show close correlation. Noted differences between the methodologies will be discussed. These results may be important in understanding the effects and side effects of beta adrenergic therapy in the human eye.

292.1

A DIURNAL COMPONENT TO STRESS RESPONSIVENESS IN THE RAT. M. Bradbury, S.F. Akana, C.S. Cascio*, K. Scribner*, C.D. Walker* and M.F.Dallman*, Division of Neuroscience, UCSF. San Francisco CA, 94143.

In most rat studies, stress-induced increases of adrenocorticotropin (ACTH) and corticosterone (CORT) are largest in the A.M. when these hormones are at their basal, or unstressed, circadian minima. These results have been commonly ascribed to circadian patterns of CORT mediated negative feedback. To study diurnal stress responses in both the presence and absence of CORT mediated negative feedback, we compared the responses of both normal and adrenalectomized (ADX) rats to restraint stress.

Male rats, ADX or sham-ADX 5 days previously, (160-180g), were stressed with restraint for 3 to 90 minutes in either the A.M. or the P.M. Plasma ACTH and CORT concentrations were measured to assess responses to both the development of and recovery from the stress.

In sham-ADX rats, the peak response to restraint stress occured at 30 minutes. The ACTH response was larger in the A.M. than in the P.M. (595 ± 55 vs 360 ± 52 pg/ml) (915% vs 396% of basal ACTH, both $p \leq 0.05$). There was no diurnal difference in the recovery from the stress. Basal P.M. CORT was 18 times higher than that in the A.M.(0.6 ± 0.1 vs 10.6 ± 1.0 µg CORT/dl). 25µg CORT/dl, a concentration of CORT sufficient to fill ~50% of the type II glucocorticoid receptors, (Reul and DeKloet, 1987), was reached at 3 minutes in the P.M., but not until 15 minutes in the A.M. This difference in the rate of receptor occupation may explain the A.M.-P.M. difference in stress responses in sham-ADX rats.

Surprisingly, the same diurnal pattern of stress-induced ACTH secretion was found in ADX rats. In ADX rats, the A.M. peak was 1407 ± 85 pg/ml ACTH (428% of basal ACTH, $p \leq 0.05$) while in the P.M., the response was negligible. A depletion of pituitary ACTH content does not explain the lack of response in the P.M. ADX rats. Therefore, we suggest that in addition to a diurnal difference in the timing of negative feed back signals, there is a neuronal input that facilitates stress-induced ACTH responses in the A.M.

292.2

STRESS FACILITATES SUBSEQUENT ACTIVITY IN THE ADRENOCORTICAL SYSTEM. S.F. Akana and M.F.Dallman*, Dept.of Physiology, UCSF, San Francisco CA 94143-0444.

Stress activates the hypothalamic-pituitary-adrenal (HPA) axis yielding increased ACTH and corticosterone (B) secretion; B alone inhibits subsequent ACTH, but B with stress does not. Logically, this suggests that stress facilitates subsequent activity in the adrenocortical system. We used cyanoketone (CK), an inhibitor of B synthesis, to impair B responses to stress in a test of whether stress-induced facilitation occurs. Restraint stress of 30 min accompanied by tail-nick blood collection (0,15 & 30 min) was imposed on separate groups of vehicle (VEH) and CK rats at different times in the 12:12 light cycle (0,3,6,9 h after lights on);rats were returned to their home cages and then decapitated at 12 h (time of peak diurnal activity) together with unstressed rats. There was a negative correlation ($r=-53; p<0.05$) between the time of restraint and ACTH or B at 12 h in VEH. By contrast, there was a positive correlation ($r=50; p< 0.05$) between the time of restraint and ACTH (no relationship to B) in CK. We conclude that when the B response to stress is blocked (by CK), an underlying, time dependent, stress-induced facilitation of subsequent HPA activity is revealed; when there is a B response to stress, both time dependent facilitation and B-induced inhibition occur (supported in part by DK28172).

292.3

PULSATILE ACTH AND CORTISOL IN GOATS: EFFECTS OF INSULIN-INDUCED HYPOGLYCEMIA AND DEXAMETHASONE. M. Carnes, M. Brownfield, S. Lent,* and Vet. School Classes of 1991-92*. VA Hospital and Univ. of Wisconsin Dept. of Medicine and School of Vet. Med., Madison, WI 53705.

ACTH and cortisol (cort) are secreted in episodic bursts. We investigated the pattern of ACTH and cort response to the stress of hypoglycemia and the effect of dexamethasone (dex) on this response. Five goats were given dex (0.1 mg/kg) and 5 were given saline 2 h before blood sampling. Blood samples were taken from jugular catheters every 2 min for 60 min before and 60 min after insulin (2.5 U/kg, i.v.). Plasma was assayed for ACTH, cort (all samples), and glucose (selected samples). Data sets were analyzed for pulses with the Cluster Analysis program. Plasma glucose was lower than pre-insulin levels at 10 min with a nadir 30-60 min post-insulin in all goats. Control goats showed a rapid rise in ACTH and cort beginning 30 ± 10 min post-insulin. Analysis of pulse parameters for 30 min segments suggested a relatively quiescent secretory period before the rapid rise in ACTH and cort with a reduction in pulse frequency and amplitude compared to preceding and succeeding time intervals. The highest autocorrelation between ACTH and cort occurred at a lag of 0 min. Dex-treated goats had lower mean ACTH and cort levels, no apparent change during hypoglycemia, and lower autocorrelation with no consistent lag.
Supported by US-DVA and NIH grant 1RO1-DK-40759.

292.4

BASAL AND STRESS HYPOTHALAMIC-PITUITARY-ADRENAL ACTIVITY IN CYCLING AND OVARIECTOMIZED-STEROID TREATED RATS. V.VIAU & M.J.MEANEY ,Douglas Hospital Research Ctr.,Dept.Psychiatry,McGill Univ.,Montreal H4H 1R3,Canada.

To determine the relationship between HPA activity and gonadal steroids we have examined the adrenocorticotrophin (ACTH) and corticosterone (CORT) responses to 20 min. of immobilization stress during different phases of the estrous cycle. Peak ACTH and CORT responses following stress were significantly higher during Proestrus (PRO) versus Estrous (EST) and Diestrous (DI) phases: ACTH; PRO > EST = DI; 432.6 ± 65.7, 188.9 ± 25.7, and 194.0 ± 19.5 pg/ml, respectively. CORT; PRO > EST = DI; 62.7 ± 2.5, 50.5 ± 3.8, and 42.5 ± 2.6 ug/dL, respectively. Basal AM HPA activity was equal between phases prior to stress. To demonstrate the role of gonadal steroids,we mimicked the estrous cycle with physiological doses of estradiol (E) and progesterone (P). Rats were OVX and either maintained on low E and P (O') for 3 days, O' plus high E (O' + E'), or with both high E and P (O' + EP'), to mimic Diestrous, Proestrous, and late Proestrus-early Estrous phases, respectively. In response to immobilization, ACTH levels were significantly higher in the E' versus the O' and EP' groups: 336.2 ± 66.4, 214.5 ± 36.0, and 169.5 ± 33.7 pg/ml, respectively. However,both the E' and EP' groups showed increased CORT levels. Taken together, it appears the HPA axis is most sensitive to stress during Proestrus. These results suggest that estrogen regulates the HPA response to stress during the esrous cycle in the rat. More specifically the data are suggestive of gonadal influence on both the activational and feedback components of HPA activity.

292.5

HYPOTHALAMIC-PITUITARY-ADRENAL ACTIVITY IN FRONTAL CORTEX LESIONED RATS. D. DIORIO AND M.J. MEANEY, Douglas Hospital Research Center, Depts of Psychiatry and Neurology and Neurosurgery, McGill Univ., Montreal, H4H 1R3, Canada.

High densities of corticosteroid receptors (CSR) are found in the frontal cortex (FC) and can be regulated by corticosterone (CORT) in a manner similar to that observed in the hippocampus; CORT treatment results in a downregulation of these receptors while an upregulation is observed following adrenalectomy. The results of earlier electrophysiological studies (Feldman and Conforti, 1985) have implicated a role for the FC in the regulation of the HPA axis. In order to examine the influence of the FC on the HPA axis, we measured ACTH and CORT levels under both basal and stress conditions in FC lesioned rats.

Following a 20-min immobilization stress the lesioned animals showed significantly ($p < 0.05$) higher plasma ACTH levels 20 and 40 min following the termination of stress. Likewise, plasma corticosterone levels were also significantly ($p< 0.05$) elevated in lesioned animals following stress. Hormone levels did not differ during immobilization. In contrast to these findings, our preliminary data indicate that lesioned animals do not differ from controls in either ACTH or corticosterone levels following ether stress. Basal values (10AM and 10PM) of both CORT and ACTH appear to be unchanged in the lesioned group. Type II CSRs in the hippocampus, which have previously been shown to regulate the HPA stress response, were unchanged in the FC lesioned animals (lesion=88 ± 11.6; sham= 99 ± 16.8; control=111 ± 16.8). Preliminary data indicate presence of both Type I and Type II CSRs in the FC. Current studies are examining the specific role of these receptors subtypes in the FC in the regulation of HPA activity. These findings suggest that the FC is involved in post-stress corticosteroid negative-feedback inhibition of HPA activity, and that this involvement may be determined by the nature of the stressor.

292.6

COMPARISON OF TWO COMMERCIAL KITS FOR MEASURING HUMAN PLASMA ACTH. E.H. Mougey, M.A. Oleshansky and J.L. Meyerhoff. Department of Medical Neurosciences, Walter Reed Army Institute of Research, Washington, D.C. 20307.

Adrenocorticotropic hormone (ACTH) is an important component of the hormonal responses to stress. Secreted by the corticotrophic cells of the pituitary it is transported via the general circulation to the adrenal glands where it initiates synthesis and release of cortisol in humans. For measuring levels of ACTH in plasma (EDTA) samples obtained during stress experiments, RIA kits obtained from INCSTAR Corp. were compared with IRMA kits obtained from Nichols Institute. Extracted and non-extracted samples were compared. PrepPAK-500/C_{18} was used for extraction of the plasma samples. The RIA antibody was generated against a conjugate of human ACTH (1-24), whereas, the IRMA is a sandwich assay involving the use of two different antibodies sensitive to opposite ends of the ACTH molecule. Overnight incubation in 12X75 mm polypropylene tubes was employed and aspiration was used to remove supernatant in both assays. The RIA tubes were maintained at $4^{o}C$ throughout the assay including the addition of second antibody. The IRMA was carried out at room temperature.

Both assays appear to detect stress-induced changes in plasma levels of ACTH. In general, the extracted vs direct assay values using the IRMA were comparable, whereas, when using the RIA the extracted values were sometimes lower than the direct assay values. The direct assay IRMA values were more similar to the extracted RIA values than to the direct assay RIA values.

292.7

N-METHYL-D-ASPARTATE (NMDA) INCREASES PLASMA LEVELS OF ADRENOCORTICOTROPIN (ACTH) IN POSTNATAL RATS. M.E. Bardgett, G.T. Taylor*, K.E. Coyne* and J.M. Farah. University of Missouri-Saint Louis and G.D. Searle & Co., St. Louis, MO 63121.

NMDA is reported to accelerate the onset of puberty in rats by precocious elevation of gonadotropin (Urbanski & Ojeda, Neuroendocrinol. 46:273, 1987). Recently, NMDA was found to increase ACTH in adult male rats (Iyengar et. al., Neuropharmacol. 29:299, 1990) indicating a role for excitatory amino acid neurotransmission in activation of the hypothalamic-pituitary-adrenal (HPA) axis. In rats, the HPA exhibits reduced responsive to stress during the postnatal period but the duration of this stress hyporesponsive period (SHRP) might be susceptable to developmental alteration by NMDA. Female and male rats at either postnatal day (PND) 10 or 21 were injected with NMDA (30 mg/kg, sc) or vehicle and trunk blood was collected 15 min after treatment. T-tests were used to compare the values of ACTH measured by radioimmunoassay. NMDA increased plasma ACTH 2-fold in both females ($p<0.002$) and males ($p<0.004$) at PND 10; similar increases were evoked by NMDA treatment at PND 21 in both females ($p<0.001$) and males ($p<0.04$). These results indicate that the postnatal quiescence of the HPA can be overcome by an excitatory amino acid receptor agonist, a finding which may facilitate studies of the SHRP during maturation of the this neuroendocrine system.

292.8

THE EFFECT OF LEARNED HELPLESSNESS BREEDING ON CORTICOSTERONE AND ACTH REGULATION., F.A.Henn, M. McKenzie* and E. Edwards . Dept of Psychiatry, SUNY at Stony Brook, NY 11794.

A selective breeding of Sprague Dawley rats for Learned Helplessness has been established in our laboratory and has reached the 19th generation of two strains of rats : LH (showing susceptibility to Learned Helplessness); NLH (a strain resistant to the development of helpless behavior).

We have examined some neuroendocrine parameters of rats from either strain LH and NLH and compared them with naive Sprague Dawley rats. Plasma corticosterone levels of LH strain rats were remarkedly low (38 ± 6.8 ng/ml) as compared to plasma corticosterone levels measured in NLH strain rats and naive Sprague Dawley rats (300 ± 27 ng/ml). There was no difference in ACTH levels measured in all three groups.

When Sprague Dawley rats and rats from the LH and NLH strain are subjected to the Learned helpless paradigm (shock training and shock escape testing), they could also be differentiated by their overall response to the stress paradigm. We measured plasma corticosterone in these three groups of rats one day after the shock escape test. While plasma corticosterone were similar to endogenous levels in Sprague Dawley rats and rats from the NLH strain, a significant increase in corticosterone levels was still present in rats from the LH strain (+42% of endogenous levels).

Changes in endogenous and post stress plasma corticosterone were seen in both male and female LH strain rats. The present data give additional support to our hypothesis that a dysregulation of the HPA axis mediates Learned Helplessness. (Supported by BNS 8614098 to E.E.)

292.9

CHANGES IN CORTISOL AND ß-END-LIKE IMMUNOREACTIVITY IN PERIMENSTRUAL SYNDROME. C.A. Cahill. University of Arizona, College of Nursing, Tucson, AZ 85748.

The cause of Perimenstrual symptoms (PS) is not clear, but the effects of changes in some hormones have been investigated. Nine women with PS like endogenous depression and 18 women without symptoms were studied over three consecutive cycles. Plasma samples were collected by acute venipuncture twice per week. ß-END-like immunoreactivity, cortisol, prolactin and progesterone were determined by RIA.

Endogenous depression has been associated with alterations in regulation of the hypothalamic/pituitary/adrenal axis. Similar differences in regulation of the hypothalamic/pituitary/ovarian axis may be associated with similar symptoms in PS. Since cortisol is secreted from the adrenal in response to ACTH and ACTH and ß-END are co-secreted from the anterior pituitary, cortisol and ß-END-like immunoreactivity were expected to be correlated. In this study, they were not correlated in either group. In the third cycle, a trend toward correlation was observed. Therefore, "stress" associated with blood collection may influence these levels.

292.10

REGULATION OF POMC PRIMARY TRANSCRIPT LEVELS IN THE ANTERIOR PITUITARY IS TIGHTLY LINKED TO PEPTIDE SECRETION. S.P. Kwak, E.A. Young, H. Akil, and S.J. Watson. Neuroscience Program and Mental Health Res. Inst. University of Michigan, Ann Arbor, MI 48109.

We have successfully detected the heteronuclear species of pro-opiomelanocortin (POMC) RNA from single anterior pituitaries by northern blot analysis. Initially, we characterized the primary POMC transcript by probing a blot containing total RNA from the anterior pituitary with an intron A specific cRNA (all genomic clones were gifts from Dr. J. Roberts). Two major bands migrating at 6.0 Kb and 4.1 Kb were detected. The size of these fragments matched the predicted length of the primary transcript (hnRNA) and the intron A containing processing intermediate of POMC, respectively, and further analysis confirmed that these two species are localized only in the nucleus. Based on the genomic sequence an intron B containing processing intermediate is predicted to migrate at 3.0 Kb. However, fragments of such size were not detectable from the nuclear fraction when reprobed with an intron B specific cRNA. Furthermore, a cRNA probe from the exon 3 region (common to all nuclear intermediates) detected only the 6 and 4 Kb bands along with the 1Kb mRNA species. The results are consistent with the data obtained from S1 nuclease assays (Autelitano et al., 1990) and suggest that intron B is spliced more rapidly than intron A in the anterior pituitary. Physiological manipulations were conducted to determine the changes in the hnRNA levels. Anterior pituitaries from adult male rats were assayed after various conditions. Acute 30 minute swim produced a 133% increase in POMC hnRNA levels over controls. Acute swim after a chronic treatment of 14 days resulted in a 240% increase over chronic rest levels. Injection of metyrapone, a steroid synthesis inhibitor, increased hnRNA levels within 1-2 hours. Finally, preliminary circadian studies indicate that there may be an upregulation of POMC transcription in the evening when ACTH secretion is increased. Parallel changes observed in hnPOMC levels and plasma ACTH suggest that POMC transcription is closely coupled to ACTH secretion in the anterior pituitary. (Work supported by NIMH 42251-04 to SJW and HA.)

292.11

EFFECT OF IMIPRAMINE ADMINISTRATION AND SWIM STRESS ON THE HYPOTHALAMIC-PITUITARY-ADRENAL AXIS.. J. F. López, D. M. Vázquez, Stanley J. Watson and Huda Akil. Mental Health Research Institute, University of Michigan, Ann Arbor, MI 48109.

Overactivity of the Hypothalamic Pituitary Adrenal (HPA) axis is a well documented phenomena in depressed patients. Depression is most commonly treated with tricyclic antidepressants, therefore the potential effect of these medications in modifying HPA activity is an important issue. We have investigated the effect of chronic imipramine (IMI) administration (10 mg/kg) in the HPA axis of rats undergoing 14 days of swim stress and in non-stressed rats. Twenty four male rats were divided in 4 groups: saline (S), IMI (I), swim + saline (SS), swim + IMI (SI). All received i.p. injections of either IMI or saline for 14 days and were decapitated on day 15. Corticosterone (CS), ACTH and Nacetyl β endorphin (NAc βE) were measured in plasma. ACTH, β endorphin (βE) and POMC mRNA content was measured in the pituitary anterior lobe (AP). NAc βE, aMSH and POMC mRNA content was measured in the neurointermediate lobe (IL). Chronic swim stress caused a significant CS increase. Chronically stressed rats treated with IMI showed a significant decrease in CS levels, but these values were still higher than the non-stressed groups. An ANOVA revealed a significant effect in plasma Nac βE for swim but not for drug treatment. No significant differences in plasma ACTH were detected. In the IL, IMI caused significant decreases in both POMC peptide content and POMC mRNA. In the AP, no differences were found in ACTH, βE content, or POMC mRNA levels, although chronic swim animals had a tendency for higher peptide content. We conclude that chronic IMI administration may alter CS levels in plasma. IMI also seems to cause an inhibition of POMC IL activation. Because of the multiple neurotransmitter effects of IMI, more specific pharmacological agents are needed to elucidate the mechanisms of this inhibition. Supported by a grant from The Robert Wood Johnson Minority Medical Faculty Development Program (JFL.) and MH422251 ((SJW, HA)

292.12

BIPHASIC CORTICOTROPH SECRETION RESPONSE TO SHORT TERM METYRAPONE. E.A. Young, S. Kwak, S.J. Watson and Huda Akil Mental Health Research Institute, Ann Arbor, MI 48109

Metyrapone is a compound that inhibits the 11-β-hydroxylase reaction in glucocorticoid synthesis, resulting in the production of 11-deoyxcorticosterone, in rats, or 11-deoxycortisol in man. When administered in humans, metyrapone blocks cortisol synthesis and results in stimulation of ACTH secretion 4-10 hours following metyrapone administration. To study the effects of short term glucocorticoid inhibition on the HPA axis in rats, metyrapone was administered by subcutaneous injection every 8 hours for 24 and 72 hours, beginning in the evening, with a final injection 1 hour before sacrifice. At 24 hours, no change in plasma β-endorphin levels were observed. At 72 hours, β-endorphin plasma levels were elevated. However, pituitary content data suggested that a secretory peak had occurred before 24 hours. To pursue this finding, shorter time periods were examined. By 30 minutes following the initial injection of metyrapone, there is clear corticotroph secretion of both ACTH and β-endorphin, which precedes the steroid inhibitory effect. This suggests that metyrapone acts as a stressor at initial injection. The ACTH plasma levels remains elevated at 1 and 2 hours, but have returned to near normal levels by 4 hours. No increases in corticotroph secretion were observed at 8, 12 or 24 hours. Corticosterone data suggest that the block of corticosterone is incomplete, and thus results in relative hypocortisolism, at 24 hours. However, at 72 hours, when ACTH levels are highest, the efficacy of the metyrapone blockade is low with 75% of the adrenal secretory product being authentic corticosterone. The mechanisms by which the secretory response to metyrapone disappears following the initial injection, then reappears at 72 hours are unclear. However, it is possible that non-steroidal feedback mechanisms may play a role.

292.13

GENOMIC EFFECTS OF COLD AND ISOLATION STRESS ON VASOPRESSIN mRNA-CONTAINING CELLS IN THE HYPOTHALAMUS OF THE RAT, J.A.Angulo, M.Ledoux*, and B.McEwen, Laboratory of Neuroendocrinology, The Rockefeller University, New York, N.Y.

We assessed the effects of cold and isolation stress on arginine vasopressin (AVP) mRNA in the paraventricular (PVN) and supraoptic nuclei (SON) of the hypothalamus. AVP mRNA levels were determined by **in situ** hybridization histochemistry. In the magnocellular neurons of the PVN isolation stress for 7 or 14 days increased AVP mRNA levels 28 and 29%, respectively, compared to group-housed controls. No significant alterations in vasopressin gene expression were observed in the SON after 7 or 14 days of isolation stress. AVP mRNA-expressing cells of the parvocellular PVN showed increases of 19 and 34% after 7 and 14 days of isolation, respectively. We also studied the effect of cold or combined cold and isolation stress on vasopressin gene expression in the PVN and SON. Cold-stress for 3 h daily for 4 consecutive days increased AVP mRNA levels in the magnocellular PVN by 15%. Cold-isolated animals showed an increase of 21%. No significant effect on AVP mRNA levels in the SON was observed. In contrast to the magnocellular PVN, cold or cold-isolation stress increased AVP mRNA in the parvocellular region of the PVN by 25 and 43%, respectively, relative to control rats. These results suggest that increased expression of AVP in the PVN may be a compensatory mechanism permitting the organism to cope with the adverse effects of stress.

292.14

Differential changes in the hypothalamic AVP mRNA and peptide levels in rats after exposure to cold and novel environments. P. Wu, D. Rougeau and G. Childs. University of Texas Medical Branch, Galveston, Texas 77550.

Arginine vasopressin (AVP) mRNA and peptide levels were studied in male rats exposed to cold and novel environment. AVP mRNA was detected in hypothalamic frozen sections with a 45-mer photobiotinylated oligonucleotide probe and streptavidin alkaline phosphatase. mRNA and peptide immunolabeling were quantified by the Cue-3 image analysis system. Exposure to +3--5 C for 30 min caused a 5.4--fold increase in AVP mRNA in the paraventricular nucleus (PVN) compared with that of control rats and a 1-4--fold elevation in serum ACTH. After 30 min of a novel, thermoneutral (24 C) environment there was a 3.6--fold increase of AVP mRNA in the PVN, and no significant changes in serum ACTH. No changes were observed in AVP mRNA in the supraoptic nucleus (SON) following exposure to either cold or novel environments. Furthermore, neither stress caused significant changes in the storage of AVP peptide in the PVN, SON, median eminence and posterior lobe of pituitary. This *in vivo* study demonstrates that PVN and SON neurons respond differentially to cold and novel environment exposures. The increased synthetic activity of AVP in the hypothalamus correlated with the elevation of serum ACTH, suggests that AVP may play a role in regulation of pituitary-adrenal responses to cold and novel environment stresses. Supported by Navy Contract # N00014-88-K-0016.

292.15

VASOPRESSIN OF SUPRACHIASMATIC ORIGIN CONTROLS CORTICOSTERON RELEASE. R.M. Buijs, A. Kalsbeek*, T.P. van der Woude*, J.J. van Heerikhuize*. Netherlands Institute for Brain Research, Amsterdam, The Netherlands.

The suprachiasmatic nucleus (SCN) is the major central nervous system pacemaker responsible for many behavioural and endocrine rhythms. The efferent projections of this nucleus are relatively well established and are largely confined to hypothalamic and thalamic brain structures. A major target area of SCN fibres appears to be the paraventricular dorsomedial hypothalamic nucleus, a major area in the control of CRF release. The physiological significance of these efferents was investigated using experiments whereby vasopressin (VP) or vasoactive intestinal peptide was infused in this SCN projection area. It appeared that in SCN lesioned rats VP was able to suppress elevated plasma corticosteron (B) levels to basal daytime values. On the other hand, infusion of VP antagonists induced a ten-fold increase of basal B levels in intact rats, but it remained without effect in SCN lesioned rats. These results demonstrate that via its peptidergic neurotransmitters the SCN controls the release of this important stress hormone and that via these peptidergic transmitters the SCN may execute its rhythmic functions.

292.16

THE PITUITARY-ADRENAL AXIS RESPONSE TO ARGININE VASOPRESSIN IN PATIENTS WITH DEPRESSION. BT Carroll, WH Meller*, RG Kathol, TL Gehris*, J Carter*, SD Samuelson, and AF Pitts. The University of Iowa College of Medicine, Iowa City, IA 52242.

Vasopressin, a hypothalamic neuropeptide, has been used to investigate hypothalamic-pituitary-adrenal axis functioning. Previous studies with arginine vasopressin (AVP) have demonstrated that cortisol response is greater in depressed patients than in controls.

AVP (0.18 pressor units/kg) was given intramuscularly to 10 patients with major depression (MD) and 15 normal controls. Plasma ACTH and cortisol levels were drawn at specific times prior to and after injection. A one mg overnight dexamethasone suppression test (DST) was also done on each subject. Six (60%) MD were DST-nonsuppressors (post-DST cortisol level > 5.0 μg/dl).

The MD group had non-significantly lower ACTH baseline, peak, delta max levels and net area under the curve (AUC) and significantly lower gross ACTH AUC when compared to controls ($t=2.12$; $p=.045$). No significant differences were observed for any of the absolute cortisol measurements. The MD group tended to have an increased cortisol response to endogenous ACTH as estimated by the gross cortisol AUC to log gross ACTH AUC ratio ($t=1.85$; $p=.078$). No significant differences were observed for ACTH or cortisol levels when groups were separated with respect to DST suppressor status.

This study replicates a previous study (Meller, WH, et al., *J. Psychiat. Res.*, 21:269, 1987) and supports the hypothesis that there is no vasopressin receptor downregulation as suggested for corticotropin-releasing hormone in depressed patients. Furthermore, we also observed an adrenal hyperresponsiveness to endogenous ACTH.

292.17

POSTNATAL DEVELOPMENT OF FMRF-NH_2-LIKE PEPTIDE, Arg^8-VASOPRESSIN AND DYNORPHIN IN PITUITARY GLANDS OF RATS. H.-Y.T. Yang and J. Rubenstein, Lab. of Biochem. Genetics, NIMH, St. Elizabeths, Washington, DC 20032.

Neuropeptide Phe-Leu-Phe-Gln-Pro-Gln-Arg-Phe-NH_2 (F-8-F-NH_2), initially detected by the FMRF-NH_2 antiserum, was isolated from bovine brain and found to have morphine modulating activity. In rats the highest level of F-8-F-NH_2 immunoreactivity (IR) has been detected in the posterior pituitary and there are studies indicating a possible interaction between F-8-F-NH_2-IR and Arg^8-vasopressin (AVP). In order to further understand this interaction, postnatal development of these two peptides in rat pituitaries was studied. Low levels of F-8-F-NH_2-IR were detected in 2 day old rats and a subsequent rapid increase of the F-8-F-NH_2-IR was observed. F-8-F-NH_2-IR was found to remain at a high level at ages around 10 to 20 days and then gradually decline to a steady level by the age of around 30 days. A similar pattern of postnatal development was observed for AVP except that F-8-F-NH_2-IR was found to reach the maximum level slightly ahead of AVP. Dynorphin, known to be colocalized with AVP in the pituitary, was found to have a developmental pattern substantially different from those of F-8-F-NH_2-IR and AVP. These results are in good agreement with the hypothesis that there may be an interaction between F-8-F-NH_2-IR and AVP.

292.18

A POSSIBLE INTERACTION BETWEEN ARG^8-VASOPRESSIN (AVP) AND MAMMALIAN FMRF-NH_2-LIKE PEPTIDE IN THE HYPOTHALAMO-NEUROHYPOPHYSEAL AXIS OF THE RAT. E.A. Majane and H.-Y. T. Yang. Lab. of Biochem. Genetics, NIMH, St. Elizabeths, Washington, DC 20032.

The neuropeptide FLFQPQRF-NH_2 (F-8-F-NH_2), initially detected by FMRF-NH_2 antiserum and subsequently isolated from bovine brain, is highly concentrated in the hypothalamo-neurohypophyseal axis of the rat. Previously, we have shown that F-8-F-NH_2-IR in the pituitary gland of the AVP deficient Brattleboro (DI) rat is below the level of detection. F-8-F-NH_2-IR in pituitary of salt-loaded rats is also greatly reduced. These experiments suggest an interaction between AVP and F-8-F-NH_2-IR in the posterior pituitary. In order to test this hypothesis, we have measured the effect of two different peripheral modes of AVP replacement therapy on F-8-F-NH_2-IR in pituitary and hypothalamus of the DI rat. While continuous infusion of 900 ng/day of synthetic AVP for six days reduces water intake of the DI rat by 50%, the pituitary levels of F-8-F-NH_2-IR are still below the level of detection. Likewise, daily subdermal bolus injections of 500 mU pitressin tannate to the DI rat for six days also fail to restore the F-8-F-NH_2 content to the pituitary although water intake is normalized. These results suggest that the interaction may occur at a central site. Alternatively, the depletion of pituitary F-8-F-NH_2-IR could be related to alterations in dynorphin or oxytocin.

292.19

LiCl INCREASES ADRENAL AMINES IN THE ABSENCE OF ARGININE VASOPRESSIN. E.F. O'Connor, S.K. Naylor* and J.E. Lawler. Physiology Program and Department of Psychology, University of Tennessee, Knoxville, TN 37996.

Arginine vasopressin (AVP) decreases locomotor activity and facilitates memory processes. In these actions AVP is similar to LiCl (Li+). Thus, the adrenal amines may mediate the actions of both agents on behavior. For example, exogenous epinephrine stimulates tissue and behavioral responses similar to those elicited by both AVP and Li+. AVP also increases adrenal amine stores, an action which we have found with Li+. Our hypothesis was that AVP may mediate this action of Li+. To test this, we gave LiCl, 3mmol/kg/day, i.p., to Long Evans rats (LE) and to Brattleboro rats (DI) for 3 weeks. Control LE and DI rats were given saline. Data are means (S.E.M.)

	N	NE(ng/gland)	E(ng/gland)
LE Na+	4	3070 (225)	11092 (1523)
LE Li+	5	3369 (157)a	13982 (1278)a
DI Na+	6	3086 (231)	10207 (874)
DI Li+	5	4497 (385)a,b	15332 (899) a

a=p<.05 vs. strain control, b=p<.05 DI vs. LE. The data suggest that Li+'s actions on the adrenal medulla are independent of AVP.

(Supported by HL-19680)

SOMATIC AND VISCERAL AFFERENTS II

293.1

REGIONAL VARIATION IN SPATIAL RESOLUTION AND SENSITIVITY OF THE HUMAN HAND. H.Hämäläinen, K.Saarhelo*, A.Antervo*and J.Kekoni*. Dept. Psychol., Univ.Helsinki, Finland.

Two-point thresholds (airpuff stimuli) and detection thresholds to mechanical vibration were determined at several locations on the hand and fingers.

Increasing 2-point thresholds were measured on both glabrous and hairy skin areas at more proximal locations. The threshold increase from the volar tip of finger to the palm well coincides with the increasing values of the cortical magnification factor (M^{-1}) determined for corresponding areas of monkey SI cortex (M.Sur et al., J. Neurophysiol. 44:295, 1980). On the glabrous skin lowest thresholds for 20 Hz vibration were measured at distal phalanges of fingers, and those for 240 Hz at palm. On the hairy skin, higher detection thresholds were obtained with all frequencies compared to those for glabrous skin. The detection thresholds for especially 240 Hz vibration were highest at distal locations. These results indicate the basic difference in innervation of the glabrous and hairy skin of the hand and also differences in proximo-distal innervation patterns of these skin areas.

293.2

MECHANOSENSORY RESPONSES FROM AXONS SERVING ALLOGRAFTED PRIMATE SKIN CONTAINING SPARSE OR NONEXISTENT MECHANORECEPTOR POPULATIONS. D.D. Samulack, R.W. Dykes and B.L. Munger*. Dept. of Physiology, McGill Univ., Montreal, PQ H3G 1Y6 and Dept. of Anatomy, Pennsylvania State Univ., Hershey, PA 17033.

The long-term survival of transplanted primate skin (baboon; *Papio h. anubis*) was achieved by host immunosuppression using cyclosporine and methylprednisolone. The stability of the histologic integrity of the allografts was dependent upon the level of immunosuppression maintained and the degree to which the immunologic processes of graft rejection led to tissue disruption. Control (autografted) tissues were compared to allografted tissues which had undergone minimal, minor or major episodes of rejection.

The cellular destruction resulting from allograft rejection, together with processes associated with nerve transection and reinnervation, led to alterations of mechanoreceptor population, structure and physical milieu in these tissues; the most dramatic changes resulted in the total destruction of hair follicles in allografted hairy skin. Yet, well-defined receptive fields for low-threshold rapid and slow adapting responses were able to be identified by recording single axons serving either allografted glabrous or hairy skin. Correlation of the histologic and electrophysiologic data derived from this study further implicates the axon as the site of mechanoelectric transduction in cutaneous mechanoreceptive afferents. The presence of the mechanoreceptor (i) may serve to lower sensory thresholds possibly by optimizing mechanoelectric transduction, and (ii) sharpen tuning curves, thereby providing frequency filtering. Axons in contact with a receptor may also be less likely to fatigue.

293.3

STIMULUS FEATURES RELEVANT TO THE PERCEPTION OF SHARPNESS AND MECHANICAL PAIN. J.D. Greenspan and S.L.B. McGillis*, Depts. of Neurosurgery and Physiology, SUNY Health Science Center, Syracuse, NY 13210

This project examined the influence of stimulus size and shape upon thresholds for the perception of sharpness and mechanically-induced, cutaneous pain. We used an ascending method of limits protocol with 26 subjects. Flat, circular probes were placed on the subject's finger, with a resting force of 0.2-0.5g. One set of probes varied in size, ranging from 0.01-5.00mm^2. A 2nd set were the same size, but varied in the angle at the tip's edge (30-90°).

The threshold for sharpness was consistently lower than the threshold for pain by 20-40g. Thresholds measured as force were similar for probes between 0.01 and 0.1 mm^2: sharpness ≈ 10g; pain ≈ 35g. These thresholds progressively increased with larger probes, but not at an even rate with area. Rather, thresholds increased in proportion to the probe circumference over the range of 0.1-1.0 mm^2. As tip angle decreased, thresholds for sharpness remained constant until 45°, at which time it increased dramatically. In contrast, pain threshold gradually increased over the same range of angles.

The thresholds for sharpness and pain are similarly influenced by stimulus area, but differentially influenced by stimulus angle. The relationship between force and area is not simple, but with flat surfaces, thresholds are more consistent when expressed as force/circumference.

This research was supported by NSF grant #BNS-8808337.

293.4

PSYCHOPHYSICAL MEASUREMENTS OF PRICKING PAIN SENSATION MECHANICALLY EVOKED BY PROBES OF DIFFERENT DIAMETERS. M.K.C. Mengel* and R.H. LaMotte. Yale University School of Medicine, Dept. of Anesthesiology, New Haven CT 06510.

The threshold and magnitude of pricking mechanical pain were determined in human subjects. The stimuli were delivered to the volar forearm by truncated epoxy cones with tip diameters of 150, 200, 250, 300 and 600 μm. The probes were applied manually with a quick indentation and immediate retraction. The cones were mounted to a handle equipped with a strain gauge in order to measure the peak force of skin indentation and the indentation velocity. Subjects estimated the magnitude of the pricking (first) pain sensation produced by each stimulus and also reported the presence of any other sensory quality such as aching and itching.

The threshold of pricking pain (minimal force for evoking pain on 50% of the trials) was found to be inversely proportional to the tip diameter. The thresholds for the smallest and the largest diameter probes differed more than tenfold. The threshold of the 600 μm probe was reached at forces that evoked pain on nearly 100% of the trials with the two smallest diameter probes. The thresholds decreased with increasing force and indentation velocity. Pricking was less frequent and aching pain more prevalent at slower indentation velocities particularly as the diameter of the probe was increased. Both the quality and magnitude of mechanically evoked pain are a function of the diameter of the probe and the force and velocity with which it is applied to the skin. (ONR Contract N00014-88-K0604).

293.5

COOLING HUMAN NERVES AFFECTS TACTILE ROUGHNESS SENSATIONS. J.R. Phillips and P.B.C. Matthews*. University Laboratory of Physiology, Parks Road, Oxford. OX1 3PT. U.K.

Cooling the arm interferes with the transmission of sensory messages from the hand by prolonging the refractory period of nerve fibres and by increasing conduction delay dispersion between fibres. We have employed cooling to investigate which features of the afferent discharge might account for the sensation of roughness. The left arm was cooled between wrist and axilla. Conduction effects were monitored by stimulating the ulnar nerve and recording the F wave in a hand muscle. Subjects scanned the little finger over plastic surfaces embossed with patterns of dots. On the uncooled side, a standard pattern was repeatedly presented. On the cooled side, a series of test patterns with different dot spacings were presented. As the test arm cooled, progressively coarser test patterns were perceived as equivalent to the standard (a 2.6 mm pattern and a 2.0 mm standard were perceived as equally rough.) The reduction in perceived roughness with cooling was not accompanied by a change in the subject's discriminative capacity on the cooled side. The longer refractory period of the cooled afferents would have prevented them transmitting any high frequency discharges evoked by the patterns, thereby reducing the peak-to-peak modulation of the afferent response. The results are consistent with the idea that roughness magnitude increases with depth of modulation of the afferent response.

293.6

SPATIAL LOCATION OF MAGNETIC TRIGEMINAL SOMATOSENSORY RESPONSE BY TACTILE STIMULATION AND CORRELATION OF THE MRI M. Nomura, U. Ribary, L. Lopez, A. Mogilner, F. Lado and R. Llinás. Dept. of Physiology, New York University Medical Center, New York, NY 10016 U.S.A.

Magnetic trigeminal somatosensory responses from human subjects were recorded using a 14-multichannel cryogenic neuromagnetic measuring system (BTi) in order to locate the evoked responses to the tactile stimulation of upper and lower lips.

The stimulation was produced by 15 ms vibration burst from a Piezo electric buzzers at frequencies of 50 Hz, 150 Hz and 250 Hz, given at a rate of 1-2 times per second. Recordings were obtained from the contralateral scalp by using the probe position indicator (Yamamoto et al, '88 Proc. Natl. Acad. Sci). 500 responses were averaged. The spatial locations are being mapped on the MRI of each subject.

The sources activated by upper and lower lips stimulation were separated by approximately 10 mm (using the response to 150 Hz), and their spatial location corresponded to the posterior wall of the central sulcus. Compared with the component of the dipole moment at each stimulating frequency, the stimulation at the frequency of 150 Hz produced the largest response. Moreover, the location of the response shifted with the change of the stimulation frequency.

These results demonstrate that a large area of the somatosensory cortex is utilized for lip representation and suggest that the spatial displacement of the trigeminal somatosensory response may be related to the discrimination of frequency.

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293.7

MECHANICAL SENSITIZATION OF HTMS AND ITS RELATION TO TISSUE COMPLIANCE. B. Cooper, M. Ahlquist*, R. Friedman, B. Loughner*, and M. Heft, Depts. of Oral & Maxillofacial Surgery, Oral Biology and Neuroscience, University of Florida, Gainesville, FL, 32610

Unit recordings from the goat trigeminal ganglion identified two subgroups of HTMs in the goat palate (Cooper et at., 1988; Friedman et al., 1988). One HTM group transduced pressure into the noxious range (intense pressure receptors or IPRs; after Burgess and Perl, 1967). The responses of IPRs to intense pressure were described by power functions that were bounded by pressures which produced a minimum or asymptotic frequency (Pressure-Frequency Threshold and Asymptote, PFT and PFA). PFTs of IPRs matched human pain thresholds (1.59 N/mm^2 vs 1.65 N/mm^2), while activation thresholds did not. PFTs and PFAs of IPRs matched the human pressure-pain range from "WEAK" to "INTENSE" pain.

After carrageenan inflammation (CI) or 5HT, IPR sensitization was manifested as: 1) decreased mean response interval (MRI); 2) increased slope: and 3) decreased PFT and PFA of power functions fit to pressure-interval data (9/12 cases). We then examined the contribution of edema to CI sensitization of IPRs. We observed that CI induced changes in tissue compliance (TC) varied with tissue site (.10 to .078 mm/g, ventral palatal zone; .144 to .173 mm/g) sulcal zone. An artificial edema was used to compare the effect of tissue fluid contesnt to antigen-induced changes in indices of IPR sensitization. These observations were made: 1) Changes in activation threshold followed tissue dependent shifts in compliance, but changes in PFT, PFA and MRI did not (5/6 cases); 2) Changes in slope produced by artificial edema were independent of TC and tissue site (6/6 cases).

In conclusion: 1) IPRs are sensitized by CI or 5HT; 2) Edema contributes to this sensitization via increased power function slope; 3) Edema decreases activation threshold only when edema produces in increases in compliance, and this depends upon tissue site; 4) Changes in PFT, PFA, and MRI are independent of edema and might be due, in part, to 5HT (NIDR DE08701, IRB 5-86, AWA 3377-01, 7136)

293.8

AXONAL ORIGIN OF PROLONGED DISCHARGES OF SOME CUTANEOUS RECEPTORS. J.M. Chung, J.W. Leem, B.S. Chung and K. Sheen. Marine Biomed. Inst., Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX 77550.

A previous study in our laboratory have shown that some afferent axons produce prolonged discharges after complete isolation. We have attempted to identify the type of afferent fibers displaying such activity.

Single unit activity was recorded from a filament dissected from the distal stump of cut sural or plantar nerve of anesthetized rat (Sprague-Dawley). After thorough identification of the receptor type, the nerve was cut at a site between the recording electrode and the cutaneous receptive field, isolating the nerve being recorded completely. Spontaneous activity was recorded up to 1 hour after sectioning the nerve.

Upon sectioning the nerve, many units showed brief injury discharges lasting only a few seconds. However, 6 of 16 units exhibited prolonged discharges at least a half hour after isolation from their receptors. These 6 units included 5 slowly adapting type II mechanoreceptors and 1 Paccinian corpuscle.

These results suggest that prolonged discharges can be produced in the middle of axons of the slowly adapting type II and Paccinian corpuscle afferent fibers. This phenomenon may explain parasthesias in patients often associated with peripheral nerve injuries. (Supported by NIH grants NS21266 and NS11255 and a grant from Bristol-Myers Co.)

293.9

SOME PROPERTIES OF MECHANORECEPTORS IN THE RAT HIND FOOT. J.W. Leem, K. Sheen, B.S. Chung, J.M. Chung and W.D. Willis. Marine Biomed. Inst., Depts. of Anat. & Neurosci. and of Physiol. & Biophys., Univ. Texas Med. Br., Galveston, TX 77550.

The aim of this study was to analyze the properties of mechanoreceptors in the glabrous skin of the rat hind foot.

Single unit activity was recorded from a filament dissected from the sural or plantar nerve of 26 anesthetized (sodium pentobarbital, 50 mg/kg) rats. The receptive fields (RF) of mechanoreceptive units were mapped and various mechanical stimuli were tested.

From a total of 68 mechanoreceptive units identified, 39 were slowly adapting type II mechanoreceptors (SA-II). The remainder were 3 slowly adapting type I, 20 rapidly adapting, and 6 Pacinian corpuscles. The discharge rate of SA-II correlated better to graded forces than to pressure. Five of the 12 SA-II units that generated a resting spontaneous discharge were found to have one or two "inhibitory fields" that situated adjacent to excitatory RF. Mechanical stimuli applied to the inhibitory field depressed the spontaneous discharge.

The results indicate that SA-II are the dominant slowly adapting mechanoreceptors in the glabrous skin of the rat hind foot. For their activation, constant force can be used as a reliable stimulus parameter. It is also suggested that SA-II afferents may play a possible role in a lateral inhibition for spatial discrimination via a peripheral mechanism. (Supported by NIH grants NS21266, NS09743 and NS11255 and a grant from Bristol-Myers Co.)

293.10

SERIAL ELECTRON-MICROGRAPHIC RECONSTRUCTION OF PRESUMED TRANSDUCER SITES IN PACINIAN CORPUSCLES. J. E. Landcastle*, N.B. Slepecky* and S.J. Bolanowski, Institute for Sensory Research, Syracuse University, Syracuse, NY 13244.

The Pacinian corpuscle (PC) is composed of a neurite surrounded by an accessory capsule. The portion of the neurite discussed here is elliptical in cross-section and has filopodial structures projecting only along the major elliptical axis. They are the presumed transduction sites. Based on physiological experiments, we have proposed that two populations of filopodia, organized in morphofunctional opposition, exist. In order to test this, PCs obtained from cat mesentery were processed for electron microscopy using standard techniques. Reconstructions were made of selected regions from several sets of serial thin sections. We evaluated filopodial location, inclusions, shape and size. The density of filopodia was found to be approximately 2.8/μm or over 1,400 per terminal neurite. While the full complement of cellular inclusions (e.g., vesicles, amorphous ground substance, mitochondria at the filopodium base, etc.) were found, there also appeared to be two different types of filopodia: T-shaped, with the arms extending over 0.75 μm (s.d., 0.12μm) parallel to the neurite's long axis and I- or Y-shaped, extending 0.24 μm (s.d., 0 μm) along that direction. The maximum perpendicular projection of both types is roughly 7.4 μm. No obvious relation between filopodial type and location along the neurite was found, although the filopodia seemed to project alternately from either side of the neurite along the proximo-distal direction. That is, filopodia seemed never to project from both sides of the neurite in the same region, although most regions of the neurite did contain a filopodium. It is possible that the two filopodial types contribute oppositely to the receptor potential during the transduction process. Supported by NS23933.

293.11

A VISCOELASTIC CONTACT-MECHANICS MODEL OF SKIN PREDICTING SPATIOTEMPORAL RESPONSE PROPERTIES OF CUTANEOUS MECHANORECEPTORS.

M.N.H. Waller* and J.R.Phillips. University Laboratory of Physiology, Parks Road, Oxford. OX1 3PT, UK.

(SPON: Brain Research Association)

The local physical conditions at the receptor terminal governing the responses of cutaneous mechanoreceptors are unknown and cannot be investigated empirically. We have used contact-mechanics theory to compute the time dependent patterns of stress and strain within the skin resulting from embossed stimuli scanned across the surface. These stress/strain patterns are then compared with neural responses recorded from the three classes of mechano-receptive afferents in order to establish which components of strain determine their response.

Embossed stimuli are modelled as collections of vertical point forces applied to the surface summed with horizontal frictional forces. The skin medium is taken as an ideal, elastic half-space in which the principle of superposition is valid. A significant development of the model is the inclusion of a viscoelastic (spring and dashpot) element. Its effect on the surface forces is expressed in two ways: an enhancement of the leading relative to trailing forces and a "dynamic lift" of the stimulus. The alterations to the surface force profile result in modifications of the stress and strain patterns, making them closely match the form of the empirical responses.

293.12

WIDEBAND SYSTEMS ANALYSIS OF TACTILE RECEPTORS. F.J. Looft, Dept. Elect. Engin., Worcester Polytechnic Institute, Worcester, MA. 01609

In a previous study, a vibrotactile indenting stimulus with a 85 Hz bandwidth was used to study the linear transfer characteristics of cutaneous mechanoreceptors (Looft et al, IEEE-Trans. BME, in press). Recently, the bandwidth of this stimulator was increased to 400 Hz and used in preliminary experiments to determine the first and second order Wiener kernels (Marmarelis et al, Biol. Cyber. 54:115-123, 1986) of cat slowly adapting T1, T2 and 'field' receptors. Further, because of the potential for a vibrotactile stimulus to tap the skin's surface, these experiments were performed when the stimulus tip was/was not glued (cyanoacrylate) to the skin surface.

Spectral analysis of the linear transfer functions confirmed our earlier results that, to varying degrees, these receptors responded as fractional order differentiators (increasing sensitivity with frequency). Cementing the tip of the vibrotactile stimulus to the surface of the skin overlying a unit had little effect on the shape of the first order kernel. By contrast, the second order kernels were modified, although the topographic changes were not major. Such changes portend interesting questions regarding the specific mechanism by which stimulus energy is transformed by skin and receptor dynamics and, ultimately, results in an AP event stream.

293.13

5-HT AND ATP MODULATE THE ACTIVITY OF CUTANEOUS TYPE I MECHANORECEPTORS IN THE RAT: AN "IN VITRO" STUDY. Hervé A. Martin, Robert P. Tuckett and Kathy English. Dept. Physiology, Univ. of Utah, 410 Chipeta Way, Salt Lake City, UT 84108.

To determine whether Merkel cells are the transducer element in the Merkel cell neuronal complex, we have studied the effects of intra-arterial injections of 5-HT and ATP contained in Merkel cell. To minimize the side effects of these putative neurotransmitters, we have developed an "in vitro" model.

Rats (n=27, 75-150 gram) were anesthetized with nembutal (35 mg/kg). The saphenous nerve and accompanying blood vessels were removed along with the dorsum skin of the leg and foot and placed in a chamber perfused with a freshly oxygenated bicarbonate saline buffer (pH=7.35; 32 °C). To inject drugs, the femoral artery was canulated. The activity of single type I units was recorded by placing the whole nerve on a silver chlorided electrode in an oil chamber from which thin filaments were dissected out and placed on a second silver chlorided recording electrode. Type I receptive fields were localized with a 1 gram von Frey stimulator. Then, mechanical stimuli were delivered using a rigidly-mounted mechanical stimulator that indented individual domes at a rate of 10 µm/sec with a 200 µm plateau for 60 sec. In a first series of experiments, we studied the effects of increasing doses of serotonin (1; 5; 10; 25; 50; 100; 250; 500 µM-100µl); each dose injected 1 min prior the mechanical stimulus. After 5-HT injections, we studied the effects of ketanserin (500 µM;200µl), a $5HT_3$ receptor antagonist. In a second series of experiments, we studied the effects of ATP (same range of concentrations), of a mixture of ATP and ketanserin (250 µM,200µl), and of ATP and serotonin (250 µM;200µl).

We have demonstrated inhibitory effects of serotonin on the tonic discharge of type I units which can be reversed by ketanserin "in vitro". ATP has been found to activate type I units and may facilitate their discharge during indentation. Evidence is suggesting that a mixture of ATP and ketanserin reverses 5-HT-induced inhibition.

PAIN: PATHWAYS I

294.1

FOS ONCOPROTEIN-LIKE IMMUNOREACTIVITY EVOKED BY NOXIOUS HEAT AND ELECTRICAL STIMULATION IN THE ANESTHETIZED RAT AND MONKEY H.H. Willcockson*, A.R.Light, E. Bullitt, and C.J.Vierck. Depts. of Physiology, Neurosurgery, UNC-Chapel Hill, Chapel Hill, NC 27599-7545.

The proto-oncogene *c-fos* is expressed in various brain regions following synaptic activation (Hunt, 1987). This study compares the effect of electrical vs heat stimulation in two species. Electrical stimuli were delivered via electrodes designed previously for psychophysical studies of pain in monkeys and humans (Vierck et al. 1983). Fifty stimuli (40 msec on, 200 msec off, 60 Hz, total 3 seconds each, intertrial interval 25 sec) ranging from 0.2mA to 4 mA were delivered. Noxious heat stimuli (paw immersed in 52^{o} water) was applied 20 times for 20 sec every two minutes. An antibody (Cambridge) against *fos* proteins revealed somatotopically appropriate labeling of cell nuclei in histological sections of lumbar spinal cord. Electrical stimulation greater than 2 mA and noxious heat evoked profuse labeling of neuronal nuclei in laminae I, II and V ipsilateral to the stimulus in rats and monkeys. Labeled nuclei were also found in lesser numbers in all other dorsal and ventral horn laminae except IX. Less than 1 mA labeled fewer neuronal nuclei which were confined to a narrow transverse region in the lumbar cord mostly in laminae II, III, and IV. Thus, noxious heat and noxious electrical stimuli evoke a similar pattern of *fos* labeling in both rat and monkey. Supported by PHS grants DA04420, NS16433,and NS14899.

294.2

C-FOS AS A MARKER FOR SUBSETS OF VISCERAL SECOND ORDER NEURONS IN THE RAT LUMBOSACRAL SPINAL CORD L.A. Birder, J.R. Roppolo, M.J. Iadarola, and W.C. De Groat, Dept. of Pharmacol., Univ.of Pittsburgh, Pgh, Pa. 15261.

C-Fos immunocytochemistry was used to identify neurons in the rat spinal cord that receive afferent input from the urinary bladder (UB). Physiological distension, and noxious stimulation (overdistension and chemical irritation, formalin 3%) were used to stimulate UB afferents in urethane anesthetized rats in which preganglionic neurons (PGN) and spinal tract neurons projecting to the rostral pons were labeled with fluorescent dyes. Noxious stimulation of the UB produced 3 times the number of c-fos cells than nonnoxious stimulation. C-fos positive cells were located in laminae I and II, laminae X and laminae VII near the sacral parasympathetic nucleus. A small % (5%) of PGN exhibited c-fos, however many cells in lamina VII dorsal to the PGN were c-fos positive, and 20-30% of dye labeled spinal tract neurons in this and other laminae were c-fos positive. Capsaicin pretreatment (70 mg/kg) blocked the c-fos response to all stimuli. Administration of hexamethonium (60mg/kg) decreased the number of c-fos cells. These findings indicate that both noxious and nonnoxious stimulation of the UB increases the number of c-fos positive cells in the spinal cord via activation of capsaicin sensitive afferents. The responsive cells include spinal tract neurons as well as PGN.

294.3

NOXIOUS AND NON-NOXIOUS COLORECTAL DISTENTION RESULTS IN C-FOS INDUCTION IN THE RAT SPINAL CORD. R.J. Traub and G.F. Gebhart, Dept. of Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

The spinal mechanisms involved in visceral pain processing are not well understood. In order to further elucidate pathways activated by visceral stimuli, immunocytochemical methods were used to localize the cellular proto-oncogene protein Fos following colorectal distention. Rats were lightly anesthetized with halothane and the descending colon and rectum were distended with pressures of 20, 40, or 80mmHg for 30s every 2min for 2h. The rats were sacrificed within 30min and the lumbosacral spinal cord was processed for Fos-like immunoreactivity (Fos-li, antisera donated by Dr. M. Iadarola). Greater distending pressures resulted in an increased number of cells with Fos-li in the L5-S1 spinal segments. In the most heavily labeled sections (>300 nuclei), Fos-li was observed bilaterally in laminae I-VII, X, and the sacral parasympathetic nucleus (SPN). The most densely stained nuclei were observed in the lateral 2/3 of laminae I/II, laminae V, VI, X, and the SPN. Very lightly stained nuclei were more prevalent in laminae III, IV, and VII. Rats that received the greatest stimulus intensities had a greater number of densely stained nuclei. These data suggest that noxious and non-noxious visceral stimulation result in the induction of Fos protein that may parallel the increase in stimulus intensity.

294.4

DISTRIBUTION OF FOS-LIKE IMMUNOREACTIVE NEURONS IN THE CAUDAL MEDULLA OF THE RAT INDUCED BY NOXIOUS STIMULATION OF FACIAL SKIN AND CORNEA. A.Strassman, J.Leite, and R.Maciewicz, Pain Physiology Lab, Dept. of Neurology, Massachusetts General Hospital, Charlestown, MA 02129.

Peripheral somatosensory stimulation can induce neuronal expression of the proto-oncogene c-fos in the spinal dorsal horn. To evaluate further the use of c-fos expression as a marker for neuronal activity in somatosensory pathways, the distribution of immunocytochemically labelled fos-positive neurons was mapped from the obex to C2 following acute noxious stimulation of the facial skin and cornea. Electrical (1 msec, 5mA pulses at 1 Hz), mechanical (repeated cutaneous pinch or corneal brushing), and thermal (50o C., 20-second pulses, every 1-2 minutes) stimuli were delivered over a period of 15 minutes to the cornea or to localized (<50 mm2) cutaneous sites within the V1, V2, or V3 dermatome in rats anesthetized with pentobarbital or urethane. Animals were sacrificed 2 or 4 hours following stimulation.

The pattern of neuronal labelling in the medullary and upper cervical dorsal horn exhibited a precise somatotopic organization, with rostral and dorsal facial sites represented rostrally and ventrally. This pattern of labelling is generally consistent with the somatotopy described previously in single-cell recording studies. For both electrical and natural forms of stimulation, the labelling in the superficial laminae (I-II) of the dorsal horn was both denser and more widespread than in the deeper laminae (III-V). Outside the dorsal horn, labelled neurons were most densely distributed in the nucleus of the solitary tract, the ventrolateral medulla adjacent to the lateral reticular nucleus, and in a highly restricted region in the ventral corner of the nucleus interpolaris at levels caudal to the obex. The distribution of labelled neurons outside the dorsal horn was bilateral and did not appear to be somatotopically organized. The results suggest fos localization may provide a useful tool to study the functional organization of central pathways involved in trigeminal nociception.

294.5

DIRECT GLUTAMERGIC INNERVATION OF PRIMATE SPINOTHALAMIC TRACT NEURONS. **K.N. Westlund, S.M. Carlton, D. Zhang, and W.D. Willis. Univ. of TX Medical Branch, Marine Biomedical Institute, Galveston, Texas 77550.**

Since glutamate excites spinothalamic tract (STT) neurons, the possibility that glutamergic terminals directly innervate STT neurons was investigated. One STT cell (lamina IV and V) in lumbar spinal cord in each of two monkeys was identified and characterized. The cells were characterized as a wide dynamic range and a brush inhibitory high threshold neuron. Following intracellular injection of the cells with HRP, the animals were perfused with mixed aldehydes. The tissues were reacted for HRP, dehydrated and embedded. Thin sections were reacted for post-embedding immunogold (10 nm) using antibodies specific for glutamate (1:20,000)(Arnel). Glutamergic synapses were observed on the soma and dendrites (lamina III-V) of the STT neurons. The synapses were numerous and of both symetrical and assymetrical type. Glutamergic profiles comprised 48% and 51% of the population of terminals apposing the soma and dendrites respectively, of one of these cells. The profiles contained round, clear vesicles and many contained a variable number of large dense core vesicles. It was concluded that glutamergic terminals comprise a large portion of the profiles apposing STT neurons. The presence of large dense core vesicles suggests that some terminals are of primary afferent origin. Supported by NS11255 and Bristol Myers-Squibb Corp.

294.6

ANALYSIS OF GABAERGIC AND GLYCINERGIC INPUT TO SPINOTHALAMIC TRACT CELLS IN PRIMATE DORSAL HORN. **H. Lekan, E.S. Hayes*, K.N. Westlund, D. Zhang, W.D. Willis and S.M. Carlton. University of Texas Medical Branch & Marine Biomedical Institute, Galveston, TX 77550.**

Inhibitory interneurons containing either gamma aminobutyric acid (GABA) or glycine are located in the dorsal horn. The relationship of GABAergic and Glycinergic profiles to spinothalamic tract (STT) cells is unknown; however, iontophoresis of either substance results in inhibition of these cells. The purpose of this study was to determine the relationship between these inhibitory systems and cells at the origin of the STT. In primates, STT neurons iontophoretically injected with HRP in the lumbar enlargement were immunostained with anti-GABA or anti-glycine using a postembedding immunogold technique.

Analysis at the EM level of cell bodies and proximal dendrites demonstrated that both GABAergic and Glycinergic terminals synapsed on lamina V STT neurons. Analysis of several levels through one STT cell demonstrated that 25% of the terminals were GABAergic and 13% were Glycinergic. These data indicate that STT cells are under considerable inhibitory control by dorsal horn interneurons. We hypothesize that disruptions of this inhibitory input may lead to aberrant pain sensations. This work was supported by NS11255, NS27910 and the Bristol Myers-Squibb Corp.

294.7

SPINAL NEURONS LIKELY TO MEDIATE LOW BACK AND REFERRED LEG PAIN. R.G. Gillette*, R.C. Kramis* & W.J. Roberts, R.S.Dow Neurol.Sci.Inst., Good Samaritan Hosp. & Med. Ctr. Portland, OR 97209.

Low back pain (LBP) typically is felt diffusely, even after focal injury, and it often radiates into the leg. We are investigating the physiological bases for LBP by recording from spinal neurons with paraspinal receptive fields in anesthetized cats. The principal goals are: to characterize the receptive fields of single units; to test for expansion of receptive fields after noxious stimulation; and to test for responses to sympathetically evoked afferent activity, which may impose a sympathetically maintained pain (SMP) component in LBP (Roberts, 1986; Brenna et al., 1980).

Recordings to date from 70 neurons indicate that: 1) many neurons with large paraspinal receptive fields receive convergent input from the zygapophysial joint capsule, paraspinal and thigh muscles and skin, and the anterior longitudinal ligament; 2) injection of an algogen into paraspinal tissues causes expansion of the receptive field into the thigh in many neurons; and 3) stimulation of sympathetic efferents activates about 75% of the WDR neurons tested.

These results demonstrate a neuronal substrate for the diffuse nature of LBP and for the tendency of LBP to radiate into the leg. They also demonstrate a mechanism through which sympathetic activity may contribute to LBP. (NIH NS13447)

294.8

A PROJECTION FROM LAMINA I TO THE LATERAL CERVICAL NUCLEUS OF THE CAT. A. Blomqvist, J. Broman and A.D. Craig, Jr. Department of Cell Biology, Faculty of Health Sciences, University of Linköping, S-581 85 Linköping, Sweden, and Divisions of Neurobiology and Neurosurgery, Barrow Neurological Institute, Phoenix, AZ 85013, U.S.A.

The lateral cervical nucleus (LCN) receives ascending input from spinocervical tract cells located in the nucleus proprius of the dorsal horn. However, the results of retrograde tracing experiments have suggested that also neurons in lamina I project to LCN. In the present study the question of lamina I input to LCN was addressed by anterograde tracing with Phaseolus vulgaris leucoagglutinin (PHAL). Injections of PHAL into lamina I at different spinal segments resulted in terminal labeling in the most medial portion of the LCN (mLCN). The labeled fibers were fine and longitudinally oriented, with reticular arborizations. They were clearly different from the large-diameter fibers with dense bursts of terminals in the topographically appropriate portions of the lateral LCN that were seen in cases with lamina III-IV injections. The observations corroborate the earlier identification of nociceptive neurons in the mLCN. The mLCN contains GABAergic neurons that are probably local inhibitory interneurons; thus, the present findings may help explain the inhibitory effects of noxious stimuli on the majority of LCN neurons and support the hypothesis that in the intact animal the spinocervicothalamic pathway does not subserve nociception.

294.9

POSTSYNAPTIC DORSAL COLUMN FIBERS IN THE CUNEATE NUCLEUS OF THE MONKEY (*MACACA FASCICULARIS*) REVEALED BY PHA-L. K.D. Cliffer and W.D. Willis, Jr. Marine Biomedical Institute, The University of Texas Medical Branch, Galveston, TX 77550

Postsynaptic cells in the cervical spinal cord of cats, the majority of which receive nociceptive input, have been previously shown to project to the non-cluster regions of the cuneate and gracile nuclei and sparsely if at all to the cluster regions. In monkeys, pars rotunda of the cuneate nucleus, considered to be the equivalent of the cluster region in cats, has been reported not to receive a projection from postsynaptic cells in the cervical spinal cord. We made multiple iontophoretic injections of the anterograde tracer *Phaseolus vulgaris* leucoagglutinin (PHA-L) into the cervical enlargement of a cynomolgous monkey (*Macaca fascicularis*). After 34 days survival the animal was deeply anesthetized and perfused, and the tissue processed for immunohistochemical detection of the PHA-L, using the avidin-biotin fluorescence technique. Many labeled fibers and varicosities were detected in a widespread distribution in the cuneate nucleus, including most of pars rotunda and part of pars triangularis throughout their lengths. Fibers and varicosities in a substantial portion of the external cuneate nucleus were also labeled. These results indicate that the postsynaptic projection to the cuneate nucleus is widespread, and includes pars rotunda and areas from which cells project in the medial lemniscus to the ventrobasal thalamus. Such projections could contribute to transmission of information originating in nociceptors through the dorsal column-medial lemniscal system to the ventrobasal thalamus. We have previously recorded nociceptive responses in cells in the gracile nucleus that project to the ventrobasal thalamus in monkeys (Ferrington et al., *J. Neurophysiol.*, 59:886-907, 1988) and cats (Cliffer et al., *Soc. Neurosci. Abs.*, 15:1190, 1989) .

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294.10

MEDULLARY DORSAL HORN PROJECTIONS TO THE DIENCEPHALIC NUCLEI IN THE RAT. K. Iwata, R.L. Nahin and D.R. Kenshalo,Jr. Neurobiology and Anesthesiology Branch, NIDR, NIH, Bethesda, MD 20892.

Previous studies revealed that a major projection of the medullary dorsal horn (MDH) is to diencephalic nuclei. However, the localization and the pattern of MDH afferent terminations in the diencephalon remains unclear. The present study employed injections of the anterograde tracer, Phaseolus Vulgaris-leucoagglutinin (PHA-L), into the MDH in rats. Injections of a 2.5% solution of PHA-L (0.01µl each point, 5-10 points) were made into the rostro-caudal extent of the MDH. Following a survival period of 14-21 days, animals were deeply anesthetized with sodium pentobarbital and perfused transcardially. Transverse serial sections (30 µm) were cut and incubated in a 1:2000 dilution of goat anti PHA-L for 48 hours and then processed with the avidin-biotin technique. Labeled fibers and varicosities were seen in the N. ventralis posteromedialis (VPM), N. submedius (SM) and N. posterior hypothalamus (PHT). Non-varicose axons were widely distributed in the caudal half of VPM, whereas the majority of axonal varicosities were found in the middle portion of VPM. In SM and PHT, axonal varicosities and non-varicose axons were intermingled and were distributed in the dorsal portions. Varicosities in SM and PHT were much smaller than those observed in VPM and were predominantly of the en passant variety. These data suggest that information originating in the MDH is processed in at least three different nuclei of the diencephalon.

294.11

FORMALIN PAIN BUT NOT ANALGESIA IN BRAIN STEM TRANSECTED RATS. B. Matthies, and K.B.J. Franklin. Psychology Dept., McGill University, Montreal, Canada.

Rats with transections of the brain stem 1-2mm anterior to the interaural line (Paxinos and Watson, 1986) were tested for pain responses and morphine analgesia in the formalin and tailflick tests. Cuts passed through the inferior colliculus dorsally and the pontine nuclei ventrally. Rats were aphagic and adipsic but regained an upright posture, locomotor activity and grooming. Animals responded to formalin injection by lifting and favoring the injured paw, but attempts to groom the paw were inaccurate and often resulted in loss of balance. Responses to pain differed from normals in several ways: a) formalin pain is normally high immediately after formalin is injected, falls to a low point 15 minutes later, then rises again to a level which remains steady for 30 to 40 minutes and then falls. In transected animals pain ratings remained high for 60 min. and then fell off as in normal rats. b) In normal rats the ED50 for morphine analgesia in the formalin test is 4 mg/kg but 8 mg/kg morphine had no effected in transected rats. c) The tailflick reflex was not impaired, but the animals response to 8 mg/kg morphine was attenuated. The results suggest that the neural mechanisms involved in protective and recuperative responses to injury produced pain lie in the brainstem, but structures critical for the analgesic effect of morphine appear to be more rostrally located.

294.12

AMYGDALOID PROJECTIONS OF THE EXTERNAL PARABRACHIAL AREA IN THE RAT: A PHA-L STUDY WITH REFERENCE TO PAIN TRANSMISSION. J.F. BERNARD* and J.M. BESSON, Unité de Recherches de Physiopharmacologie du Système Nerveux, I.N.S.E.R.M. U 161, 2 rue d'Alésia 75014 PARIS

We have demonstrated that a high proportion of neurons that were located in the external parabrachial (PBe) area (i.e. external lateral (PBel) and external medial (PBem) subnuclei) and that projected to the nucleus centralis (Ce) of the amygdala was implicated in pain processes (Bernard and Besson J. Neurophysiol. 1990, 63: 473-490). Futher investigations at the Ce level showed that regions not detailed in previous anatomical studies were also implicated in pain processes. The aim of the present study was to precisely investigate the efferent projections of PBe area to the amygdaloid complex and the adjacent areas.

In anaesthetized Sprague-Dawley rats PHA-L (5%) was electrophoretically injected into the PBe area. Two weeks later, the rats were perfused transcardially, the brain was removed and cut in serial sections which were reacted with PHA-L antibody and an ABC kit of Vector. The location of labeled terminal fibers varied dramatically with the location of the very restricted injection sites (200-400µm) in the parabrachial area. The caudal PBel subnucleus projects mainly to the peripheral region of the Ce. The caudal PBem subnucleus projects mainly to the rostrolateral portion of the Ce and the adjacent striatopallidal region. The location of these efferent projections precisely corresponds to the location of neurons responding to noxious stimuli. Thus this study details and extends the terminal area of the spino(trigemino)- ponto-amygdaloid nociceptive pathway that could be implicated in the affective-emotional aspects of pain.

294.13

THE DORSAL SPINOTHALAMIC PATHWAY IN THE MONKEY: A LIGHT AND ELECTRON MICROSCOPIC STUDY. D.D.Ralston and H.J.Ralston, III. Department of Anatomy, University of California, San Francisco, California, 94143.

A recent study by Apkarian and Hodge (J.Comp. Neurol. 288: 474, 493, 1989) showed that lamina I cells of the spinal dorsal horn (which are primarily nociceptive neurons) give rise to a dorsal spinothalamic pathway (STT). We have studied this pathway by making microinjections (0.02µl) of WGA-HRP into the cervical enlargement of anesthetized macaque monkeys, with a unilateral high cervical anterolateral cordotomy. We have found that the dorsal STT travels in the white matter at the level of the denticulate ligament and terminates primarily in the ventral caudal region of the somatosensory thalamus. This area was called Vc.pc. by Hassler, who stated that electrical stimulation of this region in humans elicited reports of contralateral burning pain. Recent studies by Dostrovsky (personal communication) support Hassler's earlier report. Nathan (Pain 40: 239, 1990) has described a patient with loss of cold and pinprick sensation following a lesion of the intermediate aspect of the lateral column white matter at the level of the denticulate ligament.

Electron microscopy revealed that dorsal STT terminals formed axodendritic synapses upon thalamic neurons without involving the GABAergic local circuit neurons which characterize lemniscal terminations. We conclude that the dorsal STT from cervical segments occupies the lateral column white matter opposite the denticulate ligament and terminates primarily in a restricted zone of the thalamus shown to be associated with pain sensation in humans. The dorsal STT information is not subject to GABAergic modulation in the thalamus. Supported by NS21445 from N.I.H..

294.14

EFFECT OF NUCLEUS SUBMEDIUS THALAMUS LESIONS ON NOCICEPTIVE RESPONDING IN RATS. Vicki J. Roberts and Willie K. Dong. Dept of Anesthesiology & Multidisciplinary Pain Center, Univ of Washington Sch of Med, Seattle, WA 98195.

The effect of nucleus submedius thalamus (SM) lesions on nociceptive responding in rats was assessed using both the radiant heat tail-flick test (TF) and the pain-induced (tail-shock) vocalization test. Results indicate that the intensity of electrical shock required for vocalization responses is significantly decreased in rats following SM lesions. The postlesion increase in nocifensive behavior is observed for vocalization responses which are briefer than the electrical stimulus as well as for vocalizations which outlast the stimulus. No changes in vocalization responses were present in the sham lesion group. In contrast, both the sham and SM lesion group exhibited a significant postlesion increase in TF latencies. This decrease in nociceptive responding is attributed to conditioned analgesia, since analgesia on TF measures has been demonstrated following exposure to environmental cues previously paired with tail-shock. We are currently assessing whether or not lesion effects on tail-flick response to radiant heat may have been masked by conditioned analgesia. Preliminary results indicate that there is no postlesion change in tail-flick latencies in either sham or lesion groups when the vocalization test is omitted. This suggests that the SM is not important for spinally mediated reflexes. The postlesion increase in nocifensive vocalization responses suggests that the SM may be important in affective reactions to nociceptive stimuli. Supported by NIH grant NSO7217.

294.15

ACTIVATION OF NEURONES IN THE ORBITAL REGION OF RAT CORTEX BY NOXIOUS STIMULATION AND BY STIMULATION OF NUCLEUS SUBMEDIUS. N. El-Yassir and J.O. Dostrovsky. Dept. Physiology, University of Toronto, Toronto, Ontario, M5S 1A8, Canada

Anatomical studies have delineated a major reciprocal connection between the Orbital Region (OR) of the cortex (specifically the lateral and ventrolateral orbital cortex) and the thalamic nucleus submedius (Sm). The objective of this study was to confirm these anatomical findings using electrophysiological techniques and to determine the characteristics of OR neurones receiving an input from Sm or projecting to Sm. Experiments were carried out on rats anesthetized with α-chloralose and urethane. Glass-coated tungsten electrodes were used both for stimulation and recording. A total of 69 OR cells activated by Sm stimulation was studied. Stimulation in Sm antidromically activated 32 OR units (mean latency of 4.5 ± 3.2 ms; range 1.7 to 15.2 ms). Most of these units had no spontaneous activity and only 3 out of 9 tested responded to cutaneous stimulation (only to noxious stimuli). Sm stimulation orthodromically modulated the firing rate of 37 cells. Many of these cells fired spontaneously in bursts and 18 of 20 tested responded to noxious cutaneous stimuli (15 excited, 3 inhibited). Receptive fields were large and usually bilateral. The effect of a train of stimuli to Sm on 2 of the 3 neurons inhibited by peripheral stimulation was inhibitory. These results confirm the presence of a reciprocal connection between the Sm and OR and further suggest that nociceptive information reaching the OR may be relayed through the Sm.

294.16

ANATOMIC EVIDENCE FOR A THALAMIC RELAY FOR NOCICEPTIVE INFORMATION TO SECONDARY SOMATOSENSORY CORTEX (SII). S.M. London and A.V. Apkarian, Dept. of Neurosurgery, SUNY Hlth. Sci. Center, Syracuse, NY, 13210.

Physiologic, behavioral, and clinical evidence implicate the second somatosensory cortex (SII) in processing nociceptive information. A small number of thalamic nuclei project to SII, some of which are known to receive spinothalamic tract (STT) terminations. In this study, the relationship between STT terminals and thalamocortical cells projecting to SII was investigated. Ketamine/nembutal anesthetized squirrel monkeys received injections of fluorescent tracers in electrophysiologically defined hand area of SII and injections of 2% wheatgerm-agglutinin conjugated HRP in the contralateral cervical enlargement. Some animals also received injections of a different fluorescent tracer in the hand area of primary somatosensory cortex (SI). Anterogradely labeled terminals and retrogradely labeled cells were plotted and digitized for every fifth section, throughout the thalamus. The number of fluorescent thalamic cells varied with the number of SII injection sites, spread from the injection site, and the tracer used. However, certain findings were common to all experiments. Labeled thalamic neurons were noted in the ventroposterior lateral nucleus (VPL), the ventroposterior inferior nucleus (VPI), the anterior pulvinar (Pa) and the posterior nucleus (Po). Labeled neurons in the ventroposterior medial nucleus (VPM) were attributed to contamination of the SI face region. Close proximity of a small number of thalamocortical neurons to STT terminals was noted in VPL, VPI and Po, but not in Pa. Overlap of the labeled cells and terminals is being quantified. Coincidence of these thalamic neurons and terminals suggests the possibility for synaptic contact and these findings indicate a sparse, though potentially significant anatomical relay pathway for pain signals from the thalamus to SII.

294.17

LASER EVOKED CEREBRAL POTENTIALS IN MONKEYS A.Beydoun*[1,3] T.J.Morrow[1,2,3], and K.L.Casey[1,2,3]. Depts of Neurology[1] and Physiology[2], U. Michigan and Neurology Research Laboratories[3] VAMC, Ann Arbor, MI.
Purpose: Cutaneous stimulation with CO2 laser pulses activates small diameter sensory afferents and evokes a pain-related potential recorded from the vertex (CZ) of humans. We here report the first successful recording of laser evoked cerebral potentials from awake monkeys. **Methods**: We delivered laser pulses (60 msec, 9mm beam, 4-12 watts) to the shaved tail of awake African green monkeys and recorded averaged responses from CZ to linked ears (sweep 500 msec, bandpass 0.4-40 Hz, sensitivity 200uV). Twenty five stimuli were delivered in each of 2 trials. Stimulus intensity was adjusted to the lowest level giving reproducible potentials. Throughout recording, monkeys occasionally oriented toward the stimulus but were otherwise quiet and did not vocalize. No evidence of tissue damage was seen. The proximal and distal tail were stimulated in order to calculate the conduction velocity of the fibers. **Results**: At low laser intensities (4-6 watts), no potentials were recorded. As the stimulus intensity was increased (8-12 watts), a tail flick response was elicited and a reproducible, triphasic positive-negative-positive potential complex (P1-N-P2) was recorded. Following stimulation of the proximal tail, the average peak latencies were as follows: P1=104 msec(98-113), N=163 msec(152-179), and P2=234 msec(217-249). Average amplitudes were: P1=6.1uV (2.0-11.3), N=6.9uV (2.3-11.5), and P2=9.0uV (4.7-16.7). In one monkey, a latency-intensity, amplitude-intensity study revealed that, as stimulus intensity increased, the peak latency of the potentials decreased. Amplitude increased up to an intensity of 8 watts, then decreased with higher stimulus intensities. The calculated conduction velocity of the fibers activated was 8 m/sec. **Conclusions**: Based on the morphology and topography of these potentials, their threshold relationship to the tail flick response and the conduction velocity of the activated afferent fibers, it is very likely that they represent nociceptive-related evoked potentials similar to those recorded from humans. Supported by VA Merit Review and Bristol-Myers Squibb.

294.18

FUNCTIONAL NEURAL SUBSTRATES INVOLVED IN PROCESSING TONIC NOCICEPTIVE STIMULATION
F.J. Helmstetter & F.Gonzalez-Lima Dept. Medical Anatomy, Texas A&M Univ., College Station, TX 77843

[14C]2-Deoxyglucose (2DG) autoradiography was used to map metabolic changes in brain and spinal cord resulting from a subcutaneous injection of dilute formalin. Unrestrained rats were injected with 15% formalin into the dorsal surface of the right hindpaw and left undisturbed for 45 min after 2DG administration. Autoradiographs were examined for regional changes in isotope uptake. A bilateral decrease in labeling was observed in a number of structures including the sulcal frontal cortex and intralaminar thalamus while a bilateral increase was observed in the paramedian raphe. Structures showing a unilateral increase included the ventrobasal thalamus contralateral to the treated hindlimb. This study represents the first functional mapping of CNS activity in a rodent model of chronic pain. (supported by NIMH grant R01-MH43353)

294.19

OPERANT RESPONSE MAGNITUDE AS AN INDICATOR OF NOXIOUS HEAT INTENSITY IN RATS. D.K. Douglass, I.G. Campbell*, E. Carstens and L.R. Watkins. Dept. Animal Physiology, University of California, Davis, CA 95616.

The present study was designed to explore the relationship between the magnitude of an operant response and the intensity of noxious heat stimuli in rats. Rats were trained to push a lever upward with their noses to terminate mild electrical stimulation of the tail. The lever was connected to a force transducer, allowing measurement of the force and duration of pushing. The electrical stimuli used for the initial training were not sufficient to cause any noticable damage to the tail. When the rats consistently pressed in response to the electrical stimulus, radiant heat trials were added to the training, using a quartz halogen bulb with thermocouple feed back. The initial radiant training stimuli were either 53 or 55°C, and the rats quickly learned to press in response to these stimuli. Rats were tested by applying noxious heat to the tail at 51, 53, 55, 57 and 59°C. It should be noted that tail-flick threshold with this radiant heat device is 51°C, and such stimuli cause no damage to the tail skin. Our earlier studies with this paradigm suggested it was useful only as a threshold test when the push terminated the test stimulus. The current study was designed such that during the actual test sessions, the stimuli were given for 5 sec, regardless of whether the rat pushed the lever or not. Each rat was used in three test sessions; on days between sessions, rats were given stimuli that could be terminated to prevent extinction of the response. The integrated wave forms, latency, force and number of pushes in response to each stimulus were recorded to disk and stored for later analysis.

There is a positive correlation between the integrated wave (magnitude) of the operant response and the stimulus temperature, as well as between the temperature and the peak force of the response, number of pushes per stimulus, and the inverse of the latency. The correlation coefficients are low however, due to wide variability between rats (integrated wave, r^2=0.27; force, r^2=0.24; latency, r^2=0.38; push number, r^2=0.23). With refinement of the training technique, one or more of the parameters measured by this method may prove a useful indicator of stimulus intensity above pain threshold. Supported by NIH NS 20037.

295.1

SUBSTRATES FOR INTERACTION OF VISUAL CHANNELS WITHIN AREA V1 OF MONKEY VISUAL CORTEX. T. Yoshioka and J.S. Lund. Department of Psychiatry and Center for Neuroscience. University of Pittsburgh, Pittsburgh, PA 15261.

Two distinct channels of visual input from the lateral geniculate nucleus (dLGN) terminate in the middle layer 4C of monkey V1 cortex. Magnocellular dLGN neurons project to layer 4C alpha and parvocellular dLGN neurons project to layer 4C beta. In turn, the spiny stellate neurons of 4C project to more superficial layers, 4C beta projecting to layers 4A and 3B and 4C alpha projecting to layer 4B. These recipient zones are known to differ in physiological response properties and efferent neuron projection patterns. We have found from study of Golgi impregnations that the layers 4B, 4A, and 3B are richly interconnected by the axons and dendritic processes of spine free or sparsely spined local circuit neurons which are usually GABAergic. This suggeststhat although visual cortex excitatory intrinsic relays may tend to preserve the separate identities of the channels of visual information presented by geniculate relays, there is a substrate for considerable functional interaction between them. Interestingly while we have previously found excitatory links to pass between these layers in a virtually one-way direction, from layer 4B to the superficial layers, local circuit neuron projections pass in both directions. Supported by EY05282.

295.2

SUBLAMINAR ORGANIZATION WITHIN LAYER VI OF THE STRIATE CORTEX IN GALAGO. M. Conley and D. Raczkowski. Depts. Psychol. & Neurobiol., Duke University, Durham, NC 27706

We studied projections from the striate cortex to the dorsal lateral geniculate (GL) and pulvinar (PUL) nuclei in the prosimian Galago using retrograde transport methods. Injections of WGA-HRP into the PUL labeled two bands of cells in the striate cortex: one in the upper half of layer V and another located in the deepest part of layer VI. The labeled cells in layer VI coincided with a distinct sublayer, VIb, which contains fewer and paler staining cells than VIa. Injections of WGA-HRP that were restricted to one or a few GL layers revealed a further refinement of the subdivisions within layer VI. Injections into the parvicellular and intercalated layers labeled neurons mainly in the upper half of layer VIa, whereas injections restricted to the magnocellular layers labeled neurons in the lower half of layer VIa and in layer VIb. To determine whether single neurons in layer VIb send axon collaterals to both the GL and PUL, we injected WGA-HRP into one nucleus and rhodamine beads into the other. In three experiments, we found only one double-labeled cell. In sum, these results show a trilaminar division of layer VI that is sufficiently discrete to propose the existence of multiple, descending pathways from layer VI of the striate cortex that complement those ascending from the GL and PUL. Aided by MH04849, BNS8519709 & EY06821.

295.3

NEUROTRANSMITTER REGULATION IN OCULAR DOMINANCE COLUMNS OF NEW WORLD MONKEYS. R.K. Carder, S.H.C. Hendry, E.G. Jones. Dept. of Anatomy and Neurobiology, University of California, Irvine, CA 92717

In Old World monkeys loss of neural activity in one retina leads to chemical changes in neurons of area 17. To determine whether chemical plasticity is a feature of area 17 in other primates, we examined a species of New World monkey *(Cebus capuchinus)* in which ocular dominance columns have been demonstrated. In normal Cebus monkeys, staining in neuronal somata and terminals for GABA and and its synthesizing enzyme, glutamic acid decarboxylase (GAD) was uniform and intense in layer IVC. Following monocular deprivation by tetrodotoxin injections, stripes of normal staining were found to alternate with stripes of light immunostaining in layer IVC. Comparison with adjacent sections stained for GABA or GAD and cytochrome oxidase, indicated that the reduced immunocytochemical staining occurred within deprived-eye columns. In these same monkeys, 5HT immunoreactivity and acetycholinesterase histochemical staining exhibited highly irregular yet complementary lattice patterns. This contrasts with normal monkeys in which 5HT immunostaining and acetylcholinesterase histochemical staining were relatively uniform within the various cortical laminae. These findings demonstrate that visual cortical neurons in both Old and New World monkeys display activity dependent changes in neurotransmitter and enzyme immunoreactivity and suggest that this mechanism of plasticity may be a feature of the primate visual cortex. In addition, in New World monkeys chemical plasticity may extend to "non-specific"cortical afferents. Supported by EY06432 and EY07193

295.4

ULTRASTRUCTURAL ASTROCYTIC LOCALIZATION OF INTRACELLULAR DOMAINS OF β-ADRENERGIC RECEPTOR IN VISUAL CORTEX: COMPARISON TO AREA POSTREMA EXPOSED TO CIRCULATING AND ENDOGENOUS CATECHOLAMINES by Chiye Aoki[1], Catherine D. Strader*[2] and Virginia M. Pickel[1],[1]Cornell Med Coll, NYC 10021 & [2]Merck Sharp & Dohme Res Labs, Rahway, NJ 07065.

Astrocytes may influence visual cortical (VC) plasticity (Muller & Best, Nature 342:427) and also mediate central action of catecholamines (CA) (Stone et al., Brain Res Rev 14:297). The CA stimulus in VC is exclusively derived from afferents whereas, in area postrema (AP), a circumventricular organ, the astrocytes are exposed to both neuronal and circulating CA. We comparatively examined the VC and AP to determine whether the localization of β-adrenergic receptor (βAR) in neurons or astrocytes might reflect these differences of CA in rat and cat brain. We used immunoperoxidase methods and antibodies directed against synthetic peptides corresponding to either the intracellular third loop (3i) or the C-terminus (C') of βAR-protein. In supragranular laminae of visual cortex, C'-immunoreactivity (ir) was most frequently seen along cytoplasmic surfaces of plasmalemma of selective populations of astrocytic processes. These were associated with dendrites, terminals and basement membranes of blood vessels. In contrast, 3i-ir was evident within axons, dendrites and Golgi apparatus of neurons but rare in astrocytes. In AP, the astrocytes showed more extensive vesicular C'-ir. Neuronal 3i-ir was similar to VC. We have established a cellular basis by which neuronally released CA may interact with blood vessels and neurons through astrocytes in VC and AP. (Supported by grants EY08055 and HL18974)

295.5

IMMUNOCYTOCHEMISTRY OF GABAERGIC NEURONS IN VISUAL CORTEX OF DOLPHINS. I. I. Glezer, C. Leranth, P.J. Morgane, K. Baimbridge and J. Solomon*. Dep. Cell Biol. Anat. Sci. of CUNY Med. Sch., New York, NY 10031, Yale Univ. School of Medicine, New Haven, CT 06510, Worcester Foundation for Exp. Biology, Shrewsbury, MA 01545, Univ. of British Columbia, Vancouver, Canada, Dep. of Biology of the City College, New York, NY 10031

An immunocytochemical technique was used for light and electron microscopic analyses of GABA localization in different layers of visual neocortex of several species of toothed whales. GAD-positive synaptic boutons both of the axosomatic and axodendritic synapses were found in all cortical layers. The highest density of the boutons per mm^3 was found in layer III and the lowest density was found in layer I. The special class of GABAergic neurons, immunopositive to Calbindin-28 (CB) were found in layers I and II of the dolphin visual cortex. Almost all CB-positive neurons are of bipolar or fusiform type. In layer I they form columns of perikarya perpendicularly oriented toward pial surface. The morphology of the perikarya and dendrites of these neurons as well as localization are similar to those found by us for CCK-positive neurons and different from mostly multipolar, CB-positive neurons in primates. (Supported by NSF grants 87-42032, and NIH grants HD-06364, NS26068, P50MH44866).

295.6

ARCHITECTONIC DIFFERENCES IN THE DISTRIBUTION OF PARVALBUMIN IN THE RAT NEOCORTEX H-Y. Tseng[1], K.G. Baimbridge[2] and A.R.Kay[1]. [1]Biophys. Dept., AT&T Bell Labs, Murray Hill, NJ. 07974. and [2]Dept. Physiology, Univ. British Columbia, Vancouver, BC, Canada V6T 1W5.

The Ca binding protein parvalbumin (PV) appears to be exclusively localized in gabaergic nonpyramidal cells in the neocortex of rat, cat and monkey. We have studied the distribution of PV-IR (immunoreactivity) in rat (Long Evans) neocortex using polyclonal antibodies raised against rat muscle PV. Both cell bodies, processes and terminals exhibit PV-IR. In contrast to monkey, PV-IR is not present in the corpus callosum, nor in any of the regions that provide significant synaptic input to neocortex. Hence most of the PV-IR evident in neocortex probably derives from intrinsic neurons. In all neocortical cortical areas PV-IR cell bodies are excluded from layer I, although some processes do penetrate this layer. Auditory cortex shows a higher density of PV-IR cells and a greater concentration of cells in layer II-III than visual cortex.

Cortical slices when viewed under low power exhibit PV-IR bands running parallel to the pial surface, which correspond mostly to PV containing terminals. In most isocortical regions of the neocortex two clear bands are found flanking layer IV. However, in most of visual cortex only the subgranular band is present. The distribution of PV-IR provides a graphic image of differences in cortical inhibition across different functional areas.

295.7

SPONTANEOUS EXCITATORY POST SYNAPTIC POTENTIALS IN LAYER V CELLS ARE PRODUCED BY ACETYLCHOLINE APPLIED IN LAYERS I-II OF RAT VISUAL CORTEX. G. Vaknin and M. Segal. Center for Neuroscience, Weizmann Institute of Science, Rehovot 76100, Israel.

Apical dendrites of pyramidal cells ascend to layer I where they branch and form synaptic contact with a horizontal fiber system containing cholinergic afferents. This study examined the effect of ACh applied in layers I-II on spontaneous and evoked (white matter stimulation) EPSP's of layer V cells using the visual cortical slice preparation. ACh was applied by pressure pulses (20-100ms, 3Kg/cm^2) to a broken micro-electrode containing ACh-chloride (20-40mM). A single microdrop of ACh in layers I-II resulted in EPSP's (2-5mv) lasting for 10-20s with no detectable change in membrane potential. Continuous application of ACh (microdrop applied every 3s for 20s-3min) produced depolarization associated with an increased input resistance. A long-lasting (up to 3min) barage of large EPSP's (5-10mv, 10-15Hz) often producing spikes, was observed. The effect of ACh on evoked EPSP's was mixed; increases, decreases, no change.

295.8

EARLY ENTRY OF GENICULOCORTICAL AXONS INTO THE CORTICAL PLATE DURING THE FORMATION OF LAYER IV IN VISUAL CORTEX OF NEONATAL RAT AND HAMSTER. Glenn H. Kageyama & Richard T. Robertson. Dept. Anatomy & Neurobiology, Univ. of California, Irvine, CA 92717.

The developing visual system in mammals is amongst the most intensely studied in neurobiological research, however, the precise temporal development of the geniculocortical (GC) projection into the cell dense cortical plate (CP) of visual cortex has not been adequately described in detail. Using eye injections of WGA-HRP, combined with transneuronal transport and TMB histochemistry, we labeled geniculocortical terminal fields as early as postnatal day 0 (P0) in rat pups and P1 in hamsters. Intraocular injections of 0.5 µl 2% WGA-HRP (10 µg) were made close to the retina with a micropipette and animals allowed to survive for 0.6-3d. Tissue sections were treated with TMB for light microscopy (Mesulam'78) or EM (Naus et al.,'85). Adjacent sections were used for cytochrome oxidase (CO) or Nissl staining. The light microscopy results are summarized below:

Event	Hamster	Rat
WGA-HRP labeled GC projection to:		
Subplate (layer SPu or VIb)	P1	(Before P0)
Cortical layers V-VI	P3	P0
Developing layer IV (top of CP)	**P4**	**P1**
First distinct layer IV (Nissl)	P6	P2
Initial elevation of CO in Layer IV	P7	P3

At the EM level, transneuronal WGA-HRP was localized within presumed GC terminals. The results demonstrate that GC terminals enter the CP much earlier than previously thought and appear to penetrate the cell dense CP prior to the appearance of a distinct granular layer IV. It is possible that GC axons may play a role in the histogenesis of layer IV. The elevation of CO in layer IV 2 days after the arrival of GC axons and one day after the histogenesis of layer IV suggests that elevated oxidative metabolic activity closely follows the development of GC input. Supported by NSF 87-08515 and NIH NS 25674.

295.9

FOCAL LASER RETINAL LESIONS REVEAL DIFFERENTIAL CYTOCHROME OXIDASE REACTIVITY IN FOUR ISOECCENTRIC REGIONS OF THE MACAQUE STRIATE CORTEX. T.C. Trusk and M.T.T. Wong-Riley Dept. of Anatomy & Cellular Biology, Medical College of Wisconsin, Milwaukee, WI 53226.

Focal blue-green argon laser lesions (3 mm in diameter) were unilaterally placed in the superior parafoveal retinae of 2 Japanese macaques so that lesioned, transitional, and normal visual cortex could be analyzed in the same hemisphere of the same monkey. After 5 and 7 weeks' survival, laser damage at the center of the lesion included the destruction of photoreceptors, outer nuclear layer, and the outer plexiform layer. While much of the inner nuclear layer was vacuolated, the inner plexiform layer, ganglion cells, and nerve fiber layer remained intact. Optical densities of cytochrome oxidase (C.O.) reaction product within 4 isoeccentric regions of the striate cortex were measured: **(A)** Within ocular dominance columns (ODC) associated with laser-damaged portions of the treated eye; **(B)** within ODC representing the homonymous retinal portions of the untreated fellow eye; **(C)** in the transitional region (no visible ODC) adjacent to the laser-affected area; and **(D)** in regions 10-20 mm away from the edge of histochemically visible ODC. Within each defined region, reaction product density was sampled in puffs and interpuffs of lamina 3B, and in lamina 4Cβ. For puffs, C.O. activity was equally high in regions B and D, moderate in C, and lowest in A. Interpuff enzyme activity was highest in region B, and lowest in A (B > D > C > A). In 4Cβ, C.O. activity was equally high in regions B and D, significantly lower in C, and still lower in A. These results suggest that: (1) Striate cortex responds metabolically to retinal insults even when ganglion cells remain intact; (2) cortical metabolic plasticity extends beyond the zone representing the core of the retinal lesion into a transitional area; and (3) C.O. activity in the interpuffs of spared-eye ODC increases above the normal when homonymous regions of the fellow retina are destroyed. [Supported by NIH EY07016 to TCT and EY05439 to MWR.]

295.10

TOPOGRAPHY OF CORTICAL EFFERENTS OF V1 IN CEBUS apella MONKEY. M.C.G.P. Piñon*, A.P.B. Sousa and R. Gattass. Depto. Neurobiologia, Inst. de Biofísica Carlos Chagas Filho, UFRJ, Rio de Janeiro, RJ. Brazil.

Cortical projection fields of area V1 in the Cebus monkey were examined by the use of radioautographic tracing technique. In five monkeys the topographic organization of the fields was determined by injecting tritiated proline-leucine into selected V1 sites. The receptive fields corresponding to these sites were electrophysiologically identified and their eccentricities ranged from 2 to 60 degrees in the upper and lower visual quadrants. The results showed that projection sites were found in homotopic regions of V2, MT and V3 following central, intermediate and peripheral injections in V1. Projections to area V4 were only found following central and intermediate injections while projections to areas PO, MST and prostriata were only observed following peripheral injections. In addition to these projecting sites we also observed projections to TVP and FST in one animal that received multiple injections at different eccentricities. In the intraparietal region we only observed projections following intermediate injections in the lower quadrant of the visual field.
(Financial support: FINEP, CNPq and CEPG/UFRJ).

295.11

TOPOGRAPHY OF INTERHEMISPHERIC CONNECTIONS THROUGHOUT STRIATE CORTEX IN THE RAT. J.W. Lewis* and J.F. Olavarria. Division of Biology 216-76, Caltech, Pasadena, CA 91125.

In mammals, interhemispheric connections are concentrated at the lateral border of striate cortex, where the vertical meridian of the visual field is represented. Presumably these connections facilitate fusion of the two halves of the visual field, and may also subserve midline stereopsis. In the rat, callosal connections are also found in infragranular layers throughout striate cortex, including medial regions representing peripheral portions of the field (Olavarria and Van Sluyters, Brain Res., 279:233, 1983). Determining the topographic organization of this widespread interhemispheric pathway may be important for understanding the function of the callosal projections.

We investigated the topography of callosal connections by charting the location of retrogradely labeled cells within striate cortex of one hemisphere after combined injections of two or more fluorescent tracers into multiple sites in the contralateral striate cortex. We also labeled the entire callosal pathway with larger injections of an additional tracer in order to relate the location of cells labeled by the small injections to the overall callosal pattern within striate cortex.

We found that injections into medial striate cortex labeled cells predominantly in medial, mirror-symmetric portions of contralateral striate cortex, and rarely in lateral portions of this area. Conversely, injections into lateral striate cortex labeled cells in lateral portions of contralateral striate cortex, with few or no labeled cells in medial portions of this area. Thus, in addition to permitting interactions between midline portions of the visual hemifields, callosal connections of rat striate cortex appear to be capable of mediating interactions between widely disparate inputs from mirror-symmetric peripheral portions of the hemifields.

295.12

INTRINSIC SYNAPSES OF CALLOSAL PROJECTION NEURONS IN MOUSE VISUAL CORTEX. D. CZEIGER AND E.L. WHITE, DEPT OF MORPHLOGY, BEN-GURION UNIV. BEER SHEVA, ISRAEL.

Neurons at the borders of area 17 with areas 18a and 18b in mouse visual cortex were labeled by the retrograde transport of horseradish peroxidase (HRP) transported from severed callosal axons in the contralateral hemisphere. "Intrinsic" terminals of the local axon collaterals of these neurons were identified in areas 17/18a and 17/18b, and their distribution and synaptic connectivity were examined. A postlesion survival time of 3 days was chosen because by this time extrinsic callosal axon terminals were all degenerating, whereas the intrinsic terminals were labeled by HRP.

Intrinsic callosal axon terminals formed only asymmetrical synapses. Analyses of serial thin sections through layers II and III in areas 17/18a and 17/18b show 96% of the intrinsic terminals synapse onto dendritic spines, likely those of pyramidal neurons. The remainder synapse onto dendritic shafts of both spiny and nonspiny neurons. The high proportion of axospinous synapses formed by intrinsic callosal axon terminals differs from the proportion of asymmetrical, axospinous synapses that occur in the surrounding neuropil; a result essentially identical to that obtained for the synaptic connectivity of intrinsic callosal axon terminals in mouse somatosensory cortex. Taken together, these results indicate that axonal pathways are highly selective for the types of elements with which they synapse. NIH 20149 and BSF 86000-41

295.13

THE RETINAL ORIGIN OF THE IPSILATERAL FIELD REPRESENTATION IN THE AREA 17/18 TRANSITION ZONE IN THE CAT. J.D. Mendola, S.G. Lomber & B.R. Payne, Department of Anatomy, Boston University School of Medicine, Boston, MA 02118.

In the cat, the transition zone between areas 17 & 18 contains a representation of the ipsilateral visual field adjacent to the vertical meridian. This representation is not equal for all elevations in the visual field, for it is smallest closest to the visual axis and it enlarges with increasing and decreasing elevations in the visual field. The purpose of this study was to relate this representation to the patterns of projections of ganglion cells in the retina. This was achieved by mapping the representation of the visual field in the cortical transition zone using standard electrophysiological recording methods and then injecting horseradish peroxidase into the ipsilateral lateral geniculate nucleus to identify ganglion cells in the contralateral temporal retina with *crossed* projections to the lateral geniculate nucleus. As has been shown previously for the lower visual field, the representation in the transition zone extends to an azimuth of 3° at an elevation of -3° and expands to an azimuth of -20° at -30° elevation. Analyses of labelled cells in the retina showed that they lay increasingly temporal in location as the vertical distance from the area centralis increased. Conversion of these locations to visual field coordinates show that the region viewed by α cells matches the representation of the ipsilateral field demonstrated in the cortex very closely for all elevations. This correspondence between the retina and the cortex suggests that the crossed projections from temporal ganglion cells may be responsible, at least in part, for the ipsilateral field representation in the cortex. (Supported by EY06404)

295.14

THE ROLE OF THE MEDIAL INTERLAMINAR NUCLEUS IN THE REPRESENTATION OF THE IPSILATERAL VISUAL FIELD IN THE AREA 17/18 TRANSITION ZONE OF THE CAT. S.G. Lomber, B.R. Payne & D.F. Siwek. Department of Anatomy, Boston University School of Medicine, Boston, MA 02118.

In the cat, the transition zone between areas 17 & 18 in the cerebral cortex and layer 3 of the medial interlaminar nucleus (MIN) both contain a significant representation of the ipsilateral visual field adjacent to the vertical meridian. The purpose of the present experiment was to determine if the representation in MIN contributes to the representation present in the cortex. This was achieved by recording the activity and plotting the receptive fields of neurons in MIN and the immediately adjacent laminated part of the lateral geniculate nucleus. Layer 3 of MIN was identified by the presence of neurons with receptive fields well into the ipsilateral field. The neurons representing an azimuth of ~-12° in the ipsilateral field and adjacent cells were then destroyed by electrolytic ablation. The representation of the visual field in the area 17/18 transition zone was then assessed immediately afterwards. Unlike the representation of the ipsilateral field in the intact cat, which extends to -3° azimuth at the horizontal meridian and to -20° azimuth at an elevation of -30°, the representation in the ablated cat was virtually eliminated, for it extended only to an azimuth of ~-3° in the ipsilateral field. In addition, no neurons were detected in cortex that responded to the region of the visual field known to be at the center of the ablation in MIN. These results show that MIN mediates, at least in part, the representation of the ipsilateral field in the area 17/18 transition zone. (Supported by EY06404)

295.15

THE REPRESENTATIONS OF THE VISUAL FIELD IN THE TRANSCALLOSAL SENDING AND RECEIVING ZONES IN CAT AREA 17. B. R. Payne. Department of Anatomy, Boston University School of Medicine, Boston, MA 02118.

The purpose of the present study was to identify the callosal sending and receiving zones in area 17 and the adjacent area 17/18 transition region by application of anterograde and retrograde pathway tracers, and to assess, by recording the activities of neurons and plotting their receptive fields, the extent of the visual field represented in each zone. The assessments of the representations were made for all elevations in the visual field and are based on receptive fields plotted for neurons recorded at over 1900 sites. The results show that extent of the representation in the callosal sending zone is not equal at all elevations in the visual field. In regions close to the visual axis, receptive fields could be plotted from the vertical meridian to an azimuth of only +4° in the contralateral hemifield. In contrast, at representations of increasingly positive and negative elevations in the visual field receptive fields could be plotted at increasingly greater distances from the vertical meridian up to +15° to +20° into the contralateral field. This representation in the callosal sending zone overlaps the representation in the callosal fiber recipient zone of the opposite hemisphere which extends into that hemisphere's ipsilateral field out to -3° azimuth close to the visual axis and out to -15° or so toward the extremes of the upper and lower fields. The congruence of these representations indicate a considerable potential for interaction between the two hemispheres and that, for positions high and low in the visual field, the interactions can be evoked by stimuli displaced away from the vertical meridian. (Supported by EY06404)

295.16

THE VISUAL FIELD MAP IN THE CORPUS CALLOSUM OF THE CAT. D.F. Siwek and B.R. Payne. Department of Anatomy, Boston University School of Medicine, Boston, MA 02118.

In the cat, the corpus callosum conveys all the fibers connecting areas 17 & 18 in the two cerebral hemispheres; areas that both contain well-ordered maps of the visual field. The purpose of the present study was to ascertain the organization of the visual map in the corpus callosum. This was achieved by injecting anterograde and retrograde pathway tracers into the callosal zones of areas 17 & 18. The positions of the injection sites were varied systematically to include all visual field elevations represented along the marginal and posterolateral gyri. Overall, the results show that callosal fibers arising: (1) from the marginal gyrus, where the lower visual fields are represented, pass through the body of the corpus callosum; (2) from the junction of the marginal and posterolateral gyri, where central fields are represented, pass through the dorsal splenium of the corpus callosum; and (3) from the ventral posterolateral gyrus, where upper fields are represented, pass through the ventral splenium. Within this pattern a finer arrangement exists, making it possible to generate a map of visual field elevations in the corpus callosum. In this map, the representations of the different elevations is not regular. In the body the map appears to reflect cortical magnification of the lower fields, whereas the map of the central and upper fields in the splenium is highly compressed. This compression is reflected in the higher packing density of visual fibers in the splenium compared to the body, where visual fibers are intermixed with axons arising from other sources such as auditory cortex. (Supported by EY06080 & EY06404)

295.17

NEURONAL DYSFUNCTION AND ASSOCIATED IMMUNOHISTOCHEMICAL CHANGES AT THE BORDER OF FOCAL LESIONS IN THE CAT VISUAL CORTEX. U.T. Eysel and R. Schmidt-Kastner.* Department of Neurophysiology, Faculty of Medicine, Ruhr-Universität Bochum, D-4630 Bochum, F.R.G.

We used small heat lesions (1 mm in diameter) produced by photocoagulation of the visual cortex to study the local effects of focal brain damage. The lesions were visualized by high resolution thermography. After survival times of 1, 2, 7 and 30 days recordings were made under halothane/N_2O/O_2 anesthesia at distances between 0.5 and 2.5 mm from the center of the lesion. Single cell activity in the borderzone was characterized by depressed activity close to the lesion and hyperactivity with bursts or continuous high frequency discharges up to 700 Hz at distances between 1 and 1.5 mm. In this region orientation tuning was weak and order in orientation columns appeared disturbed. The histology of the lesions showed coagulation necrosis surrounded by tissue with some dark stained neurons as well as neurons with cytoplasmic vacuolation. Immunostaining for parvalbumin was reduced in the borderzone. A zone of serum-protein staining indicated massive vasogenic edema in the adjacent cortex and the white matter below. This vasogenic edema and the disturbance of inhibitory interneurons might be important pathophysiological factors leading to the observed neuronal dysfunction in the surrounding of focal cortical lesions. Supported by the DFG grant Ey 8/13-1.

295.18

THE CORTICAL CONTRIBUTION TO THE VISUAL FIELD REPRESENTATION IN THE PRETECTUM OF MONKEYS. K.-P. Hoffmann, C. Distler, U. Ilg, B. Preilowsky*, Allgemeine Zoologie und Neurobiologie, Ruhr-Universitaet Bochum, Postfach 102148, 4630 Bochum 1, FRG *Universitaet Tuebingen, FRG

Receptive fields of neurons in the nucleus of the optic tract (NOT) in the pretectum of macaque monkeys include the fovea and extend up to 20° into the ipsilateral visual hemifield. In primates there is no direct retinal projection from the temporal retina to the contralateral midbrain as in most other vertebrates. The subcortical representation of the ipsilateral visual field can therefore only originate via projections across subcortical or cortical commissures.

Brain areas with extensive ipsilateral visual field representation can be found in the superior temporal nucleus of neocortex (STS). To test the hypothesis that cortical pathways are essential to create an ipsilateral visual field representation which is then projected to the pretectum we investigated two macaca mulattas with total transsection of the corpus callosum. In the pretectum of these animals all receptive fields stopped precisely at the vertical 0-meridian. Thus, the NOT-neurons were no longer able to respond to moving stimuli in the ipsilateral visual field. Other response properties remained unchanged.

In another set of experiments we electrically stimulated the NOT in normal monkeys via a microelectrode while recording with a second microelectrode from STS to antidromically identify those neurons providing the visual information from the ipsilateral hemifield. Such neurons with response properties very much like NOT neurons (receptive fields including the fovea and covering the central 20°-40°, direction specific responses to a moving bar or random dot patterns) were found in the floor of STS. The consequences of these findings will be discussed in the context of optokinetic reflex.

Supported by DFG Ho 450-19 and ESPRIT Basic Resarch

295.19

CORTICAL INFLUENCES ON VISUAL PREDATOR AVOIDANCE IN THE MONGOLIAN GERBIL. C.G. Ellard and D.G. Chapman*. Dept. of Psychology, Mount Allison University, Sackville, New Brunswick.

Previous work has demonstrated heavy involvement of subcortical visual pathways in the fleeing response of the gerbil to a sudden, overhead visual threat (Ellard and Goodale. Exp. Brain Res., 71:307, 1988). In this study, we examined the effects of lesions to some posterior cortical areas on the predator avoidance response. Gerbils received either sham operations, aspiration lesions of area 18b or of posterior cingulate cortex (areas 29c and 29d). Following recovery, animals were placed in an open field containing a refuge and videotaped during presentation of unpredictable overhead visual stimuli of varying sizes.

Shams and animals with 18b lesions responded consistently to overhead movement by running to the refuge, even when the stimulus was only slightly larger than the resolution acuity threshold for gerbils. Cingulates showed severely depressed responses to overhead threat. Comparison of behaviour both before and after threat presentation showed a post-stimulus increase in risk assessment in shams and cingulates, but a tendency for animals with 18b lesions to spend abnormally long periods of time in the refuge following the threat.

These results suggest that there are cortical modulatory influences on the tectal mechanisms for predator avoidance. In light of connectional and physiological properties of these areas, as well as the present behavioural evidence, it is suggested that these areas modulate an animal's responsiveness to threat on the basis of environmental contextual variables, rather than the properties of the threat stimulus.

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295.20

CELL CLUSTER VARIATIONS WITHIN ISOAZIMUTH LAMELLAE OF TURTLE VISUAL CORTEX. P. S. Ulinski. Dept. Organismal Biology and Anatomy, Univ. Chicago, Chicago. IL 60637.

Cerebral cortex of turtles receives a projection from the dorsal lateral geniculate complex in which individual geniculate axons course from lateral to medial across the visual area, bearing varicosities en passant (Mulligan and Ulinski, J. Comp. Neurol., 295: in press, 1990). Visual cortex, consequently, contains lamellae representing the azimuth lines of visual space. Casual observation suggests there are regional variations in neuron properties within these isoazimuth lamellae.

This possibility was explored using computer-assisted, 3-D analysis methods to reconstruct regions of visual cortex from transverse and horizontal sections of cortex. The study confirms that the lateral, or "pallial thickening", region of cortical area D2 of Desan contains clusters of many neurons with apposed somata. It also shows for the first time that the medial region of D2 consists of clusters containing smaller numbers of neurons embedded within a continuous sheet of neurons in layer 2 of this trilaminate cortex. Thus, individual geniculate axons synapse first upon neurons in the lateral, large cluster region, and then upon neurons in the medial, small cluster region of the visual area. Correlation with data on the dendritic morphology of cortical neurons and of geniculate axons in these two regions suggests an individual geniculate axon has different functional interactions within one isoazimuth lamella. Supported by PHS Grant EY08352.

SENSORY SYSTEMS—SUBCORTICAL VISUAL PATHWAYS: RETINAL PROJECTIONS AND THALAMUS

296.1

α-HERPESVIRUSES ARE DIFFERENTIALLY TRANSPORTED THROUGH THE RODENT VISUAL SYSTEM AND ASSOCIATED CIRCUITRY. J.P. Card, M.E. Whealy*, A.K. Robbins*, R.Y. Moore[1] and L.W. Enquist*. Central Res. & Dev. Dept., The Du Pont Co., Wilmington, DE 19880 and [1]Dept. of Neurology, SUNY @ Stony Brook, Stony Brook, NY 11794.

Precise, circuit-specific transport of neurotropic herpesviruses has recently been shown to result from trans-synaptic passage of virus through synaptically linked populations of neurons. We have examined the transport of two strains of pseudorabies virus through visual circuits of the rat CNS following injection of virus into the vitreous body of the eye. The viral strains included a virulent field isolate (Becker, PRV-Be) and an attenuated vaccine strain (Bartha, PRV-Ba) harboring mutations which reduce virulence. Both strains of virus were taken up by retinal ganglion cells and transported to retinal recipient regions of the forebrain. However, distinct differences were noted in the rate of transport and the distribution of infected neurons. Transneuronal passage of PRV-Be was detected within 50 hours of injection and occurred predominantly in the dorsal lateral geniculate nucleus (dLGN). By contrast, PRV-Ba could not be detected prior to 72 hours post-injection and, rather than being localized in the dLGN, was concentrated in neurons of the intergeniculate leaflet. PRV-Ba also led to pronounced labeling of neurons in all regions of hypothalamus shown to receive retinal innervation and, with increasing survival, appeared to pass through the efferent projections of these cell groups to infect their target neurons. The two distinct patterns of infectivity observed following intraocular injection of PRV-Be and PRV-Ba suggest that differences in uptake and/or transport of virus may be related to the mutations know to exist in PRV-Ba.

296.2

FURTHER DEMONSTRATION OF IPSILATERAL PREPONDERANCE OF RETINAL PROJECTIONS IN THE PRIMATE VISUAL SYSTEM. G. Mick*, M. Magnin and H. Cooper*. Vision et Motricité, I.N.S.E.R.M. 94, 16 avenue Doyen Lépine, 69500 Bron, FRANCE.

We recently reported an unusual ipsilateral preponderance of the retinal input to the suprachiasmatic nucleus (SCN) in several primate species (Magnin, M., *Brain Res.*, 488:390, 1989). The aim of the present study is to determine whether a similar ipsilateral preponderance is also characteristic of other primary visual structures. Following monocular intravitreal injections of tritiated amino acids in 12 different mammalian species the relative density of terminal label of retinal projections was measured using quantitative image analysis. In Old World monkeys (Macaca fascicularis, Hylobates concolor), a predominance of the ipsilateral retinal projection is present in the olivary pretectal nucleus (OPN). In other primates (Callithrix jacchus, Galago demidovii) and non primates (Felis domesticus, Mustela putorius, Philander opossum, Heliosciurus rufobrachium, Rousettus aegyptiacus, Erinaceus europaeus, Manis tricuspis, Bradypus pilosa) the OPN shows a mainly contralateral retinal input. Preliminary results also indicate that one subdivision of the pregeniculate nucleus (PGN) receives a predominantly ipsilateral input in macaque and gibbon, although the homologue of the intergeniculate leaflet in primates remains to be defined.

The predominant ipsilateral retinal input, the anatomical connections and the presence of luminance responsive cells in all three structures suggest that not only the SCN and the PGN but also the OPN is involved in the photic regulation of circadian rhythms.

296.3

RESPONSE OF ADULT CAT RETINAL GANGLION CELLS TO LOSS OF POSTSYNAPTIC TARGET NEURONS IN THE DORSAL LATERAL GENICULATE NUCLEUS. H.E. Pearson and D.J. Stoffler*. Department of Anatomy and Cell Biology, Temple University School of Medicine, Philadelphia, PA 19140.

To investigate the effects of target cell removal on the survival and maintenance of mature retinal ganglion cells, kainic acid (3 nmol/ul) was injected bilaterally at multiple sites within the dLGN of adult cats. Following post-operative survivals of 2,4 and 6 months, the cats received multiple injections of HRP into the dLGN. After a further 72 hr, the cats were sacrificed and the retinae were reacted for the presence of HRP and counterstained with cresyl violet. Adjacent brain sections were processed for thionin staining and HRP histochemistry. Thionin staining showed large regions of dLGN to be degenerated, as characterized by the absence of neurons and increased numbers of glia. At each survival, counts of retinal ganglion cells were made at comparable locations in peripheral nasal retina, at a site corresponding retinotopically to regions of degeneration within the dLGN. Cell densities were determined separately for cells labelled retrogradely with HRP and for unlabelled cells stained for Nissl. There was a gradual decline in overall ganglion cell density with increased survival after target neuron loss. Approximately 20% of cells survived at 6 months. The proportion of cells labelled with HRP declined more sharply, with less than 10% labelled at 6 months. These results demonstrate that postsynaptic targets are essential for the survival of mature retinal ganglion cells. In the absence of normal targets, ganglion cells will first retract their axon terminals and then subsequently degenerate. Supported by NS25196.

296.4

THE STRUCTURE OF RETINAL AND COLLICULAR AXONS THAT TERMINATE ON PRIMATE W-LIKE LGN CELLS. E.A. Lachica[2] and V.A. Casagrande[1,2]. Depts. Psy.[1] and Cell Biol.[2], Vanderbilt Univ., Nashville, Tennessee 37232

The connections of the smallest relay cells of the lateral geniculate nucleus (LGN) of primates differ from large and medium relay cells in two ways. Only the smallest relay cells receive projections from both the superior colliculus and the retina, and only the smallest cells project directly to the cytochrome oxidase (CO) blobs. In this study we examined and compared the morphology of retinal and collicular axons that terminate on small cells in the LGN of *Galago*, by injecting HRP into the optic tract, and biocytin into the superficial gray layer of the colliculus. The small LGN cells in *Galago* have W-like physiology and are found in the koniocellular (K) layers and the interlaminar zones (ILZs). Results show that distinct population of retinal and collicular axons innervate the K layers and ILZs, and may be distinguished from one another in the following way. Both retinal and collicular axons arborize in a single K layer. However, the arbors of retinal axons are larger in area, and have a greater number and density of boutons than collicular arbors. In the ILZs, retinal and collicular axons terminate in multiple patches. However, collicular ILZ arbors are larger in area and have a greater number and density of boutons than retinal ILZ arbors. The following conclusions can be made based upon these differences in arbor morphology. First, the K layers and ILZs appear to receive input from distinct population of retinal W-like cells. Second, the K layers and ILZs may also receive input from distinct populations of collicular cells. Finally, W-like cells in the K layers may be dominated by visual input from the retina, while W-like cells in the ILZs could receive their main visual input from the colliculus. The latter conclusion suggests that the CO blobs may be receiving different kinds of input from W-like LGN cells. Supported by EY01778 to VAC and MH09754 to EAL.

296.5

MORPHOLOGY OF PHYSIOLOGICALLY IDENTIFIED RETINAL AXONS IN THE CAT'S THALAMUS AND MIDBRAIN AS REVEALED BY INTRA-AXONAL INJECTION OF BIOCYTIN. N. Tamamaki[1,2], D.J. Uhlrich[1], and S.M. Sherman.[1] Dept. of Neurobiology[1] and Howard Hughes Medical Institute[2], SUNY, Stony Brook, NY 11794-5230.

The retina provides a direct projection to visual centers in the thalamus and midbrain, but the nature of this divergent innervation is little understood, particularly at the single-axon level. We thus studied this projection via intra-axonal labeling with biocytin in anesthetized and paralyzed cats. Single retinal axons were recorded from the optic tract ventral to the lateral geniculate nucleus (*LGN*), physiologically characterized, impaled, and pressure-injected with 5-6% biocytin dissolved in 0.5M KCl. After a two day survival period, we perfused the cats transcardially with an aldehyde fixative and processed the tissue with an ABC reaction procedure. Thus far, we have recovered and reconstructed 12 retinal Y axons, 5 projecting ipsilaterally and 7 contralaterally. Each of these innervates the LGN, the pretectum, and the superior colliculus (*SC*). For each axon, there is good retinotopic correspondence between the location of the terminal field in the LGN and that in the SC. The retinotopic projection to the pretectum is less evident. In the SC, the Y axons innervate the ventral portion of the retinal-recipient zone. Each axon from the ipsilateral eye tends to form a single, circular puff 250-300μm wide, while each from the contralateral eye tends to extend further mediolaterally and dorsoventrally. These termination patterns from the two eyes are thus consistent with the interdigitated pattern revealed by bulk-fill anterograde labeling studies in which segregated puffs of label from the ipsilateral eye contrast to the more continuous labeling seen contralaterally. Finally, in both the LGN and SC, arbor size and bouton number vary with the eccentricity of the axon's receptive field: axons with greater eccentricities form smaller terminal arbors. Since the size of the parent somata (i.e., of retinal α cells) *increases* with increasing eccentricity, this suggests a *negative* correlation between soma size and axon arbor, which runs counter to frequent suggestions that these two parameters would be positively correlated.

296.6

RELATIONSHIPS BETWEEN CHOLINERGIC AND GABAERGIC INNERVATION OF THE CAT'S LATERAL GENICULATE AND PERIGENICULATE NUCLEI: AN EM DOUBLE-LABELLING STUDY. C. Beaulieu and M.S. Cynader, University British Columbia, Dept Ophthalmology, 2550 Willow Street, Vancouver, B.C. CANADA.

The relationship between cholinergic axons and gamma-amino butyric acid (GABA) containing cells in the lateral geniculate (LGN) and the perigeniculate nuclei (PGN) was assessed by combining pre-embedding immunocytochemistry of choline acetyltransferase (ChAT) with post-embedding immunogold GABA localisation at the EM level. In the LGN, all 152 ChAT positive vesicle-containing profiles studied to date contacted dendritic profiles within or outside synaptic glomeruli. Only 7.1% of the recipient dendrites tested for the presence of GABA were positive. In the PGN, of the 34 ChAT synapses analyzed, the vast majority targeted dendritic profiles (94%) and only a few were found on somata (6%). In this nucleus, all targets tested were GABA-positive. This is not surprising since all PGN neurons are GABA-positive. We thus suggest that cholinergic synapses show a preference to target LGN relay neurons without a strong innervation of GABA interneurons. In the PGN however, ChAT synapses provide a powerful input to the GABA cells.

296.7

MONOCLONAL ANTIBODIES RECOGNIZE SUBPOPULATIONS OF CELLS IN THE LATERAL GENICULATE NUCLEUS OF THE CAT. N. Tumosa+, J.R. Baker+* and P.D. Spear#, School of Optometry, University of Missouri-St. Louis+, MO 63121 and Department of Psychology, Center for Neuroscience, University of Wisconsin#, Madison, WI 53706.

Several classes of cat retinal ganglion cells can be distinguished on the basis of their morphology. These cell classes form parallel functional outputs from the retina to visual areas of the brain. We previously produced monoclonal antibodies (MAbs) that recognize different subpopulations of retinal ganglion cells (Tumosa et al., 1987). The present study examined whether these MAbs recognize subpopulations of cells in the cat'sLGN. PAP methods were used to visualize antigen-antibody reactions for each of 4 IgM MAbs on fifty-micron sections.

One of the four MAbs recognized no cells in the LGN. The remaining three MAbs labeled cells in both the A and C layers. One MAb labeled cell bodies and proximal dendrites of about 40% of the layer A neurons and the labeled cells were 20-120 um^2 in area. The two remaining MAbs labeled cell bodies only. One labeled about 30% of the layer A neurons, which were 25-180 um^2 in area. The other MAb labeled about 35% of the layer A neurons, which were 20-140 um^2 in area. These results suggest that antigens that distinguish subpopulations of retinal ganglion cells also distinguish LGN cells. Future studies will examine the relationship between the classes of retinal and LGN cells labeled by a single MAb, which may have implications for understanding the molecular bases of retino-geniculate connectivity.

296.8

RESPONSES TO STIMULUS OFFSET OF GANGLION AND GENICULATE X AND Y CELLS IN THE CAT. S. Lehmkuhle, J.A. Baro*, H. C. Hughes. School of Optometry, Univ. of Missouri-St. Louis, St. Louis, MO 63121 and Dept of Psychology, Dartmouth College, Hanover, New Hampshire 03755.

Depending upon the direction of the luminance change, on- and off-center cells respond to stimulus onset or offset. These responses for ganglion and geniculate cells, however, differ in amplitude and latency. The magnitude of this difference depends upon cell site (ganglion vs. geniculate), cell type (X vs.Y), and stimulus duration.

The responses of the receptive-field center to stimulus onset and offset were measured for ganglion and geniculate X and Y cells in the anesthetized, paralyzed cat. The direction of the luminance change of the spot was set to elicit either onset or offset responses for on- and off-center cells. The maintained luminance of the spot was 11.6 cd/m^2, and modulated to either 23.2 or 5.8 cd/m^2. Stimulus duration was varied from 5 to 160 ms in octave steps. Visual latencies and response amplitudes were measured with a criterion based, trial-by -trial analysis.

The response rates of geniculate X and Y cells were equivalent for stimulus onset and offset across all stimulus durations, whereas responses were less for stimulus offset than onset for both ganglion X and Y cells. Visual latencies to stimulus offset were longer for all cell groups. The difference in latencies between stimulus onset and offset varied with stimulus duration (≈Δ60 ms at a stimulus duration of 10 msec to ≈Δ20 ms at a stimulus duration of 160 ms). The difference in the visual latencies between X and Y cells is greater for stimulus offset (≈Δ20 msec) than for stimulus onset (≈Δ5 ms).

296.9

THE CAT MEDIAL INTERLAMINAR NUCLEUS IN DIM-LIGHT VISION. D. Lee* and J. Malpeli. Neuroscience Program and Dept. of Psychology, University of Illinois, Champaign, IL 61820.

The medial interlaminar nucleus (MIN) receives inputs only from the region of retina backed by the reflective tapetum, suggesting a special role in dim-light vision (Lee et al., J. Neurophysiol., 5:848, 1984). To test this idea, we compared the sensitivity of MIN and lateral geniculate nucleus (LGN) cells over the same range of eccentricities. The contrast sensitivity of single cells was determined as a function of spatial frequency (0 to 2 cyc/deg) and adaptation level. The lowest adaptation level tested was 0.5 log units below the absolute threshold of the most sensitive cells, and adaptation levels were examined in logarithmic steps up to 6.5 log units above this level. On average, MIN cells had higher contrast sensitivity than LGN cells for low spatial frequencies (.125 cyc/deg and below) and a wide range of adaptation levels (within 5 log units of the lowest level).

Lee et al. proposed that the MIN trades off acuity for sensitivity through high convergence of retinal afferents, whereas the LGN maintains high acuity at the cost of sensitivity. Our data are consistent with the hypothesis that the retinal conflict between sensitivity and acuity is ameliorated in the central nervous system through separate thalamic relays with different degrees of afferent convergence. (Supported by NIH grant EY02695)

297.1

BIPOLAR CELL DENSITY IN THE MACAQUE MONKEY RETINA
Paul R. Martin* and Ulrike Grünert. Dept.Neuroanatomy, Max-Planck-Institut für Hirnforschung, D-6000 Frankfurt/M., W.Germany.

The macaque retina contains rod and cone bipolar cells. Several types of cone bipolar cells can be distinguished, whereas rod bipolar cells are a homogeneous population which can be labelled with an antibody against protein kinase C (PKC) (Grünert and Martin, *Inv. Ophthal. Vis. Sci.* 3: (4) p536, 1990).

We have measured the density of rod bipolar cells in the macaque monkey retina, in sections processed for PKC immunoreactivity. We compared this with the density of the total bipolar cell population, which could be distinguished in semithin Nissl stained sections and low-power electron micrographs. Our main findings are:

1) Bipolar cells outnumber ganglion cells in central as well as peripheral retina. In three monkeys where the average peak ganglion cell density (measured in temporal retina) was 50 000 cells/mm^2 , the average bipolar cell density at the same eccentricity was 92 000 cells/mm^2 . Taking post-receptoral displacement into account and integrating cell densities over retinal volume we obtain an average of 1.7 bipolar cells per ganglion cell within the first 900 μm of the fovea.

2) Rod bipolar cells are relatively scarce in the central retina. Their average density within the first 900 μm of the fovea was 6000 cells/mm^2 . Discounting these cells gives a cone bipolar:ganglion cell ratio of 1.6:1.

3) There are at least three cone bipolar cells per cone up to 5mm eccentricity. The density of cone bipolar cells is thus high enough to allow for the existence of independent populations which contact single cones up to this eccentricity.

297.2

SYNAPTIC EVENTS UNDERLYING TRANSMISSION FROM PHOTORECEPTORS TO OFF-CENTER BIPOLAR CELLS.
B.R. Maple and F.S. Werblin, Div. of Neurobiology, Univ. of California, Berkeley,Ca 94720

Bipolar cells were voltage clamped in salamander retina slices and filled with lucifer yellow. Excitatory conductances were activated by pressure ejection of glutamate at the outer plexiform layer and by ejection of hyperosmotic sucrose solutions which elicited transmitter release from photoreceptors. Miniature excitatory synaptic currents were observed and their time course fit by a difference of two exponentials.

Cells with telodendria ramifying in the distal 1/4 of the inner plexiform layer gave responses to glutamate and sucrose ejection that were sustained and resistant to cobalt. These cells displayed miniature synaptic events fit by mean time constants of .98 and 1.7 msec. Off cells ramifying more centrally in the IPL gave responses which rapidly desensitized and were reversibly abolished by cobalt. These cells displayed slower events characterized by time constants of 1.3 and 6.4 msec. Thus glutamatergic transmission to off bipolar cells may be mediated by two different receptors, possibly associated with rod and cone transmission, respectively.

The quantal structure of transmission to the distally ramifying cells in cobalt was studied. An analysis of current variance over short segments of bandpass filtered records suggested that quantal events in these cells, ranging up to 660 pS in amplitude, were composed of 60 pS subunits. The overall variance to mean ratio for osmotically induced release corresponded to an event far smaller than this subunit amplitude and was close to the variance to mean ratio for glutamate responses (2 pS). Photoreceptors may release transmitter by two mechanisms: quantal release involving the synchronous discharge of vesicles, and nonquantal release resulting in uncorrelated channel openings.

297.3

SYNAPTIC TRANSMISSION FROM PHOTORECEPTORS TO BIPOLAR CELLS (BCs).
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Synaptic transmission from photoreceptors to BCs were studied in the mudpuppy retinal slices, using dual, whole-cell recordings from synaptically connected pairs. Compared to on-BCs (n=12), off-BCs (n=24) showed significantly shorter response delay (27.8 vs. 80.6 msec) and peak delay times (50.5 vs. 159.8 msec).

Photoreceptor inputs to off-BCs were blocked by kynurenic acid (Kyn), but, rod inputs to off-BCs were more resistant to Kyn, compared to cone inputs. The rod inputs to off-BCs were indistinguishable from those to horizontal cells (HCs) in terms of Kyn sensitivity. However, the cone inputs to off-BCs were more resistant to Kyn than those to HCs. These data suggest that three pharmacologically distinct excitatory amino acid receptors are involved in the sign-conserving pathway of the outer retina.

The sign-reversing rod and cone inputs to on-BCs were blocked by 2-amino-4-phosphonobutyric acid (APB). The effect of APB was permanent. However, after, and sometimes during APB application, current injections into photoreceptors produced sign-conserving inputs in on-BCs. These sign-conserving inputs persisted after washing away the APB. With one exception, the sign-conserving inputs were never observed before the APB application. Possible mechanisms to account for these observations are considered.
(R01EY03014)

297.4

RESPONSES OF DISSOCIATED ROD BIPOLAR CELLS OF THE RAT RETINA TO 2-AMINO-4-PHOSPHONOBUTYRIC ACID (APB).
M. Yamashita* and H. Wässle. Max-Planck-Institut für Hirnforschung, Frankfurt, Germany

Rod bipolar cells were dissociated from adult rat retina and identified by PKC-immunocytochemistry. Recently a wash-out of ON-bipolar responses to glutamate was reported during conventional whole-cell patch clamp recordings (Nawy & Jahr, Neurosci. Lett., 108:279, 1990). This was prevented in the present study by using the nystatin method (Horn & Marty, J. Gen. Physiol., 92:145, 1988). Tonic inward currents (3.7-79.3pA) were observed in 29 of 57 cells held at -33mV. APB decreased the amplitudes of these currents to 58.9±25.3% of the predrug level (n=27). In cells, which showed no APB-sensitive current, this current could be evoked by application of the calcium ionophore A 23187 (1µM). The mean reversal potential of the APB-sensitive current was -10.8±4.5mV (n=7) in normal saline solution. Lowering the external [Na] changed the inward currents into outward. We conclude that the refractoriness of rod bipolar cells to glutamate reported previously (Karschin & Wässle, J. Neurophysiol., 1990) was due to a wash-out of internal Ca, and that APB closes a Ca-dependent non selective cation channel. Since APB acts selectively on ON-bipolar cells as a glutamate agonist to decrease membrane conductances (Slaughter & Miller, J. Neurosci. 5:224, 1985), rod bipolars of the rat retina are likely to be ON-bipolar cells.

297.5

EFFECTS OF GLUTAMATE ANALOGUES ON BIPOLAR AND HORIZONTAL CELLS IN THE TIGER SALAMANDER RETINA.
Samuel M. Wu and Xiong-Li Yang. Cullen Eye Institute, Baylor College of Medicine, Houston, Texas 77030.

Overwhelming evidence in recent years has suggested that L-glutamate is the photoreceptor neurotransmitter in the vertebrate retina. It is important to determine what subtypes of glutamate receptors are used to mediate postsynaptic signals in various second-order neurons. In an earlier study, we found that at least four subtypes of glutamate receptor subtypes exist in the tiger salamander horizontal cells (HCs): kainate (KA) receptors, α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) receptors, 6-cyano-7-nitroquinoxaline (CNQX)-resistant quisquate (QA) receptors, and trans-l-aminocyclopentane-1,3-dicarboxcylic acid (ACPD) receptors (ARVO abstract 2628.1990). In the depolarizing bipolar cells (DBCs), 20 μM CNQX (the satuarating dose for blocking HC light responses) exerts little action on either the resting potential or the light responses. On the other hand, 20 μM L-2-amino-4-phosphonobutyrate (L-AP4) (exert no effect on HC and HBC) abolishes the depolarizing response of the cell to whole field illumination and converts it into a hyperpolarizing response. This result suggests that L-AP4 receptors probably only exist in the photoreceptor-DBC synapses and that at least part of the HC-DBC input (antagonistic surround response) is mediated by the forward synaptic pathway (direct HC-DBC). In the hyperpolarizing bipolar cells, 20 μM CNQX reduces the light responses but rarely abolishes them completely. These results indicate that the HC, DBC and HBC in the tiger salamander retina use different combinations of glutamate receptor subtypes to process their postsynaptic responses, although they all receive inputs from the same glutamatergic synapses.

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297.6

EFFECTS OF GABA AND DOPAMINE ON THE ELECTRICAL COUPLING OF ROD-DRIVEN HORIZONTAL CELLS IN SKATE RETINA.
H. Qian,*^ R. P. Malchow,+and H. Ripps.+^ Depts. Ophthalmology+, and Anatomy & Cell Biology^, Univ. of Illinois College of Medicine, Chicago, Il. 60612.

The large receptive fields of fish horizontal cells are due primary to intercellular coupling via gap (electrical) junctions. For cone-driven horizontal cells, the degree of coupling is modifiable by light and by neuroactive substances (e.g. dopamine, GABA). For rod-driven horizontal cells, we have previously reported that background illumination does not significantly alter the receptive fields of skate horizontal cells. In this study, we investigated the effect of GABA and dopamine on the receptive field properties of rod-driven horizontal cells in the skate (*R. ocellata* and *R. erinacea*) retina.

Intracellular recordings from horizontal cells were obtained from eyecup preparations; responses to either a 60 um light slit moved across the retina or a circular spot of varying diameter was used to measure the receptive fields. The cells were identified by their positions in the retina, by their response to light, and by dye marking after recording. The receptive fields of skate horizontal cells can be described by a simple exponential equation to a light slit stimulus and by a modified Bessel function to a spot stimulus. When 500 uM GABA or 200 uM dopamine were superfused on the skate eyecup, no significant change of the receptive field characteristics of horizontal cells was observed. Further, the ratio of responses to spot vs annular stimuli did not appear to change during the time course of drug application. These observations again suggest that there may be fundamental differences between the gap junctional properties of rod- and cone-driven horizontal cells.
Supported by EY-06516.

297.7

PROTEIN CONTENT AND cAMP-DEPENDENT PHOSPHORYLATION OF FRACTIONATED WHITE PERCH RETINA. D.G. McMahon, J.C. Rischert* and J.E. Dowling, The Biological Laboratories, Harvard University, Cambridge, MA. 02138.

Dopamine modulates the activity of both gap junctional and glutamate-receptor channels in teleost retinal horizontal cells through cAMP-dependent protein phosphorylation. To identify horizontal cell-specific phosphoproteins we have fractionated the white perch (Roccus americana) retina on Percoll density gradients and studied the protein content and phosphorylation of horizontal cell enriched and non-horizontal cell enriched fractions.

Visual examination of Coomassie blue-stained SDS-PA gels from 8 fractionations revealed that proteins of ca. 76, 50, 43, and 28 kDa consistently coenriched with horizontal cells, while proteins of 75, 36 (rhodopsin), and a doublet at 30 kDa were enriched in non-horizontal cell fractions. This doublet was also enriched in 4/8 of horizontal cell fractions, but to a lesser extent than in the non-horizontal cell preparations suggesting that it is not horizontal cell in origin. The 43 kDa protein and the 30 kDa doublet were phosphorylated upon stimulation of endogenous kinase with cAMP (N=4). The 43 kDa phosphoprotein is of interest since our results suggest that it is contained in horizontal cells. While the identity of this protein remains to be determined, it is similar in molecular weight to the gap junction protein connexin43 which is also regulated by cAMP.

297.8

DOPAMINERGIC CELLS AND DOPAMINE DIFFUSION IN THE XENOPUS RETINA. P. Witkovsky and M. Schütte*. Depts. of Ophthalmology and Physiology & Biophysics, NYU Medical Center, New York, NY 10016.

Catecholaminergic cells and fibers in the Xenopus retina were characterized with an antiserum directed against tyrosine hydroxylase. TOH+ cells were interplexiform-like with an extensive network of processes in the inner retina. Fine distally directed processes emerged from TOH+ perikarya that reached the level of the outer plexiform layer (OPL) but arborized not at all or to a very limited degree within it. A second TOH+ system consisted of a few centrifugal fibers and some smooth, relatively stout processes that reached the OPL within which they coursed horizontally. It remains to be shown whether the latter processes are extensions of centrifugal fibers. These anatomical findings suggested that dopamine reaches target cells in outer retina (horizontal, photoreceptor and pigment epithelial cells) by diffusion. We examined this postulate by studying the release of ^{3}H-dopamine from isolated retinas dissected from eyecups incubated in 1 μM ^{3}H-dopamine. ^{3}H-dopamine was taken up in a time and dose-dependent manner. Specificity of uptake was checked by relase with D-amphetamine, blockage of serotonin uptake mechanisms and by autoradiography. Baseline release of ^{3}H- dopamine was low; by reference to date of Boatright et al. (Brain Res., 482:164, 1989), ^{3}H-dopamine represented 0.5-1% total retinal dopamine so that dopamine release = 1-2 pMol retina^{-1} min^{-1}. Presently we are comparing efflux of ^{3}H-dopamine across vitreal and photoreceptoral surfaces. These data will be used to estimate the extracellular concentration of dopamine in outer retina. Supported by NIH Grant EY03570 to P.W.

297.9

ORIENTATION BIAS OF HORIZONTAL CELL RESPONSES IN THE RABBIT RETINA. Stewart A. Bloomfield. Dept. Ophthalmology, NYU Medical Center, New York, N.Y. 10016.

The sensitivity of horizontal cell (HC) responses to the angle of orientation of visual stimuli was examined. Intracellular recordings were obtained from HCs in the isolated, superfused retina-eyecup of the rabbit during which a moving, rectangular slit of light was presented at four angles of orientation. Physiologically-characterized cells were injected with HRP for morphological identification.

Responses of B-type HC somas (n=12) and axon terminals (n=5) showed no sensitivity to the orientation of visual stimuli. However, 32% (n=17/53) of A-type HC responses showed an orientation bias. Of these orientation bias HCs, 82% preferred (i.e., displayed the greatest hyperpolarization) slits of light oriented parallel (90^o) to the visual streak, whereas the remainder preferred light stimuli oriented orthogonal (0^o) to the visual streak. All orientation bias HC responses were recorded within or slightly superior to the visual streak. HRP-labeled orientation bias HCs which preferred stimuli oriented at 90^o displayed asymmetrical dendritic arbors with the long axis oriented parallel to the visual streak. Oppositely, orientation bias HCs with preference for the 0^o orientation had dendritic fields oriented orthogonal to the visual streak. These cells corresponded to the elongated A-type HCs reported previously by Bloomfield & Miller (1982) and Kolb & Normann (1982). The present results suggest that the orientation bias of rabbit HCs reflects a marked asymmetry in their dendritic arbors. These cells may contribute to the orientation bias of neuronal responses in the proximal retina (Bloomfield, 1990).

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297.10

GABA INHIBITS ACH RELEASE FROM THE RABBIT RETINA: A DIRECT EFFECT OR BIPOLAR CELL FEEDBACK? David M. Linn and Stephen C. Massey*, Sensory Sciences Center, GSBS, UTHSC, 6420 Lamar Fleming Avenue, Houston TX 77030

The cholinergic amacrine cells of the rabbit retina may be labeled with ^{3}H-Ch and the activity of the cholinergic population monitored by following the release of ^{3}H-ACh. ACh release may be produced directly by exogenous glutamate analogs or physiologically via bipolar cells by light stimulation. Muscimol, a potent GABA agonist, blocked the light evoked release of ACh with an IC_{50} of 1.0 μM but the KA and NMDA evoked release was not reduced by concentrations of muscimol as high as 100 μM. Thus we have been unable to demonstrate a direct effect of GABA on the cholinergic amacrine cells.

GABA antagonists such as picrotoxin caused a large increase in the base release and potentiated the light evoked release of ACh. Both these effects were blocked by DNQX, a KA antagonist which blocks the input to cholinergic amacrine cells from bipolar cells. This implies that the dominant site of GABA inhibition is on the bipolar cell input to the cholinergic amacrine cells.

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297.11

Light evoked glutamatergic inputs to amacrine cells in the tiger salamander retina. D. B. Dixon and D. R. Copenhagen Dept. of Ophthalmology, UCSF; San Francisco, CA 94143

We investigated the excitatory synaptic inputs into amacrine cells in the salamander retinal slice preparation using standard whole cell patch clamp techniques. Light evoked excitatory currents were measured in control saline, and in salines containing 30 μM AP7, an NMDA antagonist, or 2 μM CNQX, a non-NMDA glutamatergic antagonist, or both AP7 and CNQX. Inhibitory currents were blocked by 100 μM bicuculline and 500 nM strychnine.

The sustained light responses (LR) of the ON-cells were completely blocked by CNQX. AP7 had no discernible effect on the time course or amplitude of these LRs. Current-voltage (I-V) relations of these LRs in control and AP7 salines were nearly linear between -90 mV and +30 mV. Reversal of the response was at +6.3 mV. Thus, the synaptic inputs generating these responses appear mediated exclusively by non-NMDA glutamatergic inputs.

In contrast, the transient LRs of the ON/OFF or OFF cells had concurrent NMDA and non-NMDA synaptic inputs. The I-V curve in control salines had a distinct negative slope region between -75 mV and -50 mV and reversed at +7.2 mV. AP7 produced a shortening of the LRs at potentials positive to -70 mV when compared to control responses and the I-V curve in the presence of AP7 was nearly linear between -90 mV and +30 mV. The LR in CNQX lacked a fast rising phase as compared to the controls and the I-V curves of these LRs had a distinct negatively sloped region between -75 mV and -50 mV, characteristic of NMDA-receptor activation. The combined application of AP7 and CNQX eliminated the entire LRs in these cells.

This work supported by Fight for Sight and NIH EY01869.

297.12

EFFECTS OF PICROTOXIN ON THE ELECTRORETINOGRAM B. Oakley II, B.J Katz*, R. Wen*, J. Zheng*, and Z. Xu*.
Vision Research Laboratory, University of Illinois, Urbana, IL 61801

We recorded transretinal and intraretinal ERGs, Müller cell voltage, and $[K^+]_o$ in the isolated retina of *Bufo marinus*, in response to diffuse light flashes (0.1-10 s). Picrotoxin (PTX, 10-100 μM), a $GABA_A$ antagonist, enhances K^+ increases in the IPL and Müller cell depolarizations at ON and OFF by ~250%, and leads to the appearance of an ON-M-wave and a positive OFF-response in the vitreal ERG, as well as a PNR and M-wave in the intraretinal ERG. These effects likely are due to blocking GABA-mediated inter-amacrine inhibition that occurs with diffuse flashes. By removing this inhibition, amacrine cell responses to diffuse light become equivalent to superposition of individual cells' responses to small spots, which are optimal for evoking the M-wave. Since with PTX the M-wave is generated over a large area, it contributes to the *vitreal* ERG, where it is a large, *positive* component following the b-wave. In PTX, the M-wave and OFF-response have waveforms similar to the IPL K^+ increases and these potentials are blocked by 100 μM Ba^{2+}, supporting the hypothesis that these potentials result from Müller cell current sinks that arise in the IPL in response to K^+-evoked membrane depolarization distal to the cell's neutral zone. The pattern of intraretinal reversals of these components is consistent with current sources on the Müller cell membrane both distal and proximal to the IPL. The OFF-response reverses at a depth distal to where the M-wave reverses, consistent with the OFF-K^+ increase having its amplitude maximum distal to the ON-K^+ increase. Finally, comparison of control ERGs evoked by rod-matched 600 nm and 500 nm flashes shows that a small M-wave contributes to the ERG under control conditions. Supported by NIH grant EY04364.

297.13

INTRACELLULAR ACIDIFICATION SUPPRESSES THE CALCIUM ACTION POTENTIAL OF ISOLATED HORIZONTAL CELLS FROM CATFISH RETINA.
K. Takahashi and D.R. Copenhagen. Departments of Ophthalmology and Physiology, UCSF Sch. of Med., San Francisco, CA 94143

Brief depolarizing current pulses injected into isolated horizontal cells via an intracellular microelectrode evoke sustained action potentials that can last for several minutes (Tachibana, M., J. Physiol., 321:141, 1981; Shingai and Christiansen, J. Neurophysiol., 56:32, 1986). Pharmacological manipulations suggest that these are calcium action potentials.

Changes in intracellular pH affected the duration of the action potentials: alkalinization induced by bath application of NH_4Cl prolonged the depolarization, while acidification induced by bath application of acetate or the washout of NH_4Cl shortened and often eliminated the action potential. The pH-dependency was observed in the presence of IBMX (0.5 mM), or BAPTA-AM (70 μM), or TEA (30 mM), or low sodium(0 mM). These findings suggest that the pH effects were not being mediated via pH-dependent, sodium or delayed rectifier-type potassium conductances and did not result from a pH-dependent reaction in a cyclic nucleotide second-messenger system.

We postulate that the pH effects are mediated by a direct action of H^+ ions on the calcium conductance underlying the action potential. Similar H^+ ion suppression of calcium conductances has been reported in *paramecium* (Umbach, J., Proc. R. Soc. Lond. 216:209, 1982)

297.14

CHANGES IN INTRACELLULAR CALCIUM CONCENTRATION OF DISSOCIATED RETINAL GLIA AND NEURONS OF THE LARVAL TIGER SALAMANDER MEASURED WITH FURA-2 IMAGE ANALYSIS. S.A. Keirstead & R.F. Miller. Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55455

We have examined alterations in intracellular calcium of single, dissociated cells from the neotenous tiger salamander retina using fura-2 imaging techniques. Cells were studied from acutely dissociated preparations, or from preparations that had been cultured for several days, after plating them onto a matrix of Sal-1 (kindly provided by P. Macleish). Neurons and glia were loaded with fura-2 by incubating them in a 0.5 to 1.0 uM solution of the acetoxymethyl ester of fura-2 (Molecular Probes) in amphibian Ringer's for 15 to 30 minutes and then rinsed extensively with Ringer's. Images of the intracellular calcium concentration of retinal cells were obtained using a commercially available system (Quantex, Sunnyvale, CA) configured with a Nikon Diaphot inverted microscope and a dual monochrometer (PTI, South Brunswick, NJ) with excitation wavelengths of 340 and 380 nM. An insert was placed within the culture dishes to reduce dead space and the cells were continuously superfused using a perfusion pump connected to a group of ganged syringes for changing the bathing medium.

We have observed substantial increases in intracellular calcium concentration in retinal cells as a result of increasing extracellular potassium concentration and as a result of application of excitatory amino acid agonists.

297.15

$[Ca^{2+}]_o$ GRADIENTS AND LIGHT-EVOKED $[Ca^{2+}]_o$ CHANGES IN CAT RETINA, IN VIVO. R.P. Gallemore*, F. Yamamoto* and R.H. Steinberg. Physiology and Ophthalmology, Univ. of Calif., San Francisco, CA 94143.

We report the first measurements of Ca^{2+} gradients and light-evoked Ca^{2+} changes in the mammalian retina, in vivo. Double-barreled Ca^{2+}-selective microelectrodes were used to record intraretinally from the intact cat eye. Depth profiles in the dark revealed $[Ca^{2+}]_o$ in the subretinal space surrounding rod outer segments to be 1-2 mM higher than in the vitreous, with $[Ca^{2+}]_o$ highest near the RPE. In addition, a trans-RPE gradient was observed with choroidal $[Ca^{2+}]_o \geq$ 1 mM higher than subretinal $[Ca^{2+}]_o$.

Light-evoked $[Ca^{2+}]_o$ changes were maximal at two retinal depths. Deep in subretinal space, near the RPE, maintained illumination evoked a sustained $[Ca^{2+}]_o$ *decrease* that peaked in 30-45 sec after light-onset and was as large as 1 mM. At light-offset, Ca^{2+} returned to baseline with a similar time course. The dark-adapted sensitivity of this response indicated its dependence on rods. These results were unexpected since, in vitro, measurements in other species have revealed a light-evoked increase in subretinal Ca^{2+} of $\leq$ 50 uM, followed by a decrease of similar magnitude at light-offset and these responses are thought to reflect changes in light-dark Ca^{2+} fluxes across rod outer segments. In the inner plexiform layer, light-evoked a transient *increase* in Ca^{2+} that peaked in ~20 sec and was as large as 0.5 mM. At light-offset, Ca^{2+} underwent a transient decrease similar in time-course and amplitude. Experiments are in progress to elucidate the origins and mechanisms of the observed Ca^{2+} gradients and light-evoked Ca^{2+} changes. Given the exquisite sensitivity of photoreceptors to $[Ca^{2+}]_o$ and the proposed roles of Ca^{2+} in light-adaptation and the transduction process, these results may have important implications for photoreceptor function in vivo. (NIH grant EY01429)

297.16

Time-Domain Models of the Horizontal Cell Network Implemented on a Massively Parallel Processor R. L. Winslow, A. L. Kimball*. Department of Physiology & Army High Performance Computing Research Center, University of Minnesota, Minneapolis, MN 55455.

Previous studies have investigated the ways in which voltage-dependent membrane currents, synaptic input from photoreceptors, and gap junction coupling between neighboring horizontal cells interact to determine the steady-state response properties of isolated horizontal cells and horizontal cell networks (J. Neurophysiol., 62(3): 738; J. Neurophysiol. in press). We have extended these results to time-domain models of the horizontal cell network of the whiteperch retina. Hodgkin-Huxley type models of membrane current kinetics based on data from isolated whiteperch horizontal cells (provided by A. Knapp) have been formulated. Ca mediated inactivation of the Ca current is modeled using the approach of Eckert & Chad. Network models are constructed by interconnecting cells to form a square mesh of typically 128x128 cells. Gap junction coupling between neighboring cells is adjusted to match the space constant measured in horizontal cell bodies of the carp, and the point-to-point transfer impedance measured in catfish retina. Light responses (e.g., photoreceptor inputs to the network) are modeled by adjustment of the synaptic conductance value within the region of the network illuminated by the stimulus. State variables are computed by mapping individual cells in the network onto a single processor of a massively parallel computer known as the Connection Machine. A fourth order Runge-Kutta adaptive step-size algorithm is used to update values of the ten state variables describing each horizontal cell concurrently. The model has been used to investigate the effects of cell-to-cell coupling on reponse to light spot, slits, and drifting sinusoidal gratings. Decoupling of cells, as has been reported to occur during dark adaptation, increases spatial resolution at the expense of greatly reduced temporal resolution. (Supported by The Whitaker Foundation, NIH Grant PO1NS17763-07, The Northeast Parallel Architecture Center, Syracuse University, and Thinking Machines Corp., Cambridge, MA).

SENSORY SYSTEMS—AUDITORY SYSTEM: CENTRAL PATHWAYS I

298.1

EMBRYONIC DEVELOPMENT OF THE MAMMALIAN HINDBRAIN AUDITORY DECUSSATION.
J.K.Brunso-Bechtold, C.K.Henkel, and S.L.Vinsant, Department of Neurobiology and Anatomy, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, N.C. 27103.

The proper formation of fiber decussations is essential for normal auditory function which depends upon the convergence of information from the right and left ears. In order to begin to address the issue of mechanisms which may be involved in regulating the development of decussating auditory fibers, we have studied the development of the trapezoid body decussation in ferrets. In Bodian silver-impregnated material, fibers can be observed crossing midline in the hindbrain during embryonic development. In order to determine whether the fibers observed at midline in the embryonic hindbrain are actually trapezoid body fibers, we have placed gelfoam pellets impregnated with the carbocyanine dye, DiI, into the cochlear nucleus of embryonic brains which had been immersion-fixed in 2% paraformaldehyde. The brains were stored in the dark at room temperature for 1-2 months, cryostat-sectioned, and viewed with epifluorescent microscopy. As early as embryonic day 34, fibers extend from the cochlear nucleus to enter the ipsilateral superior olive, often in discrete fascicles. In addition, labelled fibers cross the midline and enter the contralateral superior olive. Thus converging ipsilateral and contralateral cochlear nucleus inputs are present in the superior olivary complex at least three weeks before the major period of synaptogenesis and five weeks before the onset of hearing. Supported by DC00335 and March of Dimes.

298.2

LATERALITY OF SUPERIOR OLIVE PROJECTIONS TO THE INFERIOR COLLICULUS IN ADULT AND DEVELOPING FERRET. Craig K. Henkel and Judy K. Brunso-Bechtold. Department of Neurobiology and Anatomy, Wake Forest University, Bowman Gray School of Medicine, Winston-Salem, NC 27103.

The connections of the lateral superior olivary nucleus (LSO) with the inferior colliculus (IC) were investigated in adult and immature ferrets. The proportion of cells labeled in LSO on each side in retrograde tracing experiments depended on both the specific marker and location of the injection in adult IC. Large wheat germ agglutinin-HRP injections labeled nearly all LSO cells on both sides. When HRP alone was injected in adult IC even in very large amounts, two populations of cells were found in each LSO, one labeled and the other unlabeled. Large, bilateral injections of two fluorescent markers in IC resulted in two populations of single-labeled cells, supporting the conclusion that nearly all cells in LSO have ipsilateral or contralateral projections to IC but not bilateral projections. In the HRP experiments the contralateral lateral limb of LSO contained 61-75% of the labeled cells when compared to the ipsilateral lateral limb, suggesting that areas of different frequency representation project differently to the two sides. At birth LSO projections have arrived at IC based on similar retrograde experiments. As in the adult in neonatal ferrets there appears to be a contralateral bias in the LSO projection to IC and this bias is greater in the lateral limb than in the nucleus overall.

Supported in part by NIH Grant DC00335.

298.3

AGE-RELATED CHANGES IN GABA AND ACH IN THE BRAINSTEM AUDITORY NUCLEI OF FISCHER-344 RATS. A. Raza, D.M. Caspary & S.P. Arneric. Dept. of Pharmacology, Southern IL Univ., Springfield, IL 62794-9230

Our previous studies have suggested a selective, age-related deficit of GABA immunostaining, release and tissue levels in the central nucleus of the inferior colliculus (CIC) (Soc. Neurosci. Abstr., 1989, 15, 115; J. Neurosci., 1990, In Press). This study sought to determine whether there are additional age-related alterations in the biosynthetic enzyme, the degradative enzyme and the uptake system for GABA in the CIC. The cochlear nucleus (CN) and nuclei of the lateral lemniscus (NLL) were also examined for comparison. The cholinergic neuronal system was studied concurrently. Results were compared in young (3-7 mo), intermediate (15-17 mo) and aged (24-26 mo) Fischer-344 rats. In young animals glutamic acid decarboxylase (GAD) activity ranged 6-fold and was highest in the CIC (219 nmol/mg protein/h; N=5). Choline acetyltransferase (ChAT) activity was highest in NLL and CN, while GABA-transaminase (GABA-T) activity showed a more uniform distribution. Age-related reductions in GAD activity were seen in the CIC of intermediate (-31%) and aged (-30%) rats when compared to young controls, $p < 0.05$ (N=5). Neurotransmitter selectivity of this deficit in CIC is supported by the modest, non-parallel changes in ChAT activity (-22%, aged vs. intermediate, $p < 0.05$). High-affinity uptake processes (K_m and V_{max}) for ^{14}C-GABA and ^{3}H-D-Asp were not significantly altered in the CIC synaptosomes with aging. Similar to the CIC, the NLL showed remarkable age-related deficits in GAD and ChAT activities, but these deficits were more substantial for the cholinergic system (ChAT activity: -56% aged vs. young). There was no age-related change in GAD or ChAT activity in the CN. No area examined showed a significant loss of GABA-T with aging. Taken together these data suggest: 1) The CIC of Fischer-344 rats shows consistent, age-related loss of GABA as reported previously which is not attributable to changes in uptake or degradation; 2) loss of GABA and ACh is regionally selective in that it apparently affects the midbrain auditory nuclei but not the CN; and 3) these age-related impairments in central auditory structures may contribute to abnormal processing seen in neural presbycusis. (Supported by NIH DC00151-10; NIA N01-AG-2104 and CRC funds from SIU).

298.4

THE OCTOPUS CELL AREA OF THE PVCN IS NOT INNERVATED BY TUBERCULOVENTRAL NEURONS AND COULD ENCODE ECHOES. Robert E. Wickesberg* and Donata Oertel. Dept. of Neurophysiology, Univ. of Wisconsin, Madison, WI 53706

Tuberculoventral neurons in the deep layer of the dorsal cochlear nucleus (DCN) inhibit bushy and stellate cells in the anteroventral cochlear nucleus (AVCN) topographically. To determine whether they also innervate the posteroventral cochlear nucleus (PVCN), extracellular injections of horseradish peroxidase (HRP) were made in brain slices. HRP injections into the multipolar cell area of the PVCN labeled tuberculoventral neurons in the deep layer of the DCN within the band of auditory nerve fiber terminals labeled by the same injection. Injections into the octopus cell area failed to label cells in the deep DCN.

The inhibition from the deep DCN to the AVCN could suppress echoes monaurally (Wickesberg and Oertel, *J. Neurosci.* 9:, 1990). While echoes are suppressed monaurally in localization tasks (Harris *et al.*, J.A.S.A., 35:672, 1963), they do contribute to the perception of "timbre and loudness." If tuberculoventral neurons suppress echoes, then octopus cells, which are not innervated by tuberculoventral neurons, could carry information concerning echoes and thus contribute to the encoding of timbre and loudness.

298.5

SOME ASPECTS OF THE ORGANIZATION OF THE AUDITORY FOREBRAIN AND MIDBRAIN IN THE PIGEON. J.M. Wild, B.J. Frost and H.J. Karten. Dept. of Neurosciences, Univ. of California, La Jolla, CA 92093.

Telencephalic projections of thalamic auditory nuclei ovoidalis (Ov) and semilunaris parovoidalis (SPO) define a cytochrome oxidase positive field L2a and an adjacent, wider, dorsolateral end-zone, L2b. Regions flanking L2 dorsally (L1) and ventrally (L3) are not labelled by these methods. Ov and SPO are represented within L with an inverted dorsoventral topography. For instance, SPO, which receives a major input from lateral lemniscal nuclei and a lesser input from the mesencephalon (MLd), is retrogradely labelled specifically by injections in L2b. Within L2a there is a tight tonotopicity with higher frequencies (<4kHz) represented ventromedially and lower frequencies (>200Hz) progressively more dorsolaterally. In contrast, L2b is not tonotopically organized and units are broadly tuned. These two regions, L2a and L2b, together with L1 and L3, are further functionally distinguished by 2-DG autoradiography following acoustic stimulation with white noise. Some efferents of the L region terminate in a diffuse neostriatal zone dorsolateral and caudal to L (Nd) and the medial paleostriatum, but not Ov or SPO. Retrograde labelling from Nd, however, is confined to L1 and L3. Nd projects to the ventromedial archistriatum (Avm), which then projects bilaterally to L. Avm also supplies a major descending projection to peri-ovoidal regions, and to intercollicular regions surrounding MLd which may be characterized by their reactivity to antibodies directed against nicotinic acetylcholine receptors. Together, these studies indicate the existence of at least two parallel auditory streams ascending through the forebrain, but how far they remain separate remains to be determined. Supported by NIH grant NS24560-04 and ONR Contract N00014-88-K-0504 (HJK).

298.6

PREFERRED ORIENTATION OF BASKET CELL AXONAL ARBORS IN RABBIT AUDITORY CORTEX: THREE DIMENSIONAL RECONSTRUCTION USING COMPUTER MICROSCOPY Nathaniel T. McMullen. Department of Neurology, University of Arizona College of Medicine, Tucson, Arizona 85724

Current network formulations of cerebral cortical circuitry feature GABA-ergic "basket cells" whose axonal fields mediate lateral inhibitory interactions. Although several examples of computer-aided reconstructions of cortical basket cell axons have appeared, no quantitative population studies exist. During the course of Golgi studies of auditory cortical development (McMullen et al, 1988, ICN:278), we encountered examples of basket cells whose axons were exceptionally well-impregnated. The present study is based on the reconstruction of 22 basket cells from lamina III/IV of the auditory cortex of NZW rabbits 12-30 days of age. The dendritic and axonal territory of each cell was digitized from 300-400 um thick Golgi-Cox sections using a computer microscope system equipped with oil-immersion optics. Tangential (parallel to the pial surface) projections and spatial analyses revealed a preferential orientation of axonal arbors parallel to the dorsal-ventral axis of the brain. In some cases, a distinct slab-like axonal orientation was present. In fortuitous sections with two adjacent cells impregnated, their tangential orientations were nearly identical. Because the orientation of the presumptive inhibitory fields is roughly orthogonal to the isofrequency bands in this species (McMullen & Glaser, 1982, Exp. Neurol: 72), these cells may mediate feedforward lateral inhibition, and participate in the formation of isofrequency strips (Supported by The Deafness Research Foundation).

298.7

Auditory fibers are present in the cochlear nucleus during inward migration of precochlear neurons of the opossum, *Monodelphis domestica*, F.H. Willard, Department of Anatomy, University of New England, Biddeford, Maine, 04005.

The mammalian cochlear nucleus (CN) contains multiple cell types, of which at least three, principal and giant cells of dorsal CN and large multipolar neurons of ventral CN, are derived embryologically from a migrating, precochlear group found in the dorsomedial brainstem (Willard and Martin, J. Comp. Neurol., 248:119, 1986). Developmental interactions between precochlear neurons and central processes of the auditory nerve are being examined in the opossum, *Monodelphis domestica*, a species born 14 days after conception with its auditory system in a very immature state (Willard and Munger, Neurosci. Abst., 14:425, 1988; Willard, Neurosci. Abst., 15:742, 1989). In Monodelphis, the precochlear cells are seen approaching the CN on postnatal day (PND) 4; from PND-6 through 9, they enter the nucleus passing over the presumptive dorsal acoustic stria; subsequently, cytoarchitectural organization develops during PND-10 to 19. Bifid central processes of the auditory nerve, labelled by HRP placement into the cochlea, are present throughout ventral CN and extend into the subventricular zone of presumptive dorsal CN, at least by PND-6 (the youngest age examined to date). On PND-8 labelled axons extend through the nucleus to the ventricular zone and are present in between precochlear cells as they inhabit the subventricular zone of presumptive dorsal CN. We conclude that auditory axons are present in the CN as precochlear neurons arrive and, as such, could play a role in the final positioning of these neurons. (NIH 1-R15 NS25978-01)

298.8

MOLECULAR MARKERS IDENTIFY NEURONAL SUBSETS IN GERBIL AUDITORY BRAINSTEM NUCLEI. L.R. Schwartz[1,2], P. Eager[1]* and J. R. Naegele[3], Sections of Otolaryngology[1], Neuroanatomy[2], and Dept. of Opthalmology and Visual Sciences[3] Yale University School of Medicine, New Haven CT. 06510.

A panel of immunocytochemical markers used previously to identify functionally distinct subsets of neurons in the visual system, has now been used to identify subsets of auditory neurons, particularly those containing GABA. Serial sections of adult gerbil brains were incubated in antibodies to: 1) an N-linked carbohydrate associated with certain integral membrane proteins (monoclonal antibody VC1.1); 2) parvalbumin (PV) (an intracellular calcium binding protein); 3) GABA or, 4) in a lectin specific for N-acetylgalactosamine (VVA). Each marker showed a characteristic and selective labeling pattern of distinct neuronal subsets. VC1.1 staining patterns were generally similar to those reported for Cat-301 (Schwartz & Hockfield, Soc. Neurosci. Abstr. 15: 110, '89). Like VC1.1 and Cat-301, VVA labeling was limited to the surfaces of cells. It labeled posterior ventral cochlear nucleus (PVCN) octopus cells and large multipolar subcollicular neurons but, in contrast to VC1.1 and Cat-301, it also stained neurons in the lateral external nucleus of the inferior colliculus (IC) and a different subset of anterior ventral cochlear nucleus (AVCN) neurons. Like GABA, PV immunoreactivity was restricted to cartwheel and Golgi neurons in the dorsal cochlear nucleus (DCN). PV antibody also labeled small neurons throughout the lateral extent of the central nucleus of IC, but large multipolar subcollicular neurons and octopus cells of the PVCN were unlabeled. Patterns of co-localization with these markers may demonstrate new relationships between auditory neuron populations and their transmitters. (Supported by DC00132, EY07119, EY05206 and the Klingenstein Foundation).

298.9

DESCENDING INPUT FROM THE CENTRAL NUCLEUS OF THE INFERIOR COLLICULUS TO THE MEDIAL OLIVOCOCHLEAR SYSTEM IN RAT: A COMBINED PHA-L AND CT-HRP STUDY.
D.E. Vetter and E. Saldaña. Lab. of Neuromorphology, Univ. of Connecticut, Storrs, CT 06269-4154.

The descending input to the superior olivary complex (SOC) from the central nucleus of the inferior colliculus (ICC) is tonotopic (Saldaña, Soc. Neurosci. Abstr., 1990) and terminates in the ventral nucleus of the trapezoid body (VNTB), which contains neurons of the medial olivocochlear (OC) systems. Rajan (Hearing Res.,1990) has shown that electrical stimulation of the inferior colliculus (IC) reduces temporary threshold shifts in the cochlea, apparently via the OC system. We have combined anterograde transport of *Phaseolus vulgaris*-leucoagglutinin (PHA-L), which delineates axonal arbors, and retrograde transport of HRP conjugated to cholera toxin B subunit (CT-HRP), which delineates dendritic arbors, to investigate where and how the IC can influence the OC systems in the rat. PHA-L was injected unilaterally into the ICC of adult rats 7-9 days prior to injection of CT -HRP into the contralateral cochlea. The animals were perfused 2-3 days later with an appropriate aldehyde fixative. Sections of the SOC were reacted sequentially for the presence of CT-HRP and PHA-L. PHA-L labelled axons originating in the ICC entered the ventral portion of VNTB. Most labelled fibers were observed ipsilateral to the PHA-L injection site, and they were not found near LSO. The collicular fibers intermingled with CT-HRP labelled cell bodies and dendrites. PHA-L labelled boutons only rarely seemed to contact CT-HRP labelled cell bodies, but they were often observed in close apposition to labelled dendrites, suggesting that synapses between these two elements may be present. (Supported by PHS grant NS-09904).

298.10

THE RAT COLLICULO-OLIVARY PROJECTION IS TONOTOPIC.
E. Saldaña. Dept. of Cell Biology and Pathology, Univ. of Salamanca, 37007 Salamanca, Spain.

It is generally accepted that the descending input to the superior olivary complex (SOC) originates mostly in the external cortex of the inferior colliculus (Faye-Lund, 1986, Anat. Embryol.). In an attempt to determine: 1) to what extent the central nucleus of the inferior colliculus (ICC) innervates the SOC, and 2) what is the topography of this projection, *Phaseolus vulgaris*-leucoagglutinin (PHA-L) was iontophoretically injected unilaterally into the ICC of adult rats. After 7-12 days, the animals were transcardially perfused with an appropriate aldehyde fixative. Brainstem sections were reacted for PHA-L immunocytochemistry.

From the ICC, the labelled axons descend in the lateral and rostral aspects of the ipsilateral lateral lemniscus (LL). At the ventral border of the LL the fibers curve caudally to innervate the ventral nucleus of the trapezoid body (VNTB), particularly the ventral half. The collicular fibers run rostrocaudally, in parallel to the main axis of the nucleus, creating a dense terminal plexus with abundant *en passant* and terminal varicosities. After injections in the dorsolateral, low frequency, areas of the ICC, the terminal field in the VNTB occupies a lateral position, whereas injections in the ventromedial, high frequency, regions of the ICC produce a medially located terminal field. This clear tonotopic arrangement of the colliculo-olivary projection suggests that the VNTB could have a tonotopic organization not previously described.

In each case a few fibers innervate the ipsilateral superior periolivary nucleus and the contralateral VNTB; only rarely were labelled fibers seen in other nuclei of the SOC.

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298.11

GLYCINE IMMUNOREACTIVE PROJECTIONS FROM THE DORSAL TO THE ANTEROVENTRAL COCHLEAR NUCLEUS.
R. L. Saint Marie, C. G. Benson, E. -M. Ostapoff and D. K. Morest. Department of Anatomy and Center for Neurological Sciences, University of Connecticut Health Center, Farmington, CT 06032

Neurons in the deep layer of the dorsal cochlear nucleus (DCN) have axons that ramify in the three principal subdivisions of the cochlear nucleus. They are ideally situated to influence all of the outputs of the cochlear nucleus and, consequently, to influence monaural and binaural processing throughout the auditory brainstem. The aim of the present study was to determine if projections from the DCN to the anteroventral cochlear nucleus (AVCN) use either of two inhibitory transmitters, glycine or GABA. Retrograde HRP labeling of DCN-to-AVCN projection neurons was combined with postembedding immunocytochemistry in the DCN of guinea pigs. Following injections of HRP in the anterior or posterior divisions of AVCN, large numbers of neurons were labeled in the DCN. All of these were located in the deep layer, except for a few granule cells. Nearly all (96%) of the projection neurons were immunoreactive for glycine and most had dendritic and somatic morphologies corresponding to those of elongate neurons (so-called "corn" cells). A few may have been small stellate neurons. Few retrogradely labeled neurons (3%) were immunoreactive for GABA. The results suggest that projections from the DCN to AVCN are formed primarily by glycinergic elongate neurons and, therefore, could have inhibitory influences on the output of neurons in AVCN. Supported by NIH grants DC127 & DC199.

298.12

A PHAL-L STUDY OF THE DISTRIBUTION OF THE IPSI- AND CONTRALATERAL CORTICO-CORTICAL PROJECTIONS OF THE AUDITORY CORTEX OF THE ALBINO RAT.
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(1) Laboratorio de Neuroanatomia Comparada, Instituto de Neurobiologia "Santiago Ramón y Cajal".

In the work PHA-L was injected iontophoretically into the primary auditory cortex. The ipsilateral cortico-cortical projections are arranged in a structure of bands formed of axons and axonic endings. These bands are predominantly oriented in the antero-posterior direction and extend from the site of injection in the anterior and ventral sense until the parietal cortex (for a distance of about 1800 microns). In the posterior sense, they extend to the Te 2 area (for a distance of approximately 1600 microns). The number of bands varies, depending on the animal injection, but is usually 2 to 3 bands in the anterior sense situated ventrally to the area of injection, and 1 to 2 bands in the sense posterior to the injection and dorsal to the site of injection. The bands form a continuous structure constituted of axons and axonic endings that course throughout the six layers of the cortex. Among them there are spaces with few or no labelled fibres. Contralaterally, the projection is disposed in the contralateral auditory cortex, also forming from 1 to 2 bands in the anteroposterior sense, although its extension on this axis is less than that of the homolateral bands (some 2200 microns).

298.13

FINE STRUCTURE OF GABA-LABELED AXONAL ENDINGS IN THE INFERIOR COLLICULUS OF THE CAT. IMMUNOCYTOCHEMISTRY ON DEPLASTICIZED ULTRA-THIN SECTIONS.
D.L. Oliver and G.E. Beckius*. Dept. Anatomy, Univ. CT Health Center, Farmington, CT 06032.

Numerous GABAergic endings in the inferior colliculus (IC) arise from several sources that include the dorsal nucleus of the lateral lemniscus and neurons within the IC itself. Despite this, little is known of their fine structure and synaptic organization.

GABAergic axonal endings in the IC were identified at the EM level with affinity-purified (Wenthold, NIH) and Pel-Freez polyclonal antisera against protein-conjugates of GABA. Serial ultra-thin sections were used to compare immunostaining and normal fine structure in the same axonal endings. Most GABA-labeled endings had a similar morphology. Most endings contained pleomorphic synaptic vesicles and made symmetrical synapses. This morphology is consistent with inhibitory function. Although most GABA-labeled axonal endings synapsed on dendrites, a number of labeled axo-somatic synapses also were found. These might be related to different postsynaptic cell types or different receptor types.

Sponsored by Deafness Research Foundation and NIH grant DC00189.

298.14

COLOCALIZATION OF GABA-LIKE AND GLYCINE-LIKE IMMUNOREACTIVITY IN THE COCHLEAR NUCLEI OF THE BABOON (PAPIO ANUBIS).
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GABA and glycine (Gly) have been described as the major inhibitory neurotransmitters in the cochlear nuclei. In the present study, pairs of adjacent 0.5 µm Durcupan-embedded sections were treated with antibodies raised against GABA- and Gly-glutaraldehyde-protein conjugates (O.P. Ottersen and J. Storm-Mathisen, Trends Neurosci. 10:250-255, 1987) and treated with the PAP technique. GABA-LI and Gly-LI was observed in neuronal somas, axons and terminals. The relative staining intensity of cell somas was evaluated by optical densitometry.

In baboon, as in other mammals, immunoreactive cells are concentrated in the dorsal cochlear nucleus (DCN) and in the cap area of the ventral cochlear nucleus (VCN). The majority of the reactive cells in these areas show colocalization of GABA-LI and Gly-LI. In DCN neurons, the baboon appears to show a higher degree of colocalization of GABA-LI and Gly-LI than is seen in nonprimate mammals. More colocalization is also seen in fascicles of fibers running between DCN and VCN and in terminals in the central part of the VCN. These structures may constitute a system of association projections from the DCN to the VCN. In the magnocellular part of the VCN, the few immunoreactive cells tend to be large and to show only Gly-LI. These cells may give rise to the population of large Gly-positive axons in the acoustic stria.

298.15

STRONG PROJECTION TO THE PONTINE GRAY FROM THE INFERIOR COLLICULUS OF THE MUSTACHED BAT. Jeffrey I. Wenstrup. Dept. of Neurobiology, Northeastern Ohio Universities College of Medicine, Rootstown, OH 44272 and Div. of Neurobiology, Dept. of Mol. and Cell Biol., University of California, Berkeley, CA 94720.

Anterograde tracing methods were used to examine projections to the pontine nuclei from physiologically defined regions in the central nucleus of the inferior colliculus (ICC). The goal was to describe how isofrequency representations in ICC, analyzing major components of the bat's biosonar echoes, project to the cerebellar relay nuclei of the pontine gray. Multiple tracer deposits of horseradish peroxidase (HRP) or [^{3}H]leucine were placed at sites in ICC tuned near 25, 30, 54, 61, 80, and 93 kHz.

The ICC projected strongly to the pontine gray; only its projection to the medial geniculate body was stronger. Each frequency representation in ICC projected to multiple loci in the ipsilateral pons, where the lateral and dorsolateral nuclei were principal targets of ICC input. There were clear differences in the target zones of different frequency representations; however, some overlap may occur. When one or a few HRP deposits were placed in restricted regions of an ICC isofrequency representation, labeled regions in the pons were subsets of those observed with larger or more numerous deposits in the same representation. Single or restricted deposits nonetheless labeled multiple pontine loci.

The results demonstrate that the ICC is an important source of auditory input to the pons, and they suggest a patch-like representation of sound frequency within the pontine nuclei as a result of the ICC projection.

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298.16

EM OBSERVATION OF PHA-L-LABELED INPUTS TO THE GUINEA PIG SUPERIOR OLIVARY COMPLEX. A.M. Thompson and C.J. Haaksma*, Dept. of Otorhinolaryngology, Univ. of Oklahoma, Hlth. Sci. Ctr., Oklahoma City, OK 73190.

We have previously found that after injection of *Phaseolus vulgaris*-leucoagglutinin (PHA-L) into the guinea pig ventral cochlear nucleus (VCN) or inferior colliculus (IC), PHA-L-labeled swellings, associated with labeled fiber segments, were located in the superior olivary complex (SOC). This finding was interpreted as evidence that VCN and IC neurons provided inputs to the SOC. This EM study was done to determine whether the labeled swellings are indeed indicative of synaptic inputs.

PHA-L was injected into either the VCN or IC (7-10 day survival time) and after perfusion-fixation and sectioning, brain sections were treated immunohistochemically to detect the PHA-L using DAB or TMB/DAB/Cobalt as the chromagen. Following osmication, the sections were flat-embedded in Polybed-812, thin-sectioned, and viewed with a JEOL 2000-FX EM scope.

Within the SOC, positive staining was observed in structures containing typical synaptic specializations such as synaptic vesicles. Pre- and post-synaptic membrane densities were also observed in association with many of the labeled endings. These results suggest that the PHA-L-labeled swellings observed in previous studies indicate synaptic inputs to the SOC originating in VCN and IC. (This research was supported by the Deafness Research Foundation)

298.17

CORTICAL AND THALAMIC AFFERENTS OF THE TEMPORAL AUDITORY FIELD OF THE CAT. E.M. Bowman and C.R. Olson. Lab. Sensorimotor Res., NEI, Bethesda, MD. 20892 and Dept. Psychol., George Mason Univ.,Fairfax, VA. 22032.

Feline auditory cortex consists of a core of tonotopically organized areas and a surrounding ring of auditory association cortex . The degree to which the different sectors of the nontonotopic association ring constitute distinct areas is poorly understood. One sector of the ring - "the auditory belt" of the posterior ectosylvian gyrus - is connected to established auditory structures but also to the lateromedial-suprageniculate nucleus of the thalamus and to frontal, parietal and cingulate areas (Bowman & Olson, J. Comp. Neurol., 272:15-42). The experiments described here characterized the inputs of an adjacent auditory association area, the "temporal auditory field" (Area Te). We placed emphasis on the question of whether the afferents of Area Te justify regarding it as a distinct entity.

We analyzed thalamic and cortical retrograde labeling following deposits of multiple distinguishable tracers at restricted cortical loci in four cats. Area Te receives its strongest thalamic input from neurons forming a caudal cap on MGd, the dorsal division of the medial geniculate nucleus. These neurons are caudal to neurons projecting to the auditory belt and to Area AII. The projection from caudal MGd to Area Te is topographically organized, as indicated by the fact that tracers deposited at separate loci within Area Te label segregated groups of neurons within MGd. Cortical projections to Area Te arise primarily from other auditory association areas and from insular and perirhinal cortex. Projections from tonotopic auditory areas and other association areas are minor. These findings justify regarding Area Te as a distinct entity. They suggest that the Area Te is not a portal through which auditory information passes to supramodal cortical centers of the frontal, parietal and cingulate districts.

298.18

CHEMICALLY DISTINCT MEDIAL GENICULATE CELLS PROJECT TO DIFFERENT LAYERS OF MONKEY AI CORTEX. T. Hashikawa*, E. Rausell, M. Molinari and E. G. Jones Neural Systems Lab., Frontier Research Program, RIKEN, Wako 351-01, Japan, and University of California, Irvine, CA 92717

Immunocytochemistry shows that the medial geniculate complex of M. fuscata contains three principal cell types: GABA immunoreactive interneurons and neurons immunoreactive for either of the calcium-binding proteins, parvalbumin or 28Kd calbindin. The three are distributed throughout all subnuclei of the complex but parvalbumin and calbindin cells show differential concentrations. Parvalbumin cells dominate the ventral nucleus and the large-celled part of the magnocellular nucleus, while calbindin cells dominate the posterodorsal nucleus and small-celled part of the magnocellular nucleus.

Retrograde tracing studies, using fluorescent dyes in combination with immunocytochemistry, show that cells containing the calcium binding proteins are thalamocortical relay cells. Among ventral nucleus cells projecting to AI, parvalbumin cells project to middle cortical layers and calbindin cells to layer I. These observations suggest the presence of parallel thalamocortical projection systems based upon chemically distinct cell types with differential cortical projections.

298.19

CHEMICAL IDENTIFICATION OF PHYSIOLOGICALLY CHARACTERIZED MONKEY AI CORTEX. S. H. C. Hendry, L. M. Kitzes & M. N. Semple Dept. of Anatomy & Neurobiology, Univ. of California, Irvine, CA 92717

The tonotopic organization of auditory fields in the monkey (Macaca fascicularis) cerebral cortex was correlated with immunocytochemical staining for the calcium binding protein, parvalbumin. In the superior temporal gyrus (STG), parvalbumin immunoreactivity is present in numerous nonpyramidal somata and in dense bundles of axons in the underlying white matter. The axons can be traced from the medial geniculate nucleus to the posterior half of the STG, where they invade the cortex, intermingle with the immunostained neurons and form a band of intense immunostaining in layers IIIB and IV. The cortical region which contains this band is flanked first by narrow regions that are much less intensely stained and then by secondary zones of moderate immunostaining. Physiological mapping of best frequency responses in the STG indicates that the region of the intense parvalbumin immunostaining corresponds to a cortical field in which there is a complete, tonotopically organized representation of the cochlea. We interpret this to be the primary auditory area (AI). Secondary zones of parvalbumin staining appear to represent surrounding, non-primary auditory areas. These data suggest that monkey AI is a chemically distinct region that can be accurately localized by the intense parvalbumin immunoreactivity of its afferents and interneurons. Supported by NS 25674.

299.1

INFERIOR COLLICULUS FUNCTIONAL PLASTICITY FOLLOWING NEONATAL ABLATION OF THE CONTRALATERAL COCHLEA AND COLLICULUS. N. K. Woolf and A.F. Ryan. Div. of Otolaryngology, UCSD Medical Center and Veterans Administration Medical Center, La Jolla, CA 92161.

Early unilateral cochlear destruction in the Mongolian gerbil leads to limited anatomical and functional plasticity in the auditory central nervous system [e.g., Kitzes, L.M., Brain Res. 306:171, 1984], but does not alter broad patterns of neural activity in IC as reflected by sound evoked 2-deoxyglucose (2-DG) activity [Ryan, A.F. and Woolf, N.K., Neurosci. Abstr., 13:79, 1987].

To determine whether the loss of a CNS neuronal target increases the extent of developmental plasticity, we destroyed one cochlea and the ipsilateral IC at 0-2 days after birth (DAB). At 45 DAB, acoustic stimulation of the surviving cochlea with pure tones inappropriately increased 2-DG uptake in the ipsilateral IC. This suggests that extensive developmental plasticity can be induced in the CNS when a population of neurons is deprived of its normal target and, simultaneously, a homologous target is deprived of its normal functional inputs.

Supported by DC139 & the VA Research Service.

299.2

RESPONSE PROPERTIES OF NEURONS IN AUDITORY CORTEX OF THE BIG BROWN BAT. S.P. Dear, T. Haresign, M. Ferragamo*, J. Fritz*, C.F. Moss[1] and J.A. Simmons. Dept. of Psychology and Section of Neurobiology, Brown University, Providence, RI 02912 & [1]Dept. of Psychology, Harvard University, Cambridge, MA 02138

The big brown bat, *Eptesicus fuscus*, uses temporal and spectral cues to determine target range (Simmons, J.A., Cognition, 33:155, 1989). As a prelude to investigating the neural basis of this echolocation behavior, we have mapped the response properties of neurons in the auditory cortex to tones, FM sweeps and pulse-echo pairs. Using single and multi-unit recordings with carbon-fiber electrodes in awake bats, most auditory neurons exhibited phasic responses to single tones or FM sweeps and were not delay-tuned to pulse-echo pairs. We found a tonotopic distribution of auditory neurons covering an area of 5 mm^2. Paradoxical latency shift was observed in some of these neurons, while other neurons showed a facilitated response to the combination of pulse and echo but were not delay-tuned.

Our preliminary data indicated that delay-tuned neurons were diffusely distributed in the cortex, exhibited columnar organization and formed two populations. Neurons with longer best delays (>10 ms) had short latencies (9.2 $\pm$ 2.3 ms) and were found in the dorsal region. Some of these delay-tuned neurons responded to a narrow range of pulse and echo amplitudes, suggesting coding for both target range and size. Neurons with shorter best delays (<10 ms) had long latencies (24.7 $\pm$ 6.9 ms) and were found in the ventral region. Together, the organization of these two populations suggests a neural axis of echo delay-tuning orthogonal to the tonotopic neural axis. (Work supported by NIMH grant MH19118 and ONR grant N00014-89-J-3055)

299.3

THE COMPUTATION OF SOUND ELEVATION IN THE BARN OWL: MODEL AND PHYSIOLOGY. J.C. Pearson[1], C.D. Spence[1*], R. Adolphs[2]. [1]David Sarnoff Research Center, CN5300, Princeton, NJ 08543. [2]Div. of Biology 216-76, CalTech, Pasadena, CA 91125.

In the barn owl, the elevation of sound source direction is largely determined by the interaural level difference (ILD). We present a network model of the ILD processing of nucleus ventralis lemnisci lateralis pars posterior (VLVp) and the lateral shell of the central nucleus of the inferior colliculus (ICL). The model explains several of the most significant physiological findings (*J. Neurosci.* 8: 2665; SN-89 50.5), and makes strong predictions about the anatomy of these nuclei: (1) criss-cross pattern (with respect to the dorsal-ventral axis) of reciprocal inhibition between the right and left VLVp; (2) dorsal-ventral gradient (high ventral, low dorsal) of cell density in the VLVp; (3) excitatory contralateral input from nucleus angularis to the VLVp and ICL that is uniform within an iso-frequency band; (4) inhibitory topographic projection from the contralateral VLVp to both the sensitive and tuned cells of the ICL; (5) inhibition from the sensitive to the tuned ICL cells, such that a sensitive cell with a given ILD threshold inhibits tuned cells with a smaller optimal ILD (ILD defined as contra. - ipsi. sound pressure level.).

We also present the results of experimental tests that are consistent with computer simulations of the model: (1) injections of bicuculline in the VLVp cause the optimal ILD of ICL neurons to increase, while injections of lidocaine have the opposite effect; (2) there are transient responses to all non-optimal ILD's in the ICL, but not in the VLVp. ILD's less than the optimal produce a more pronounced transient reponse than ILD's greater than the optimal.

[1]Supported by AFOSR F49620-89-C-0131; [2]Howard Hughes Medical Institute Fellow.

299.4

AUDITORY DIRECTIONAL SENSITIVITY OF SINGLE NEURONS IN THE PONTINE NUCLEI OF THE FM BAT, EPTESICUS FUSCUS. H. Teng and P. H-S. Jen Div. Bio. Sci. University of Missouri-Columbia, MO 65211

Auditory directional sensitivity of 82 pontine neurons of the FM bat, Eptesicus fuscus, was studied under free field stimulation conditions. For each pontine neuron, its best frequency (BF) and lowest minimum threshold (MT) were first determined with a sound (4 msec duration, 0.5 msec rise-decay times) broadcast at a specific azimuthal angle (the best angle). Then the neuron's MT, discharge rate and response latency to pure tone and/or FM sound(s) delivered at 1-3 intensities (10, 20 dB re MT and best intensity at which the neuron discharged maximally) from 9 chosen azimuthal angles within $\pm 80^0$ were recorded. Directional sensitivity of each neuron appeared similar when determined with both stimuli. Most pontine neurons had their angles of lowest MT (81%) and maximal discharge rate (67%) at $0\text{-}40^0$ contralateral (relative to the recording site). The discharge rate of one-third neurons changed drastically over a limited range of azimuthal angle regardless of stimulus intensity. Thus the directional sensitivity curves of these neurons obtained at all intensities were almost congruent. Conversely, the discharge rate of remaining two-third neurons varied with both sound direction and intensity resulting in different profiles of directional sensitivity curves. While high BF neurons had their angles of lowest MT and maximal discharge rate located more medially than low BF neurons did, the former might not necessarily have a sharper directional slope (dB/deg or impulse/deg) than the latter. The response latency of pontine neurons also varied with sound direction. Most (90%) neurons had a shortest latency at $0\text{-}40^0$ lateral. Beyond this azimuthal range, the latency increased drastically resulting an inverted bell-shaped latency directional sensitivity curve. Compared with previous studies in the inferior colliculus (IC), our present observations suggest that the pontine neurons preserve, if not integrate, the directional sensitivity of IC neurons before relaying it to the cerebellum.

299.5

STIMULUS CODING OF COMPLEX SOUNDS BY SMALL GROUPS OF NEURONS IN THE COCHLEAR NUCLEUS. D.R. Kipke*, B. M. Clopton and D. J. Anderson. Kresge Hearing Research Institute, University of Michigan, 1301 E. Ann St., Ann Arbor, MI 48109.

The cochlear nucleus, receiving direct inputs from the cochlea via the auditory nerve and possessing a number of primary cell types and interneurons, is thought to have a significant role in the encoding of complex sounds. In this research we investigated the relative influences of common-stimulus driving and intranuclear connectivity in the spike activities of small groups of neurons in the dorsal cochlear nucleus and the anteroventral cochlear nucleus. Solid-state, thin-film microelectrodes were used to simultaneously record the spike activities of groups of up to five neurons. Short-duration tonebursts, tone sweeps, and periodic pseudo-random white noise sequences were used for acoustic stimulation. Standard toneburst-based single unit characterizations were generated (peri-stimulus time histogram at unit's characteristic frequency and/or response map). Single unit characterizations derived from the reverse-correlation of a unit's spike train to a time-frequency representation of the noise provided an estimate of the unit's stimulus encoding properties (spectro-temporal receptive field, STRF). Cross-correlation analysis of the spike trains of each pair of units within a group was used to identify functional relationships between the units. The shift-predictor cross-correlogram was used to estimate common-stimulus driving effects, while the algebraic difference between the raw cross-correlogram and the shift-predictor was used to estimate connectivity effects. It was found that the relative influences of common-stimulus driving and connectivity were sensitive to stimulus context. For tonebursts, the functional relationships depended on frequency and intensity. For noise stimulation, specific features in cross-correlograms were associated with relationships between the units' STRFs yielding an estimate of the encoding of specific features of sound by a group of neurons. This work was supported by NIH grant NS-05785 and contract NS-7-2397, and NSF equipment grant BN8609850.

299.6

EFFECTS OF CONTINUOUS TONE MASKERS ON SPECTRO-TEMPORAL RECEPTIVE FIELDS FOR WIDEBAND NOISE IN GUINEA PIG COCHLEAR NUCLEUS. P.M.Backoff* and B.M.Clopton. Kresge Hearing Res. Inst., U. of Mich., Ann Arbor, MI 48109.

Studies of lateral supression and masking of tonal stimuli for single unit responses have utilized either tone or noise maskers to suppress firing rate. This study of neurons in the ventral(VCN) and dorsal cochlear nuclei(DCN) used continuous tones as the masker, and examined their effect on unit responses to wideband noise stimuli, estimated in the spectrotemporal receptive field(STRF). Masking tones at the unit best frequency(BF), and 1/4 octave above and below BF were presented at approx. 10dB above the spectral level of the noise and/or at 0-5dB below.

Masked and unmasked STRFs were derived for characterized neurons using a set of periodic wideband noises and a reverse-correlation analysis. Period histograms(PHs), collected for 150-200 repetitions of each noise, were cross-correlated with the corresponding time-frequency representations of the noise stimuli. Taken across the noise set, this estimated the time-frequency energy patterns in the stimuli which preceded spike occurrence, represented as peak and trough areas on the STRF display. In the unmasked condition, all neurons showed at least one restricted peak region, with a maxima at the unit BF. Presumably, these peaks reflect short latency, excitatory response areas. Troughs were often seen for DCN units, which are known to have numerous inhibitory inputs. The effect on average spike rate of introducing the masker tones varied with unit type: e.g., onsets showed little change in rate with addition of the continuous tone. Maskers disrupted temporal patterning in the PH and altered response areas on the STRFs for all units, especially for higher level BF tones. The findings for representative unit types from DCN and VCN will be summarized and discussed.

299.7

DIFFERENT PATTERNS OF UNIT ACTIVITY SUPPORT DISCRIMINATION OF A CLICK CS FROM HISS DS IN DIFFERENT AREAS OF THALAMUS. X.F. Wang, V. Chizhevsky*, E. Gruen*, O. Melamed* and C.D. Woody. UCLA Med. Ctr., MRRC, BRI, Los Angeles, CA 90024.

Patterns of unit activity were recorded during conditioned blinking produced by 70 db click CS (forward paired with glabella tap and hypothalamic electrical stimulation; 570-10ms ISI; see Hirano et al., Br. Res. 1987) but not by a backward paired hiss DS of comparable intensity. Different averaged patterns of response were found in anterior, LD (lateralis dorsalis), CL (centrolateral), and posterior thalamus. After conditioning, the following changes were observed relative to prior baseline findings:

Region	Spont. firing	Resp. to CS	No. CS resp. cells
ant.	decrease	increase	increase
LD	no change	sl incr.	no change
CL	increase	increase	no change
post.	decrease	no change	no change

Differences in magnitudes of response to CS, background activity, and the proportions of CS responsive cells were the major variants. The CR was discriminatively elicited by the click CS as opposed to the hiss DS, and so was the unit activity after conditioning. (Supported by NS25510.)

299.8

TEMPORAL AND SPATIAL PATTERNS OF NEURONAL RESPONSES TO AMPLITUDE MODULATED AND HARMONIC SOUNDS IN FREQUENCY BAND LAMINAE IN THE INFERIOR COLLICULUS OF CAT.

G. Langner and C.E. Schreiner*
Zool. Inst., THD, 61 Darmstadt, FRG; *Coleman Lab., UCSF, San Francisco

A relation between the topographical representation of characteristic frequency (CF) and best modulation frequency (BMF) of neurons has been previously demonstrated in the central nucleus of the inferior colliculus of cat (Langner,G. and Schreiner, C.E., *Soc. Neurosci. Abstr.*, Vol. 15, Part 2, p. 1116, 1989). Reconstruction of the location of single and multiunit recordings provided evidence that the anatomically defined neuronal laminae of the ICC are not equivalent to 'isofrequency planes' but represent small frequency bands with a tonotopic fine structure which may be described by isofrequency lines running from rostral to caudal. Temporal selectivity results in isoperiodicity lines which are approximately orthogonal to the isofrequency lines.

The current investigation shows how responses to sinusoidal amplitude modulations (AM) and harmonic complexes are represented with respect to the tonotopic and periodotopic maps. The spatial distribution of the responses to these signals corroborate the measured periodicity maps, since the response maxima are located at positions where neurons have adequate BMFs. Q_{10dB}, characterizing the bandwidth of tuning curves, is also systematically distributed over the laminae and maximal near the place where neurons with maximal BMFs were recorded. The map for onset-latency is in line with the finding that this parameter correlates statistically with 1/BMF (Langner,G. et al., *Hearing Res.* 31, 197, 1987). The spatial distribution of response rate and synchronization of neurons in a frequency band lamina during stimulation with harmonic complexes shows significant fluctuations during the first 30 ms of response. *(Supported by DFG, SFB 45)*

299.9

A COMPARISON OF THE DIRECTIONAL PROPERTIES OF NONMONOTONIC NEURONS IN THE AUDITORY THALAMUS AND PRIMARY AUDITORY CORTEX (AI) OF CAT. P. Barone, W. A. Irons*, J.C. Clarey, and T.J. Imig. Dept. Physiol., Univ. Kansas Med. Ctr., Kansas City, KS 66103.

Data on the azimuthal and sound pressure level (SPL) sensitivity to free-field noise-burst stimuli were collected from single neurons in the medial geniculate body (ventral division and posterior nucleus: N=325; BFs ranging from 1.8 to 24 kHz) and AI (N=333; BFs ranging from 1.5 to 28 kHz) of barbiturate-anesthetized cats. To provide some indication of cells' sensitivity to azimuth, they were arbitrarily classified as either "high directional" (HD) or "low directional" (LD). The average azimuth functions of HD cells showed greater than 75% reduction from maximum response. Cells were classified as nonmonotonic (NM) if their average rate-level functions showed a maximum response at a single SPL, and a decrease in discharge rate of greater than 50% from maximum at higher SPLs. NM cells were less frequently encountered in thalamus than cortex (16% and 40%, respectively).The proportions of HD and LD nonmonotonic cells in the thalamic and cortical populations differed. HD response were observed in 55.8% of NM thalamic cells and 82.4% of NM cortical cells, and a Chi-squared test revealed that these two populations differed significantly ($Pr<0.01$). The distributions of azimuthal preferences of HD cells (i.e., contralateral, ipsilateral or midline) were similar in the two areas. There are two general possibilities to explain these findings. First, a subset of the HD nonmonotonic responses are generated at the level of cortex. Second, a subset of the nonmonotonic thalamic cells that show LD responses project to cortical areas other than AI.

299.10

FUNCTIONAL ORGANIZATION OF SOUND DIRECTION AND SOUND PRESSURE LEVEL (SPL) IN PRIMARY AUDITORY CORTEX (AI) OF CAT. J.C. Clarey, P. Barone and T.J. Imig. Dept. Physiol., Univ. Kansas Med. Ctr., Kansas City, KS 66103.

The present study was undertaken to examine the organization of neurons' responses (CFs from 3 to 26 kHz) to free-field noise-burst stimuli that varied in frontal azimuthal location and SPL. Extracellular recordings were made in barbiturate-anesthetized cats from single and/or multiple neurons at successive locations within microelectrode penetrations oriented parallel to the cortical surface and isofrequency contours (N=19), and normal to the cortical surface (N=22). The majority of tangential penetrations (15: 79%) showed evidence of clustering of neuron/s with the same azimuthal sector preference, i.e., contralateral, ipsilateral, or midline. Three of these penetrations showed evidence of a systematic shift of azimuthal preference covering the entire frontal sound field. The remaining tracks (4) showed no clear organization and were often associated with a mixture of broad and/or complex responses. The majority of normal penetrations (14: 64%) showed greater than 80% registration in sector preference and sound location that elicited the maximum response; 8 of these tracks showed 100% registration, and little variation in the best azimuthal range. All tangential tracks showed evidence of long sequences of successively recorded neuron/s (from 5 to 21) with the same SPL response (monotonic or nonmonotonic), often segregated by regions of mixing of response type. Half of the normal penetrations showed greater than 80% registration in SPL response type. These data suggest segregation of both azimuthal and SPL response along an isofrequency band, and evidence for radial homogeneity of these properties, especially apparent in the middle cortical layers.

299.11

EFFECTS OF SUPERIOR OLIVARY COMPLEX LESIONS ON BINAURAL RESPONSES RECORDED FROM THE RAT'S INFERIOR COLLICULUS. S. L. Sally and J. B. Kelly. Laboratory of Sensory Neuroscience, Carleton University, Ottawa, Ontario, K1S 5B6

This study was undertaken to determine the extent to which binaural responses in the central auditory system survive destruction of the brain stem superior olivary complex (SOC). Cells in the SOC of the adult albino rat were selectively destroyed by micro-injections of kainic acid. Following a period of approximately one month, the binaural response properties of neurons in the inferior colliculus were examined using microelectrode recording techniques. Both summation and suppression binaural responses were found in animals with complete unilateral or bilateral lesions of the SOC. Interaural intensity difference (IID) sensitivity was assessed by comparing near threshold contralateral intensity with the lowest ipsilateral intensity that unequivocally produced a summation or suppression response. Interaction IIDs for animals with unilateral SOC lesions did not differ from those previously obtained from normal rats; however, IIDs for the bilateral lesion cases differed significantly. Modal IID values for bilateral lesion cases were elevated in comparison with normals. Thus, binaural interactions were affected but not eliminated by bilateral destruction of the SOC. (Supported by NSERC).

299.12

CHARACTERIZATION OF CELLS IN THE CORTEX OF THE RAT INFERIOR COLLICULUS USING THE BRAIN SLICE TECHNIQUE. P. H. SMITH. Dept. of Anatomy, University of Wisconsin, Madison, WI 53706.

Coronal brain slices from 24-78 day-old hooded rats have been utilized to begin to characterize the intracellular responses of cells in both the external and dorsal cortices of the inferior colliculus (IC). Shock stimulation of the commissural fiber tract (CC) connecting the left and right IC often produced a characteristic sequence of synaptic events distinguished by a short latency ipsp which could last for 20-40 ms and was interrupted by a slightly longer latency epsp. Kynurenic acid, but not APV, significantly reduced the amplitude of the EPSP and eliminated the late portion of the IPSP indicating that the excitatory crossed connection activates non-NMDA type glutamate receptors not only on the cell being recorded from but on other cells that provide the late inhibitory input to the recorded cell. The persistence of the early IPSP indicates a direct CC inhibitory component as well. Both the early and late inhibitory components are GABAergic. Picrotoxin not only blocked all inhibitory drive from the CC but, as described previously in guinea pig (P. Smith, '86) and rat (Pierson et al., '89) a large NMDA-mediated depolarizing event (PDS) could be elicited by much lower shock strengths applied to the CC. That this event was NMDA-mediated was shown by its occurence when the slice was bathed in 0 Mg^{++} ringers, in ringers containing NMDA, and its significant reduction in ringers containing APV where GABA inhibition had previously been blocked. I have used biocytin to intracellularly label cells with the responses described. Those labeled thus far are medium sized multipolar cells. Their dendrites are moderately spiny and can extend superficially up to the cortical surface. Their thin axon gives rise to collaterals which branch in the superficial layers of the cortex while the main axon heads laterally along the circumference of the IC in a fashion resembling the descending corticopontine system described by Hashikawa ('83).

299.13

EXCITABILITY AND FREQUENCY TUNING IN THE GUINEA PIG MEDIAL GENICULATE BODY. Robert C. Lennartz and Norman M. Weinberger, Centr. Neurobiol. Learning and Memory and Dept. Psychobiol., Univ. California, Irvine, Ca. 92717

Excitability changes following an acoustic stimulus involve periods of inhibition and facilitation in the medial geniculate body (MGB) but their relation to other major features of auditory information processing is largely unknown. To determine if frequency tuning is related to tone-evoked excitability patterns, measures of both were obtained from single neurons in the MGB of anesthetized guinea pigs. Tuning was determined by measuring bandwidth (20 dB above threshold, square-root transform of range to eliminate frequency bias, Calford et al, Hear. Res., 11:395,1983). Excitability was determined by presenting pairs of tones at or near the characterisitic frequency (20 dB above threshold, 50 ms, 15-2000 ms intervals). As revealed by responses to the second tone, the first tone usually produced varying duration periods of suppression and facilitation. For each neuron, an index of excitability change was computed and correlated with tuning bandwidth. A significant negative correlation was obtained: cells with the greatest excitability (sometimes including no suppression even at the 15 ms interval) had the smallest frequency bandwidth. These findings suggest that the most narrowly tuned neurons can process frequency information with the highest degree of reliability and that two-tone excitability functions may provide a convenient index of the degree of "lemniscal" properties. Supported by ONR #00014-87-K-0433 and the Monsanto Co. (NMW)

299.14

EARPHONE SIMULATION OF A FREE-FIELD SOUND SOURCE AND ITS APPLICATION TO STUDIES OF CORTICAL MECHANISMS OF SOUND LOCALIZATION IN THE CAT. J.C.K. Chan , R. Kochhar*, A. Musicant*, R.A. Reale*, J.E. Hind, and J.F. Brugge. Department of Neurophysiology and Waisman Center on Mental Retardation and Human Development, University of Wisconsin, Madison, WI 53706.

Studies of the neural mechanisms of sound localization have been limited by the difficulty of generating and analyzing the spectrally complex, critically timed acoustical signals reaching the two tympanic membranes as the direction of a sound source is varied. Recently, we measured free-field to eardrum acoustic transfer functions in anesthetized cats in an anechoic chamber using a broad-spectrum stimulus (click) whose location was varied over 360° in azimuth and 120° in elevation (Musicant et al., J. Acoust. Soc. Am. 87:757, 1990). Using these data, which reflect the effects of the head, pinnae and ear canals, we have synthesized the signals required to drive a pair of specially designed insert earphones so as to reproduce the same sound pressure waveforms at the eardrums as had been produced for a particular source location in the free field. We have presented these dichotic stimuli to the barbiturate-anesthetized cat while recording from single neurons in the primary auditory cortical (AI) field. Preliminary experiments have shown that AI neurons respond robustly to many of these broad-band signals. Systematic presentation of the 1800 synthesized pairs of signals that represent the azimuth and elevation coordinates reveals that certain AI neurons exhibit spatial receptive fields. The shapes and positions of these fields vary among neurons in the same animal.

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299.15

MEASUREMENT OF THE STRENGTH OF SYNCHRONY OF CHINCHILLA COCHLEAR NUCLEUS UNITS IN RESPONSE TO COS+ NOISE. W.P. Shofner, Parmly Hearing Institute, Loyola Univ. of Chicago, Chicago, IL 60626

Rippled noise produces the perception of pitch in humans. Cos+ rippled noise is generated when a wideband noise is delayed and added to the undelayed noise. Some cochlear nucleus units can synchronize to the delay of cos+ noise as shown by a peak in the renewal density (autocorrelogram) [Shofner, W.P., Abstr. ARO, p. 400, 1990). The strength of synchrony in response to cos+ noise can be measured as the height of the peak in the renewal density. However, correlations that may exist among higher-order intervals will contribute to the height of this peak. In order to evaluate the contributions that correlations may have on the peak in the renewal density, a new spike train is generated by randomly shuffling the intervals. Random shuffling of the intervals removes any correlations among higher-order intervals, but does not affect the first-order interval distribution [Moore et al, Ann. Rev. Physiol., 28:493, 1966]. Single units were recorded primarily from the AVCN of barbiturate anesthetized chinchillas using tungsten or indium electrodes. Units that show phase-locking at BF generally have a peak in the renewal density at the delay of cos+ noise; for these units, a slightly reduced peak remains in the renewal density computed from the shuffled intervals. The presence of peaks in the shuffled renewal densitites which are only slightly reduced suggests that the heights of these peaks in the unshuffled renewal densities predominately reflect contributions of first-order intervals, but that correlations among higher-order intervals can also contribute to the measured strength of synchrony. It may, therefore, be more appropriate to use shuffled renewal densities to measure the strength of synchrony.

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299.16

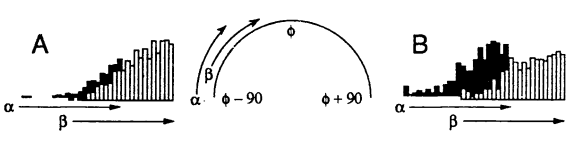
MEDIAL GENICULATE BODY UNIT RESPONSES TO KITTEN CALL. J. Buchwald, J. Harrison, L. Dickerson* and C. Hinman. Department of Physiology, Brain Research Institute and Mental Retardation Research Center, UCLA, Los Angeles, CA 90024

We have been investigating central processing of behaviorally significant sounds. The medial geniculate body, pars principalis (MGBp) in the cat projects to cortical areas AI, AII and Ep, the pars magnocellularis (MGBm) projects to insular cortex, and the dorsocaudal division (MGBd) projects to temporal cortex. Lesions of AI do not cause a deficit in discrimination of vocal stimuli or temporal sequences of auditory stimuli, whereas lesions of insular and temporal cortex do. To test whether particular thalamocortical systems preferentially respond to certain stimulus types, we compared unit responses in the 3 subdivisions of the MGB to various sounds. Units were recorded in awake cats. Stimuli included digitized vocalizations derived from a kitten isolation call, tone pulses and clicks. Of 809 units studied, only 23% responded to any of the auditory stimuli. In MGBp, 39% of responsive cells responded only to clicks. In contrast, 39% of responsive cells in MGBd were pure call responders, while only 12% of responsive cells in MGBp and MGBm responded only to the kitten call. These data support behavioral and anatomical data which suggest functional separation of auditory forebrain projections, with vocal stimuli selectively activating a dorsocaudal MGB-temporal cortex subsystem. (Supported by USPHS HD-05958.)

299.17

NEURAL RESPONSES TO INTERAURAL PHASE MODULATION IN THE INFERIOR COLLICULUS . M.W. Spitzer, M.N. Semple. Dept. of Anatomy and Neurobiology, Univ. of California, Irvine, CA 92717.

We used interaural phase modulation (IPM) to study the responses of single isolated neurons in the central nucleus of the inferior colliculus to dynamic manipulations of interaural phase. Digitally synthesized stimuli were presented dichotically to anesthetized gerbils. The phase angle of a pure tone stimulus presented to one ear was linearly modulated relative to the stimulus at the other ear. Modulation depth varied from 10 to 360°.



Two types of response to IPM stimuli were observed. The discharge rate of some neurons was strictly dependent on absolute interaural phase. Such units responded to two overlapping IPM sweeps (α and β) toward some phase angle, ϕ, with congruent discharge profiles (see fig., A). For the majority of neurons studied, however, over-lapping IPM sweeps generated incongruent discharge profiles (B). Over a wide range of phase angles, discharge of these units depended upon the depth of modulation relative to the starting point of a sweep, with much less dependence on absolute phase angle. The former class of neurons may encode information about sound source location; whereas, the latter may encode information about sound source motion.

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299.18

POPULATION RESPONSES OF AUDITORY NERVE (AN) FIBERS TO PURE TONES: ANALYSIS OF d_e' MEASURE FOR INTENSITY DISCRIMINATION. K. Parham and D.O. Kim. Div. Otolaryn., Surg. Res. Ctr., Ctr. Neurol. Sci., Univ. Connecticut Health Ctr., Farmington, CT 06032.

The d_e' is a sensitivity measure used in signal detection theory (Green and Swets, 1966). This measure was recently applied to AN population responses by Kim et al. (J. Acoust. Soc. Am., 1990). In the present study, we examined the d_e' as a measure of intensity discrimination performance as applied to populations of AN fibers in unanesthetized decerebrate cats. At each of several stimulus levels of 1 and 5 kHz pure tones and for each of an array of AN fibers, we analyzed the mean and standard deviation, σ, of spike counts which occurred during 0.2 sec tone bursts. From two sets of mean and σ corresponding to a pair of stimulus levels, we computed the d_e' measure for discrimination between the stimulus levels. We found that: (1) for discrimination between two low stimulus levels, d_e'-versus-CF profiles of both low and high spontaneous rate (SR) fibers (SR < or > 15 spikes/sec, respectively) exhibited distinct peaks of high d_e' scores at the characteristic place (CP); (2) for discrimination between two high stimulus levels, d_e'-versus-CF profiles of high-SR fibers exhibited quite low scores of d_e' in a region around the CP reflecting saturation of spike counts but the profiles of low-SR fibers exhibited a valley of low d_e' at the CP with region(s) of high d_e' closely adjoining on one or both sides. These findings suggest that microscopic spread of excitation within a relatively limited region around the CP, e.g., spanning about 1 octave of CFs, may play a role in the psychoacoustically measured intensity discrimination of pure tones.

[Supported by NIH-R01-DC00360 and HCRAC, Univ. Conn H.C.]

299.19

PHYSIOLOGICAL MECHANISMS OF DIRECTIONAL SELECTIVITY IN THE CAT'S PRIMARY AUDITORY CORTEX (AI) REVEALED BY EAR OCCLUSION. F. R. Samson* and T. J. Imig, Dept. of Physiol., Kansas University Med. Ctr., Kansas City, KS 66103.

Neurons in AI are selective to sound source location in the horizontal plane (azimuth). To elucidate mechanisms of azimuth selectivity we evaluated the effects of unilateral ear occlusion on the responses of 67 single units (CFs from 6 to 28 kHz). Single unit recordings were obtained from barbiturate-anesthetized cats. Noise bursts stimuli varying from 0 to 80 dB SPL, were delivered from various azimuths by loudspeakers mounted on a horizontal hoop encircling the animal's head. Ear occlusion effected a frequency-dependent attenuation of 20-60 dB and revealed both binaural and monaural mechanisms of azimuth selectivity. The most common binaural interaction was of the E/I type in which one ear was excitatory and the other inhibitory (48%). In some cases E/I responses were strongly nonmonotonic with respect to SPL and they received nonmonotonic input from the excitatory ear (28%). A less frequently encountered binaural interaction was mutual facilitation (21%). These cells were often tuned to a limited range of azimuth and SPL, and their responses appeared to be the product of nonmonotonic excitatory inputs from both ears. The azimuth selectivity of some cells was determined by monaural cues (6%). Occlusion of the excitatory ear abolished the response, and occlusion of the other ear had no effect. Azimuth selectivity in these cells is presumably derived from spectral cues as they were much less selective to tones than noise. Many cells showed evidence of both monaural spectral and binaural mechanisms of azimuth selectivity.

Supported by NIDCD grant # DC00173.

SENSORY SYSTEMS—AUDITORY SYSTEM STRUCTURE: FUNCTION OF IDENTIFIED CELLS

300.1

INTRACORTICAL DISTRIBUTION OF HORIZONTAL COLLATERALS OF PYRAMIDAL NEURONS IN CAT PRIMARY AUDITORY CORTEX. H. Ojima, C.N. Honda and E.G. Jones, Neural Systems Laboratory, Frontier Research Program, RIKEN, Wako, Japan

Eight pyramidal neurons in primary auditory cortex (AI) were intracellularly injected with HRP (2 cells) or biocytin (6 cells). Each neuron was functionally characterized relative to the antero-posterior sequence of best frequencies that form a tonotopic map in AI. All labelled somata were in layers II or III and gave rise to typical apical and basal dendritic arbors as well as extensive axonal systems.

Two major collateral systems emerged from the main axon, one ending in the vicinity of the cell and the second at a distance. Superficial recurrent collaterals originating in layers II and III coursed vertically or obliquely towards the pial surface. Upon reaching layers I or II, these ran parallel to surface. Deep collaterals given off in layer V ran roughly parallel to the pial surface and were confined to a region of layer V immediately below the soma. A few collaterals were given off in layer IV and distributed to other layers.

Each cell also gave rise to 2 to 4 thick long-range collaterals in layers III and V. These ran parallel to the pial surface for several millimeters. At several points along these long horizontal collaterals, vertically directed branches emerged to form a columnar pattern of collaterals distributed in layers I through V. When viewed in the tangential plane, most of the horizontal collaterals were oriented dorsoventrally. This may correspond to the orientation of isofrequency bands as described in cat. Boutons were found sparsely on the horizontal branches, but were densely distributed in column-like arrays in the vicinity of the cell and at a distance.

300.2

SPONTANEOUS DISCHARGE PATTERNS OF DORSAL COCHLEAR NUCLEUS NEURONS IN RAT BRAIN SLICES. H. J. Waller and D. A. Godfrey, Depts. of Neurological Surgery, Otolaryngology, Physiology & Biophysics, and Anatomy, Medical College of Ohio, Toledo, OH 43699.

Neuron recordings *in vivo* have shown that spontaneous firing of dorsal cochlear nucleus (DCN) neurons persists after deafferentation, while ventral (AVCN and PVCN) neurons become silent (Koerber et al., Exp. Neurol. 16:119 (1966)). We have identified rhythmic, irregular, and bursting spontaneous firing patterns of neurons within or close to the fusiform soma layer of DCN by extracellular recordings from slices of rat brainstem (Abstr. 13th Midwint. Mtg., ARO, 1990, 13:398). The present study more systematically identifies the distribution and possible origins of the spontaneous discharge. No spontaneously active neurons were found in multiple penetrations of AVCN or PVCN in experiments showing active DCN neurons. DCN activity probably does not depend upon inputs from other structures because a high density of active DCN neurons was found in slices containing no discernible AVCN or PVCN, trapezoid body, dorsal or intermediate acoustic stria.

When K^+ of the slice medium (ACSF) was lowered from 6.25 to 3.25 mM, spontaneous firing rates of rhythmic neurons decreased 21-48% and showed greater variability, but the rhythmicity persisted. When Ca^{++} and Mg^{++} were changed from 2 mM to 0.2 and 3.8 mM, respectively, firing rates of rhythmic neurons usually increased (up to 3.7-fold) and those of bursting neurons always increased (2.5- to 4.6-fold); interval variability of rhythmic neurons decreased, while bursts were prolonged and more frequent. The results suggest that spontaneous firing is generated within DCN, probably does not require synaptic activity, and may reflect specific membrane characteristics of DCN neurons. (Supported by NIH grant DC00172)

300.3

AMINO ACID CONCENTRATIONS IN COCHLEAR NUCLEUS OF PERFUSED RAT BRAIN SLICES. D. A. Godfrey, H. J. Waller and W. B. Farms, Depts. of Otolaryngology, Neurological Surgery, Physiology & Biophysics, and Anatomy, Medical College of Ohio, Toledo, OH 43699.

Although *in vitro* slices have been extensively used for electrophysiological and pharmacological studies, there has been relatively less chemical evaluation of the slice metabolism. We have begun a chemical evaluation of slices from which electrical activity was recorded in the cochlear nucleus (Waller and Godfrey, Soc. Neurosci. Abstr., 1990). Following the recording session, the slices were removed from the perfusion chamber and frozen within 5 seconds. Frozen sections were cut at 20 μm thickness and alternate sections either freeze dried overnight or mounted onto slides for staining with thionin. Freeze-dried sections were dissected into samples, which were weighed (0.2 - 2 μg) and loaded to the bottoms of plastic tubes for measurement of amino acid concentrations using high performance liquid chromatography (HPLC). Data were analyzed for 10 amino acids: aspartate, glutamate, glycine, γ-amino-butyrate (GABA), serine, glutamine, threonine, arginine, taurine, and alanine.

Slices containing dorsal and posteroventral cochlear nucleus, which had been incubated for 7-8 hours and displayed many active neurons throughout this incubation period, showed differences in several amino acid concentrations from values in cochlear nucleus of rat brain removed and immediately frozen. The averages of the concentrations for the slices as percentages of those for control brain were: aspartate 48%, glutamate 25%, glycine 95%, GABA 29%, serine 168%, glutamine 12%, threonine 98%, arginine 79%, taurine 24%, alanine 95%. The results suggest that several aspects of amino acid metabolism, notably glutamate metabolism including its precursor glutamine and products aspartate and GABA, may be quite different in perfused brain slices than in intact brain. (Supported by NIH grant DC00172)

300.4

ELECTRICAL PROPERTIES AND MORPHOLOGY OF SOME NEWLY IDENTIFIED AUDITORY RESPONSIVE UNITS FROM THALAMUS OF CONSCIOUS CATS. C.D. Woody, E. Gruen*, V. Chizhevsky*, O. Melamed*, and X.F. Wang. UCLA Med. Ctr., MRRC, BRI, Los Angeles, CA 90024.

Electrical properties of 366 neurons (resting potentials averaging -55 mV) were measured intracellularly: 172 from LD and CL thalamus, 103 anteriorly from areas bordering dorsal thalamic peduncle, and 91 from pulvinar and LP nucleus. Over half the cells of these areas responded to 70 db click or hiss with increased discharge; 65% had naturally occurring bursting (sustained) discharges, and 17% had spindle waves or sequences. Low threshold spike discharges were observed in <3% of cells given $\pm$1nA intracellular current pulses of 40 ms duration. Over one-third of all cells had non-bursting, unsustained firing responses during 1nA depolarizing pulses.

Very large (400-600 um^2) cells with thick, primary dendrites were marked with PHA-L in the anterior thalamus that responded to auditory stimuli and sent projections toward the rostral cortex through the dorsal thalamic peduncle. These cells constituted about one-fourth of those marked in that area; the remaining cells of the anterior thalamus had morphologies that corresponded with those previously described (Chizhevsky et al. Soc. Neurosci. Abstr. 1989). (Supported by NS25510.)

300.5

AMPA RECEPTORS, NOT KAINATE RECEPTORS, MEDIATE SYNAPTIC TRANSMISSION IN THE AVIAN AUDITORY NUCLEI MAGNOCELLULARIS AND LAMINARIS. N. Zhou and T. N. Parks, Dept. of Anatomy, Univ. of Utah Sch. of Med., Salt Lake City, UT 84132.

Because kainic acid (KA) is more potent than other excitatory amino acid (EAA) agonists in blocking synaptic transmission, previous reports have concluded that primary afferent neurotransmission to the cochlear nucleus in birds and mammals is mediated by KA receptors (Neurosci. Lett. 59:297,1985; Hear. Res. 17:153, 1985; Brain Res. 486:39, 1989). We studied the pharmacology of synaptic transmission between the cochlear nerve and nuc. magnocellularis (NM) and between NM and nuc. laminaris (NL) in chickens, using previously-described methods for bath application of drugs and recording of electrically-evoked field potentials in brain slices (Neurosci. 16:171,1985). Postsynaptic responses in NM and NL, but not compound action currents in the afferent axons, were blocked fully and reversibly by the broad-spectrum EAA antagonist kynurenic acid (2 mM) but not by the selective NMDA antagonists MK-801 (3 μM) or CPP (50 μM). The selective non-NMDA antagonist DNQX also blocked transmission, with IC_{50}'s of 7 μM for NM and 10 μM for NL. KA, domoic acid, quisqualic acid and AMPA also produced complete, dose-dependent and reversible transmission blockade, presumably by desensitization or depolarization. At 0.5 mM, the EAA antagonist g-D-glutamylaminomethylsulphonate (GAMS) prevented or reversed the antagonist action of KA in NM and NL but did not affect either synaptic transmission itself or the antagonist action of AMPA. Since these data rule out NMDA or KA receptors as mediators of synaptic transmission, we suggest that normal transmission in NM and NL is mediated by AMPA receptors, with KA receptors probably acting as modulators of transmission.

Supported by USPHS grant DC-00144.

300.6

WITHDRAWN

300.7

INTRACELLULAR RECORDINGS FROM CELLS IN THE RAT SUPERIOR OLIVARY COMPLEX (SOC) LABELLED WITH BIOCYTIN. M.J. Banks and P.H. Smith. Neurosciences Training Program and Depts. of Anatomy and Neurophysiology, Univ. of Wisconsin, Madison, WI., 53706.

Cells in the lateral superior olive (LSO) and the medial nucleus of the trapezoid body (MNTB) are believed to be involved in computing interaural level differences as a cue for sound localization. We have recorded intracellularly from cells in the LSO and MNTB using 400μ brain slices from 3-5 week old hooded rats. Cells were either injected with biocytin or the recording location was marked to verify their location.

LSO cells usually have linear current-voltage (IV) curves for both depolarizing and hyperpolarizing currents and high input resistances (typically > 70 MΩ). When stimulated with small sustained depolarizing currents, LSO cells often fire repetitively only after a long delay. At higher currents, a short latency spike is often followed by a pause, then a resumption of the discharge. Synaptic potentials can be evoked by stimulating both the ipsilateral trapezoid body (TB) and the TB at the midline. LSO cells have small, elongated somata (typically 24μ x 14μ) and long, thin, aspinous, bipolar dendrites often stretching >300μ from the soma in the dorso-ventral direction and a significant distance rostro-caudally. One LSO cell whose axon could be followed a considerable distance had collaterals within the SOC. One cell injected in the hilus of the LSO that responded similarly to cells in the LSO had axon collaterals terminating in the medial superior olive (MSO).

Principal cells in the MNTB respond to depolarizing current with only a single onset spike, have lower input resistances (typically <50MΩ) and IV curves that rectify for depolarizing current. Synaptic potentials can usually be elicited only by stimulating the TB at the midline. MNTB cell morphology resembles that of their principal excitatory inputs, cochlear nucleus bushy cells. The axon of one MNTB cell sent collaterals to the MSO and superior para-olivary nucleus, before entering the LSO.

300.8

CALBINDIN AND PARVALBUMIN REACTIVITY IN GRAFTED AND INTACT INFERIOR COLLICULUS. J.R. Coleman, B. Pinek*, M.C. Zrull, A.J. McDonald and K.G. Baimbridge. Depts. of Psychology, Physiology and Anatomy, Univ. of South Carolina, Columbia, SC 29208; Dept. of Physiology, Univ. of British Columbia, Vancouver, BC V6T 1W5.

The rat inferior colliculus is an excellent site for study of neural grafting into the central auditory system. Immunohistochemical labeling was used to identify major structural proteins in normal and graft tissues of the colliculus. Discoid and stellate neuron populations of the central nucleus were infrequently immunostained by calbindin, as were large portions of the core of graft tissue. Stellate and other neuron classes of dorsal and lateral cortex commonly labeled with calbindin as did graft margins. In contrast, discoid neurons in central nucleus and in graft tissue were reactive for parvalbumin which labeled few neurons of the dorsal and lateral nuclei.

Immunolabeling for tubulin-glutamate was pervasive in the inferior colliculus, but most intense in the central nucleus. Pure tone stimulation (8 kHz) enhanced activity of central nucleus tubulin-glutamate immunoreactivity in discoid and stellate neurons of the normal central nucleus.

These results show that neuron immunoreactivity patterns for calbindin, parvalbumin and other structural proteins provide important markers for identifying architectonic features of host and graft tissue in the inferior colliculus.

(Supported by the Deafness Research Foundation, the Fyssen Foundation and NIH R01 NS19733)

300.9

PROPERTIES OF FETAL TECTUM GRAFTED TO ADULT AND NEONATAL RAT INFERIOR COLLICULUS. M.C. Zrull and J.R. Coleman. Depts. of Psychology and Physiology, Univ. of South Carolina, Columbia, SC 29208

Whole fetal tecta were grafted to lesion sites in inferior colliculus (IC) of adult (HA) and neonatal (HP) rats. Under anesthesia, tecta were taken from Long Evans rat fetuses at 17 to 19 days of gestation, bisected, and the caudal half injected into lesion cavities in HA or HP conspecifics. Host were used in one of three procedures: 1) HRP-WGA injection to medial geni-culate body (MGB) ipsilateral to graft (0.1 μl; 24 h); 2) white noise stimulation (85 dB, 1 h) following ^{3}H-2-deoxy-glucose (200 μCi/100 g ip), and autoradiography; 3) postmortem tracing of graft connections with the carbocyanine dye DiI.

At 2 months post-implant, graft survival was 73% in HA and 80% in HP rats. In 60% of HA and HP tectal grafts neurons with fusiform somata, similar to discoid principal cells of normal central nucleus of IC (CNIC) were retrogradely labelled by HRP-WGA injections to MGB. Preliminary investigation of autoradiograms indicated 75% of HA and 50% of HP rats showed differential activity in tectal grafts driven by auditory stimulation. At present, only HP rats have been processed for DiI tract tracing. In 75% of these hosts, DiI diffused along fiber tracts coursing rostrally and ventrocaudally from crystals placed directly in grafts located above remnants of host IC. The visualized tracts appear to correspond to the location of brachial and lemniscal fibers that exit and enter the normal rat IC and CNIC.

Whole tectal grafts survive in both young and mature rat IC. The grafts contain neurons similar to normal CNIC cells that comprise the midbrain component of the central auditory pathway, and these neurons make connections with the target of CNIC in thalamus. Finally, early evidence suggests graft neurons receive afferents from lower brainstem nuclei and are responsive to auditory stimulation.

300.10

DEAFENING DECREASES STAINING FOR THE NERVE TERMINAL PROTEIN NT75 IN AUDITORY NUCLEI. T.C. Ritchie, S. Angeli*, S. Anastaplo* and P.J. Abbas*, Depts. of Anat., Otolaryngol. & Head & Neck Surg., and Speech Path. & Audiol., Univ. of Iowa, Iowa City, IA 52242.

NT75 is a 75 kD membrane protein concentrated in nerve terminals of selected CNS pathways including the auditory system. During development, NT75 staining in axons and then in nerve terminals of the auditory pathway correlates with synaptogenesis and the onset of hearing. In this study, NT75 staining was performed in deaf animals to determine the effect of lowering synaptic activity on the level of NT75 in auditory nuclei. For comparison, the synaptic vesicle protein synaptophysin was also localized. Animals (guinea pigs, rabbits) were deafened unilaterally by intracochlear injection of neomycin, or bilaterally by peripheral injection of kanamycin and ethacrynic acid. These ototoxic drugs deafen primarily by destroying cochlear hair cells. Brainstem auditory responses evoked by click stimuli were normal prior to the injections, but were markedly decreased or absent afterward. Immunocytochemical staining for NT75 with the S-7B8 antibody and for synaptophysin with the SY38 antibody was performed after survival periods of 3 days to 4 months. In normal animals, brainstem nuclei of the auditory system, including the cochlear nuclei (CN), the superior olive complex, the inferior colliculus (IC) and the medial geniculate nucleus, contain moderate to dense NT75 immunoreactivity. Three to 10 days after unilateral deafening, a small decrease in NT75 staining is detectable in the ventral CN ipsilateral to the deaf ear and in the medial nucleus of the trapezoid body on the contralateral side. In bilaterally deaf animals at longer survival periods (3-4 months), larger and more widespread decreases are seen, with decreases detectable in more rostral nuclei of the auditory pathway, particularly the IC. In contrast, very little or no difference is detectable between synaptophysin staining in deaf and normal animals. The results indicate that the level of NT75 staining in auditory system nerve terminals may be regulated by synaptic activity. Staining for NT75 may thus be used as an anatomical probe to evaluate the efficacy of electrical stimulation of the auditory nerve in maintaining synaptic activity through the ascending auditory pathway in deaf animals. Supported by NS23783 and by a grant from Iowa Lions.

300.11

CELLULAR GENERATORS OF THE BRAINSTEM AUDITORY EVOKED POTENTIAL IN CAT. J.R. Melcher*, B.C. Fullerton, J.J. Guinan, N.Y.S. Kiang, I.M. Knudson*. Eaton Peabody Lab., Mass. Eye and Ear Infirmary, Boston, MA 02114 and Dept. Elec. Eng., M.I.T., Cambridge, MA 02139.

We tested the hypothesis that individual peaks in the brainstem auditory evoked potential (BAEP) are generated by specific cell populations. The BAEP (vertex to ipsilateral earbar) was recorded in barbiturate-anesthetized cats before and after creating lesions in cochlear nucleus (CN) by injecting kainic acid which destroys cells near the injection site. Since different parts of the CN contain different cell populations, we could create lesions limited to specific populations by varying the injection site. The BAEP following N1 was virtually eliminated when a lesion involved almost all of the CN. When a lesion was confined to the dorsal (DCN) and posteroventral (PVCN) cochlear nuclei, there was no significant change in the BAEP. Lesions that included posterior AVCN, but largely avoided anterior AVCN (4 cases), resulted in greater changes in P2 than in P4. In one case P2 was reduced by 90% while P4 was unchanged. Furthermore, P2 was reduced more for the three cases with large posterior AVCN lesions than in the one case with a small lesion. In contrast, lesions confined to anterior AVCN (2 cases) resulted in larger reductions in P4 than in P2. In one case, P4 was reduced by 48% and P2 by 13%. These results, combined with previous measurements of single unit response latencies and our results for kainic acid lesions in the superior olivary complex, are consistent with three hypotheses: I) DCN and posterior PVCN cells do not contribute significantly to the BAEP, II) globular bushy cells located in posterior AVCN are the primary generators of P2, and III) cells that receive projections from spherical bushy cells in anterior AVCN are major generators of P4. (NIH P01 DC00119, Unisys Corp. Fellowship for JRM)

300.12

MEDIAL AND LATERAL SUPERIOR OLIVES RECEIVE PROJECTIONS FROM THE MEDIAL AND LATERAL NUCLEI OF THE TRAPEZOID BODY. N.Kuwabara and J.M.Zook. Dept. of Zool. & Biomed. Sci. & COM, Ohio Univ., Athens, OH 45701.

The medial (MSO) and lateral (LSO) superior olives are the first cell groups of the auditory brainstem which show binaural interactions. The MSO receives excitatory inputs bilaterally from the ventral cochlear nucleus (VCN). The LSO receives excitatory input from ipsilateral VCN and inhibitory input from the ipsilateral medial nucleus of the trapezoid body (MNTB). The MNTB functions to relay signals from the contralateral VCN to the LSO. Histochemical and ultrastructural studies suggest that there are other sources of input to MSO and LSO in addition to the aforementioned major inputs (Clark '69, Schwartz '80, Cant '84).

We have characterized the axonal inputs to the MSO and LSO by intracellular recording and dye labeling of cells in the MNTB and in the lateral nucleus of the trapezoid body (LNTB). This work was based on a tissue slice preparation of the auditory brainstem using two bat species, *Pteronotus parnellii* and *Eptesicus fuscus*, as well as the mouse, *Mus musculus*, and the gerbil, *Meriones unguiculatus*. In addition to the main projection, axon collaterals from MNTB principal cells were traced to MSO cell soma. Axons from large cells in the LNTB formed perisomatic terminals in both the MSO and LSO. These findings suggest that the MSO and LSO may have similar local circuits and patterns of activity. (Supported by NIH Grants NS 26304, NS 01394 and the OUCOM)

300.13

AUDITORY PONTINE GREY: CONNECTIONS AND RESPONSE PROPERTIES IN THE HORSESHOE BAT. E. Covey, J.H. Casseday and G. Schuller*. Dept. of Neurobiology, Duke University Medical Center, Durham, NC 27710 and Zoologisches Institut, Munich, Federal Republic of Germany.

To investigate the role of the pontine grey (PG) as a link between the auditory system and the cerebellum, we recorded response properties of single units in the PG and injected WGA-HRP in areas responsive to sound. The stimuli were pure tones and frequency modulated (FM) sounds. Retrograde transport identified two sources of auditory input to the pons: 1) The inferior colliculus, mainly the central nucleus, contained a large number of labeled cells; 2) An area of neocortex responsive to FM sounds contained some labeled cells. There was little evidence either of tonotopy or topographic distribution of binaural response types. Units responsive to pure tones had best frequencies that fell into one of two classes, both related to the harmonic structure of the biosonar signal: 1) near the first harmonic (35 kHz), or 2) near the second harmonic (78 kHz). Although all but 17% of the units could be driven by a pure tone, the most effective stimulus for 70% of all PG auditory units was some type of FM sound. Taken together, these results indicate that the PG processes specialized information about the biosonar signals used by this bat. (Supported by the Deutsche Forschungsgemeinschaft (SFB-204) and NIH Grant DC00287)

300.14

PROJECTIONS OF SPHERICAL BUSHY CELLS TO MSO IN THE CAT: EVIDENCE FOR DELAY LINES. P.X.Joris*, P.H. Smith, and T.C.T. Yin. Dept. of Neurophysiology, Univ. of Wisconsin, Madison.

In order to characterize projections of cochlear nucleus spherical bushy cells, we did intracellular recording and labeling of axons in the trapezoid body (TB) of the cat. Single fibers showing a primary-like or phase-locked response pattern were iontophoretically injected with HRP or biocytin, and their projection fields were reconstructed.

Characteristic frequencies (CFs) of labeled fibers were biased to low frequencies. For CFs below 1 kHz, the degree of phase-locking was often much higher than that found in the auditory nerve. Spontaneous rate was high (mean: 60 spks/sec). The axons labeled were thin compared to globular bushy cell axons, and coursed in the dorsal component of the TB. Consistent projections were found bilaterally in MSO and ipsilaterally in LSO. The ipsilateral projection to the lateral limb of the LSO consisted of several branching collaterals from the main axon or from collaterals projecting to the ipsilateral MSO. Profusely branching collaterals projected to the lateral side of the ipsilateral MSO and to the medial side of the contralateral MSO, spanning a considerable rostrocaudal extent. The contralateral projection gave off collaterals projecting caudally to the MSO. Their branching pattern is reminiscent of the delay lines proposed in Jeffress' model of azimuthal sound localization (Jeffress, J. Comp. Psychol., 41:35-39, 1948). Ipsilateral fibers showed a more variable pattern: the parent axon tended to give off axons anteriorly as well as posteriorly. A consistent contralateral projection was present to VNLL. Sparse projections were occasionally found in DMPO and the nuclei of the TB (LNTB, VNTB, MNTB).

The projections to MSO are consistent with extracellular MSO recordings (Yin & Chan, 1990, J. Neurophys., in press) showing a predominance of positive characteristic delays, indicating a longer conduction time for the contralateral input, at the caudal pole of the MSO.

300.15

CYTOMORPHOLOGICAL DIFFERENCE OF 2 TYPES OF FM CELLS IN THE RAT IC. P.W.F. Poon*, X.Y. Chen* and J.C. Hwang. Dept. of Physiology, Medical Faculty, Univ. of Hong Kong, Sassoon Rd., HONG KONG.

The cytomorphology of electrophysiologically identified inferior collicular (IC) neurons in the pento-barbital anaesthetized rat was studied using intracellular HRP techniques. Prior to the impalement and dye injection, extracellular activities of single units were analysed in terms of their response to triangular frequency modulated (FM) tones and pure tones. Of the 30 cells so labelled, 20 were 'mixed' (responded to both FM and pure tones), and 10 'FM specialized' (responded to FM but not pure tone).

All cells were multipolar with large dendritic fields. The 'FM specialized' cells showed profuse dendritic spines, whereas the 'mixed' cells were practically non-spiny. Results suggest that the cytomorphological difference may account for the functional specialization of FM cells.

(supported by Hong Kong University and Medical Faculty research grants)

300.16

PREVENTION OF DEAFNESS-RELATED SYNAPTIC CHANGES BY COCHLEAR PROSTHETIC STIMULATION. R.A. Altschuler, J.W. Horn*, E.A. Plattner*, and J.M. Miller. Kresge Hearing Research Institute & Dept. Anatomy & Cell Biology, Univ. of Michigan, Ann Arbor, MI 48109.

Studies have shown deafness-related changes in auditory nerve synapses with chemically induced and genetic deafness and acoustic deprivation (Gulley et al,'78; Rees et al,'85). Changes included flattening of the active zone and a decreased number of synaptic vesicles. Interestingly, such changes were not observed in the genetically deaf white cat (Larsen '88,89). We have investigated whether chronic stimulation with a cochlear prosthesis could prevent or reduce auditory nerve synapse changes in chemically deafened guinea pigs.

A cochlear implant was unilaterally placed in the scala tympani of adult guinea pigs. They were bilaterally deafened with systemic kanamycin and ethacrynic acid and then stimulated for 2 hr/day, 5 days/wk for 9 weeks. Evaluation of cochleae showed loss of the inner and outer hair cells and a large loss of spiral ganglion cells. In the rostral anteroventral cochlear nucleus (AVCN) contralateral to the implant, auditory nerve synapses on spherical cells had flattened active zones and a somewhat reduced number of vesicles. Other spherical cells completely lost their primary afferent input. In the rostral AVCN ipsilateral to the cochlear implant (stimulated side), most auditory nerve synapses on spherical cells had the normal pre-synaptic invagination and post-synpatic evagination of the active zone and numerous large round vesicles. Some active zones, however, were flattened and had a decreased number of vesicles. Occassionally a flattened active zone was seen in the same terminal as an active zone with normal morphology. Cochlear prosthetic stimulation therefore appeared to reduce but not completely prevent deafness related changes in auditory nerve synapses.

supported by NIH, NIDCD grant #PO1 DC 00274-06

301.1

INTERACTIONS OF TWO NEUROPEPTIDES IN MODULATING THE CARDIAC SAC RHYTHM IN LOBSTERS. P.S. Dickinson, W.P. Fairfield* and E. Marder. Dept. of Biology, Bowdoin College, Brunswick ME 04011 and Dept. of Biology, Brandeis University, Waltham, MA 02254

In the intact stomatogastric nervous system of the spiny lobster Panulirus, the cardiac sac rhythm can be activated by application of either of two neuropeptides, proctolin or Red Pigment Concentrating Hormone (RPCH), to the stomatogastric ganglion (STG). However, when the STG is isolated, proctolin can no longer initiate a cardiac sac rhythm, although RPCH can still do so. If, however, proctolin is applied shortly after an RPCH application (but after the RPCH-induced cardiac sac rhythm has ceased), it does initiate a cardiac sac rhythm, suggesting the possibility that RPCH "primes" the proctolin response. In addition, both proctolin and RPCH cause an increase in the size of the PSPs from the IV cells, which drive the rhythm, onto the cardiac sac dilator neuron 2 (CD2). This increase is much more pronounced in RPCH than in proctolin and is dose-dependent. When RPCH and proctolin concentrations are adjusted to give the same increase in PSP amplitude, a cardiac sac rhythm is still induced in RPCH but not in proctolin. These data suggest that, although both the increase in PSP amplitude and the initiation of rhythmicity involve the IV cells, they are separately controlled.

301.2

CONTROL OF THE CRUSTACEAN PYLORIC PATTERN GENERATOR BY AN IDENTIFIED MODULATORY NEURON: MODULATION OF BURSTING PROPERTIES IN PYLORIC NEURONS ISOLATED IN SITU. T. Bal*, F. Nagy and M. Moulins, Lab. Neurobiologie et Physiologie Comparées, CNRS, Univ. Bordeaux I, 33120 Arcachon, France.

The pyloric central pattern generator (CPG) of the crustacean stomatogastric nervous system displays a spontaneous rhythmic activity, which depends on the intrinsic bursting properties of the pyloric neurons. It has been shown (see Nagy *et al.* in this issue) that this spontaneous activity is controlled by identified modulatory inputs from higher centers. A brief discharge of such a neuron (APM) induces a complex and long lasting alteration of the ongoing activity of the CPG. To better understand this complex effect, we study the neuromodulatory control exerted by APM on the bursting properties of individual pyloric neurons, by isolating them from their counterparts in the CPG (photoinactivation or pharmacological blockade of presynaptic neurons).

We found that: 1) APM controls the regenerative properties of every pyloric neuron but to different extent depending on the neuron. Thus APM discharge induces oscillations in all previously non oscillating neuron, but with a different latency for each neuron. Also, characteristics of oscillations induced (period, plateau duration,...) and duration of effects are different for each type of neuron. 2) A brief discharge of APM induces a prolonged increase in input resistance of isolated pyloric neurons, which could mediate the long term modulation of their bursting properties. This increase of input resistance is associated with a long lasting decrease of a K^+ current.

Taken altogether, these different modifications of bursting properties of individual pyloric neurons, account for the complex changes of the pyloric motor pattern, provoked by APM's discharge when the network is intact.

301.3

EVIDENCE FOR DOPAMINERGIC MODULATION OF CRUSTACEAN VENTILATORY PATTERN GENERATOR AND ITS ACTIVATION BY NICOTINE. K.P. Rajashekhar and J.L. Wilkens, Department of Biological Sciences, University of Calgary, Calgary, AB, T2N 1N4, Canada.

Dopamine (DA) is known to elevate ventilatory rates in intact shore crab, Carcinus maenas. When perfused into isolated thoracic ganglia DA increases the burst frequency of the ventilatory pattern generator (CPG). Perfusion of domperidone, a potent dopamine D2 receptor antagonist, first eliminates some motor units from bursts and after a few s depresses the frequency of the rhythm. This action is reversible. Nicotine which is known to release DA from neural tissue in mammals, dramatically enhances the burst rate in isolated ganglia, an action not elicited by acetylcholine or blocked by curare. The excitatory action of nicotine is reversed by domperidone and nicotine fails to activate the CPG in the presence of domperidone. The CPG recovers from domperidone induced depression when washed for several min.

These observations suggest that DA is involved in the generation and maintenance of the ventilatory motor pattern in crabs. Nicotine appears to activate this CPG through a dopaminergic pathway, while it stimulates the vertebratemedullary ventilatory rhythm via cholinergic pathways. Characterization of DA receptors is under progress. Supported by NSERC, Canada.

301.4

AN ENDOSCOPIC ANALYSIS OF GASTRIC MILL MOVEMENTS PRODUCED BY THE PEPTIDE CHOLECYSTOKININ. M.E.T. Boyle*, G.G. Turrigiano, and A.I. Selverston. Dept. of Biology, UCSD, La Jolla, Ca 92093.

The gastric mill of lobster is composed of three teeth in the stomach, two lateral and one medial, which masticate food. The neural circuit which controls movements of these teeth is highly flexible, and can be modulated by a number of neuropeptides, including cholecystokinin (CCK). There is evidence that an endogenous CCK-like peptide is necessary for the feeding-induced activation of the gastric mill, and injections of CCK into the haemolymph of intact lobsters can activate the gastric mill (G.G. Turrigiano and A.I. Selverston, 1990, Nature, in press). The goal of this study is to use an endoscope to observe and quantify the gastric mill movement patterns produced by CCK.

Intact lobsters were restrained, and an endoscope was inserted through the esophagus and into the cardiac sac (the stomach sac) until the gastric mill was in view. Peptides were dissolved in saline and injected into the dorsal heart sinus, and the resulting gastric mill movements recorded on video cassette. Injections of CCK (doses between 5 and 200 μg) were able to activate a quiescent gastric mill in 18 of 19 cases. In some preparations CCK was able to activate the cardiac sac rhythm as well. The most common chewing pattern produced by CCK was a "cut and squeeze" mode, in which the lateral teeth closed strongly (cut), opened while moving backward, and then closed again to meet the cusp of the medial tooth (squeeze). The "cut" phase of the chew was commonly associated with contractions of the cardiac sac. Several variations on this basic pattern were observed. This pattern differs from that produced by proctolin, as reported by Heinzel (J. Neurophy. 59:2, 1988) and as observed by us; proctolin produced either a "cut and grind" movement in which the lateral teeth remain closed as they move backward to meet the medial tooth cusp, or a "squeeze" mode in which there is no strong lateral tooth closure. We are currently quantifying and comparing the movement patterns produced by these two peptides.

301.5

THE STRENGTH OF A SYNAPSE BETWEEN TWO NEURONS IN THE LOBSTER STOMATOGASTRIC GANGLION CAN BE CONTROLLED IN A GRADED MANNER N. Herterich and A.I. Selverston, Dept. of Biology, UC San Diego

We are developing a technique to control the strength of connections between neurons in a quantifiable manner over a broad range of numerical values. This technique will give us greater flexibility in "sculpting" circuits, enabling us to explore the relationship between circuitry and neural activity to a degree not previously possible. Our aim is to make predictions about the neural circuitry underlying neural function.

We report a simple means to successfully emulate the functional effects of a synapse between two cells in the pyloric CPG. We stimulate the PD cell with current pulses that are triggered by spikes in the LP cell, which normally makes a strong inhibitory synapse onto PD. The synapse can be blocked pharmacologically with picrotoxin (PTX), thereby increasing the pyloric pattern frequency. Hyperpolarizing pulses restore the cycle frequency, as well as the intracellular voltage timecourse in PD, effectively mimicing the normal synapse. Conversely, depolarizing pulses delivered prior to PTX increase the pattern frequency to the same extent as PTX, and therefore effectively cancel the influence of the normal synapse. The pulses which cancel the synapse are the same amplitude as the pulses which mimic the synapse, but of opposite sign. The pulses are therefore functionally equivalent to normal synaptic input.

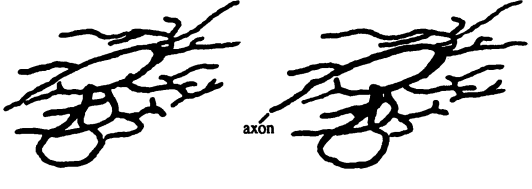
The strength of the connection from LP onto PD can be controlled by changing the amplitude of the pulses. The pattern period varies roughly linearly with pulse amplitude over a range at least twice as great as the pulse amplitude which is equivalent to normal synaptic function, and the slope of the relationship is independent of the presence of PTX. We conclude that pulse amplitude is a good measure of connection strength.

301.6

CONFOCAL MICROSCOPY OF CRAB STOMATOGASTRIC NEURONS. D. Baldwin* and K. Graubard, Dept. of Zoology, University of Washington, Seattle, WA 98195.

Pyloric neurons of *Cancer irroratus* and *C. borealis* were injected with Lucifer Yellow, fixed and examined as whole mounts with a confocal microscope. Data were analyzed as serial images through the ganglion and as stereo pair reconstructions. In agreement with the electron microscopic results of D. King on *Panulirus interruptus* (*J. Neurocytology* 5:207-237, 1976), we find that the crab ganglion has a central core containing only major processes and a surround containing fine neurites, which is largely dorsal and lateral in the crab. Preliminary studies have not shown any sign of regional differences in the area of fine neuropil. Instead, pyloric neurons have branched widely, covering much of the peripheral neuropil with fine neurites.

Supported by NIH Grant NS25505.



301.7

COMBINED ENDOSCOPIC AND ELECTROPHYSIOLOGICAL RECORDINGS OF MULTIPLE STG RHYTHMS IN INTACT CRABS H.G. Heinzel and J.M. Weimann. Inst. of Zoology, Univ. Cologne, 5 Köln 41, FRG; Biology Dept., Brandeis Univ., Waltham, MA 02254.

A method was developed to correlate the motor patterns produced by the stomatogastric ganglion (STG) and the movements of the gastric mill of the crab, Cancer pagurus.

An endoscope was inserted via the oesophagus into the stomach thus allowing video analysis of the movements of the gastric teeth. Intracellular recordings from STG neurons and extracellular recordings of the nerves exiting the STG were simultaneously displayed with the movements of the gastric teeth. These methods allowed elucidation of the role of many of the gastric motor neurons during ongoing behavior.

Intra/extracellular recording of the intact preparation were remarkably similar to the in vitro recordings. Importantly, many of the switches between CPGs seen in vitro (Meyrand et al.,1988 Neurosci Abs) produced relevant movements of the gastric mill. For example, the pyloric timed IC neuron not only controls the cardio-pyloric valve but also cause the anterior movement of the lateral teeth during gastric activity. When the gastric LG neuron fires in pyloric time the lateral teeth produce a mixing movement possibly to stir the contents of the stomach.

Supported by GRF Hel118/7 & NS17813 (E. Marder).

301.8

MODELING THE PYLORIC RHYTHM OF THE STOMATOGASTRIC GANGLION. L. Abbott, T. Kepler, E. Marder and S. Hooper. Center for Complex Systems, Brandeis Univ., Waltham MA; Center for Neurobiology and Behavior, Columbia Univ., NY.

The pyloric network of the crustacean stomatogastric ganglion produces a three-component rhythm with approximately constant phase relationships over a wide range of frequency. In the network, the anterior burster (AB) cell, which acts as a pacemaker for the rhythm, exhibits a constant duty cycle (burst duration proportional to oscillation period) when its frequency is manipulated by external current injection. However, when current is injected into an AB cell which is isolated from the rest of the network, the AB bursts show constant duration (non-constant duty cycle) as the frequency varies. Modeling studies are revealing the mechanisms by which network interactions are able to produce this modification of behavior which is vital for proper network function. Supported by MH 46742 and T32 NS07292.

301.9

PERIODIC INHIBITION OF PACEMAKER NEURONS: LOCKING, CHAOS, INTERMITTENCY, AND WALK THROUGHS; NON-STATIONARITIES; RATE DEPENDENCY. Altshuler, E.*, Garfinkel, A., Segundo, J.P., Stiber, M.* UCLA: Anatomy and C.B., Comp. Sciences & Kinesiology Depts.; BRI.

Nonlinear Dynamics has clarified obscurities of pace-maker driving by periodic IPSPs.Preparations: crayfish SAO, simulated (e.g., v.d. Pol). Methods: Poincare sections, spectra, etc. RESULTS. a. POSTSYNAPTIC DISCHARGES MAY BE PERIODIC AND CHAOTIC; they also show INTERMITTENCIES, WALK-THROUGHS AND ACCELERATIONS. b. SITUATIONS MAY BE NON-STATIONARY along unchanging arrivals (apart from initial settlings). E.g.: cross-intervals vary even during invariant discharges; steady epochs alternate. c. BEHAVIOR DEPENDS ON PRE-SYNAPTIC AND ON POST-SYNAPTIC NONDRIVEN RATES. E.g.: chaos differ with fast or slow drivings; fast pacemakers accelerate because soma spikes re-excite rebounding trigger zones. CONCLUSIONS. The above, compatible with canonical formulations for non-linear oscillators, allows deeper, broader biological and formal understandings. Achieved are norms for underlying mechanisms and testable hypothesis. Network comprehension benefits, for periodicity and arrhythmias pervade Physiology and Pathophysiology. SUPPORT: T.J. Wells, Jr., Inc.; UCLA Sch. of Medicine (BRSG), Department of Anatomy and Cell Biology.

301.10

REAL-TIME DISCREPANCIES IN THE CONTROL OF SLOW LOCOMOTOR ACTIVITY BY A CENTRAL PATTERN GENERATOR, Jeffery Lee Johnson, Dept. of Phys/Pharm., USD School of Medicine, Vermillion, SD 57069

Slow crawling movements in segmented vertebrates are regulated by a central network of interneurons which control the activity in small motoneurons which distribute to the body wall musculature. In order to understand how such a neuromuscular system operates, it becomes necessary to discern how the discharge rate initiated in the motoneuron by the central pattern generator(s) can be correlated with the slow contractile response. In this study it was learned that the slow contractile response in the longitudinal muscle of the earthworm over time could be described by a **parabolic equation.** Most importantly, the **slope** of the parabolic equation describing the end organ response could be precisely indexed to the amount of interneuronal tract fibers that were activated as well as the motoneuron discharge rate. The slope for the parabolic contraction curve displayed a **23X variation** between the weakest and strongest response. Thus, the central network of interneurons may primarily fuction to control the slope of the parabolic contraction curve in the end organ merely by inducing slight changes in the motoneuron discharge rate. In addition, each given slope value for contraction finally attained a stable **plateau level** of tone which may be associated with a **specifically indexed modifiction** of the **central pattern generator.** SUPPORTED BY PARSON'S TRUST FUND.

301.11

EFFECTS OF AFFERENT STIMULATION AND OF LONG TERM DEAFFERENTATION ON THE CRAB VENTILATORY MOTOR PATTERN GENERATOR (CPG). J.L.Wilkens, Biological Sciences, University of Calgary, Calgary, AB T2N 1N4, Canada

Acutely deafferented CPGs produce coordinated rhythmic bursts of motoneuron spikes typical of the output in intact animals but at much reduced rates (120 vs > 400 bpm, resp.). Stimulating the mixed sensory/motor levator nerve (LN) increases burst rates to intact levels and can activate silent CPGs. LN stimulation also eliminates some motor units from both levator and depressor bursts. Trains of stimuli reset and can entrain the CPG rhythm. Stimulation of the purely motor depressor nerve has no effect on the rhythm.

The ability of LN stimulation to enhance burst rate decreases as afferent axons degenerate following gill bailer ablation. Five d after ablation LN effectiveness is reduced 50%; at 22 d there is no increase. At 60 d LN stimulation inhibits the rhythm and phase resetting by train stimuli is largely lost. After 6 mo the motoneuron collateral arborizations are reduced by half while the motor rhythm remains robust.

Afferent input has both phasic and tonic influences on this CPG; however, this input is not necessary for long term stability of the motor program. Supported by NSERC of Canada.

301.12

THE HELISOMA "CYBERCHRON" NETWORK: IS IT PART OF THE FEEDING PATTERN GENERATOR? A. D. Murphy. Dept. of Biol. Sci., University of Illinois at Chicago, Chicago, IL 60607

A network of premotor neurons called the cyberchron and thought to drive and time the rhythmic activity of feeding motoneurons was described by Kater and colleagues. The cyberchron was supposed to excite retractor neurons and inhibit protractor neurons which then fired due to post-inhibitory rebound. There are three phases of activity in the standard buccal pattern and three pattern generator subunits (S1, S2, & S3) provide excitation to motoneurons active during each phase. S1 and S3 interneurons have been identified but not S2. S2 PSPs in motoneurons were attributed to cyberchron activity. However, rhythmically active cyberchron neurons may sustain rhythmic activity in the pattern generator without phase linkage to it and neuromodulators that activate the pattern generator do not activate monitored cyberchron neurons. I suggest that the "cyberchron" is an electrically coupled network of sensory neurons or modulatory interneurons that can trigger and sustain rhythmic activity in the buccal pattern generator. (Supported by NIH grant NS26145).

301.13

COMPUTER MODELING OF THE RESPIRATORY CENTRAL PATTERN GENERATOR (CPG) OF LYMNAEA USING HODGKIN-HUXLEY EQUATIONS. P.J. Kruk, N.I. Syed and A.G.M. Bulloch. Dept. of Medical Physiology, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

In vitro reconstruction of the respiratory central pattern generator (CPG) of Lymnaea was recently reported (Syed et al., 1989, Soc. Neurosci. Abstr.15: 1047). This CPG is comprised of three identified interneurons: R.Pe.D1, Ip.3.I and V.D4. In the present study, using Hodgkin-Huxley-like equations we modeled the electrical activities of this network. When interconnected, the electrical stimulation of R.Pe.D1 elicits a series of alternating bursts of action potential in previously quiescent Ip.3.I and V.D4. When cultured as single cells, both Ip.3.I and V.D4 which are conditional bursters produced only a single burst of APs upon their electrical stimulation and no repetitive bursts were observed. R.Pe.D1, on the other hand is a low frequency pacemaker and the injection of depolarizing currents increases its firing rate. These intrinsic properties of identified neurons were modeled in the present study using three voltage-dependent membrane currents: (1) sodium, (2) potassium, and (3) a low-voltage-activated (LVA) Ca^{2+} current. In our model system Ca^{2+} current is relatively low, yet allows one AP to evoke another thus producing a burst. Channel inactivation leads to stepwise attenuation of this Ca^{2+} current with each AP, and eventually terminates the burst. Excitation is transmitted within this network by two mechanisms: (1) depolarization of the postsynaptic cell, and (2) postinhibitory rebound. Both mechanisms are simulated by the same set of differential equations. This suggests that just three currents (Na^+, K^+ and LVA Ca^{2+}) can account for some aspects of CPG activity.

Supported by AHFMR.

301.14

IN VITRO STUDIES ON THE INTRINSIC PROPERTIES OF RESPIRATORY CENTRAL PATTERN GENERATING (CPG) NEURONS OF LYMNAEA STAGNALIS.
K. Lukowiak, N.I. Syed, R.L. Ridgway and A.G.M. Bulloch. Department of Medical Physiology, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

We recently reconstructed the respiratory CPG of Lymnaea in culture and described the network properties of this neural ensemble. In the present study we explore the intrinsic properties of these respiratory interneurons. The network that comprises the respiratory CPG of Lymnaea is composed of three interneurons: Ip.3.I, V.D4 and R.Pe.D1. Interneurons Ip.3.I and V.D4 contain FMRFamide, whereas R.Pe.D1 is dopaminergic. Ip.3.I and V.D4 are conditional bursters and have reciprocal inhibitory connections with each other. Weak mutual inhibitory connections also exist between R.Pe.D1 and V.D4. The dopamine cell R.Re.D1 can initiate the respiratory rhythm by postinhibitory rebound excitation (PIR) of Ip.3.I. In turn Ip.3.I, in the presence of R.Pe.D1 activity, excites V.D4 by PIR and the cycle is repeated. Ip.3.I and V.D4 were cultured in vitro as single cells and their intrinsic properties were examined. Upon release from hyperpolarizing currents, both Ip.3.I and V.D4 fired only one burst of action potentials before becoming quiescent. Further trials with hyperpolarizing currents of even higher intensity failed to evoke the PIR in most cells tested. However, the presence of exogenous dopamine (applied via pressure pulses) decreased the threshold for PIR. Phasic application of dopamine evoked PIR in Ip.3.I, but not in V.D4. Similarly, phasic application of FMRFamide on V.D4 did not cause PIR in this cell. However, when both dopamine and FMRFamide were applied simultaneously at short intervals, V.D4 exhibited PIR. These results suggest that PIR plays an important role in this neural network and that *both* dopamine and FMRFamide are critically required for rhythm generation.

Supported by MRC (Canada).

301.15

NEURONAL NETWORK GENERATING LAMPREY LOCOMOTION - EXPERIMENTS AND SIMULATIONS - SUPRASPINAL, INTERSEGMENTAL, SEGMENTAL AND SENSORY MECHANISMS.
S. Grillner, P. Wallén*, L. Brodin*, A. Lansner*, Ö. Ekeberg* and H. Tråven*, T. Matsushima and J. Christensson*, Nobel Institute of Neurophysiology, Karolinska Institute and Computer Science Department at Royal Institute of Technology, Stockholm, Sweden

The isolated nervous system of the lamprey, a lower vertebrate, can produce the motor pattern underlying locomotion. Ventral root bursts alternate between left and right side in a range between 0.2-10 Hz in each segment (Wallén and Williams 1984, Brodin et al 1985). Along the spinal cord the segments are coordinated with a constant phase lag around 1% of the cycle duration (Grillner 1974) generating a rostro-caudal travelling wave. The motor pattern is elicited by reticulospinal projections (McClellan and Grillner 1984), and it is modulated by sensory input (Viana di Prisco et al 1989). A neuronal network of supraspinal, sensory and segmental neurones have been described in terms of connectivity, membrane properties and transmitters (Buchanan and Grillner 1987, Ohta and Grillner 1989, Viana di Prisco 1989, Wallén and Grillner 1987, Dubuc et al 1989, Alford et al 1989). It is shown by realistic simulation that the experimentally established network can account for the locomotor behaviour in the entire physiological range including the initiation, sensory modulation and the intersegmental coordination (see also Grillner et al 1988,1989). The connectivity can create a constant excitability gradient between adjacent segmental oscillator networks from a leading segment anywhere in the spinal cord. The significance of the different neuronal mechanisms for the operation of the network is evaluated.

301.16

EXTRACELLULAR RECORDINGS FROM THE MOTORNERVOUS SYSTEM OF THE NEMATODE ASCARIS. **R.E. Davis and A.O.W. Stretton. Department of Zoology and Neuroscience Training Program, Univ. of Wisconsin - Madison. Madison, WI 53706.**

Previous intracellular recordings from Ascaris motorneurons and some interneurons revealed that these cells do not show the classical voltage-sensitive action potentials characteristic of other nervous systems (Davis, R.E. and A.O.W. Stretton, J. Neurosci., 9:415, 1989; Angstadt, J.D. et al., J. Comp. Neurol., 284:374, 1989). The existence of discrete EPSPs and IPSPs in some classes of motorneurons, however, suggests that spikes are probably being generated in presynaptic interneurons.

Extracellular suction electrode recordings made over the nerve cords and over muscle cells have revealed fast spike activity. There are two general categories of spikes associated with the ventral nerve cord of Ascaris: (1) small amplitude, short duration, relatively simple biphasic spikes and (2) larger amplitude, longer duration, typically complex multiphasic spikes. The small spike population can show a variety of patterns of firing activity, has been correlated with motorneuron PSPs, and is not correlated with intracellularly recorded ventral muscle events. It is likely that this population of signals originates in interneurons responsible for the motorneuron PSPs. The large spike population is correlated with intracellularly recorded muscle events and can be manipulated by polarizations of presynaptic motorneurons as would be expected if this population originates in muscle. Pharmacological experiments indicate that the spikes are Ca^{++} based signals. We are in the process of further characterizing these various spike events and assessing their role in locomotory behavior.

This work supported by USPHS Grant 15429.

301.17

Optical techniques can be used to identify neurons and their patterns of activity during rhythmic motor programs in the buccal ganglion of *Aplysia*. D. W. Morton, H. J. Chiel, J.-Y. Wu, and L. B. Cohen. Depts. of Biology and Neuroscience, Case Western Reserve University, Cleveland, OH 44106, Dept. of Physiology, Yale Univer. Sch. of Med., New Haven CT. 06510, and Marine Biological Lab., Woods Hole MA 02543.

We have used optical techniques to observe the activity of buccal ganglion neurons in *Aplysia californica* during patterned activity induced by stimulating the esophageal nerve. Ganglia were stained with a voltage-sensitive absorbance dye, JPW1124 (2 mg/ml) dissolved in *Aplysia* L15. Extracellular activity from all six buccal neurons projecting from one side of the ganglion was measured while optical data were obtained from an array of 124 photodiodes (recording time: 16 seconds). Data were obtained from 6 different ganglia. Ganglia were then photographed in place to aid in cell identification. Of these ganglia, one was selected for detailed analysis because its rhythmic pattern of activity most closely resembled that observed in isolated buccal ganglia not exposed to the dye. Action potentials were grouped using a template matching technique, and a total of 48 distinct neurons showing different patterns of activation were identified from the optical data. In order to identify projection neurons, extracellular action potentials on buccal nerves were grouped using the template matching technique. In each group, the time of occurrence of each extracellular action potential was used to search the optical record for the locations in the ganglion displaying action potentials. Using this technique, it was possible to reliably associate intracellular optical activity with extracellular action potentials for several neurons (2 on buccal nerve 1, 6 on buccal nerve 2, 3 on buccal nerve 3, 1 on the cerebral-buccal connective). Furthermore, neurons with similar patterns of activity were found in similar locations in two other ganglia. Analysis of other nerves was complicated by the high level of activity on the extracellular record. We also determined the location of 19 neurons showing intracellular activity during the rhythmic pattern, but showing no obvious correspondence to the neural activity on the extracellular record. We conclude that, if a neuron has a large or distinctive action potential, and the extracellular record is not overly complex, optical techniques can be utilized to identify putative projection neurons and their dynamic pattern of activity during rhythmic motor programs. [Supported by NSF grant BNS-8810757 and PHS grants NS08437, 2 T32 GM07250-14]

302.1

DEVELOPMENT OF SEROTONERGIC PROJECTIONS AND SEROTONIN-INDUCED DEPOLARIZATIONS IN THE SPINAL CORD OF RAT EMBRYOS. L. Ziskind-Conhaim, and P.D. Newcomer*. Department of Physiology, University of Wisconsin, Madison, WI 53706.

Development of serotonergic projections and motoneuron responses to serotonin (5-HT) were studied in isolated lumbar segments of the spinal cord of rat embryos. We determined the increase in 5-HT innervation using intracellular recordings and immunocytochemistry. 5-HT projections were first detected in the lateral and ventral funiculi at 16-17 days of gestation. By Day 18, 5-HT fibers invaded the ventral horn and the intermedio-lateral column. At this stage, 5-HT application (10-50 μM) induced membrane depolarizations <10 mV and spontaneous subthreshold potentials. After birth, the number of 5-HT projections significantly increased around motoneurons, and 5-HT application generated membrane depolarizations >10 mV and high frequency spontaneous action potentials. These depolarizations were blocked by methysergide (20-50 μM). In the continuous presence of 5-HT, the frequency of the spontaneous potentials was gradually reduced. 5-HT induced membrane depolarization was associated with either a small decrease (< 25%) or no measureable change in membrane conductance. Depolarizations evoked by 5-HT were unaffected by blocking presynaptic activity with high Mg^2/low Ca^2 or TTX, which suggests that 5-HT acts on motoneuron membranes. Supported by NIH Grants NS23808 and NS01314.

302.2

MODULATION BY GLYCINE AGONISTS AND ANTAGONISTS OF THE EFFECTS OF NMDA AND L-HOMOCYSTEIC ACID IN THE HEMISECTED RAT SPINAL CORD PREPARATION. F.Brugger*, M.F. Pozza*, U. Wicki*, H.-R. Olpe*, G.E. Fagg. Res. and Dev. Dept., Pharm. Div. CIBA-GEIGY Ltd., CH-4002 BASEL/Switzerland.

The NMDA receptor is a multiple domain transmitter-gated neuronal membrane channel. The most potent antagonists currently defined for this receptor are CGP 37849 (competitive), MK-801 (channel blocker), 7-chlorokynurenic acid (7CKA) and HA-966 (allosteric modulators), with K_i values of about 35nM, 5nM, 0,2μM and 3μM as determined using receptor binding techniques. We have compared the actions of these substances on NMDA- and L-homocysteic (L-HCA) acid-evoked responses on the hemisected spinal cord preparation of the rat (extracellular root recordings). NMDA and L-HCA evoked depolarizations of the ventral roots which could be potentiated by the addition of D-serine (a glycine like agonist). 7CKA (10μM), HA-966 (100μM) and CGP 37849 (1μM) antagonized the effects of NMDA and L-HCA (CGP 37348 > 7CKA > HA-966). Unlike CGP 37849, the antagonistic effect of 7CKA and HA-966 could be overcome by the addition of D-serine. Our results indicate that L-HCA induces its major effects in the spinal cord via an action at the NMDA receptor.

302.3

BRAINSTEM GLUTAMATE RECEPTORS CONTROLLING MUSCLE ATONIA AND LOCOMOTION. Y.Y. Lai and J.M. Siegel. UCLA and VAMC, Sepulveda, CA 91343.

We have demonstrated previously that L-glutamate (Glut) participated in muscle atonia. We also found that electrical stimulation in rostral brainstem produced not only atonia but also locomotion. In the present study, we examined the effect of different Glut agonists on muscle activity in the decerebrate cat. NMDA and non-NMDA agonists microinjected into the same points within the inhibitory area of the pons and nucleus magnocellularis (NMC) produced opposite effect. Non-NMDA agonists produced muscle tone suppression, while NMDA produced muscle excitation and locomotion. This chemical effect was consistent throughout the survival period. In contrast, electrical and mechanical (by needle insertion) stimulation of these same points produced only atonia when administered within 10 h of decerebration, but produced atonia followed by locomotion when administered > 10 h after decerebration. We hypothesize that 1) non-NMDA receptors mediate muscle suppression, and that NMDA receptors participate in locomotion and muscle facilitation, 2) degeneration of axons severed by decerebration removes a net inhibition of the brainstem locomotor control system. Pathological dysfunction of these descending motor systems in the REM sleep behavior disorders may similarly disinhibit brainstem locomotor systems, and produce motor activity in response to the glutamate release of REM sleep.

302.4

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

A GABAergic innervation of the trigeminal motor nucleus is suggested from physiological studies which investigated the inhibition of masseter motoneurons following peripheral and cortical stimulation. Following stimulation of the inferior alveolar, hypoglossal and masseter nerves an early and late hyperpolarization is observed in masseter motoneurons. The late hyperpolarization is blocked by a systemic application of picrotoxin suggesting that the hyperpolarization is due to GABA (Nakamura, Y. et al., Brain Res., 57:29, 1973). Following repetitive stimulation of the masticatory cortex, rhythmical jaw movements are observed. Most of the hyperpolarization seen in the masseter motor neurons is blocked by strychnine, yet a residual component persists which is strychnine resistant (Enomoto, S. et al., Neurosci. Res., 4:396, 1987). This latter component is possibly GABAergic.

We initiated a series of experiments to identify GABAergic inputs to the trigeminal motor nucleus. A retrograde tracer, colloidal-gold bound WGA-HRP (gWGA-HRP) was injected into the trigeminal motor nucleus of male albino guinea pigs. Following a 72 hour survival time, the animals were transcardially perfused with 4% paraformaldehyde and 0.1% glutaraldehyde. GABA and GAD immunohistochemistry was performed using polyclonal antibodies. ABC histochemistry with DAB as a chromagen was used to label the immunoreactive complex. Neurons which contained the retrograde tracer and displayed a positive immunoreactivity were identified in the parvocellular reticular formation, peritrigeminal region, supratrigeminal region and spinal trigeminal nucleus oralis. Immunoreactive GABA and GAD cells, not containing the retrograde marker, were identified in the superior olive, nucleus of the lateral lemniscus, dorsal cochlear nucleus, trapezoid body, nucleus gigantocellularis, lateral reticular formation and spinal trigeminal nucleus interpolaris. We are providing anatomical evidence for the existence of GABAergic inhibitory trigeminal premotoneurons which contribute to jaw closure motoneuron hyperpolarization during reflex and centrally produced jaw movements. Funded by NIH-NIDR grant DE01693.

302.5

BURSTING PATTERNS, IN VITRO, IN NEURONS WITHIN THE SWALLOWING AREA OF THE NUCLEUS TRACTUS SOLITARII : ROLE OF ENDOGENOUS PROPERTIES. A. JEAN and F. TELL*. Laboratoire de Neurobiologie fonctionnelle. CNRS URA 205. Faculté Saint-Jérôme 13397 Marseille Cedex 13. France.

The activity of nucleus tractus solitarii (NTS) neurons was recorded on rat brainstem slices within the swallowing area. Extracellular recordings showed that repetitive stimulation of afferent fibers, in tractus solitarius, elicited in some neurons a synaptic response followed by a burst of spikes (350-450 ms ; 16-35 Hz). Under bath application of NMDA (30-120 μM), these neurons exhibited a pattern of rhythmic bursting characterized by trains of action potentials (400-450 ms ; 30-35 Hz) occuring at a regular frequency (0.5-1.5 Hz). Intracellular recordings indicated that these rhythmic activities were subtended by endogenous properties : NMDA elicited TTX-resistant membrane potential oscillations with a voltage-dependent frequency. These findings suggest that some NTS neurons might have conditional pacemaker properties. The patterns of these bursting activities in vitro being very similar to those of swallowing neurons, it may be suggested that the endogenous properties of NTS neurons are involved in the generation of the swallowing motor activity.

302.6

MODULATION OF THE ACOUSTIC STARTLE REFLEX AFTER DIETARY FALSE CHOLINE PRECURSOR NADE. M.-F. Wu , D. J. Jenden, M. D. Fairchild, R. S. Szymusiak, D. J. McGinty, and J. M. Siegel. Neurobiol. Res., VAMC, Sepulveda, CA 91343 & Depts. Psychiat., Psychol., & Pharmacol., UCLA, Los Angeles, CA 90024.

The role cholinergic systems play in modulating the startle reflex mechanism is unclear (Davis, M., Neurosci. Biobehav. Rev., 4:241, 1980). It was recently reported that rats treated with chronic diet of cholinergic false precursor N-amino-N,N-dimethylaminoethanol (N-aminodeanol, NADe), which replaced 65-75% of the endogenous acetylcholine in certain brain regions, showed enhanced startle reflex (e.g., Newton, M. W., Crosland, R. D., & Jenden, D. J., Brain Res., 373:197, 1985). In the present study the acoustic startle reflex and its modulation by a preliminary stimulus (prepulse inhibition) was examined in the choline deficient NADe rats.

Weanling rats were fed ad libitum a choline free basic diet in pellet form. Either NADe chloride (experimental group, n=7) or choline chloride (control group, n=6) at a concentration of 35.8 mmol/kg was added to the diet. Startle tests were performed following 100-120 days of diet. The basal startle level was determined with a 20 msec noise pulse of either 115 or 100 dB (SPL). In half of the trials a 65 db noise pulse was presented 100 msec before the eliciting stimulus (prepulse trials). Basal startle level was facilitated by 160-200% in the NADe rats as compared to the choline controls. Prepulse inhibition was greatly reduced in the NADe rats (12% reduction of basal startle level) as compared to the controls (75%). Reducing the intensity of the eliciting stimulus to 100 dB decreased the basal startle level of the NADe rats to about that of the controls with 115 dB but did not change the deficit in prepulse inhibition. It is concluded that cholinergic systems not only modulate the elicitation of the startle reflex but also play a role in prepulse inhibition of the startle reflex . (Supported by PHS grants MH17691 & MH43811 and the Medical Research Service of the Veterans Administration)

302.7

LOCOMOTION IN THE IN-VITRO MUDPUPPY (NECTURUS MACULATUS). M. Wheatley*, M. Edamura*, R.B. Stein. Division of Neuroscience, University of Alberta, Edmonton, Alberta, Canada, T6G2S2.

Previous investigations have demonstrated the viability of the Mudpuppy as a suitable in-vitro model of vertebrate locomotion (Wheatley and Stein, Can. J. Physiol. Pharm. 67, 1989). Locomotion in the in-vitro preparation is initiated by bath application of the excitatory amino acid N-methyl aspartate (NMA 100 μM). Further investigations on this isolated brainstem - spinal cord - forelimb preparation have shown that the locomotion in-vitro requires the presence of Mg^{2+} in the solution. The locomotor rhythm appears to require a basal level of glycine in the media and is potentiated by 1 μM glycine.

The mudpuppy shows many of the reflex responses commonly seen in higher vertebrates. In particular we have investigated the cutaneous reflex elicited with an electrical stimulus to the dorsal aspect of the foot. This reflex is modulated throughout the step cycle in both cats (Forssberg, J. Neurophysiol. 42:963-953) and humans (Yang and Stein, Soc. Neurosci. Abstr., 14:1303, 1988). The mudpuppy shows a similar reflex modulation. The most consistent response seen is an excitation of the elbow flexor muscle (Brachialis) during the swing phase and no response in the Brachialis during the stance phase. The response seen in the elbow extensor muscle (Extensor Ulnae) is less consistent but is also modulated. (M.W. is an AHFMR student, M.E. is an Alberta Parapelegic Foundation fellow, Research is supported by MRC of Canada.)

302.8

EXCITATORY AMINO ACID-MEDIATED RESPONSES IN THE PONTINE RETICULAR FORMATION OF THE RAT. D.R. Stevens, R.W. McCarley, and R.W. Greene. Dept. of Psychiatry, Harvard Medical School/VAMC, Brockton, Mass 02401.

The medial pontine reticular formation has been implicated as a site important in the induction of REM sleep and other behavioral processes. As a prelude to studies of the role of excitatory amino acids in synaptic transmission in the pontine reticular formation, we have examined the actions of excitatory amino acid receptor agonists on neurons in brain slices containing the pontine reticular formation.

Application of glutamate or more selective agonists led to depolarizations associated with inward current in 95% of the reticular neurons examined. Glutamate and N-methyl-D-aspartate (NMDA) treatment resulted in mixed conductance changes while kainate and quisqualate receptor agonists caused clear conductance increases. NMDA currents were voltage-dependent and were depressed in physiological concentrations (1.3 mM) of magnesium. Currents evoked by NMDA were blocked by the NMDA antagonist 2-amino-5-phosphono-pentanoic acid (AP5, 40 uM). Currents activated by kainate and amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid, a quisqualate agonist, were voltage-insensitive and were blocked by kynurenic acid.

These results demonstrate the presence of the three subtypes of excitatory amino acid receptors on neurons of the pontine reticular formation. The responses associated with the actions of NMDA and non-NMDA agonists are similar to those reported for these agents in mammalian cortical and spinal neurons.

302.9

NMDA RECEPTOR ANTAGONISTS BLOCK BRAINSTEM-EVOKED LOCOMOTION IN DECEREBRATE CATS. J.R Douglas*, X. Dai*, B.R. Noga and L.M. Jordan. Dept. of Physiology, Univ. of Manitoba, Winnipeg, Man. R3E 0W3.

The excitatory amino acid agonist NMDA has been implicated in the generation of locomotion in the frog embryo (Dale, N. and Roberts, A., J. Physiol. 348:527 (1984)), lamprey (Grillner, S. et al, TINS 10:34 (1987)) and newborn rat in vitro (Smith, J.C. et al, FASEB J. 2:2283 (1988)). The present study was designed to extend these findings to an adult mammalian preparation.

Pre-collicular, post-mammillary decerebrations were performed on adult cats. An intrathecal cannula was inserted at the seventh lumbar segment and advanced rostrally under the dorsal roots for 2-3 segments. A string was tied around the twelfth thoracic segment securely enough to occlude the subarachnoid space. The mesencephalic locomotor region (MLR) was electrically stimulated (less than 200 μA) to produce locomotion as monitored by electromyograms in treadmill locomotion experiments or electroneurograms in fictive locomotion experiments.

The amplitude of hindlimb steps began to decrease within 20 minutes of intrathecal administration of the specific NMDA antagonist 2-amino-5-phosphono-valeric acid (APV) to the lumbar region of the spinal cord, with no changes in forelimb locomotion. The strength of MLR stimulation required to elicit hindlimb locomotion gradually rose in the first hour, until no hindlimb movements were seen even at high stimulation strengths. Full recovery was possible within 2 hours of APV administration. These results are consistent with previous findings that NMDA receptors play a role in locomotion. However, they do not indicate the mechanism of their involvement. Possible sites of action of NMDA include interneurons involved in the pattern generation circuitry, the last order interneurons which produce locomotor drive potentials in motoneurons, or the motoneurons themselves. (Supported by the Medical Research Council of Canada)

302.10

PHENTOLAMINE REDUCES MOTONEURON DEPOLARIZING RESPONSES TO LOCUS COERULEUS STIMULATION AND IONTOPHORETICALLY RELEASED NOREPINEPHRINE. S.J. Fung, J.Y.H. Chan, D. Manzoni* and C.D. Barnes. Department of VCAPP, Washington State University, Pullman, WA 99164-6520.

It has previously been shown that synaptically (via electrical stimulation of the locus coeruleus, LC) and iontophoretically released norepinephrine (NE) induced spinal cord motoneuron (MN) depolarizations accompanied by enhanced excitabilities in cats. The present work was aimed at determining an α-adrenergic role of NE-LC actions on spinal MNs of decerebrate cats. Placements of a stimulating electrode in the LC, with subsequent histological verifications, were aided by exploring the low-threshold sites for inducing EPSPs from antidromically identified lumbar MNs. Low-intensity (15-150 μA) cathodal train stimuli (4-34 pulses at 500 Hz) were delivered to the LC while intracellular recordings plus extracellular iontophoresis of NE and phentolamine were made from MNs. In MNs with antidromic spike height above 60 mV, phentolamine (15-40 nA, 2-5 min) was found to significantly reduce the NE- (45-60 nA, 1-2 min) and LC-induced membrane depolarizations. Multiple discharges of MNs in response to LC stimuli, and NE in combination with subthreshold current injections, were both inhibited by concurrent phentolamine application. These data indicate the involvement of α-adrenoceptor-mediated NE and LC activations of ventral horn MNs in cats. Supported by NIH grant NS 24388.

302.11

TRH AUGMENTS THE FREQUENCY OF RAT RESPIRATION *IN VITRO* IN BRAINSTEM - SPINAL CORD PREPARATIONS FROM NEONATES. D.F. Russell and M. Takenoshita*. Dept. of Anesthesiology, Washington Univ. Med. School, St. Louis, MO 63110.

The site of action of thyrotropin-releasing hormone (TRH) on the breathing rhythm is controversial. Modulation of respiratory rhythms by TRH was characterized using *in vitro* preparations from 0-2 d old Long-Evans rats that included the medulla and cervical spinal cord (MC), usually with the pons (PMC), or sometimes with the hypothalamus (HPMC). Fictive respiratory discharges were recorded from a phrenic nerve. The bath was always partitioned near C_{2-3} for selective bath application of ~100 nM TRH and other agents to the brainstem. The action of TRH depended on the rhythm pattern. If it had stopped or consisted of single bursts at 8-20 s intervals, TRH restarted rhythmic bursting or accelerated its frequency in a marked tachypnea. Conflicting results came from fresh active preparations in which the rhythm pattern was episodic, consisting of 2-23 bursts at ~1 Hz with ~10 s apneas between episodes; TRH tended to slow the average burst frequency and convert the pattern to regular single bursts, in a seemingly "inhibitory" manner. This may be due to a performance ceiling of the *in vitro* preparation. To illustrate the inverse relation between TRH stimulation and the basal rhythm rate, 60-500 μM GABA or glycine was applied to the brainstem to experimentally slow the rhythm. Simultaneous application of TRH then consistently evoked 2-3x tachypnea in PMC preparations. Transection at the level of n.VI yielded MC preparations, which also showed tachypnea under 100-500 nM TRH. Removal of the cerebrum and transection at the optic chiasm left the hypothalamus attached; the effects of 100 nM TRH were not statistically different from those in PMC preparations. In sum, we obtained no evidence for a rostral tachypneic site of TRH action, but did confirm that TRH can augment the respiratory rhythm frequency at the level of the medulla. Supported by NIH grant NS23028 to D.F.R..

302.12

GABA-MEDIATED DEGLUTITIVE MECHANISMS IN THE RAT NUCLEUS TRACTUS SOLITARII (NTS). D. Bieger and Y.T. Wang. Faculty of Medicine, Memorial University of Newfoundland, St. John's, Newfoundland A1B 3V6

The role of central GABA neurons in swallowing is still poorly defined despite suggestive clinical evidence. In the present study, the GABA receptor antagonist, bicuculline (BIC) was used as a tool to characterize the role of solitarial GABA synapses in deglutitive control.

When applied to the NTS surface in urethane-anaesthetized rats BIC (5-200 picomol) evoked robust dose-dependent rhythmic complete swallows. Pressure ejection of BIC at solitarial s-glutamate responsive loci in the subnuclei intermedialis and centralis gave rise to buccopharyngeal and oesophageal peristalsis, respectively, accompanied by an enhancement of glutamate-evoked responses. Furthermore, subthreshold BIC doses injected into the subnucleus centralis caused a transient disinhibition of primary deglutitive peristalsis of the esophagus, evident when buccopharyngeal swallowing was elicited by chemical (kainic acid or noradrenaline) or electrical stimulation of the NTS.

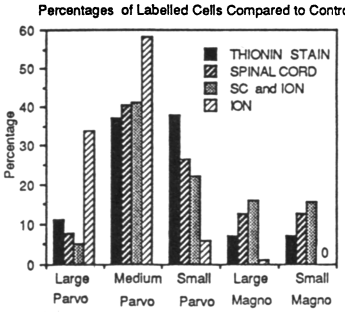
We conclude that solitarial GABA neurons exert a tonic inhibition of the medullary deglutitive pattern generator and control buccopharyngeal - esophageal coupling. Supported by MRC (Canada)

ANATOMICAL EVIDENCE FOR AXON COLLATERALS OF BRAINSTEM NEURONS PROJECTING BILATERALLY TO AREAS IN AND AROUND THE TRIGEMINAL MOTOR NUCLEUS IN GUINEA PIGS. D.M. Phillips*, J.E. Turman, Jr., M.C. Woo*, T.V. Trank*, L.J. Goldberg and S.H. Chandler. Dept. of Kinesiology, School of Dentistry, and The Brain Research Institute, UCLA, L.A., CA 90024.

Previous anatomical and electrophysiological experiments from our lab suggest that trigeminal pre-motoneurons are distributed throughout the lateral reticular formation in the guinea pig brainstem. The present study was undertaken to determine if brainstem reticular neurons have axon collaterals projecting bilaterally to the trigeminal motor nucleus or areas adjacent to the motor nucleus. Retrograde fluorescent tracers were injected into the right and left trigeminal motor nucleus with either a Hamilton syringe or with a glass pipette via pressure. Red beads were injected into the left side, while either a 3% solution of fluorogold in saline or green beads were injected into the right side. Following a transcardial perfusion of 4% paraformaldehyde and post fixation in 4% paraformaldehyde and solutions of increasing concentrations of sucrose, the brainstem was cut from the obex through the inferior colliculus into 30µm sections using a freezing microtome. Labeled neurons were observed using an epifluorescent unit on a Nikon Optiphot microscope. Neurons containing red beads were found in clusters with neurons containing the other retrograde fluorescent tracer. These bilateral clusters were best observed in the parvocellular reticular formation, particularly in that portion around the caudal 1/3 of the facial motor nucleus and around the descending branch of the facial nerve. Occasional double labeled neurons were observed in these clusters of singly labeled cells. The clusters of singly labeled reticular formation neurons suggest that projections to areas in and around the trigeminal motor nucleus are primarily unilateral and allow for independent control of jaw musculature on the left and right sides. The double labeled neurons may be used in bilateral coordination of synchronous jaw movements, such as occurs in jaw opener muscles during reflex jaw opening, mastication or vocalization. Funded by NIH-NIDR grants DE 06193 and DE 04166.

303.2

RE-DEFINING RAT RED NUCLEUS: CYTOARCHITECTURAL ANALYSIS OF RED NUCLEUS NEURONS SINGLY AND DOUBLY LABELLED FROM SPINAL CORD AND INFERIOR OLIVARY NUCLEUS. C. L. Tucker* and P. R. Kennedy. Neuroscience Laboratory, Georgia Institute of Technology, Atlanta, GA 30332.



Having reported last year (Soc. Neurosci. Abstrs. 15: (1), 405) that both parvocellular and magnocellular neurons in rat red nucleus project to the spinal cord and inferior olivary nucleus (ION), we determined the types of parvos (large, medium, or small) and magnos (large or small) that project to these targets and their distributions within the nucleus. Thionin stain was used to visualize neurons in direct light (controls), and fluorogold and/or diamidino yellow was used to fluorescently label RN neurons from these targets. The unexpected results (graphed above) show that parvos and magnos are labelled from the spinal cord, whereas parvos almost exclusively are labelled from ION. Double label is found in both cell types. Supported by NIH grant NS24602.

303.3

NUCLEUS PREPOSITUS HYPOGLOSSI PROJECTS TO THE DORSOLATERAL PERIAQUEDUCTAL GRAY (PAG): A LINK BETWEEN VISUOMOTOR AND LIMBIC SYSTEMS. G. Holstege[1], R.J. Cowie[2] and P.O. Gerrits[1]* Depts. of [1]Anatomy Groningen, The Netherlands, and [2]Howard University, Washington D.C.

The PAG forms part of the limbic system. Stimulation in the PAG elicits behavioral patterns used for defense in situations which represent a threat to the animals' survival (such as antinociception, postural and vocal signals and cardiopulmonary responses). Many neurons in the PAG project to the caudal brainstem and receive profuse afferent connections from various limbic structures. However, this is not true for the neurons in the dorsolateral wedge-shaped portion of the PAG (PAGdl). In order to clarify the role of this part of the PAG, it was injected with WGA-HRP in 4 cats to identify its connections. The injection-sites extended into the adjacent superior colliculus (SC). In all 4 cases, a specific group of labeled neurons was found in the contralateral prepositus hypoglossi nucleus (PPH) and ventrally adjacent reticular formation. To determine the exact dorsal midbrain target of these medullary neurons, ^{3}H-leucine was injected into the PPH and adjoining areas in 3 additional cats. In these cases, a pronounced termination of labeled fibers was observed in the contralateral PAGdl, while other parts of the contralateral PAG were devoid of label. Only sparse labeling was found in the intermediate layer of the contralateral SC.

It has been shown that the PAGdl receives afferents from the deeper layers of SC, substantia nigra and various parts of the prefrontal cortex. However, this specific PPH projection to the PAGdl has never been demonstrated. The PPH is known to be involved in control and adaptation of eye- and head-movements. The present results show that the PPH not only projects to visuomotor structures, but also to the PAG. This PPH-PAG connection may play a role in informing the limbic system about ongoing visuomotor activity.

303.4

TELENCEPHALIC EFFERENT PROJECTIONS IN BUDGERIGARS. D.M.S. Webster. School of Rehabilitation Medicine, University of British Columbia (UBC), Vancouver, B.C., - V6T 2B5.

Previous retrograde tracing studies have shown no differences in the origins of descending brainstem-spinal pathways, or supraspinal afferents to the medullary reticular formation in prehensile (parrots) compared to non-prehensile (duck, goose) birds that could account for the pedal dexterity of parrots. The purpose of this study is to extend those findings using anterograde tracers in other members of the parrot family, the budgerigar (Melopsittacus undulatus). Fluorescent anterograde axonal tracers fluorescein-conjugated dextran-amines (FDA) or rhodamine- conjugated dextran-amines (RDA) were injected (0.05-0.1 µL) into the intermediate archistriatum. Dense labelling of fibres and terminals was found in the diencephalon, tectum, nucleus intercollicularis, lateral mesencephalic reticular formation, locus coeruleus, dorsal and ventral subcoeruleus and caudal pontine reticular formation. In the medulla, labelling was largely confined to the parvocellular reticular nucleus, subtrigeminal nucleus and dorsal column nuclei. There was little or no labelling within the medial medullary reticular formation. At the level of the spinomedullary junction, labelling in the descending nuceus and tract of the trigeminal nerve and dorsal column was equivocal; there was no clear evidence of a fibre tract emanating from the archistriatum that descended into the spinal cord. These results confirm that there are little or no differences in the efferent projections onto brainstem reticular formation neurones in parrots, compared to previous reports in non-prehensile birds. Confirmation of other efferent projections from the hyperstriatum are being investigated. Supported by NSERC of Canada.

303.5

FOS PROTEIN-LIKE IMMUNOREACTIVITY IN RAT AND CAT SPINAL NEURONES EVOKED BY PERIPHERAL ELECTRICAL STIMULATION. I. Barajon*, H. Hultborn* and J.-P. Gossard. Dept. Neurophysiology, Univ. Copenhagen, DK-2200 Copenhagen, Denmark.

The intracellular level of the translational product of the fos protooncogene may be enhanced by direct or synaptic activation of neurones. After electrical stimulation of peripheral nerves and spinal roots we evaluated the expression of fos-like immunoreactivity in the dorsal and ventral horn of rat and cat spinal cord. Cambridge polyclonal primary antibody 0A-11-823 "fos-family" was used in both species.

Stimulation including both noxious and non-noxious afferents (20-50 xT at 10 Hz for 1 hour) gave rise to a fos nuclear labelling more densely distributed in the superficial layers and deeper regions of the dorsal horn, but to a lesser extent present also in the ventral horn and controlateral grey matter. These data support the results reported in the rat after other kinds of peripheral sensory stimulations. (Hunt, S.P., Nature, 328:632, 1987. Menetrey, D., J. Comp. Neurol., 285:177, 1989).

When we attempted a more selective activation of subsets of neurones in the intermediate zone and ventral horn (by stimulation of muscle group I afferents, by antidromic volleys in motor axons and by activating the long-latency long lasting flexor reflexes following i.v. administration of DOPA to acute spinal cats) the labelling of neurones in the intermediate zone and in the ventral horn was still present, while much fewer positive cells were seen in the dorsal horn (despite that the initial surgery must have activated pain afferents).

These preliminary results show the possibility to extend the use of fos immunohistochemistry to the CNS of the cat. Furthermore they suggest the use of this technique as a tool in the investigation of cells not only involved in sensory pathways but also of neurones that are more directly part of the network underlying motor behaviour.

303.6

TERMINATION OF CORTICOSPINAL EFFERENTS WITHIN THE CERVICAL CORD OF NEW WORLD PRIMATES. G.A. Bortoff and P.L. Strick. VA Med. Ctr. and Depts. of Neurosurg. & Physiol., SUNY-HSC @ Syracuse, Syracuse NY, 13210.

Anterograde transport of 2-10% wheat germ agglutinin conjugated to horseradish peroxidase was used to examine the pattern of termination of efferents from the primary motor cortex within cervical segments of the spinal cord in cebus (Cebus apella) and squirrel (Saimiri sciureus) monkeys. We have compared the termination patterns in these monkeys because of marked differences in their manipulative abilities. Both primates have pseudo-opposable thumbs; however, only cebus monkeys use independent finger movements to pick up small objects. We found that cebus and squirrel monkeys differ in the extent of corticospinal terminations within the ventral horn. As others have noted, efferents from the primary motor cortex of squirrel monkeys terminate densely within the intermediate zone of the spinal cord and have, at best, only sparse terminations within the ventral horn. In contrast, our results show that efferents from the primary motor cortex of cebus monkeys have dense terminations within both the intermediate zone and the ventral horn. The projection to the ventral horn in these monkeys is particularly dense at C8-T1 segments, where terminations form a 'ring' which encircles the lateral motoneuronal cell group. These observations provide further evidence for the concept that cortical projections to the ventral horn provide the neural substrate for independent movements of the fingers. Support: VA Med. Res. Serv. and Rehab. R&D; USPHS 2957, 24328.

303.7

PRIMARY AFFERENT PROJECTIONS FROM INTRINISIC HAND MUSCLES TO THE SPINAL CORD AND BRAINSTEM OF THE MONKEY. D. L. Schulmann, C. L. Martin-Elkins* and C. J. Vierck. Dept. of Neuroscience, University of Florida, College of Medicine, Gainesvile, Fl 32610.

The most severe and enduring sensorimotor deficit associated with dorsal column lesions is an inability to perform fine, coordinated finger movements. The intrinsic hand muscles, including the lumbricals, play an important role in these movements. We used WGA-HRP and free HRP placed into selected lumbrical muscles to study the organization of primary afferent terminations specific to these muscles. In one animal, tracer injection of a single lumbrical labeled terminals in a discrete column in lamina 9 of segments C7, C8 and T1 of the spinal cord gray matter, while more superficial ventral horn laminae and intermediate gray contained a diffuse pattern of labeling. In the brainstem, terminal patchy labeling was located within the main cuneate nucleus, but not the external cuneate. Unilateral injection of two separate lumbrical muscles in another animal resulted in two distinct patches of terminal labeling in the main cuneate. Supported by NS-17474 and NS-07261.

303.8

ORGANIZATION OF THE MOTORPOOLS INNERVATING EPAXIAL MUSCULATURE IN THE MACAQUE. M. Kramer and T.W. Deacon. Biological Anthropology, Harvard University, Cambridge, MA 02138.

Although much is known about the organization of the motorpools that innervate appendicular muscles, little is known about the innervation of axial musculature. Previous studies indicate interspecific differences in the organization of the motorpools that innervate dorsal neck muscles in the cat and the rat (Richmond et al., JCN, 181:451,1978; Abrahams & Keane, JCN, 223:448,1984; Callister et al., JCN, 255:369,1987).

In this study the organization of the motorpools that innervate individual epaxial muscles in the macaque (M. fascicularis) is described. Approximately 2 µl of WGA-HRP was injected into five epaxial muscles: the iliocostalis, the longissimus, and the transversospinalis (at thoracic and lumbar levels); as well as two tail muscles, the extensor caudae lateralis and medialis (ECL and ECM). The spinal cords were serially sectioned and the tissue was processed with TMB. For each injection a single composite diagram illustrating the locations in the ventral horn (VH) of all the HRP-labelled motoneurons was constructed.

Motoneurons that innervate the individual epaxial muscles are found in localized regions within the ipsilateral VH. The transversospinalis motorpool is confined to the extreme medial border of the VH. The longissimus motorpool is centrally located in the ventral third of the VH and extends into the tip of the VH. Motoneurons that innervate the iliocostalis are found along the lateral border of the ventromedial nucleus. The motorpools of the longissimus and iliocostalis muscles exhibit a small amount of overlap. The motorpools that innervate the tail muscles, ECL and ECM, are the direct caudal continuations of the motorpools that innervate the longissimus and transversospinalis, respectively. These results in the macaque are similar to those reported in the rat (Brink et al., Brain Res., 170:23,1979; Smith & Hollyday, JCN, 220:16,1983).

303.9

THREE-DIMENSIONAL ANALYSIS OF DENDRITE DEVELOPMENT IN PHRENIC MOTONEURONS. W.E.Cameron, He F., P.Kalipatnapu and R.D.Guthrie. Department of Pediatrics, Magee-Womens Hospital and Center for Neuroscience, Univ. of Pittsburgh, PA 15213.

Fifteen kitten phrenic motoneurons labeled by intracellular injection of HRP were reconstructed using a personal computer and the *Eutectic Neuron Tracing System*. Five cells were analyzed in each of three postnatal age groups: 2 wks, 1 and 2 mos. We found no change in mean total surface area of phrenic motoneurons between 2 wks ($68,224\pm10,135um^2$) and 1 mo ($62,351\pm11,373um^2$) while the surface area at 2 mos ($123,879\pm22,487um^2$) was twice the area found at 1 mo. In contrast, the area of influence (defined by the surface covering the distalmost points of the terminal dendrites) increased throughout postnatal development. At 1 mo, the expansion of the dendritic field without a concomitant increase in total membrane surface area was achieved by an elongation of the dendrites and a reduction in the complexity of the dendritic tree. There was an increase in segment length of 4-6th order with a loss of most 7th and all 8th order dendrites at 1 mo when compared to 2 wks. Subsequent development (between 1 and 2 mos) was characterized by an increase in the numbers and lengths of the more distal segments (7-9th order dendrites). The mean dendritic diameter which had remained constant in early development was found to have increased in all segments of the dendritic tree between 1 and 2 mos. Analysis of the distribution of dendrites in three-dimensional space revealed an increase in the proportion of terminal branches found in the rostral and caudal hexants with age.

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303.10

FLUORESCENT DYE LABELLING OF NEURONS IN THE MESENCEPHALIC NUCLEUS IN PIGS AND FERRETS. R. E. Druzinsky, S. W. Herring*, T. J. Drolsum*, and E. H. Polley. Depts. of Oral Anatomy, and Anatomy and Cell Biology, Univ. of Illinois, Chicago, IL 60612.

The cell bodies of primary and secondary afferents from spindles of the jaw adductor muscles are found in the mesencephalic nucleus of the fifth nerve (Mes V). In order to map the positions of cell bodies in preparation for electrophysiological studies in the Mes V and trigeminal motor nucleus, dyes were injected into the masseter muscle in pigs (Sus scrofa) and ferrets (Mustela putorius furo). Although Fast Blue (Sigma F-5756, 2.5% sol.) and TRITC (Sigma T-2018, 2.5% sol.) were tried, the most consistent labelling was obtained using Fluoro-Gold (2-5% sol.). In both species cells were labelled throughout the entire rostro-caudal length of the Mes V. The labelled cells exhibited a variety of cell types, including some which are clearly multipolar. Our results are consistent with the findings reported in other recent studies of the Mes V (e.g., Gottlieb, et al., J. Comp. Neurol., 228:273-283, 1984, in cats) and demonstrate that the topological arrangement of primary afferents from the masseter in two members of the Carnivora studied (cats and ferrets) is similar to the arrangement found in the pig, a member of the Ungulata.

Supported by NIDR NRSA DE05562 to R.E. Druzinsky.

303.11

NUCLEUS RETICULARIS INTERMEDIALIS (NIR) - POSSIBLE CHOLINERGIC SOURCE SUBSERVING OESOPHAGEAL PERISTALSIS IN THE RAT. D. Vyas, Y.T. Wang and D. Bieger, Faculty of Medicine, Memorial University of Newfoundland, St. John's, NF A1B 3V6

Cholinergic mechanisms in the rat brainstem play a crucial role in the control of oesophageal peristalsis at the levels of both premotoneurons in the subnucleus centralis of solitary complex (NTS_c) and motoneurons in the compact formation of the nucleus ambiguus (AMB_c). In this study, the origin of cholinergic afferents to those neurons was investigated by the combination of retrograde tracing and ChAT-immunocytochemistry. Deposits of fluorogold into glutamate-responsive oesophageal loci in NTS_c resulted in bilateral labelling of a discrete population of cells extending 200-300 µm medial and up to 600 µm rostral to AMB_c and corresponding to the nucleus intermedialis reticularis of the medulla. Most of the fluorogold-labelled cells were ChAT-immunopositive. Smaller numbers of double-labelled cells were also revealed in the NIR after injection of fluorogold into the AMB_c. The present study implicates the NIR as a major cholinergic afferent input to the brainstem pattern generator for oesophageal peristalsis.

Supported by MRC (Canada)

303.12

HETEROGENEITY OF THE MORPHOLOGY OF SINGLE VESTIBULOSPINAL COLLATERALS IN THE UPPER CERVICAL SPINAL CORD OF THE CAT. A. Donevan, S. Vohra* and P.K. Rose, Department of Physiology, Queen's University, Canada, K7L 3N6.

Recent studies using the anterograde tracer PHA-L have demonstrated that the funicular paths and terminations of vestibulospinal axons projecting to the upper cervical cord are more extensive than had been appreciated using classical neuroanatomical techniques. Following PHA-L injections into regions of the medial and descending vestibular nuclei, boutons were observed bilaterally in lamina II through IX. The goal of the present study was to determine the contribution of individual collaterals to this widespread projection.

The structure and bouton distribution of single PHA-L labelled collaterals arising from axons travelling in the ventromedial funiculi were examined from serial reconstructions. Some collaterals had one termination zone in a small region of the ventral horn or intermediate zone. Although the termination zones of some of these collaterals overlapped, no two were identical. Other collaterals projected to several discrete sites. These sites included small regions as dorsal as lamina IV and as ventral as lamina IX. The unexpected structural diversity of vestibulospinal collaterals suggests that vestibulospinal cells, even those whose axons travel in the same funiculi, may serve different functions. (Supported by MRC).

303.13

IS THE ROLE OF THE TECTOSPINAL TRACT IN THE CONTROL OF HEAD MOVEMENT OVERRATED? P.K. Rose, J. MacDonald* and V.C. Abrahams, Department of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

The tectospinal tract (TST) together with the tectoreticular spinal system provide two routes by which the superior colliculus can influence the activity of neurons in the upper cervical spinal cord and hence, play a role in the control of head movement. In the present experiments we re-examined the projections of TST neurons using the anterograde tracer PHA-L. Despite the proven sensitivity of this technique, PHA-L labelled axons and collaterals in the upper cervical spinal cord were sparse following two or more injections of PHA-L in the superior colliculus. All labelled axons and collaterals found were contralateral to the site of injection. In agreement with previous studies, boutons were found in the lateral part of laminae VI and VII. Unexpectedly, boutons were also found in the medial part of laminae VII and VIII with most in lamina VII. Boutons belonging to single collaterals were usually clustered in a small zone and thus, only occupied a small fraction of the grey matter receiving TST projections as a whole. These results suggest that the TST plays little or no direct role in the control of head movement, but may influence the activity of neck motoneurons via a selective action on a small number of segmental interneurons. (Supported by MDAC).

303.14

PROJECTIONS FROM THE LATERAL VESTIBULAR NUCLEUS TO THE UPPER CERVICAL SPINAL CORD OF THE CAT - AN ANTEROGRADE TRACING STUDY USING PHA-L. K. Wainwright*, M. Neuber-Hess* and P.K. Rose, Department of Physiology, Queen's University, Kingston, Ontario, Canada, K7L 3N6.

Use of the sensitive anterograde tracer PHA-L has recently shown that spinal projections originating from the medial and descending vestibular nuclei terminate in regions of the spinal cord previously not thought to receive direct vestibulospinal input. In the present experiments, we have extended these studies to include the spinal projections from the lateral vestibular nucleus (LVN). Injections of PHA-L in the LVN labelled similar number of axons in the medial vestibulospinal tract (MVST, bilateral) and lateral vestibulospinal tract (LVST, ipsilateral) at the C1/C2 junction. Many boutons were found in laminae VIII and IX and the ventral part of lamina VII ipsilateral to the injection site. Contralateral projections were sparse and the boutons were widely scattered in laminae V through IX. More caudally (C3/C4 junction), few boutons and axons were found contralaterally. Ipsilaterally, the number of axons travelling in the MVST was smaller, the frequency of axons in the LVST was unchanged, and the number of boutons decreased. These results indicate that descending axons from the LVN take a variety of routes in the upper cervical spinal cord, but the major termination zone is in the ipsilateral ventral horn of C1 and C2. (Supported by MRC).

303.15

EXTENSIVE AXON COLLATERALS REVEALED BY INTRACELLULAR NEUROBIOTIN OR HRP SUGGEST SPINAL INTERNEURONAL FUNCTIONS FOR SACRAL PGN. **C.W. Morgan, W.C. deGroat, S.J. Zhang*, and L.A. Felkins*** Depts.Anatomy and Neurobiology, and Urology, Eastern Virginia Med. School, Norfolk, VA 23501.

The morphology of sacral preganglionic neurons (PGN) in the cat was examined using intracellular injections of neurobiotin or HRP. Among 21 filled PGN extensive axon collaterals from parent axons were identified in laminae I, V, VII, VIII, X, and the lateral funiculus bilaterally and in lamina IX ipsilaterally. Labeled boutons were located in proximity to: (1) areas of visceral primary afferent termination, (2) the soma and dendrites of PGN, (3) interneurons near the central canal, (4) motoneurons and interneurons in the ventral horn, and (5) descending axons in the lateral funiculus. Patterns and locations of boutons suggested that a single PGN could directly contact as many as 40 neurons in the cord. These results indicate that PGN have *integrative, interneuronal functions in the central nervous system* in addition to their traditional efferent role of conveying information to ganglion cells in the peripheral nervous system. Supported by NINDS RO1 NS26585

303.16

AFFERENT CONNECTIONS OF THE PARVICELLULAR RETICULAR FORMATION, STUDIED IN THE RAT BY ANTEROGRADE AND RETROGRADE TRANSPORT METHODS. S.J.Shammah-Lagnado*,M.S.M.O. Costa*,S. Nicastri* and J.A.Ricardo. Dept. of Physiology, Inst. Biomed. Sci.,Univ. of Sao Paulo,01000 Sao Paulo, SP,Brazil.

The afferent connections of the parvicellular reticular formation (RFp) were studied in the rat with either HRP or WGA-HRP tracer techniques; tetramethylbenzidine was the chromogen. Injections of the tracers into the RFp led to retrograde labeling in several structures that included the primary somatosensory and motor cortical areas, the granular insular cortex, the central amygdaloid nucleus, hypothalamic districts, the field H_2 of Forel, the superior colliculus, the central gray substance, the red nucleus, mesencephalic and magnocellular pontomedullary reticular structures, the contralateral RFp, the lateral, 'dorsolateral hump' and medial cerebellar nuclei, the mesencephalic, principal and spinal trigeminal nuclei, the supratrigeminal region, the vestibular complex, the nucleus of the solitary tract and the spinal cord. Deposits of WGA-HRP in several territories, that included the above-mentioned cortical and cerebellar structures, the central amygdaloid nucleus, several reticular districts, and the principal and spinal trigeminal nuclei, gave rise to anterograde labeling in the RFp. The present results may contribute to the elucidation of the anatomical substrate of the functionally demonstrated involvement of the RFp in several domains that conspicuously include forms of oral behavior. (Supported by FAPESP, FINEP and CNPq grants.)

303.17

SOURCES OF NORADRENERGIC AFFERENTS TO THE HYPOGLOSSAL NUCLEUS IN THE RAT. L.D. Aldes, Department of Structural and Cellular Biology, University of South Alabama, College of Medicine, Mobile, AL 36688

The hypoglossal nucleus (XII) in rat receives a topographically organized noradrenergic (NE) projection that terminates preferentially among protrusor motoneurons in the caudoventromedial quadrant (Aldes et al., Brain Res. Bullet., 21:305-312, 1988). The present study investigated the source(s) of this innervation with double-labeling histochemical/immunocytochemical and lesion/degeneration methods. Following injection of WGA-HRP into XII, sections through the rostral pons (RP) were reacted with tetramethylbenzidine (Mesulam, J. Histochem. Cytochem., 26:106-117, 1978), stabilized with DAB-$CoCl_2$ (Rye et al., J. Histochem. Cytochem., 32:1145-1153, 1984), and incubated in antisera to tyrosine hydroxylase (TH-Aldes et al., Brain Res. Bullet., 21:305-312, 1988). Double labeled-neurons (HRP+/TH+) were found in the nucleus subceruleus (nSC-63%) and A7 (24.6%) and A5 (12.3%) cell groups (N=68 neurons). The projections were mainly ipsilateral. Confirmation of these data and identification of the course taken by descending NE-XII projections was accomplished by placing lesions in the RP or in the medullary catecholamine bundle (MB). After 6-18 days survival, a marked decrease in TH immunoreactivity was observed ipsilaterally in XII that was most pronounced caudoventromedially. These results demonstrate that NE-XII afferents are derived from multiple cell groups in the RP, descend the brainstem in the MB and terminate topographically in XII. That the nSC provides a substantial NE innervation to XII is consistent with a recent report that the nSC projects to XII and terminates in the same pattern as NE (Aldes, J. Comp. Neurol., in press). On the basis of these data, coupled with findings in other cranial nerve nuclei (Grzanna et al., J. Comp. Neurol., 263:76-91, 1987), it is proposed that NE cell groups in the RP comprise part of an integrative network involved in controlling oral motor behavior. Supported by NIH grant NS-22686.

303.18

AFFERENTS AND EFFERENTS OF A HEAD MOVEMENT AREA IN THE PONTOMEDULLARY RETICULAR FORMATION OF THE MACAQUE. R. J. Cowie, D. L. Robinson, G. B. Stanton, and M. K. Smith.* Department of Anatomy, Howard University, Washington, D.C. and Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892.

Little is known about the supraspinal centers for the control of head movements and the interconnections of such areas. We investigated the organization of head movements by electrically evoking them in awake monkeys and then injecting WGA-HRP into physiologically defined areas. Three monkeys were studied. We identified a region within the medial pontomedullary reticular formation where stimulation with currents of less than 40 μA would reliably evoke head movements. Horizontal, vertical, as well as tilting movements were elicited. This region was bounded by tissue where stimulation evoked ear, mouth, snout, shoulder, and arm movements. The head movements were saccade-like. The amplitude of head movements was dependent on the initial starting position as well as current intensity.

At physiologically identified head movement sites, we microinjected WGA-HRP. Retrogradely-labeled cells were located in the head and face regions of frontal cortical areas 4 and 6, the deeper layers of the superior colliculus, and the cervical spinal cord. Orthograde transport was found in the spinal cord, rostral interstitial nucleus of the medial longitudinal fasciculus, and interstitial nucleus of Cajal.

We conclude from these experiments that signals which originate within the deeper layers of the superior colliculus and motor cortices impinge on this head movement center. After integration within this area, such signals are transmitted to neck motoneurons as well as other premotor centers for the generation of ballistic head movements.

304.1

AN OPEN FIELD ACTIVITY ANALYSIS OF UNILATERALLY LABYRINTHECTOMIZED RATS. J.D. Porter, S.M. Pellis, M.E. Meyer. Dept. of Psy., Univ. of FL, Gainesville, FL 32611.

Classification of the neural deficits such as vestibulo-ocular and vestibulo-motor deficits following destruction of one of the labyrinths is a common experimental theme. This study provides a detailed analysis of the behavior of unilaterally labyrinthectomized rats. Labyrinthectomies were performed by injecting sodium arsanilate (Abott, 100 mg/ml 0.9% saline) intratympanically into the middle ear cavity of 20 animals (10 left, 10 right). Two days following surgery the animals were placed into a Digiscan Activity Monitor where their behaviors were automatically scored and placed into twelve individual categories. Compared to control, the labyrinthectomized animals spent a significantly greater amount of time in motion and in repetitive behavior. The hyperactivity and stereotypy of the labyrinthectomized animals appears to reflect their inability to adequately explore the novel environment.

304.2

SEMICIRCULAR CANAL HAIR CELL RESPONSE TO ACh A. Ricci*, C. Norris, P. Guth. Departments of Otolaryngology, Pharmacology, and the Neuroscience Training Program, Tulane University, New Orleans, LA 70112-2699

Acetylcholine (ACh), the major efferent transmitter of the vestibular end organ, elicits a biphasic response from hair cells as measured in the afferent nerve endings of an isolated semicircular canal preparation. The calcium dependency and voltage dependency of the response were studied. In one set of experiments calcium was replaced by magnesium, in another calcium channels were blocked with diltiazem, Cd or Co respectively. The ACh response was not affected by any of these manipulations indicating that ACh can cause the release of intracellular stores of calcium. A constant DC field was used to demonstrate the lack of voltage dependence of this ACh response, supporting the hypothesis of intracellular release of calcium. Other experiments have shown ACh to preferentially increase spontaneous afferent firing over mechanically evoked firing. It is therefore suggested that the regulation of free intracellular calcium is critical in determining the level of afferent nerve firing. The mechanisms involved in regulating this concentration vary for spontaneous, mechanically evoked and ACh induced afferent nerve firing. (This work was supported by grant USPHS-DC 00303.)

304.3

DYNAMIC POLARIZATION VECTOR OF OTOLITH NEURONS. D.E. Angelaki. Dept. of Physiology, Univ. of Minnesota, Minneapolis, MN 55455.

The study of the dynamic properties of otolith vestibular neurons has been difficult previously because of the differing response sensitivities of individual fibers to specific stimulus directions. A method for estimating both the spatial and temporal properties of neurons like the otolith afferents that are spatially tuned to different stimulus directions is presented. At each stimulus frequency, a *response ellipsoid* can be constructed from the neural responses elicited by stimulation along three linear independent axes. The response ellipsoid then predicts the gain and phase properties of a neuron at the same frequency along any other direction in the three dimensional space. The gain of the neuron for stimulation along any axis is represented by the intersection of that axis with the surface of the ellipsoid. The phase during stimulation along that axis can also be estimated from the parametric equations of the ellipsoid. Further, the major axis of the ellipsoid will specify the direction of maximum sensitivity (polarization vector) of the neuron, while the minor axes will provide the neural sensitivity in the perpendicular (null) directions. The predictions of the method for non-zero lengths of the minor axes (i.e., broadly tuned neurons) is qualitatively the same with the experimentally observed dependance of response phase on stimulus orientation (Baker et al., Brain Res., 294:138, 1984; Bush and Perachio, Soc. Neurosci. Abstr., 14:330, 1988). Supported in part by Shevlin and A.P.Anderson fellowships, Univ. of Minnesota.

304.4

DYNAMIC RESPONSES OF MEDIAL VESTIBULAR NUCLEUS NEURONS TO HEAD ROTATION AND TRANSLATIONAL MOTION. G.A. Bush, A.A. Perachio, Depts. of Otolaryngology and Physiology & Biophysics, Univ. TX Med. Br., Galveston, TX 77550.

We have previously demonstrated that type I and type II medial vestibular nucleus (MVN) cells may also exhibit responses to time-varying translational head acceleration. In those studies, responses to horizontal head rotation were only qualitatively described. We have now characterized, quantitatively, the dynamic responses of both types of MVN neurons in decerebrated rats to single harmonic frequency head acceleration in the form of rotations in the plane of the horizontal semicircular canals and to purely linear accelerations in the same head plane or off-vertical axis constant velocity rotation. Horizontal canal-related MVN neurons were identified by their responses to electrical stimulation of the ipsilateral vestibular nerve and to horizontal head rotation at 0.05, 0.1, 0.5, 1.0 and 2.0 Hz (50 deg/sec peak velocity). Linear head acceleration was applied in different vectors spaced at 30^{0} intervals across the horizontal head plane and at single frequencies ranging from 0.2 to 1.4 Hz, peak acceleration 0.1g. For type I neurons that responded to linear acceleration, responses to head rotation were characterized by decreasing gains and increasing phase lags re velocity with increasing frequency (0.1 to 1.0 Hz mean gain difference = -6.0 dB, mean phase at 1.0 Hz = -32.2^{0}). For cells without otolith input, over the same rotation frequency range, gains increased (mean gain difference = +3.6 dB) and phase leads decreased (mean phase angle at 1.0 Hz = -9.2^{0}). Though the sample size is at present small for type II cells, the same frequency dependent effects were not observed. (Support by NIH DC00385, NASA NAG 2-26 and NGT-50165).

304.5

GLUTAMATE-LIKE IMMUNOREACTIVITY IN VESTIBULO-OCULAR CELLS IN GERBILS, G.A. Kevetter, R.D. Hoffman*, Dept. Otolaryngology and Anatomy and Neurosciences, Univ. TX. Med. Br., Galveston, TX 77550.

Cells in the vestibular nuclei that project to the oculomotor nuclear complex and also stain for the excitatory amino acid neurotransmitter, glutamate, were studied. Gerbils were anesthetized with pentobarbital and ketamine. HRP was injected stereotaxically into the oculomotor nuclear complex. After 48 hours, the gerbils were reanesthetized and perfused. Sections, 30 μm thick, were reacted with an intensified nickel acetate diaminobenzidine procedure that stained retrogradely transported HRP black. The sections were then incubated with rabbit anti-glutamate (Chemicon 1:200; 1.500; Arnel 1:10,000) antibodies for twenty-four hours. The sections were then incubated using an avidin-biotin-peroxidase procedure, and reacted with diaminobenzidine producing a brown product. Retrogradely labeled cells were found in the medial (M) and superior (S) vestibular nuclei and cell group Y. Cells labeled with glutamate-like immunoreactivity (GLU-lir) were found in all major vestibular nuclei. Cells labeled with both HRP and GLU-lir were found in M, S, and cell group Y. More than half of the HRP labeled cells in the M contralateral to the injection site were also GLU-lir. Less than half of the retrogradely labeled cells in the S ipsilateral to the injection site were stained with GLU-lir. (Supported by a John Sealy and Deafness Research Foundation grant).

304.6

PHYSIOLOGY AND BRAINSTEM MORPHOLOGY OF SINGLE HORIZONTAL SEMICIRCULAR CANAL AFFERENTS IN THE TOADFISH, OPSANUS TAU: A BIOTIN DYE STUDY J.P. Carey and S.M. Highstein. Wash. U. Sch. Med., Dept. Oto., St. Louis, MO 63110.

Individual horizontal semicircular canal afferents were impaled with glass microelectrodes filled with Biocytin or Neurobiotin to study their morphology and physiology. Responses to sinusoidal rotations were classified as low-gain, velocity-sensitive (LG); high-gain, velocity-sensitive (HG); or acceleration-sensitive (AC) (Boyle and Highstein, 1990). Fibers were then injected, and their morphology characterized with light microscopy. Brainstem projections of these three classes differ. AC and HG fibers project to ventral n. anterior octavus (AO), all parts of n. descending octavus (DO), n. magnocellularis (M), n. tangentialis (T), and emenentia granularis (EG, the vestibulocerebellum). In contrast, LG fibers project heavily to ventral DO, lightly to M and T, but not to AO or EG. Afferent distributions in the horizontal canal crista corroborate previous findings (Boyle and Highstein, 1986), namely, AC and HG fibers occupy the middle 2/3 of the crista, with AC fibers more central, and LG fibers are confined to the distal 1/6. Microinjections of HRP into the distal 1/6 of the crista confirmed the restricted central distribution of LG fibers found with biotinylated dyes. Thus, peripheral parcellation of velocity and acceleration information is partly maintained centrally. Differential central projections may be the first stage of anatomic separation of information underlying the functional diversity of vestibular actions. (Supported by NIH NS21055 and AHA MA/MD Fellowship.)

304.7

VELOCITY STORAGE AND THE OCULAR RESPONSE TO MICROSTIMULATION OF VESTIBULAR NUCLEI IN ALERT MONKEY.

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Velocity storage holds activity that produces slow phase eye velocity during vestibular nystagmus, OKN and OKAN.The neuronal circuits underlying this mechanism are still unknown. Functions attributable to velocity storage are lost after lesion of the medial part of the medial vestibular nucleus (MVN; Uemura & Cohen,1973) and midline section of vestibular commissural connections caudal to the abducens nucleus (Katz et al.,1989). Thus, the velocity storage integrator appears to be represented in the vestibular nuclei. In this study we electrically stimulated the vestibular nuclei of the cynomolgus monkey to determine whether eye movements could be evoked, and if the characteristics of the nystagmus were related to vestibular nystagmus, OKN and OKAN. Eye movements were monitored with search coils. In darkness currents less than 40 uA evoked horizontal or rotatory nystagmus with contralateral slow phases, followed by after-nystagmus in the same direction. The rising time course of the slow phase velocity was similar to the slow rise in OKN. The maximum velocity of the steady state nystagmus was almost same as that of OKAN, and the falling time course of the after-nystagmus corresponded to that of OKAN and per- or post-rotatory nystagmus.In some tracks,the time course of rise and fall was shorter, and the peak velocity was higher. Positive stimulus sites for inducing nystagmus were located in or near the boundary between MVN, LVN and rostral DVN, just caudal to the abducens nuclei and 3-4mm from the midline,and in the white matter lateral and dorsal to the vestibular nuclei.These data suggest that the contribution of velocity storage to the VOR,OKN and OKAN can be elicited by stimulation of the vestibular nuclei in the alert monkey. Supported by NS00294, EY01867.

304.8

RESPONSES OF NEURONS IN THE RAT VESTIBULAR NUCLEI TO STATIC TILT AND AFFERENT ELECTRICAL STIMULATION.

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To elucidate central information processing of the otolith system, extracellular single unit recordings were made from the medial, lateral and superior vestibular nuclei of the urethane-anaesthetized rat. Responses to anteroposterior static tilt and to electrical stimulation of the ipsi- and contralateral vestibular nerve were analysed in 72 neurons. Thirty-two neurons showed sustained changes in their activity in response to tilt. Nineteen of the 32 cells (59 %) displayed reciprocal responses to anteroposterior tilt, i.e., excitation to nose-down tilt and inhibition to nose-up tilt and vice versa. A difference in resting discharge was seen between tilt-responsive cells (n=32) and non-responsive cells (n=26). The frequency of resting discharge of the former cells was significantly lower (mean±standard deviation, 19.1±11.8 Hz) and more irregular than that of the latters (37.3±17.2 Hz). All the cells responded with short latency to ipsilateral vestibular nerve stimulation, and about 20 % of them responded also to contralateral stimulation. A possible relationship between the excitatory convergence from both sides and responsiveness to tilt is discussed.

304.9

EYE AND HEAD MOVEMENT-RELATED DISCHARGE OF SECONDARY VESTIBULOSPINAL NEURONS IN THE ALERT SQUIRREL MONKEY.

R. Boyle. Depts. Oto. & Physiol., Oregon Health Sci. Univ., Portland, OR 97201.

In the vestibular nuclei of the alert squirrel monkey, spinal-projecting neurons were identified by their antidromic response to pulses applied to wires implanted in the ventromedial funiculi at C1; each cell's short-latency input from the ipsilateral VIIIth nerve (Vi) was determined by shocks applied to middle ear wires. The cell's discharge was examined during spontaneous eye movements, pursuit tracking of visual targets, optokinetic stimuli, and to sinusoidal head movement in the dark and during suppression of compensatory eye movements. Using a paradigm of Minor and Goldberg (1986), 100 μA anodal (inhibitory) current was applied to Vi during head rotation in some cases to assess the possible contribution from low threshold (irregular) afferents to the secondary cell's response modulation. Of the 143 neurons studied in one monkey, 19 were vestibulospinal (10 monosynaptically related to Vi). Cells encountered were: type I only (1), type I + eye position (2), type I + pursuit velocity with or without eye position (5), type II only (5), type II + pursuit velocity + eye position (2), and 4 were related to vertical head/eye movements. The discharge of 60% of the vestibulospinal cells related to horizontal head movement was modulated by the position and/or movement of the eyes in orbit and, most frequently, the head and eye velocity component of the individual responses were in the same (anticompensatory) direction. Curiously, none of the horizontal PVP (11), the vestibular-pause (12) or -burst (5) cells recorded were C1-activated in this study.

These results show that i) vestibular neurons carry varied gaze signals to the spinal cord and ii) the position/movement of the eye in orbit can strongly influence the cell's head velocity modulation; and suggest that vestibulospinal neurons may participate in different operations of gaze control.

304.10

COMPARATIVE TRANSDUCTION MECHANISMS OF HAIR CELLS IN THE BULLFROG UTRICLE.

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Previous studies have shown that the response dynamics of utricular afferents are correlated with both the epithelial location and hair bundle morphology of their innervated hair cells. To clarify the contribution of hair cell transduction mechanisms to the response properties of utricular afferents, we measured the responses of hair cells to intracellular current and mechanical stimulation to determine their membrane properties and response dynamics, including the extent and time course of adaption to long-duration stimuli and the contribution, if any, of an electrical resonance in the hair cell membrane.

Hair cells in the extrastriola have similar hair bundle morphology, exhibit little or no active currents following current steps, adapt to neither intracellular current nor mechanical stimulation, and respond only to low (< 5Hz) frequencies. Striolar hair cells, while differing in hair bundle morphology, display active currents to both depolarizing and hyperpolarizing steps, rapidly adapt to step displacements of their hair bundles, and respond to much higher (>50 Hz) frequencies. An electrical resonance, similar to that observed in bullfrog saccular hair cells, is seen in striolar hair cells with bulbed kinocilia. With the exception of this resonant behavior, the response dynamics of utricular hair cells correlate with their epithelial location rather than their hair bundle morphology and probably reflect the kinetics of voltage- and ion-sensitive conductances in the hair cell membrane. Research supported by NIDCD 00355, NASA NCC 2-651, & OLSHF.

304.11

PHYSIOLOGICAL PROPERTIES OF VESTIBULAR AFFERENTS PARTICIPATING IN THE PLASTICITY OF THE VESTIBULO-OCULAR REFLEX.

H. M. Brontë-Stewart and S. G. Lisberger. Dept. of Physiology UCSF, San Francisco, CA 94143.

Our goal is to determine the role of different classes of vestibular afferents in the plasticity of the vestibulo-ocular reflex (VOR). We use an electrical method of stimulating the VOR with an electrode implanted in the superior semicircular canal. Single electrical pulses elicit a twitch of eye velocity, the amplitude of which increases with increasing current intensity and plateaus at high currents.There is a relationship between the electrical activation of afferents and their physiological properties. Afferents with irregular spontaneous discharge are recruited at low currents that elicit little eye movement. Afferents with a more regular firing pattern are recruited over a wider range of current intensity. As the current is increased those with a higher coefficient of variation (CV) and greater sensitivity to head turns are recruited first. Afferents with very low values of CV are activated only at current values beyond the saturation of eye velocity. We tested the role of different afferents in plasticity by examining the eye velocity elicited by 200Hz trains of electrical pulses at different current intensities.We compared the responses before, during and after plasticity achieved with magnifying and miniaturizing spectacles. At low currents, trains evoke an eye velocity that does not vary with plasticity. As the more regularly firing afferents are recruited, the eye velocity evoked by trains varies with plasticity, concordant with the variation seen using natural vestibular stimuli. At higher currents the evoked eye velocity shows no further change with plasticity even though the most regular afferents have not been activated yet. It appears that plasticity in the VOR is driven largely by a group of afferents with physiological properties in the intermediate range of discharge regularity and sensitivity to head turns. (Supported by NIH grants K11 EY00302 and RO1 EY03878).

304.12

HORIZONTAL AND VERTICAL VOR LATENCY BEFORE AND AFTER CROSS-AXIS VOR PLASTICITY.

T.T. Khater, B.W. Peterson, K.D. Powell, K.J. Quinn, and J.F. Baker, Northwestern Univ. Medical School, Chicago, IL 60611.

When a monkey wears magnifying lenses for a few days, his VOR adapts to minimize the lens-induced retinal slip. The adaptive component of the VOR has a latency as short as 19 msec (Lisberger, Science 242:771), suggesting a relatively direct pathway is responsible for the learned response. To measure comparable latencies for plastic changes in VOR ***direction***, we recorded eye movements evoked by random velocity transitions between ±19°/sec earth-horizontal whole body rotation (HWBR) in total darkness with a CNC eye coil system in 4 cats before and after "cross-axis" training. Training consisted of 2 hrs of HWBR coupled with vertical pitch rotation of a projected random dot pattern with identical phase and 1.5X the amplitude. HWBR was done with the cat in the prone position, or lying on its left side (onside adaptation).

VOR latencies to ±19°/sec transitions in the plane of the HWBR were 8-11 msec both before and after adaptation to a orthogonal optokinetic stimulus. Orthogonal (vertical) responses (OVOR), which were absent before the training, usually had a latency of 35-45 msec. In 5 of 18 cases, however, two cats produced responses in the 17-21 msec range. Furthermore, a second eye velocity step (increasing velocity) often occurred at 100-120 msec latency in the adapted OVOR trace. Similar latencies were found in our onside cases. Our short (20 msec) latencies suggest a direct (intra-brainstem) pathway similar to that proposed by Lisberger. Latencies of ~ 40 msec are long enough for more complex (eg. trans-cerebellar) pathways to be involved. We believe that the most rapid learning occurs in these pathways. Short latency OVOR would appear only when significant learning occurred in longer time constant brainstem pathways within our 2 hr training period. Supported by EY05289, EY06485, and EY07342.

304.13

FUNCTIONAL CONSIDERATIONS IN MODELING VOR PLASTICITY. K.J. Quinn, N. Schmajuk, S.A. Rude, T.T. Khater, J.F. Baker and B.W. Peterson. Northwestern University Medical School, Chicago, IL 60611

We are developing a model of the vestibuloocular reflex (VOR) using neural network techniques. Structure of the model is constrained by known anatomy and physiology of the reflex. Initial efforts concentrate on computational capabilities of the basic 3 neuron arc. Activation levels of individual model elements are calculated in a way similar to that described by Grossberg (Stud.Appl.Math.,52,p213). Semicircular canal input is modeled as a head velocity (**HV**) signal and eye/muscle plant activation is based on the laplace transform described by Robinson (Ann.Rev.Neuro.,4,p463). The current model contains a total of 14 units and simulates a perfectly compensatory horizontal VOR at fixed frequencies (0.5 & 1.0 Hz). Frequency response analysis indicates that the model is much more narrowly tuned than the normal VOR, suggesting that units simulating a neural integrator producing an eye position (**EP**) signal (Arnold & Robinson, Soc. Neuro. Abstr,416.20) may be required to match normal VOR behavior.

A signal related to retinal image slip velocity (**RSV**) is thought to be the principal means by which the CNS detects errors in the VOR. How can reflex adaptation correct errors in *gain and/or phase* if only a **RSV** signal is provided? It can be demonstrated that the product **RSV*HV** is differentially sensitive to gain errors while **RSV*EP** is differentially sensitive to phase errors. Modeling a multiplicative learning process at sites where these signals converge (vestibular and prepositus nuclei?) may therefore simulate adaptive performance of the VOR. Supported by EY05289, EY06485, EY07342, NS07223.

304.14

VESTIBULO-OCULAR REFLEX (VOR) DIRECTION ADAPTATION TO MULTIFREQUENCY AND SINGLE FREQUENCY STIMULI. K.D. Powell, K.J. Quinn, S.A. Rude, B.W. Peterson, J.F. Baker, Dept. of Physiology, Northwestern Univ., Chicago, IL 60611.

Coupling horizontal vestibular rotation (HVR) with vertical optokinetic pattern rotation (VOKR) causes the VOR to acquire an adaptive vertical component (AVOR). When HVR and VOKR are .25Hz sinusoids, the AVOR is maximal at .25Hz with smaller, phase advanced responses at lower frequencies. Here we examine how this tuned frequency response depends on the frequency(ies) in the training stimulus. Two classes of cross axis adaptations were performed in 4 cats. Vertical and horizontal VOR at .02-2.5Hz were measured with EOGs in the dark before and after training.

1) Single frequency adaptation (SFA): Whole body HVR at .05, .1, .25, .5, or 1Hz was coupled to VOKR for 2hr.

2) Multifrequency adaptation (MFA): A multifrequency stimulus containing .2, .3, .5, .7, 1.1, 1.7Hz was used in synchrony for the HVR and VOKR for 2hr.

After 2hr. of MFA, vertical VOR gain during horizontal rotation was increased to .1-.2 across the training frequencies with no preferred frequency and little or no phase shift at low frequencies. This broad band adaptation in MFA contrasts with the frequency tuning seen in SFA after which adaptive vertical VOR consistently showed bandpass characteristics tuned near the adaptation frequency. Preliminary results from 1Hz SFA and adaptation to a combination of .25Hz & 1Hz suggest the presence of a high frequency stimulus decreases the phase advance at low frequencies. Supported by EY05289, EY06485, EY07342.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: VESTIBULAR SYSTEM II

305.1

AN *IN VITRO* BRAINSTEM-CEREBELLUM PREPARATION TO STUDY VESTIBULOCEREBELLAR INTERACTIONS.

L.J. Larson-Prior and N.T. Slater. Department of Physiology, Northwestern University Medical School, Chicago, IL 60611.

Vestibulo-cerebellar interactions regulate reflexes which are involved in the maintenance of posture, balance, and gaze, and have been implicated in the development of reflex plasticity. To better understand the cellular basis of these interactions, we have utilized an *in vitro* preparation of the turtle brainstem-cerebellum [1,2]. This preparation takes advantage of the extreme anoxia resistance of turtle brain, and the similarity of the reptile vestibulocerebellar system to that of mammals. The preparation included the intact cerebellum, one-half of the metencephalic tegmentum and the ipsilateral eighth nerve (nVIII). This insured that all stimuli were carried through a single cerebellar peduncle. nVIII was stimulated by a suction electrode placed either on the anterior or posterior branch of the nerve. The vestibular nuclear complex (VNC) was stimulated via a bipolar concentric electrode placed just lateral to the sulcus limitans at the level of nVIII entry.

In turtle, the auricular lobe of the cerebellum is reciprocally connected to the vestibular system and constitutes the "vestibulocerebellum". Consistent with a projection to the granule cell layer of the auricle, VNC stimulation produced epsp-ipsp sequences at latencies of 5-7 msec in intracellularly recorded auricular Purkinje cells. Higher stimulation intensities frequently evoked complex spike activation. Somewhat unexpectedly, stimulation of either branch of nVIII evoked short latency (<0.5 msec) action potentials, consistent with antidromic activation. Such responses followed stimulus presentations at frequencies of up to 100 Hz, and increased intensity of stimulation did not result in an increased number of simple spikes. It therefore appears that in turtle, as in frog [3], auricular Purkinje cells send direct afferents through nVIII to the vestibular apparatus. Supported by NS 17489 and NS 25682.

[1] Larson-Prior, L.J., McCrimmon, D.R. and Slater, N.T. (1990) *J. Neurophysiol.*, 63:637-650. [2] Keifer, J. and Houk, J.C. (1989) *Neurosci. Lett.*, 97:123-128. [3] Llinas, R., Precht, W. and Kitai, S. (1967) *Science* 158:1328-1330.

305.2

SOURCES OF CHOLINERGIC INNERVATION OF THE VESTIBULAR NUCLEI IN THE RAT R.W. Sikes, B. Litofsky*, P.A. Ullucci*, Department of Physical Therapy, Northeastern University, Boston, MA 02115.

Histological, neurochemical and physiological studies have established that the vestibular nuclei (VN) receive a prominent extrinsic cholinergic input. Although cholinergic afferents are thought to be important in vestibular compensation following uni-lateral labyrinthectomy, the source of this input has not been identified. By combining sensitive immunohistological and retrograde tracing methodologies, this study identifies several putative cholinergic inputs to VN.

Injections of transportable fluorescent dyes were made into the VN in anesthetized rats using standard stereotaxic procedures. Sections from the brains of these animals were processed to show the location of neurons containing choline acetyltransferase-like immunoreactivity (ChAT-L) using a fluorescent tag. The location of tracer dye, ChAT-L and double-labeled neurons were charted.

Overlapping populations of ChAT-L positive cells and vestibular projecting cells were observed in the prepositus nucleus, cervical spinal cord and medial medullary reticular formation. Thus, these regions may play a key role in vestibular compensation.

305.3

RESPONSES OF BILATERAL VESTIBULAR NUCLEAR NEURONS OF ACUTE HEMILABYRINTHECTOMIZED CATS TO OFF-VERTICAL AXIS ROTATIONS. Y.S. Chan, Y.M. Cheung and C.W. Chen*. Department of Physiology, Faculty of Medicine, University of Hong Kong, Sassoon Road, Hong Kong.

To explore whether vestibular nuclear neurons on the lesioned side and the labyrinth-intact sides of acute hemilabyrinthectomized cats can encode spatial orientation during 360° off-vertical axis rotations (OVAR), responses of these units were studied in decerebrate cats during constant velocity OVAR in the clockwise (CW) and counterclockwise (CCW) directions. On both the lesioned and the labyrinth-intact sides, some units showed position-dependent discharge modulation only to OVAR of one direction but not to both. Such asymmetric response was not observed in animals with intact bilateral labyrinths (Chan et al., 1988). On both the lesioned and labyrinth-intact sides, other units were found to respond to OVAR in both directions with comparable response gain. For each side, the averaged position of the CW/CCW discharge maxima of each of these units was plotted in a map to show the orientations of the best response. For units showing best response along the transverse plane, there was a predonderance of type α over type β units on both the lesioned and labyrinth-intact sides, suggesting the existence of a predominant crossed otolith contribution from type β units. For units showing best response along the antero-posterior plane, a greater proportion of units showed type 2 than type 1 response on both the lesioned and labyrinth-intact sides; a reverse pattern was observed in cats with intact bilateral labyrinths. These results suggest that inputs from the intact otoliths in acute hemilabyrinthectomized cats are operative at the level of the vestibular nuclei bilaterally to encode spatial orientation of the head.

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305.4

SEMICIRCULAR CANAL AFFERENT RESPONSE TO EFFERENT STIMULATION. J.D. Dickman and M.J. Correia. Depts. of Surgery and Anatomy, Univ. of Mississippi Medical Center, Jackson, MS 39216; Depts. of Otolaryngology and Physiology & Biophysics, Univ. of Texas Medical Branch, Galveston, Texas 77550.

We recorded extracellular single fiber responses from horizontal semicircular canal afferent (HCA) fibers in pigeons elicited by stimulation of the *contralateral* horizontal semicircular membranous duct using a mechanical micropusher (Dickman & Correia, *J. Neurophysiol.*, 1989, 62, 1090-1101). Step displacements (±10 micron) of the exposed contralateral membranous duct were delivered in order to stimulate efferent fibers projecting to the ipsilateral horizontal canal neuro-epithelium. Experiments were conducted in awake, decerebrate animals that were paralyzed (Flaxedil) and ventilated (250 ml/min O_2/CO_2). To date, complete protocols have been recorded in 19 HCA fibers. Of these, 53% (10/19) responded with clear *decreases* in the firing rate below the resting discharge level as step displacements of the contralateral membranous duct were delivered, followed by increases in firing rate above the resting discharge level at the cessation of the step displacement. This response pattern is opposite to that observed with ipsilateral membranous duct mechanical stimulation. The magnitude of the elicited unit responses (ranging 4 - 30 spikes/sec, n = 6) appeared to vary with the spontaneous firing rate and the coefficient of variation (CV) of the individual neuron. The largest responses were elicited in HCA fibers with high CV values. Several HCA fibers were rectified with contralateral stimulation. Supported in part by NIH grant 2S07RR05386 and ONR grant N00014-88-K-0015.

305.5

VESTIBULAR PRIMARY AFFERENT AND VESTIBULOOCULAR CHANGES IN RHESUS MONKEY FOLLOWING 14 DAYS OF MICROGRAVITY. M. J. Correia, A. A. Perachio, J. D.Dickman^, I. B. Kozlovskaya*, M. G. Sirota*, S. B. Yakushin* & I. N. Beloozerova*. Depts. of Otolaryng. & Physiol. & Biophys., UTMB, Galveston TX, 77550, ^Dept. of Surg., Univ. of Miss. Med. Sch., Jackson MS, 39216, & *Inst. of Biomed. Prob., Moscow, U.S.S.R.

Two Rhesus monkeys (782 & 2483) were tested before, during and after (14.5 hrs-9 days) they orbited the earth for 14 days on Cosmos Biosatellite 2044. Seven control monkeys were also tested. Nystagmus was recorded using indwelling corneo-retinal potential electrodes and single unit extracellular action potentials were recorded from the vestibular nerve in the alert animals. During pre-flight studies, 27 units were recorded from five monkeys, including 8 from the flight animals. During post-flight studies, 102 units were recorded from 4 monkeys including 41 from the flight animals. We report that during yaw pulse rotations ($\pm$ 60 deg/s for 30 s) of both the flight monkeys on post-flight day 1, the mean ($\pm$SEM) horizontal semicircular canal primary afferent excitatory time constant, τ_e = 3.3*$\pm$0.5s, and inhibitory time constant, τ_i = 3.5$\pm$0.6s were *shorter* than during pre-flight and control monkey tests (τ_e = 6.0$\pm$0.4s, τ_i = 4.8$\pm$0.6s). The gains were also *greater* (G_e = 0.83*$\pm$0.12, G_i = 0.85*$\pm$0.10) during post-flight day 1 compared with pre-flight and control monkey tests (G_e = 0.49$\pm$0.05, G_i = 0.39$\pm$0.03), (* = $p<0.01$, ANOVA, d.f. = 1,41; 1,41 & 1,30, respectively). Yaw pulse rotations ($\pm$ 60deg/s for 60 s) produced horizontal slow phase eye velocity of longer duration (1.6 times) on post-flight day 1 in both flight monkeys when the plane of their horizontal semicircular canals was pitched down 45 deg relative to a vertical axis of rotation. No difference was noted when this plane was not pitched or pitched up 45 deg. This work was supported in part by a NASA grant, NAG-2-446.

305.6

CHARACTERIZATION OF THE THREE DIMENSIONAL STRUCTURE OF VELOCITY STORAGE IN THE MONKEY. MJ Dai*, T Raphan, D Sturm, B Cohen. Dept. of Computer & Inf. Sci., Brooklyn College, CUNY, N.Y., & Dept. of Neurol. Mt. Sinai Sch. of Med., N.Y.

When subjects are tilted, OKN slow phase velocity along the subject's yaw axis generates SP Vel along the pitch or roll axis during OKAN. This "cross coupling" has been related to the 3-D characteristics of velocity storage in terms of the eigenvalues and eigenvectors of the system matrix re gravity. The eigenvalues and eigenvectors of OKAN around yaw and pitch axes were determined for 4 monkeys as a function of head roll-tilts (0-150 deg) using a modified Marquardt algorithm. For cross coupling that produced upward SP Vel the yaw axis eigenvector changed from -10.3 deg to 23.8 deg (mean) re the spatial vertical, showing Muller & Aubert-like effects. Eigenvalues (the inverse of the Tc) associated with the yaw axis increased from .051-.135/s, and pitch axis eigenvalues decreased from .23-.056/s. They were about the same whether cross coupling or pure pitch OKN stimulation was given ($p<0.34$, mean). For oblique stimulation along the eigenvector, the direction of the OKAN remained along the eigenvector as predicted. Under all conditions the pitch axis eigenvector rotated with the head. Thus, the upward yaw axis eigenvector associated with velocity storage is closely linked to gravity. Supported by EY04148, EY01867 & NS00294.

305.7

SYNAPTIC INPUTS TO BEHAVIORALLY CHARACTERIZED NEURONS IN THE MACAQUE VESTIBULAR NUCLEI. D. M. Broussard and S. G. Lisberger. Dept. of Physiology, UCSF, San Francisco, CA 94143.

The brainstem neurons mediating the vestibulo-ocular reflex show a variety of discharge patterns during head turns, but little is known about the relationship between a neuron's discharge pattern and the synaptic inputs it receives. We tested central vestibular neurons using standard behavioral paradigms in alert, trained rhesus monkeys. We then applied single, brief current pulses via chronically implanted stimulating electrodes in the flocculus and in the ipsilateral and contralateral labyrinths. Initially we have focused on flocculus target neurons (FTNs) and tonic-vestibular-pause neurons (TVPs). Horizontal TVPs paused during saccades, carried ipsilateral head velocity and contralateral eye velocity signals, and were not inhibited from the flocculus. Horizontal FTNs were defined as neurons which are strongly inhibited, within 2 msec, by stimulation of the flocculus; they usually were contralateral eye movement cells. Following stimulation of the ipsilateral vestibular labyrinth, both TVPs and FTNs were strongly excited, usually at monosynaptic latencies. When the contralateral labyrinth was stimulated, FTNs received an excitatory input, followed by inhibition. We expected TVPs to be inhibited via the vestibular commissure, and some were; however, in other TVPs, the crossed input was purely excitatory. In one animal whose ipsilateral horizontal canal was blocked, TVPs apparently lost their head velocity signals, suggesting that the commissural input to TVPs may not be related to head turns. Supported by NIH grant EY03878.

305.8

EFFERENT MODULATION OF SYNAPTIC NOISE IN LAGENAR AFFERENTS OF THE TOADFISH R.E. Locke, S.M. Highstein. Dept Oto. Washington Univ. Sch. Med., St. Louis, MO 63110

Glass microelectrodes were visually guided into primary afferents of the labrynthine lagena in anesthetized toadfish. Spontaneous miniature EPSPs (mEPSPs) of hair cell origin were recorded before and during electric pulse train stimulation of the efferent vestibular nucleus (EVN) in the floor of the IV ventricle. Control unitary mEPSPs ranged from 0.3-8.5 mV with an amplitude distribution that was skewed to the left (mode 0.375 mV). Time to peak of mEPSPs ranged from 0.12-2.06ms (mode 0.28ms). EVN stimulation shifted the amplitude histogram to the left, eliminating the largest EPSPs. The shape of the amplitude distribution did not change, but the average time to peak was shortened such that fewer medium and long duration (>.56ms) EPSPs were seen. This reduction of synaptic noise from hair cells is correlated with a reduction in firing rate. In some afferents single, double, triple, etc. pulse stimulation of the EVN evoked large (ca. 10mV) monosynpatic EPSPs which summated. Thus the EVN exerts a dual control over lagenar afferents via efferent innervation of both hair cells and afferents. We propose that the axo-axonic synapses are responsible for increased firing rates while the axo-somatic synapses on hair cells may be responsible for reducing spontaneous EPSPs and evoked firing rates. (Work supported by NIH NS21055)

305.9

TWO SUBCLASSES OF BOUTON-TYPE VESTIBULAR PRIMARY AFFERENTS IN A TURTLE, Pseudemys scripta. A. M. Brichta and E. H. Peterson. Neurobiology Program, Ohio University, Athens, OH 45701.

Classical descriptions divide vestibular primary afferents into three morphological classes. One class, the bouton-type afferent, is found in all vertebrate groups. We conducted a quantitative analysis of 69 bouton afferents supplying the posterior semicircular canal that had been filled with HRP and reconstructed from serial sections. Our analysis suggests that there may be two subclasses of bouton afferents in this species.

Compared with other (α) bouton afferents, beta (β) bouton afferents have large diameter somata and axons, and large varicosities spread over extensive collecting areas. They comprise 12% of bouton afferents in our sample. Multivariate statistical analyses reveal that the structure of these β primaries is significantly different from that of α bouton afferents at the same location. Alpha and beta afferents also differ in spatial distribution. Alpha afferents are homogeneously distributed over the surface of the hemicrista, but the density of β primaries is greatest near the center of the canal and decreases toward the canal wall. This gradient parallels the gradient of structural features observed previously for total bouton afferents and for vestibular hair cells in this species (Brichta et al., 1989 and Peterson et al., 1989, Soc. Nsci. Abs. 15: 517). It provides further evidence that bouton afferents in this species are organized around a canal-centered coordinate frame.

Our data suggest that bouton afferents in Pseudemys may be divisible into two subclasses that differ in both structure and spatial organization, and they extend our earlier findings of spatial heterogeneity within the vestibular neuroepithelium. Supported by NIH-NINCDS (NS23498), NIH-NINCD (DC00618), and the College of Osteopathic Medicine.

305.10

STRUCTURAL AND SPATIAL DIVERSITY OF TYPE I AND TYPE II HAIR CELLS FROM THE VESTIBULAR EPITHELIUM OF A TURTLE, Pseudemys scripta. L. L. DiCaprio, A. M Brichta and E. H. Peterson. Neurobiology Program, Ohio University, Athens, OH 45701.

To understand vestibular control of head movement in P. scripta we are characterizing the organization of its vestibular neuroepithelium. We distinguished between Type I and II hair cells by differential interference contrast imaging of sectioned cristae that had previously been loaded with HRP to visualize calyx endings around Type I hair cells, and we compared these images with scanning electron micrographs of sonicated and unsonicated cristae.

Type I hair cells are sequestered in a central region of the epithelium that occupies approximately 35% of its total surface. They typically are housed in complex calyces that bear 4-5 hair cells. Type II cells are densely packed in a peripheral limbus, and they are larger and less numerous in the central region. The ratio of Type I to Type II cells is 5:1 or higher in the center of the epithelium. Apical profiles of peripheral (Type II) hair cells vary in size and shape, from small and elliptical near the wall of the canal to large and round near the torus. All bear a cluster of approximately 20-40 stereocilia near the kinocilial end of the apical surface. Apical profiles in the central region (Types I and II) are larger and structurally more diverse, including (1) elliptical profiles with as few as 30 stereocilia clustered near the kinocilium, (2) large round profiles with 60-100 stereocilia covering most of the apical surface, and (3) very small profiles that appear to be erupting from the epithelium. The ratio of the first two varieties is approximately 5:1 in the middle of the central region and decreases toward the periphery.

Our data suggest that Type I and II hair cells in Pseudemys differ in ciliary bundle morphology and that they are organized around distinctive spatial coordinate frames. Supported by NIH-NINCDS (NS23498), NIH-NINCD (DC00618), and the College of Osteopathic Medicine.

305.11

HORIZONTAL AND VERTICAL VESTIBULAR AND EYE MOVEMENT SENSITIVITY OF VESTIBULAR NUCLEAR NEURONS. J.O. Phillips and A.F. Fuchs. Regional Primate Research Center and Depts. of Psychology and Physiology and Biophysics, University of Washington, Seattle, WA,98195

Cells of the vestibular nuclei are known to carry both vestibular and eye movement signals. It is not known, however, to what extent individual primate vestibular neurons carry signals related to both vertical and horizontal vestibular stimulation and/or vertical and horizontal eye movements. To address this question, we recorded the activity of vestibular neurons (located primarily in the medial and lateral vestibular nuclei) that responded to a horizontal search stimulus (i.e.,the animal tracked a spot of light that moved in counterphase to a yaw rotation). We then tested the responsive units with a velocity series of horizontal and vertical rotations, and during horizontal and/or vertical smooth pursuit .

Preliminary analysis of 39 such units shows that most horizontally sensitive vestibular neurons (24/33) also responded to vertical vestibular stimulation. The vast majority of horizontally sensitive Type II neurons (17/21) responded to vertical stimulation, with many of the vertical responses meeting or exceeding the response to horizontal stimulation. In contrast ,Type I neurons responded weakly (7/12), or not at all (5/12), to vertical stimulation. In addition, cells were observed with eye movement sensitivities orthogonal to a given vestibular sensitivity. These data suggest that one should exercise caution in suggesting a role for the Type II neuron in horizontal vestibular responses.

This study was supported by National Institutes of Health grants EY00745 and RR00166.

305.12

EFFECT OF ROLL TILT ON EYE MOVEMENTS DURING 5 HZ INTERAURAL LINEAR OSCILLATION. J.Clifford*, G.D. Paige, D.L.Tomko (SPON:C.Somps) Vest. Res. Fac., NASA Ames Res. Ctr., Moffett Field, CA 94035.

Interaural (IA) or dorsoventral (DV) linear oscillations produce visually compensatory horizontal (IA) or vertical (DV) eye movements, the linear VOR. During Earth-horizontal (E-H) oscillations in the frontal plane, these two LVORs should add to produce behaviorally appropriate eye movements (parallel to motion axis) when the head is reoriented relative to oscillation by statically rolling it from upright. Eye movements were recorded (coil technique) in three squirrel monkeys during E-H oscillatory motion (5 Hz, 0.36g pk) along the IA axis, or rolled by 10, 20, or 30°. Continuous records of digitally sampled (200 Hz) eye position in the frontal plane were plotted. Oscillatory eye movement orientation in 2-D was calculated from these plots. In upright IA oscillation, E-H eye movements occurred; When rolled 10° to either side, eye movements were also parallel to the motion axis, i.e., tilted appropriately relative to the head. For 20 and 30° tilts in either direction, eye oscillations were 27 and 39°, respectively, indicating over-compensation.

Support: NASA Space Med. Tsk 199-16-12-02 & -03.

305.13

VERGENCE RESPONSES DURING NASO-OCCIPITAL LINEAR HEAD OSCILLATIONS IN SQUIRREL MONKEY. David L. Tomko and Gary D. Paige. Vestibular Res. Fac., NASA Ames Res. Center, Moffett Field, CA 94035

Horizontal and vertical eye movements were recorded binocularly (scleral search coil technique) from squirrel monkeys during naso-occipital (NO) linear oscillations (5.0 Hz, ±0.354 g peak). Vergence was also measured by subtracting the right from the left horizontal eye position signals. Responses were measured in the dark (LVOR) or while viewing a head-fixed visual surround (VSLVOR) with target distances of 9 and 22 cm. Vergence responses were observed during both LVOR and VSLVOR trials in the two animals tested. Vergence responses (θ) were time-locked to the 5 Hz stimulus with near 0° phase. Their amplitudes depended on the point of binocular fixation (vergence) independently of visual input, and were adequately characterized by:

$$\theta = \arctan (PD/F_0+H_x)$$

where PD is the interpupillary distance, F_0 is the initial fixation distance (1/vergence), and H_x is the linear displacement along the naso-occipital axis. These responses are consistent with the kinematics required of a compensatory vergence LVOR.

Support: NASA Space Med. Tsk 199-16-12-02 & -03.

HIPPOCAMPUS AND AMYGDALA: NEUROPHYSIOLOGY I

306.1

GABA BLOCKADE IN THE REGION OF THE ANTERIOR BASO-LATERAL AMYGDALA ELICITS A DEFENSE REACTION. S.K. Sanders and A. Shekhar. Dept. of Psychiatry and Program in Med. Neurobiology, Indiana Univ. Med. Ctr., Indianapolis, IN 46202.

Although electrical stimulation of the amygdala has been known to elicit cardiovascular and behavioral changes suggesting a "fight-or-flight" reaction, the specific neurotransmitters involved in this response have not been studied in detail. The present study was conducted to determine the effects of microinjecting GABA antagonists into the different regions of the amygdala in awake and freely moving rats and measuring the changes in heart rate (HR) and blood pressure (BP). Male Sprague-Dawley rats with arterial and venous catheters placed for physiological measurements were implanted with chronic microinjection cannula in the anterior baso-lateral nucleus of the amygdala (ABL) under pentobarb anesthesia. After recovering, rats were injected bilaterally with saline and bicuculline methiodide (BMI, 25 ng/250 nl) which gave the following results:

Treatment	n	Incr. in HR (bts/min)	Incr. in BP (mm/Hg)
Saline	4	6 ± 5	1 ± 3
BMI	4	76 ± 13*	24 ± 4*

(Significantly different from saline, *P 0.05)

Locomotor activation was also observed with BMI. These results suggest that GABA blockade in the region of ABL elicits changes suggesting a defense reaction in rats.

(Supported by R29 MH 45362-01).

306.2

SELECTIVE SEROTONERGIC INNERVATION OF A SPECIALIZED TYPE OF GABAERGIC INTERNEURON IN THE RAT HIPPOCAMPUS. T.F.Freund[1], A.I.Gulyás[1],L.Acsádi[1],T.Görcs[1],R.G.Bickford[2],K.Tóth[1]. [1]Inst. Exp.Med.Budapest,H-1450, [2]Dept.Neurosci.,UCSD,La Jolla, CA.

Serotonergic axons were identified in the hippocampal formation by anterograde transport of Phaseolus vulgaris leucoagglutinin (PHAL) following median raphe injections, and by immunostaining for serotonin. Different types of interneurons were visualized in the same material by immunostaining alternate sections for calbindin D_{28K}, parvalbumin, CCK and somatostatin. The relative distribution of serotonergic afferents was identical in the PHAL transport and the serotonin immunocytochemical experiments, they were most numerous in str.radiatum and lacunosum moleculare of the CA1-CA3 regions. In the double-stained sections the calbindin-containing interneurons of these strata received multiple contacts from the serotonergic axons, whereas other types of interneurons were largely avoided. The serotonin-immunoreactive varicosities established symmetrical synaptic contacts with dendritic shafts some of which were GABAergic, as shown by postembedding immunogold staining of serial ultrathin sections for GABA. The calbindin-containing interneurons in stratum radiatum terminate in the dendritic region of pyramidal cells, and are suggested to participate in GABA-B receptor-mediated feed-forward inhibition. Thus, the serotonergic afferents can - via these interneurons - selectively modulate a specialized inhibitory circuit in the hippocampus.

306.3

DIRECTION AND LOCATION CORRELATES OF CELL FIRING IN THE RAT SUBICULUM. P. E. Sharp, J. B. Ranck, Jr., R.U. Muller, SUNY Health Sciences Center, Brooklyn, N.Y. 11215

Firing patterns of cells in each hippocampal formation region studied so far show distinctive spatial correlates. Cells in both entorhinal cortex and hippocampus show location specific firing, with that in entorhinal cortex being less crisp (Muller *et al.*,J. Neurosci.,7,1987; Quirk & Ranck,Soc. Neurosci.Abstr.,12,1986). In contrast, cells in postsubiculum fire as a function of head direction, regardless of location (Taube *et al.*, J.Neurosci.,10,1990). We here present data on spatial correlates of cells in subiculum. As in earlier studies, activity was examined while rats performed a pellet-chasing task in a cylindrical apparatus with a white card on the wall. A headstage with two light-emitting diodes allowed continuous monitoring of head direction and location.

The 14 cells fell into three categories; *1)* 8 cells fired as a function of both place and head direction. They had well-defined location-specific fields like those in the hippocampus, but firing within the field was strongly modulated by head direction, unlike that observed for hippocampal cells in this environment (Bostock *et al.*,Soc.Neurosci.Abstr.,14,1988). 2) 4 cells fired strictly as a function of head direction, regardless of location. 3) One cell had a sloppy place field, apparently not modulated by head direction.

Probe sessions showed that these cells were similar to hippocampal place cells in that their spatial firing properties rotated along with rotations of the cue card, yet were maintained if the card was completely removed.

Thus, spatial signals in subiculum are from a heterogeneous population and provide both locational and directional information.

306.4

RAPHE STIMULATION: THE ROLE OF SEROTONIN AND ACETYLCHOLINE IN NEOCORTICAL ACTIVATION AND IN HIPPOCAMPAL ACTIVATION AND SUPPRESSION. B.K. Peck* and C.H. Vanderwolf. Dept. of Psychology, Univ. of Western Ontario, London, Ont., Canada, N6A 5C2.

In chronically prepared rats, electrical stimulation (100 Hz, 0.1 ms pulses) of the dorsal raphe nucleus, some sites in the median raphe nucleus (MR), and adjoining regions of the midbrain produced locomotion accompanied by hippocampal rhythmical slow activity (RSA) and neocortical low voltage fast activity (LVFA). Both the behaviour and the cerebral waveforms persisted after injection of scopolamine HBr (5mg/kg, s.c.). MR stimulation in 14 rats produced behavioural freezing or an unnatural forced movement accompanied by RSA and LVFA. However, after scopolamine treatment, stimulation produced low amplitude, irregular (suppressed) hippocampal activity but did not alter the behavioural response or the LVFA. P-Chlorophenylalanine (PCPA, 500mg/kg/day x 3, i.p.) reduced the RSA and LVFA normally present after scopolamine but did not reduce the hippocampal suppression produced by MR stimulation in scopolamine-treated rats. Hippocampal suppression and LVFA in response to MR stimulation were also present in urethane (1.0-1.5g/kg, i.p.) anesthetized rats, whether pretreated with PCPA or not. Stimulation at most other midbrain sites produced RSA and LVFA in the urethane condition. RSA was abolished in the urethane+scopolamine condition.

The data support the view that scopolamine-resistant RSA and LVFA are dependent on serotonergic projections. The hippocampal suppression produced by MR stimulation may be dependent on non-serotonergic neurotransmission.

This work was supported by an NSERC grant to C.H.V.

306.5

SIMULATION OF NMDA RECEPTOR-MEDIATED DENDRITIC ACTIVITY IN CA1 HIPPOCAMPAL PYRAMIDAL NEURONS. G.M. Shepherd, N.P. Poolos, F. Pongracz* and J.D. Kocsis. Sect. of Neuroanatomy and Dept. of Neurology, Yale Univ. Sch. of Med., New Haven, CT. 06510.

We have constructed a compartmental model in SABER of the rat CA1 pyramidal neuron and its responses to repetitive synaptic activation. The model represented apical and basal dendritic trees, and included g_{Na}, g_K, g_{Ca} and $g_{K(Ca)}$; excitatory glutamatergic (NMDA and nonNMDA) and inhibitory (g_{Cl} and g_K) synaptic conductances; and changes in $[K^+]o$ in a restricted extracellular space.

The model reproduced intradendritic and intrasomatic responses to single excitatory inputs. Brief repetitive inputs elicited NMDA-mediated potentiation of successive EPSPs. Maximal depolarizing charge in the dendrites occurred with 10-100 Hz. The results suggest that the Ca^{2+} component of the NMDA-mediated synaptic current can be a significant factor in increasing $[Ca^{2+}]i$ at postsynaptic sites. Elevation of $[K+]o$ depolarized the membrane during repetitive activity and enhanced NMDA-mediated responses, leading to increased neuronal excitability and increased $[Ca^{2+}]i$ at postsynaptic sites. Large increases in $[K^+]o$ led to excessive depolarization and repetitive discharge resembling convulsive activity, which was prolonged by the NMDA-mediated synaptic conductance. Blockade of dendritic $g_{K(Ca)}$ increased the EPSP amplitude, leading to slow depolarization and facilitation of impulse activity. The results support the notion that the NMDA conductance functions as an amplifying component underlying short-term potentiation of responsiveness of CA1 pyramidal neurons to repetitive synaptic activation. Supported by ONR and NIDCD.

306.6

INTRADENDRITIC RECORDINGS OF NMDA RECEPTOR-MEDIATED POTENTIALS IN CA1 HIPPOCAMPAL PYRAMIDAL CELLS. N.P. Poolos, J.D. Kocsis, F. Pongracz*, and G.M. Shepherd. Dept. of Neurology and Section of Neuroanatomy, Yale University, New Haven, CT 06510.

Intracellular recordings in the distal dendrites of rat hippocampal CA1 pyramidal neurons were obtained in order to study N-methyl-*D*-aspartate (NMDA) receptor-mediated potentials during repetitive synaptic stimulation. CA1 pyramidal cells in the hippocampal slice preparation were impaled in the distal stratum radiatum using high impedance (120-250 Mohm) glass microelectrodes.

Intradendritic recordings disclosed features of the dendritic response to synaptic stimulation which were not observed in standard intrasomatic recordings. The dendritic response to a single stimulus consisted of up to 45 mV EPSPs on which were superimposed multiple fast Na-mediated action potentials of varying amplitudes. These action potentials could summate to form larger composite spikes of graded amplitude. Brief periods of repetitive stimulation (2-10 stimuli at 2-100 Hz) produced robust short-term potentiation of the dendritic EPSP, and elicited an increase in dendritic spike activity. Much of this increased postsynaptic response was mediated by NMDA receptors, as shown by application of NMDA receptor antagonists. The magnitude of NMDA receptor-mediated activity increased with increasing number of stimuli, and was maximal at frequencies around 10-20 Hz. Further, dendritic NMDA receptor-mediated activity was greatly augmented by small changes in extracellular K concentration, or by partial blockade of K conductances with low concentrations of TEA. Under these conditions, repetitive stimulation led to NMDA receptor-mediated burst firing as well as activation of intrinsic Ca spikes.

These results demonstrate that NMDA receptors in CA1 hippocampus contribute to the postsynaptic response and short-term synaptic plasticity in hippocampal pyramidal cells at a broad range of afferent stimulus frequencies. Activation of NMDA receptors is highly sensitive to changes in repolarizing K currents. These data also show that intradendritically-recorded physiology may be distinct from that seen at the cell body of highly arborized neurons.

306.7

SPECTRAL ANALYSIS OF CORTICAL EEG ACTIVITY DURING AN AUDITORY ATTENTION TASK. L.C. Oatman. U.S. Army Human Engineering Lab., Aberdeen PG, MD 21005.

Hippocampal theta during behavioral immobility was significantly larger during visual attention than non-attention (Oatman, L.C., Physiol. Psych., 10:336, 1982). Since sensory stimulation can also elicit hippocampal theta, perhaps the increase in theta was the result of increased sensory stimulation rather than attention mechanisms. This study determined whether an increase in hippocampal theta occurred during auditory attention and/or during increased irrelevant sensory stimulation.

Four chronically implanted cats learned an auditory attention task consisting of two tones (S1, 1000 Hz and S2, 800 Hz) presented successively using food reward. Auditory clicks (irrelevant stimuli) were presented at 1/sec at each 10-dB step from 35 to 125 dB SPL as background before, during, and after the presentation of the S1-S2 task. Sample epochs of cortical EEG activity from attention and nonattention periods were subjected to the fast Fourier transform. Theta (4-8 Hz) was significantly greater when the cats' attention was focused on the auditory attention task than when they were nonattentive. The increased intensity of the irrelevant auditory clicks had no systematic effect on theta (4-8 Hz). The results suggest that increased auditory stimulation had no increased arousal or alerting effect on the hippocampal theta, and significant amounts of hippocampal theta were produced only during auditory attention.

306.8

BICUCULLINE- AND PHACLOFEN-RESISTANT HYPERPOLARIZATIONS EVOKED BY GLUTAMATE APPLICATIONS TO STRATUM LACUNOSUM-MOLECULARE IN CA1 PYRAMIDAL CELLS OF THE HIPPOCAMPUS IN VITRO. S. Williams and J.-C. Lacaille. Centre de recherche en sciences neurologiques, département de physiologie, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

In the hippocampus, different types of interneurons may mediate distinct GABA responses, i.e. early and late inhibitory postsynaptic potentials (IPSPs). To verify this hypothesis, intracellular recordings were obtained from CA1 pyramidal cells (n=57) in rat hippocampal slices. Glutamate (1mM) was locally ejected in str. lacunosum-moleculare (L-M) to active interneurons in this region. Hyperpolarizations induced with glutamate (glutamate IPSPs) were characterized and compared to the early and late IPSP elicited by str. radiatum electrical stimulation. Several characteristics of the glutamate IPSPs were similar to the late IPSP: their amplitude was small (-3.4 vs -4.9 mV, respectively), each was associated with a small conductance increase (5.0 vs 9.3 nS), their peak latency was slow (124.4 vs 129.8 ms) and in the majority of cells, each displayed little response reversal. However, the equilibrium potential of the glutamate IPSP (-76.5 mV) was similar to that of the early IPSP (-73.8 mV). Perfusion with a low Ca^{2+}(0.5 mM)/high Mg^{2+}(8 mM) medium that blocked synaptic transmission also reduced the glutamate IPSP. Therefore the glutamate IPSP may be mediated indirectly by inhibitory interneurons. The $GABA_A$ antagonist bicuculline (10μM) blocked the early IPSP but not the glutamate IPSP. The $GABA_B$ antagonist phaclofen (1mM) reduced the late IPSP but not the glutamate IPSP. In conclusion, glutamate stimulation of interneurons in str. L-M evoke a slow IPSP different from the early ($GABA_A$) or late ($GABA_B$) IPSPs in CA1 pyramidal cells of hippocampus.

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306.9

SYNAPTIC POTENTIALS IN NONPYRAMIDAL CELLS OF STR. PYRAMIDALE OF THE CA1 REGION OF RAT HIPPOCAMPUS *IN VITRO*. J.-C. Lacaille. Centre de recherche en sciences neurologiques, département de physiologie, Université de Montréal, Montréal, Québec, Canada, H3C 3J7.

Synaptic responses of nonpyramidal cells were studied with intracellular recordings in the str. pyramidale region of hippocampal slices. Nonpyramidal cells (n=27) were impaled and identified by their characteristic electrophysiological properties: short duration action potential (≤ 1.0 ms at base), presence of a fAHP after an action potential, and little spike frequency adaptation during depolarizing pulses. Synaptic responses were evoked by electrical stimulation of str. radiatum. In most cells, stimulation evoked a short latency EPSP followed by a biphasic IPSP. The amplitude of the fast EPSP increased with membrane hyperpolarization (n=7 cells). The IPSPs consisted of early (mean latency 19.8 ms; 9/12 cells) and late (mean latency 145.7 ms; 12/12 cells) components. The early IPSP was associated with a 16.4 nS conductance increase (n=8), an equilibrium potential of -68.9 mV (n=9), and was blocked by bicuculline (2/2 cells). The late IPSP was accompanied by a 2.6 nS conductance increase (n=7) and its equilibrium potential was -116 mV (n=7). In some cells (6/12), a longer latency (30-80 ms) EPSP appeared at increased stimulation intensities.

The balance of excitation-inhibition resulted in the production of a single action potential in 50% of cells (6/12) or of bursts (≥ 2 action potentials) in the other 50%.

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306.10

SYNAPTIC CONNECTIONS DETERMINED BY SIMULTANEOUS INTRACELLULAR RECORDINGS OF DENTATE GRANULE CELLS AND HILAR NEURONS, OR INTRACELLULAR DYE INJECTION, IN RAT HIPPOCAMPAL SLICES. H.E. Scharfman, D.D. Kunkel, and P.A. Schwartzkroin. Dept. Neurological Surg., Univ. of Washington, Seattle, WA 98195

To examine the circuitry of the fascia dentata, paired intracellular recordings were made from granule cells and non-granule cells in transverse and longitudinally cut hippocampal slices. Many of the non-granule cells were identified morphologically by recording with dye-filled electrodes. Other non-granule cells were identified by their electrophysiological properties.

Granule cells strongly excited spiny hilar "mossy" cells; a single action potential (AP) of a granule cell, elicited by brief intracellular current injection, could evoke more than one AP in a mossy cell. Granule cells also excited aspiny cells, located in the hilus or granule cell layer. Very few connections were observed between aspiny cells and other neurons; those that were found were inhibitory and prone to fatigue. Electron microscopy of dye-filled mossy cells showed that mossy cells made synapses on aspiny dendrites in the hilus, but no clear effect of mossy cells has yet been found in any of the paired recordings. These studies are fundamental steps in unraveling the complex circuitry of the dentate region.

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306.11

COMPUTATION OF UNOBSERVED HIPPOCAMPAL ELEMENTS R.J.Sclabassi, T.Biedka*, J.Solomon*, D.Krieger, G.Barrionuevo, and T.W.Berger. Center for Neuroscience, University of Pittsburgh, Pittsburgh, PA 15213.

The nonlinear input/output properties of major nodes within the hippocampus can be characterized experimentally by recording electrophysiological responses to random impulse train stimulation and by expressing the relationship between the input and output activity as the kernels of an orthogonalized functional power series, which may be interpreted as n^{th} order impulse responses. In the hippocampal formation, there are elements which cannot be isolated and whose input/output properties cannot be measured directly. The transformational properties of these elements must be inferred from their effect on the other directly observable groups of neurons. To accomplish this the n^{th} order Laplace transforms of the impulse responses are computed and algebraically manipulated. Models of the observable and unobservable subsystems, as well as the entire system, may thus be computed in terms of n^{th} order convolution operators.

An example is presented utilizing data from three preparations: the intact hippocampal formation, with the dentate granule cell layer considered as a feed-forward element, and the remainder of the ipsilateral hippocampus as a feedback element; a partially closed-loop system, where the contralateral hippocampus has been removed; and an open-loop system, where the contralateral hippocampus has been removed, the ipsilateral trisynaptic pathway is open, and the local GABAergic pathways have been blocked. Three results are presented: (1) the n^{th} order Laplace transforms; (2) the results of the algebraic manipulation of the n^{th} order transforms used to calculate the unobservable elements; and (3) the results of simulations used to verify the estimated kernels. The solutions allow computation of the kernels for both the GABAergic pathways and the contralateral hippocampus. neither of which is available by direct measurement. Supported by NIMH, the Office of Naval Research and the Air Force Office of Scientific Research.

306.12

INTRACELLULAR ANALYSIS OF THE NONLINEAR RESPONSE PROPERTIES OF HIPPOCAMPAL DENTATE GRANULE CELLS. T.P. Harty, X. Xie, R.J. Sclabassi, T.W. Berger and G. Barrionuevo. *Departments of Behavioral Neuroscience, Neurological Surgery and Psychiatry, University of Pittsburgh, Pittsburgh, PA 15260.*

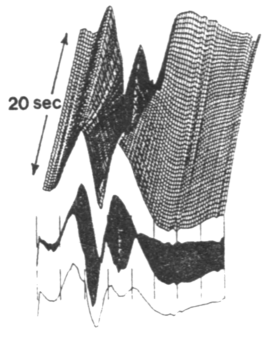
We have used nonlinear systems analytic techniques to compare the input/output dynamics of single cell and population responses of hippocampal dentate granule cells. Intracellularly recorded action potentials and extracellularly recorded population spike responses evoked by perforant path input were recorded simultaneously from sites less than 1 mm apart in the granule cell body layer of rabbit hippocampal slice preparations. The perforant path was stimulated with a train of 4064 electrical impulses having randomly distributed inter-impulse intervals (Δ's). First, second and third order kernels were computed to characterize the relationship between Δ's in the stimulus train and either population spike amplitudes or action potential probabilities. Results revealed that the nonlinear response properties of granule cells expressed at the population and single cell levels were highly similar. With respect to second order nonlinearities, both population spike amplitude and action potential probability were strongly facilitated for Δ < 100 ms. In addition, both measures revealed that granule cell responses were suppressed when Δ = 100-600 ms. Third order nonlinearities showed that both population spike amplitude and the probability of single cell discharge were decreased markedly when the intervals associated with two preceding reponses (Δ_1 and Δ_2) were less than 200 ms. This research was supported by AFOSR, ONR, MH00343 and NS01196.

306.13

CHARACTERIZATON OF TIME-VARYING BEHAVIOR IN NONLINEAR SYSTEMS. D Krieger[1], T Berger[2], D Weisz[1,2], and RJ Sclabassi[1,2]. Dept's. of Neurosurgery[1] & Beh'l Neuroscience[2], U. Pittsburgh, PA 15213.

A nonlinear system may be completely characterized (Wiener, Wiley & Sons. 1958) using functional power series (FPS). A technique is reported for identification of time-varying FPS from stimulus evoked neural activity. For each point in a FPS, regression on a closed form function of time expands the FPS to capture time-varying behavior. Evaluation of these functions at a specified time point produces an instantaneous system characterization.

Demonstration of the Technique. Potentiation was induced in the granule cell layer of the dentate gyrus by a stimulus train to the perforant path. (A) The averaged response is shown at the bottom. There is a negative going population spike (PS) at 7.0 msec post-stimulus. 2nd order polynomials were used to capture time-varying behavior. Two views of the instantaneous characterization are shown: (B) superimposed - center, and (C) isometric projection - top. The latency and amplitude of the PS changes over the 20 sec course of stimulation. A 2nd PS appears beginning 5 sec following the onset of the first stimulus. Timing marks = 3.0 msec.



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306.14

ALPHAXALONE-ENHANCED IPSPs ALTER NONLINEARITIES OF HIPPOCAMPAL DENTATE GRANULE CELLS. X. Xie, T.P. Harty, R.J. Sclabassi, T.W. Berger and G. Barrionuevo. *Depts. of Behavioral Neurosci., Neurological Surgery, and Psychiatry, Univ. of Pittsburgh, Pgh., PA 15260.*

Second order nonlinearities of hippocampal dentate granule cells to perforant path input *in vitro* are characterized by facilitation of population spike amplitude in response to interstimulus intervals (ISIs) of 10-50 ms. In contrast, suppression of spike amplitude is observed *in vivo* to the same ISIs. This difference between the *in vivo* and *in vitro* preparations suggests that basket cell inhibition is reduced in the slice, an hypothesis supported by the finding that the $GABA_A$ allosteric agonist, alphaxalone, selectively induced suppression of spike amplitude for ISIs <100 ms.

In the present study, we used intracellular recordings obtained from rabbit dentate granule cells maintained *in vitro* to study alphaxalone-induced changes in input resistance, ortho- and antidromic IPSP amplitude (evoked by stimulation of the perforant path and mossy fibers, respectively). In addition, we have characterized the resulting changes in nonlinearities of dentate granule cell action potential response to perforant path stimulation. Bath applied alphaxalone at 2, 3, 4 and 6 μM, produced similar reductions in input resistance (23.6%, n=7). At these same concentrations, alphaxalone increased IPSP amplitudes on average by 102% (orthodromic IPSPs, n=5); and 197% (antidromic IPSPs, n=3). Second order kernels revealed that alphaxalone produced a suppression in action potential discharge in response to ISIs <100 ms. These results indicate that GABA-mediated inhibition plays a major role in determining the dynamic response characteristics of dentate granule cells. Supported by AFOSR, ONR, MH00343 and NS01196.

306.15

NONLINEAR RESPONSE PROPERTIES OF HIPPOCAMPAL DENTATE GRANULE CELLS: DUAL INPUT STIMULATION OF MEDIAL PERFORANT PATH AND COMMISSURAL AFFERENTS.

T.W. Berger, C.L. Weikart* and R.J. Sclabassi. *Depts. of Behavioral Neuroscience, and Neurol. Surgery, Univ. of Pittsburgh, Pgh., PA 15260.*

We have investigated functional interactions between medial perforant path (*mpp*) and commissural (*comm*) inputs to the hippocampal dentate gyrus using nonlinear systems analytic methods. The *mpp* was stimulated electrically with a series of 4064 impulses; interstimulus intervals (Δs) were determined by a Poisson distribution with a mean of 2.0 Hz. This sequence then was repeated, and *comm* afferents (contralateral hilus) were stimulated at a constant frequency of 2.0 Hz. Amplitude of the population spike evoked to each *mpp* stimulus was measured. First, second, and third order kernels for *mpp* input were calculated using cross correlation techniques.

When the *mpp* was stimulated alone, the average population spike amplitude to all 4064 impulses (first order kernel) was approximately 1-3 mV; the influence of any preceding impulse (second order kernel) was inhibitory when Δ=10-60 ms and facilitatory when Δ=100-400 ms. Simultaneous stimulation of *comm* input decreased the first order kernel, consistent with previous reports of an inhibitory effect of *comm* afferents on *mpp* input to granule cells. In addition, however, second order kernel facilitation for Δ=100-400 ms was decreased markedly; second order kernel inhibition was reduced to a lesser degree, occurring in response to a smaller range of Δs. Simultaneous stimulation of *comm* afferents did not affect second order kernel values for any other Δs. These results are the virtual complement of the effects of unilateral hippocampectomy: an increase in the first order kernel average, a small increase in second order inhibition, and a robust increase in second order facilitation. Thus, inhibitory *comm* input to the dentate primarily modulates facilitation of granule cell response to *mpp* input.

Supported by AFOSR, ONR, and NIMH (MH00343).

306.16

TWO-LEVEL FIELD THEORY INTERPRETATION OF HIPPOCAMPAL EXTRACELLULAR FIELD POTENTIALS.

G. Chauvet and T.W. Berger. *Lab. of Theoretical Biology, School of Medicine, Angers, France, and Depts. of Behavioral Neuroscience and Psychiatry, Univ. of Pittsburgh, Pgh., PA 15260.*

From a model based on a two-level field theory, an interpretation is given for extracellular field potentials generated by the action potential firing of hippocampal dentate granule cells, as a function of the magnitude of perforant path input. It is shown that this input/output (I/O) relationship depends on: (i) the anatomical structure, as defined by the densities of input fibers, and the synaptic density-connectivity of postsynaptic neurons; (ii) the dynamics of the neural network, which is determined by six physiological parameters:

One parameter, the *source term* in the field equations, plays a major role in firing, and imposes the latency onset. It depends on the internal biochemical state of the stimulated neuron that becomes active.

Three parameters describe the *dynamics of firing*, i.e., the time evolution of firing as determined by the dynamics of the entire synapse-cell body system. This dependence is represented accurately by a linear function.

Two parameters describe the influence of *synaptic activation* upon the generation of extracellular potentials when cells are not active. This resting state depends on stimulation intensity according to a function that can be estimated with experimentally determined I/O curves.

Finally, the mathematical model can be simulated with a computer. In the specific case of the hippocampus, a geometrical model describes the interaction between the set of active perforant path afferents assumed to be a cylinder with a diameter depending on stimulation intensity, and a conical shape of the granule cell dendritic tree. An assumed topographical distribution of *en passage* synapses should permit calculation of the number of stimulated and activated granule cells according to the values of the above parameters.

Supported by DRET, INSERM (France) and ONR, AFOSR, MH00343 (USA).

BRAIN METABOLISM AND BLOOD FLOW: EXOGENOUS FACTORS

307.1

BLOOD FLOW TO CHOROID PLEXUS DURING HYPERCAPNIA.

John L. Williams, S.C. Jones, and Robert M. Bryan, Jr. Cleveland Clinic Foundation, Cleveland, OH 44195 and Milton S. Hershey Medical Center, Univ. of Pennsylvania, Hershey, PA 17033.

The response of choroid plexus blood flow (CPBF) during hypercapnia is controversial. In this study, we examined effects of hypercapnia on CPBF in unanesthetized, Long-Evans rats and in rats pretreated with phentolamine, an alpha-adrenergic antagonist. Rats were first prepared surgically during nitrous oxide-halothane anesthesia. After recovery, unanesthetized rats breathed air or a 5-8% CO_2-air mixture. CPBF was measured with [^{14}C]isopropyliodoamphetamine and quantitative autoradiography. CPBF in hypercapnic rats ($PaCO_2$ = 61.6±1.6 mmHg, mean±SE) was similar to CPBF in normocapnic, control rats (525±39 ml·min^{-1}·100g^{-1}; $PaCO_2$ =42.7±0.6 mmHg; n = 7). In contrast, blood flow to cerebral cortex increased 67% during hypercapnia (P<0.01). CPBF in normocapnic rats that were treated with phentolamine (n = 7) was similar to untreated normocapnic and hypercapnic rats. However, during hypercapnia, CPBF in phentolamine-treated rats (n = 6) increased 29% (P<0.001).

Our findings indicate that hypercapnia has no effect on CPBF when alpha-adrenergic receptors are intact. In contrast, after blockade of alpha-adrenergic receptors, hypercapnia increases CPBF. These findings suggest that, during hypercapnia, catecholamines are released and prevent increases in CPBF.

307.2

CEREBRAL BLOOD FLOW (CBF) DURING HYPOGLYCEMIA IN REGIONS WITH AND WITHOUT A BLOOD-BRAIN BARRIER (BBB).

R.M. Bryan, Jr., J.C. Reichert*, S.C. Jones, and J.L. Williams. Dept. of Surgery, Hershey Medical Center, Hershey, PA 17033.

CBF was measured in rats during mild hypoglycemia in order to compare CBF in regions with and without a BBB. Male Long-Evans rats were fasted overnight and surgically prepared with halothane/nitrous oxide. The hindquarters of each rat was immobilized with a plaster cast and the rats were allowed to recover from the anesthesia. CBF was measured using ^{14}C-isopropyliodoamphetamine after iv saline injection in controls or iv insulin injection (15 units/kg) in experimental rats. Plasma glucose was 8.2 umol/ml (148 mg/dl) in normoglycemic control rats and 1.7 umol/ml (31 mg/dl) in hypoglycemic rats. In general brain regions lying behind the BBB showed significant increases in CBF during hypoglycemia. The increase ranged from 19% to 120% with the mean increase of 22 regions being 70%. Regions not lying behind the BBB (median eminence, neural lobe, chloride plexus, and pineal) had higher resting flows than most other brain regions and showed no tendency to increase their flow during hypoglycemia; in fact, there was a tendency for some of these regions to decrease their rates of blood flow. We conclude that the presence or the absence of the BBB for a given region is an important factor in the regulation of blood flow during hypoglycemia. The fact that CBF did not increase in regions without a BBB may be due to their ability use substrates other than glucose.

307.3

CEREBRAL VASODILATORY FUNCTION IN THE STREPTOZOTOCIN (STZ) DIABETIC RAT.

D.A. Pelligrino and A.J. Sharp*. Dept. of Anesthesiology, Univ. of Illinois/Michael Reese Hosp., Chicago, IL 60616.

It has been suggested that a cerebral microangiopathy develops in the diabetic (D) to the extent that cerebral vasodilatory function may be impaired. To investigate this, we measured in the chronically hyperglycemic D rat (~6 mo. post-STZ), compared to age-matched non-diabetic (ND) controls, the regional cerebral blood flow (rCBF) changes accompanying hypoglycemia (HG), hypoxia (HX), or hypercapnia (HC). For surgical preparation, rats were maintained on 0.7% halothane/70% N_2O/30%O_2, paralysis, artificial ventilation, and local anesthesia. For study, the halothane only was discontinued (≥1 h). rCBF was measured, via radiolabeled microspheres, in the cortex (CX), subcortex (SC), brainstem (BS), and cerebellum (CE). In all rats, initial rCBF determinations were made at a normal P_aCO_2 (35-40 mmHg) and P_aO_2 (>90 mmHg). For HG studies, rCBF was measured prior to i.v. insulin and at a plasma glucose ~1.8mM. For HX, a second rCBF evaluation was made with P_aO_2=30-40 mmHg (normal P_aCO_2). With HC experiments, a second rCBF measurement was performed at a P_aCO_2=70-80 mmHg (P_aO_2 normal). When comparing D to ND rats, the cerebral hyperemic response to HG was lost in 3 of 4 regions (CX,SC,CE), but no changes were observed in the responses to HX or HC. These results might be a result of a diminished cerebrovascular ß-adrenergic (ß-ad) receptor density in D rats and that ß-ad influences play a far greater role in the hyperemia associated with HG, as opposed to HX or HC.

307.4

SOMAN INDUCES UNCOUPLING OF CEREBRAL BLOOD FLOW AND METABOLISM IN CEREBRAL CORTEX.

O.U. Scremin, T. Shih and K.D. Corcoran* V.A. Medical Center, Albuquerque, NM 87108 and USAMRICD, Aberdeen Proving Ground, MD 21010-5425.

Regional cerebral blood flow (rCBF) and glucose utilization (rCGU) were studied in conscious rats with a quantitative, double label, autoradiographic technique. Animals were treated with saline or soman (33-55 ug/kg) by subcutaneous route and rCBF/rCGU measurements were performed 1 hr later. Animals showing signs of toxicity were excluded. No change in mean arterial blood pressure was induced by soman with the doses used (before drug=112(1.7) after drug=105(3.9), n=7). A significant increase in rCBF was observed in motor, somatosensory, temporal, occipital, olfactory and cingulate cortex of soman treated animals but no changes were observed in rCGU in these same regions. A similar phenomenon was recorded in claustrum and anterior thalamus. In contrast, no changes in rCBF or rCGU were observed in posterior thalamus, hippocampus, striatum, preoptic area, hypothalamus or brain stem. A parallel increase in rCBF and rCGU was only observed in the superior colliculus. In conclusion, in the absence of signs of toxicity, soman uncouples blood flow and metabolism in select areas of the brain that show significant hyperemia.

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307.5

EFFECTS OF HEMIBRAIN X-IRRADIATION ON CEREBROVASCULAR AND METABOLIC FUNCTION. E. H. Lo, D. M. Kunis, G. K. Steinberg, K. A. Frankel*, R. L. DeLaPaz*, A. Poljak*, N. B. Manley and J. I. Fabrikant*. Department of Biophysics, Univ. California, Berkeley, CA94720; Stanford University School of Medicine, Palo Alto, CA

It has been demonstrated that the rabbit brain is a suitable experimental model for investigating the delayed reaction to radiation injury in the brain (Lo, E. H.; *Brain Res.*, 504:168-172, 1989). In this study, alterations in blood-brain barrier (BBB), regional cerebral blood flow (rCBF), and metabolic function were examined following high-dose (60 Gy, 225 keV X-rays) irradiation to the left hemisphere of the rabbit brain. GdDTPA-enhanced magnetic resonance imaging (MRI) demonstrated focal regions of BBB disruption in irradiated brain 2.5 mo post-irradiation. These BBB disruptions were primarily limited to the corona radiata, hippocampus, and fimbria. There were no changes in T1-weighted or T2-weighted scans. Radioactive microsphere flow studies were conducted to measure radiation-induced alterations in rCBF. At 2.5 mo post-irradiation, significant decreases in rCBF were demonstrated in the irradiated hemisphere ($p<0.05$). Alterations in cerebral metabolism were measured *in vivo* using positron emission tomography (PET). Initial results suggest that profound alterations in cerebrovascular and metabolic function occur following high-dose irradiation to the rabbit brain. These alterations may play important roles in the potentiation and development of delayed radiation injury in the brain. Research supported in part by the OHER, U.S. DOE Contract DE-AC03-76SF00098, and NIH Biomedical Research Support Grant RR05918.

307.6

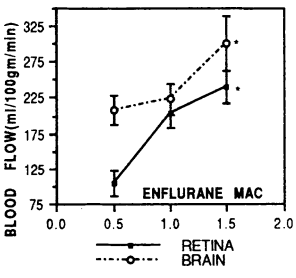
OUTCOME FOLLOWING COOLING TO NEAR THE FREEZING POINT IN TOTALLY BLOOD-SUBSTITUTED DOGS. M.L.Leavitt*, J.E.Bailes*, T.Shih*, A.M.Elrifai*, E.Teeple*, W.E.Hoffman, J.C.Maroon*. Allegheny-Singer Research Inst., Pittsburgh, PA 15212.

A new technique to maximally suppress metabolism by combining total blood substitution with whole body perfusion at near the freezing point was tested in 11 beagles. At 23°C esophageal temp. (E) the heart was stopped with a cardioplegic blood substitute. Whole body perfusion of K15 aqueous blood substitute (Cryomedical Sciences, Inc.) was continued at a mean blood press. of 30mmHg for the next 155 min. with E < 10°C and a hematocrit of < 1%. The E fell to 2°C or less for 1 hr. Upon rewarming to 10°C replacement of the perfusate with whole blood began. Mean duration of cardiac arrest was 169 min. Two dogs died due to technical errors during cardiac defibrillation. Of the 9 survivors, one died at 3 days and the other at 10 days post-op of hepato-renal failure. Creatinine, BUN, SGOT, SGPT, LDH, and alkaline phosphatase were elevated in the first post-op week but returned to normal by the second. Transient hind limb weakness occurred in half the dogs and transient circling in one dog. Two experiments are currently ongoing; all of the other 5 long term survivors behaved normally upon sacrifice at 32 to 51 days post-op. All visceral organs,the visual system and samples from the cranial and peripheral nerves, the spinal cord and 7 brain regions were microscopically normal. These results suggest that near freezing point temperatures can be tolerated in the bloodless state for prolonged periods of time.

307.7

RETINAL AND CEREBRAL BLOOD FLOW DURING GENERAL ANESTHESIA. S. Roth,* A.P. Crittenden,* and S. Glusman. Department of Anesthesia and Critical Care, University of Chicago, Chicago, IL 60637

We have previously shown that enflurane anesthesia increases retinal blood flow (ARVO, 3282, 1990). We performed this study to test the hypothesis that cerebral (CBF) and retinal blood flow (RetBF) are autoregulated in a similar manner during general anesthesia. With approval of our Animal Care Committee, we studied 13 adult cats anesthetized with enflurane, air, and oxygen. We placed bilateral femoral artery and venous catheters, a left atrial catheter (via a thoracotomy), and bilateral intraocular pressure cannulae. The P_{CO_2} was controlled between 25 and 30 mmHg (normocarbia). Cats were anesthetized with three different concentrations of enflurane (0.5, 1.0, and 1.5 MAC; MAC = a standard anesthetic measurement) administered in a random sequence. Blood flows were measured by injection of radiolabelled microspheres into the left atrium. After completion of the experiment, cats were killed and tissues counted for radioactivity using standard methods.



RetBF and CBF (ml/100gm/min) increased significantly during enflurane anesthesia Figure) despite significant decreases in perfusion pressure. The percent of change in RetBF vs CBF was significantly greater at 1.5 MAC than at 0.5 MAC. Both RetBF and CBF are increased in a dose-dependent manner by enflurane although the effect on RetBF is more pronounced.

* = $p < .05$ vs 0.5 MAC

307.8

BIPHASIC EFFECT OF CP 55,940, A THC ANALOG, ON METABOLIC ACTIVITY IN RAT BRAIN. J. E. Margulies, L. S. Melvin, M. R. Johnson and R. P. Hammer, Jr. Dept. of Anatomy & Reproductive Biology, Univ. of Hawaii, Honolulu, HI 96822 and Central Research, Pfizer Inc., Groton, CT 06340.

CP 55,940, a synthetic cannabinoid with high enantioselectivity and potency, has recently been used to identify, characterize and localize a cannabinoid receptor. In this study, regional metabolic activity was measured in male Sprague-Dawley rats following acute treatment with CP 55,940 using the quantitative 2-[^{14}C]deoxy-D-glucose (2DG) autoradiographic method. CP 55,940 (0, 0.02, 0.05, 0.1 and 1.0 mg/kg, i.p.), solubilized in 5% propylene glycol and 0.5% Tween 80, was administered 1 hour before 2DG injection. Timed arterial blood samples were collected, and local cerebral glucose utilization (lCGU) was determined. CP 55,940 altered lCGU in a biphasic, dose-dependent manner in hippocampus and cortical regions. Low doses (0.02 and 0.05 mg/kg) significantly increased lCGU in selected brain regions, while higher doses (0.1 and 1.0 mg/kg) produced a generalized decrease of metabolic activity relative to vehicle treatment. In particular, low CP 55,940 doses increased lCGU (33-65%) in motor, somatosensory, cingulate, auditory, visual and entorhinal cortices. All layers of hippocampal region CA1 showed increased lCGU (35-74%) following 0.02 mg/kg however, CA3 was relatively less affected. In contrast, lCGU was unaffected in thalamic regions. This biphasic response is consistent with the metabolic effects of acute delta-9-THC (Margulies and Hammer, *Brain Res.*, submitted) wherein maximal effects are observed in brain regions known to contain high densities of cannabinoid receptors. (Supported by USPHS Awards DAO4081 and NSO1161.)

307.9

LARGE REVERSIBLE HYPOXIA-INDUCED DECREASES IN PHOSPHOCREATINE IN THE DEVELOPING BRAIN. D. Holtzman, M. Offutt, F. Jensen, L. J. Neuringer. Dept. of Neurology, The Children's Hospital, Boston, MA 02115 and the F. Bitter National Magnet Laboratory, MIT, Cambridge, MA 02139.

Reversible hypoxia in the mature rodent brain produces up to a 30% decrease in brain phosphocreatine (PC) concentration, a doubling of inorganic phosphate (P_i), and no change in ATP. Using ^{31}P nuclear magnetic resonance spectroscopy, similar studies have been carried out in immature rats made hypoxic with either intraperitoneal cyanide (KCN) or hypoxic gas mixtures (3 or 4% O_2). Hypoxia in 10-12 day-old animals produced up to a 90% decrease in PC concentration and much larger increases in P_i concentration than seen in the adult. The brain ATP concentration, measured by the change in nucleoside triphosphate signal, decreased up to 20%. These changes in NMR spectra were associated with electrocortical seizure activity, which occurs during acute hypoxia in 100% of rodent pups at this age (F. Jensen, Neuroscience Abstracts, 1989). All the spectral changes were reversible within minutes of returning the animals to a normoxic gas mixture. The large decrease in high energy phosphate concentrations in the hypoxic immature brain may be due to the seizure activity, which is not seen in the mature animal. The limited capacity for increased ATP synthesis during states of high energy demand in the immature brain also may contribute to the large decreases in brain PC concentration. These animal studies are relevant to the pathogenesis of neurologic sequelae of prematurity and of conditions such as cardiopulmonary bypass in the neonate. [Supported by a Mental Retardation Center Grant from NICHD (P30-HD 18655), an NIH Resource Center Grant (RR-00995), a grant from NICHD (HD 00807), the Kaplan Foundation, and the Cardiovascular Surgery Research Fund of the Children's Hospital).

307.10

BRAIN VASOACTIVE EFFECTS OF PHENOBARBITAL DURING HYPERTENSION AND HYPOXIA IN NEWBORN PIGLETS. J. Goddard-Finegold and L.H. Michael.* Depts. of Pediatrics (Neurol.), Physiol., and Med., Baylor College of Medicine, Houston, Tx. 77030.

Phenobarbital (PBS) at anti-convulsant levels lowers cerebral blood flow (CBF) during hypertension (HT) in newborn dogs (in press). We proposed that hypoxic dilatation of brain vessels might overcome this effect. 12 control and 8 PBS-treated piglets (1-3 days postnatal) had microsphere determinations of CBFs during (1) steady-state (SS), (2) phenylephrine-induced HT, and (3) HT plus 5 mins. of hypoxia (HTHY). PBS was infused after SS in 1 ml. volume (20mg/kg);levels were 27.5±10ug/ml. pO2s were reduced significantly from SS during hypoxia, and pCO2s ranged from 30 to 37 mm Hg (n.s. within or between groups). SS CBFs were 37±16(C) and 40±20(PBS) ml/min/100g. During HT CBFs were 61±21(C)[p=.006 comp. to SS] and 49±14(PBS)[n.s. comp. to SS]. During HTHY, CBFs were not signif. different from those during HT. We conclude that (1) in the newborn piglet, as in the newborn puppy, PBS attenuates CBF during HT, and (2) this PBS effect persists during HT plus five minutes of hypoxia without hypercarbia. In addition, hypoxia does not increase CBF further during HT. (Supp. by NS28388 to JGF).

307.11

CHANGES IN MEMBRANE PROPERTIES AND SURVIVAL STRATEGIES FOR TURTLE BRAIN DURING ANOXIA. M. Pérez-Pinzón, C. Chan*, M. Rosenthal and T. Sick. Dept Neurology, Univ Miami Med Sch, Miami, FL 33101, *Dept Physiology, CUNY Med Sch, NYC 10031

Turtle brain survives prolonged anoxia during which ion homeostasis and ATP levels are maintained. A key to such survival may be decreased energy consumption from depression of electrical activity, and changes in membrane physiology which decrease reliance on ion transport. To examine these suggestions, it was necessary: a) to determine if an *in vitro* preparation of turtle brain survived anoxia; and b) by intracellular recording, to define changes in synaptic transmsission, spike activity and resting membrane properties during anoxia. In cerebellum isolated from freshwater turtles, extracellular potassium ion activity $(K^+)o$ was maintained for at least six hours of anoxia when superfused with 20 mM glucose. Anoxia exacerbated oscillations in transmembrane potentials (TMP) with a general pattern being that TMP became relatively more depolarized in most Purkinje cells. However, TMP was never depolarized to levels associated with energy failure. Sodium (and likely calcium) spike thresholds were increased, postsynaptic responses from the major afferent input pathways to Purkinje cells were depressed but input resistance underwent no consistent pattern of change among cells. Although preliminary, this latter finding suggests that maintenance of ion homeostasis remains linked to ion transport rather than to changes in membrane conductances. We conclude that during anoxia, synaptic depression and spike threshold increases occur which likely contribute to the sparing of energy for ion transport and perhaps other functions which may be more directly related to cell survival.

307.12

CEREBROVASCULAR RESPONSIVENESS TO HYPEROXIC-HYPERCAPNIA AFTER BRAIN MISSILE WOUNDING (BMW) IN PENTOBARBITAL ANESTHETIZED CATS. D. Torbati M.E. Carey* and J.F. Davidson.* Dept. Neurosurg. LSU Med. Ctr., New Orleans, LA 70112.

We have previously studied the regional cerebral blood flow (rCBF) responsiveness to normoxic-hypercapnia (5% CO_2) and hyperoxic-normocapnia (100% O_2) in pentobarbital anesthetized ventilated cats subjected to BMW. After BMW the vasodilatory effect of hypercapnia was entirely abolished and the vasoconstrictive effect of hyperoxia was enhanced in all structures. Since these conditions may prevent adequate brain oxygenation, we tested the hypothesis that a hyperoxic-hypercapnic mixture may ameliorate the enhanced vasoconstrictive effect of hyperoxia after wounding. The rCBF responsiveness to 95% O_2 + 5% CO_2 breathing was determined before and after BMW, inflicted by a 2mm steel sphere penetrating fronto occipitally through an intact cranium. Our preliminary results (n=3), as compared with previous data, indicate that before BMW the vasodilatory effect of hypercapnia predominated the vasoconstrictive effect of hyperoxia. After BMW however, the vasodilatory effect of hypercapnia was completely abolished, whereas the vasoconstrictive effect of hyperoxia was ameliorated in all brain areas except in the damaged tissues. Thus, brain tissue oxygenation after wounding in undamaged tissues may be improved by a hyperoxic-hypercapnic mixture rather than by hyperoxia alone. Supported by contract No. DAMD17-86-C-6098 LAIR, USMRDC.

307.13

BRAIN CAPILLARY PERFUSION AND BLOOD VOLUME DURING ACUTE HYPOXIA, HYPERCAPNIA OR PENTOBARBITAL ANESTHESIA. P. Robinson, N. Villacreses*, S. Rapoport and Q. R. Smith, Lab. of Neurosciences, NIA, NIH, Bethesda, MD 20892.

Several studies have reported that only a fraction of brain capillaries are perfused under normal conditions and that the fraction varies with cerebral blood flow (CBF), metabolism and physiologic state. To determine whether changes in blood flow result in measurable changes in perfused brain capillary surface area or blood volume, regional blood-brain barrier permeability-surface area (PA) products for [14C]sucrose and [14C]urea, and brain "blood" volumes to [14C]inulin and [3H]dextran were measured in normal awake rats, in rats made acutely (10 min) hypercapnic (10% CO_2) or hypoxic (6% O_2), and in rats anesthetized with sodium pentobarbital (50 mg/kg, i.p.). CBF was quantitated using [14C]IAP. As expected, CBF increased markedly (2-3 fold) during acute hypoxia or hypercapnia and decreased 30-50% with pentobarbital anesthesia. However, there were no statistically significant changes among groups in PA or "blood" volume for any of the four tracers. Differences generally were 30% or less. The results indicate that, if capillary recruitment does occur in brain, it may not be through the addition of previously unperfused, collapsed capillaries, and that small tracer penetration into unperfused capillaries is likely not the explanation for the difference in brain "blood" volumes between large and small tracers.

HORMONAL CONTROL OF BEHAVIOR II

308.1

CHRONIC FIGHTING IS FOLLOWED BY INCREASES IN PLASMA PITUITARY-ADRENOCORTICAL HORMONES AND DECREASES IN TESTOSTERONE AND IMMUNE RESPONSIVENESS IN SUBMISSIVE MALE HAMSTERS. **K.L. Huhman, T.O. Moore, C.F. Ferris[1], E.H. Mougey, E.W. Bernton*, & J.L. Meyerhoff. Dept. Medical Neurosci., Walter Reed Army Institute of Research, Washington, D. C. 20307-5100; [1]Dept. Physiol., U. Mass. Med. School, Worcester, MA 01605.**

This experiment was designed to examine the hormonal and immune responsiveness of dominant and submissive male hamsters following chronic exposure to social conflict. Hamsters were housed in double cages divided by mesh guillotine doors which allowed the animals constant visual, olfactory, and auditory communication with one another. The animals were paired (guillotine doors lifted) twice a day for 15 minutes on five consecutive days. Two observers rated the animals as dominant or submissive. Following the final encounter, we measured plasma adrenocorticotropin (ACTH), cortisol, and testosterone. We also examined splenic lymphocyte proliferation *in vitro* following mitogenic challenge.

Submissive, but not dominant, hamsters exhibited increases in plasma ACTH and cortisol and a decrease in plasma testosterone. Lymphocyte proliferation following challenge with Concanavalin-A was decreased in submissive hamsters. Further study of immune responsiveness following fighting is planned.

308.2

CHANGES IN DISTRIBUTION OF LUTEINIZING HORMONE RELEASING HORMONE (LHRH)-LIKE IMMUNOREACTIVITY FOLLOWING SEXUAL ACTIVITY IN DOVES. **C. Ramos and R. Silver. Barnard College of Columbia Univ.,New York, NY 10027**

LHRH immunoreactivity was examined throughout the brain of ring doves using LR-1 (gift of R. Benoit). Immunoreactive perikarya and/or fibers were consistently observed in the following regions: lining the ependymal wall, medial to the ventriculus olfactorius (VO),medial to the ventriculus lateralis (VL), nucleus septalis lateralis (SL), nucleus preopticus anterior (POA), nucleus preopticus medialis (POM), bed nucleus pallial commissure (nCPa), nucleus septalis medialis (SM), lateral hypothalamic area (LHy),in the midline region coursing towards the decussatio supraoptica (DS), dorsal to DS, nucleus dorsomedialis anterior thalami (DMA), nucleus dorsomedialis posterior thalami (DMP), median eminence (ME), and central gray (GCt). All immunoreactivity was adsorped with mammalian LHRH.

In contrast to isolated doves, birds which had been paired with a novel partner for two hours prior to sacrifice expressed LHRH-like immunoreactivity in the lateral habenula (HL) and in the right hemisphere of a localized region of Field L (FL). Immunoreactivity in the lateral habenula was adsorped with both cGNRHI and cGNRHII (gift of R.Millar) but not with mammalian LHRH. (Supported by NIMH 029380-14).

308.3

SEXUAL CYCLICITY IN MONOGAMOUS PRIMATES. B. Ingelett*, J. French*, and S. Hendricks. Department of Psychology, University of Nebraska at Omaha, Omaha, NE 68182

The distribution of sexual behavior across the ovulatory cycle was examined in adult heterosexual pairs of golden lion tamarins (Leotopithecus r. rosalia). Urinary estrogen and luteinizing hormone were assayed in urine samples obtained from females (N=6) to monitor the ovarian cycle throughout the study. Behavioral interactions between males and females were recorded using a pair test paradigm. Thirty pair tests were conducted in each of the following conditions: 1) partners were randomly assigned immediately prior to each test; 2) partners were specifically assigned but had no interaction outside of the pair tests; and 3) partners were permanently housed together but could not interact sexually except during pair tests. Affiliative behaviors such as remaining in close proximity, sniffing, allo-grooming, and huddling were higher in Conditions 2 and 3 compared to Condition 1. However, sexual behavior (female solicits, sexual mounts, and intromissions) occurred more often between partners in Conditions 2 and 3 than between partners in Condition 1. Sexual behavior was differentially distributed across the ovulatory cycle in each of these conditions. The patterns of social sexual activity will be discussed with regard to sexual cyclicity and monogamous mating systems.

308.4

HORMONAL BASIS OF FEMALE AGGRESSION AND MALE PARENTAL CARE IN SEX-ROLE REVERSED BIRDS. A.J. FIVIZZANI and L.W. ORING* Biology Dept., Univ. of North Dakota, Grand Forks, ND 58202.

A few avian species such as the spotted sandpiper and Wilson's phalarope display reversed sex roles, i.e., females aggressively compete for mates and primarily males incubate eggs and care for chicks. Plasma levels of prolactin, the hormone associated with parental care in birds, is also reversed being greater in concentration in incubating males than in females. The aggressiveness of females in competing for mates, however, is not based upon a reversal of the typical avian pattern of gonadal steroid hormones. Testosterone levels are 6 to 10-fold greater in males than in females, whereas estradiol levels are greater in females. Female phalarope aggression is not based upon atypical brain steroid conversion enzymes as hypothalamic/preoptic aromatase activity was greater in males than in females and reductase activity was equivalent. Female aggressiveness may be dependent upon unusual densities of steroid hormone receptors in adult neural tissue or upon exposure of embryonic brain tissue to atypical hormone profiles. Supported by NSF Grant DCB 8608162.

308.5

EFFECTS OF CHRONIC STRESS ON SEXUAL BEHAVIOR IN OLFACTORY BULBECTOMIZED FEMALE RATS. G.W. Williams*, M.Y. McGinnis and A.R. Lumia*. Biopsychology Prog., Skidmore College, Saratoga Springs, NY 12866 and Dept.Cell Biology/Anatomy Mt. Sinai Sch. Med., CUNY, New York, NY 10029.

Chronic stress activates the hypothalamo-pituitary-adrenal system and alters serum glucocorticoids. Chronic stress also alters ovarian cyclicity suggesting it may affect sexual behavior. Because bilaterally olfactory bulbectomized (BOB) rats are commonly used as a model for studying stress, and show behavioral extrasensitivity to estrogen (E_2), we examined the effects of a subthreshold E_2 dose on chronically stressed, BOB females. Ovariectomized Long-Evans rats received either BOB or sham surgery. Beginning a week later, half the rats in each group received 21 days of predictable chronic stress (strobe light and siren). On day 22 all rats received a 5 mm E_2-filled Silastic capsule for 4h. The next day 500ug progesterone was given and rats were tested for sexual behavior. Chronic stress significantly increased both lordosis and proceptivity regardless of surgical condition. Results suggest that predictable chronic stress induces hypersexuality in both BOB and sham operated female rats.

308.6

SEX DIFFERENCES IN THE SPONTANEOUS LOCOMOTOR ACTIVITY OF THE MEADOW VOLE (MICROTINAE): REVERSAL OF THE USUAL LABORATORY RODENT PATTERN. D.M. Tysdale*, E.L. Hargreaves, M. Kavaliers, K.-P. Ossenkopp, and D.P. Cain. Div. Oral Biology and Dept. Psychology, Univ. Western Ontario, London, Ontario, CANADA N6A 5B7.

The locomotor behavior of a polygynous microtine rodent, the meadow vole, Microtus pennsylvanicus, was examined in the laboratory through a multivariate measurment of spontaneous activity with a Digiscan Animal Activity Monitor. The locomotor activity variables that were measured over 0.5-1.0 hour periods included: total distance travelled (TD), average distance (AD), average speed (AS), number of movements (NM), and time in movement (MT). Significant sex differences in activity were observed, with adult male voles exhibiting greater levels and amounts of activity than adult female voles. These sex differences in activity were present in both wild and laboratory bred voles and were evident across recording sessions and breeding conditions. These sex differences in the spontaneous activity of meadow voles are the reverse of what has been reported for laboratory rats, whereby females are indicated to be more active than males. The present male-female differences in the laboratory activity of meadow voles do, however, agree with the sex differences in activity that have been observed in field studies and are consistent with the ecology of meadow voles. (Supported by Grants from NSERC to M.K., K.P.O. and D.P.C.)

308.7

DEVELOPMENTAL CHANGES IN THE SPONTANEOUS LOCOMOTOR ACTIVITY OF JUVENILE MALE AND FEMALE MEADOW VOLES. M. Kavaliers, D.M. Tysdale*, E.L. Hargreaves, K.-P. Ossenkopp and R.R. Shivers. Div. Oral Biology and Dept. Psychology, Univ. Western Ontario, London, Ontario, CANADA N6A 5B7.

Results of both field and laboratory studies have revealed sex differences in the locomotor activity of adult meadow voles, Microtus pennsylvanicus. Adult males of this polygynous microtine rodent have been shown to have greater levels and patterns of activity than adult female voles. Little is known, however, about the development of these sex differences and the emergence of the adult patterns of activity. In the present study developmental changes in the locomotor behavior of juvenile (7-36 days of age) male and female meadow voles were examined in the laboratory through a multivariate measurement of spontaneous activity with a Digiscan Animal Activity Monitor. The locomotor activity variables that were measured over 0.5-1.0 hour periods included: total distance travelled (TD), average distance travelled (AD), average speed (AS), number of movements (NM) and time in movement (MT). It was found that the spontaneous activity of the juvenile meadow voles differed from that of the adults. The juvenile voles showed no evident sex differences in their spontaneous activity, as well as, no consistent levels or patterns of activity through early (7-21 days of age) juvenile development. These findings indicate that male-female differences in activity are not present during early development in juvenile meadow voles. (Supported by Grants from NSERC to M.K. and K.P.O)

308.8

SEX DIFFERENCES IN THE SPONTANEOUS LOCOMOTOR ACTIVITY OF THE LABORATORY RAT: MULTIVARIATE AND TEMPORAL PATTERNS. E.L. Hargreaves, D.M. Tysdale*, D.P. Cain, K.-P. Ossenkopp, and M. Kavaliers. Dept. Psychology and Div. Oral Biology, Univ. Western Ontario, London, Ontario, CANADA N6A 5C2.

The locomotor behavior of the laboratory rat (Royal Victorian Hooded), Rattus norvegicus, was examined in the laboratory through a multivariate measurment of spontaneous activity with a Digiscan Animal Activity Monitor. Three samples were collected per session with sessions run serially on 4 consecutive days. In one experiment the session had a duration of 0.5 hours, in another, the duration was only .15 hours. Variables examined included: total distance travelled (TD), average distance travelled (AD), average speed (AS), number of movements (NM), time in movement (TM), number of vertical movements (VM) and time in vertical movement (VT). The 0.15 hour sessions revealed significant sex differences on the following variables: TD, AD, AS, and VT, with the female rats exhibiting higher levels of activity. A similar, but not identical pattern of sex differences was seen during the 0.5 hour sessions, where differences were observed on TD, AD, and AS. Within a session across the three samples activity declined for both sexes, with the activity of the females declining faster than that of the males. The only exception to this was observed during the 0.15 hour sessions where NM actually increased across the three samples. The observed sex differences and within session trends were consistent across days. These results support and expand previous findings from wheel running and open-field studies. (Supported by Grants from NSERC to D.P.C., K.P.O. and M.K.)

308.9

MULTIVARIATE ANALYSES OF SPONTANEOUS LOCOMOTOR ACTIVITY OF THE LABORATORY RAT ACROSS THE ESTROUS CYCLE: A PRELIMINARY REPORT.
D. Sauvé*, E.L. Hargreaves, D.J. Stewart and D.P. Cain. Dept. Psychology, Univ. Western Ontario, London, Ontario, CANADA N6A 5C2.

The spontaneous locomotor behavior of the laboratory rat (Royal Victorian Hooded), Rattus norvegicus, was monitored across the estrous cycle through multivariate measurement of activity with a Digiscan Animal Activity Monitor. Three samples were collected per session with each session having a duration of 0.15 hours. Activity sessions were run invariantly between 17:30-18:30 approximately 3 hours prior to the onset of the dark phase. Weights and vaginal smears were taken immediately after each session. The experiment was run for 40 days with a minimum of 5 cycles collected from each animal. Males were simultaneously run to account for non-hormonal influences on spontaneous activity. Variables examined were selected for their past ability to show sex differences. For similar reasons only data from the first sample of the session were analysed. Results indicate that spontaneous locomotor activity across all testing days was higher for females, but failed to indicate consistent changes across the estrous cycle. However, similar analyses of body weight across the cycle indicated that it was lowest at estrous and highest at diestrous. These results concur with previous findings of weight changes and sex differences, but fail to replicate those of activity across the estrous cycle. Analyses continue on the remaining samples and unexamined variables. (Supported by Grants from NSERC to D.P.C.)

308.10

INTRACRANIAL ANDROGENIC AND ESTROGENIC STIMULATION OF MALE-TYPICAL BEHAVIORS IN HOUSE MICE.
John Nyby[1], John Matochik[2] & Ronald Barfield[2]. [1]Dept. of Psychology, Lehigh University, Bethlehem, PA, 18015, [2]Dept. of Biological Sciences, Rutgers University, New Brunswick, NJ, 08903.

Two experiments were conducted to examine the neural sites of action of steroid hormones in the activation of male-typical behaviors. Male mice were castrated and randomly assigned to 6 treatment conditions. In Experiment 1, four groups bilaterally received 27 ga. cannulae containing testosterone (T) into either the SEPTUM (N=9), MPO (N=9), AHA (N=8), or VMH (N=7). The two control groups received silastic capsules of T (TSIL, N=7) or empty silastic capsules (ESIL, N=7).

The TSIL males performed all behaviors at male-typical levels while the ESIL were unresponsive. MPO males emitted ultrasonic mating vocalizations at high levels while few vocalizations were seen in the other groups. The VMH and MPO males urine-marked at higher levels than the ESIL but did not exhibit the high levels of the TSIL. Aggression and mounting were rare in males from any of the brain implant groups.

In the second experiment, the hormone activity of the implants was increased by using testosterone propionate (TP) or a 50% mixture of estradiol (E2) and cholesterol. The six groups were: SEPTUM TP (N=9), SEPTUM E2 (N=9), MPO TP (N=10), MPO E2 (N=10), TPSIL (N=9), and ESIL (N=9). TP was effective at restoring vocalizations and urine marking only when placed in the MPO, however E2 was effective at both sites. Again aggression and mounting were rare in the implant groups.

In conclusion, implants of T and TP were effective in restoring ultrasonic mating vocalizations and urine marking when placed into the MPO. The restorative effects of E2 were less localized which is probably related to the greater hormonal activity of this treatment.

(Supported by a sabbatical grant from Lehigh and NIH grant HD-04484.)

308.11

PERIPHERAL SEXUAL DIMORPHISM WITHOUT CORRESPONDING CNS DIMORPHISM IN A HORMONE-SENSITIVE NEUROMUSCULAR SYSTEM.
C.M. Seiwert and E. Adkins-Regan Department of Psychology, Cornell University, Ithaca, NY, 14853.

The foam gland complex (FGC) of Japanese quail (Coturnix japonica) is a large sexually dimorphic structure located in the dorsal cloaca. The FGC consists of an aggregate gland whose units are interdigitated with fibers of the sphincter cloacae muscle (mSC). The gland secretes a fluid that is whipped into a stiff foam, possibly by the undulation of mSC (Klemm et al., 1973), which is transferred to the female during copulation. Although the FGC is present and androgen-sensitive in both sexes, in females it is much smaller and no foam is produced (Nagra et al., 1959). To investigate possible neural correlates of this peripheral sex difference, the dendritic field of mSC motor neurons was characterized in male and female Japanese quail. 0.5µl of 0.2% cholera-toxin conjugated horseradish peroxidase (CT-HRP) was injected into the dorsal midline of mSC and the FGC was measured externally. Because it was not possible to assign arbor to specific neurons, the dendritic field of the entire population of neurons was measured. Analyses in the medial-lateral and rostral-caudal planes revealed no evidence of sex differences in the mean, minimum, or maximum length or width of the dendritic field [length: mean, $t(7)=0.6$, $p>0.1$; minimum, $t(7)=0.736$, $p>0.1$; maximum, $t(7)=.044$, $p>.01$; width: mean, $t(7)=.049$, $p>.01$; minimum, $t(7)=.038$, $p>.01$; maximum, $t(7)=.32$, $p>.1$]. A more detailed analysis of dendritic length also provided no indication of sexual dimorphism [$F(1,8)=1.362$, $p>0.25$]. These data, in combination with our earlier finding that there is no sex difference in soma size of mSC motor neurons, are in striking contrast to the large sex difference in FGC area (males: x±sem= 165±15mm^2; females: x±sem=49±6mm^2; $t(14)=7.18$, $p<.0005$]. Future work will address the possiblity of a sex difference in the number of motor neurons innervating mSC and will further characterize the dimorphim in mSC itself. [Supported by NSF #BNS 88-09441.]

308.12

DETERMINATION OF AROMATASE ACTIVITY AND ESTROGEN RECEPTOR LEVELS IN DISCRETE BRAIN REGIONS FROM INTACT AND CASTRATED HYBRID B6D2F1 MALE HOUSE MICE THAT COPULATE AND THOSE THAT DO NOT.
Sinchak, K.[1], C.E. Roselli[2] and L.G. Clemens[1]. [1]Dept. Zoology, Michigan State Univ., E. Lansing, MI 48824. [2]Dept. of Physiology, Oregon Health Sciences Univ., Portland, OR 97201.

After castration, most males of the B6D2F1 hybrid strain of house mouse (Mus musculus) continue to exhibit an ejaculatory reflex (continuers) for six to twelve months while others do not (noncontinuers). Retention of the ejaculatory reflex in castrated B6D2F1 continuer males appears to be estrogen dependent. In the present study we wished to determine if neural aromatase activity and estrogen receptor levels were different between castrated continuer and noncontinuer and intact B6D2F1 males. Discrete brain regions, preoptic area (POA), hypothalamus (HYP) and amygdala (AM), were microdisected out and radioassayed to measure aromatase acitivity (AA) and estrogen receptors levels. In general, aromatase activity and estrogen receptors were the same in both castrated continuers and noncontinuers. However, castration significantly reduced without abolishing aromatase activity in the POA and HYP ($p < 0.01$). The AM was not affected when compared to intacts. Compared to intact males castration did not affect nuclear estrogen receptors (nER)(fmol/mg DNA) in the POA and HYP, but reduced AM nER. Cytosolic and total estrogen receptors (fmol/mg DNA) increased after castration in all three brain areas with significant increases in the POA and AM ($p < 0.05$). In conclusion, although estrogen is important for the expression and maintenance of the ejaculatory reflex after castration, we cannot distinguish between castrated continuer and noncontinuer B6D2F1 male mice based on levels of aromatase activity or estrogen receptors in the POA, HYP, and AM.

308.13

INFLUENCE OF THE VASOPRESSIN-DEFICIENCY OF BRATTLEBORO (BB) RATS ON A SEXUALLY DIMORPHIC BEHAVIOR.
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We have examined the interaction of gender with vasopressin (VP)-deficiency using a conditioned taste aversion (CTA) paradigm. The extinction of CTAs has been shown to be steroid-dependent such that male rats retain a taste aversion longer than females. VP induces a long-term inhibition of extinction of an avoidance response whereas VP-deficient BB rats are not able to retain such a response. The expression of the VP gene and VP immunoreactivity in the brain is also steroid-dependent. We reasoned that gonadal steroids might influence the retention of a CTA by acting on the VP system and that homozygous BB rats therefore would not exhibit sex differences in a CTA.

Three rat strains were used: BB homozygous (BB-HO), heterozygous (BB-HET), and Long-Evans (LE). Male and female LE and BB-HO and males of BB-HET strains were given 10% sucrose to drink, then injected with LiCl to create a taste aversion to the sucrose. Rats that developed aversions were given ad lib access to water and sucrose solution for 3 weeks and their fluid intake was monitored.

Overall, the strain comparison showed that BB males extinguished faster than LE males with the BB-HETs showing intermediate retention. The gender comparison indicated a reverse pattern of extinction. While LE females extinguished quickly, BB-HO females maintained their aversions for much longer. The results of these preliminary studies suggest that expression of the sexually dimorphic retention of CTA is dependent on an intact VP system.

308.14

INSULIN-INDUCED ANESTRUS IN SYRIAN HAMSTERS.
J. E. Schneider and G. N. Wade. Dept. of Psychology and Neuroscience and Behavior Program, Univ. of Massachusetts, Amherst, MA 01003.

In previous experiments manipulations that decreased the availability of metabolic fuels interrupted estrous cycles in Syrian hamsters. Low body fat content, food deprivation, pharmacological inhibition of both glucose and fatty acid utilization, and housing at cold ambient temperatures all increased the incidence of anestrus. If estrous cycles are sensitive to the general availability of metabolic fuels it would be predicted that anestrus would result from enhanced energy storage. In the present experiment, energy storage was promoted by treatment with protamine zinc insulin (PZI, 5U every 12 hr starting on day 1 of the estrous cycle). PZI hamsters fed *ad libitum* increased food intake, body weight and body fat levels, however, there were no effects on estrous cycles. PZI hamsters limited to 110% of their pretreatment food intake also increased body weight and fat content and showed decreased plasma glucose levels but, in contrast to *ad lib*-fed hamsters, showed a significant increase in the incidence of anestrus. We suggest that in the absence of hyperphagia, PZI-enhanced energy storage leads to a shortage of oxidizable metabolic fuels with the result that reproduction is inhibited in favor of processes critical for survival such as cellular maintenance, thermoregulation, or foraging. It is unlikely that anestrus was due to a pharmacological action of PZI unrelated to its effects on metabolic fuel partitioning, since PZI had no effect on estrous cycles in the *ad lib*-fed hamsters. These findings also raise the possibility that infertility associated with some types of obesity could be due in part to a disorder of macronutrient partitioning. Supported by NS10873-17, AM32976-06 from NIH, MH00321-09 from NIMH, and BNS8719361 from NSF.

308.15

LUTEINIZING HORMONE (LH) RELEASE IS ATTENUATED IN PRENATALLY-STRESSED (P-S) MALE RATS EXPOSED TO SEXUALLY-RECEPTIVE FEMALES. C.H. Kinsley[1,2], P.E. Mann[2] & R.S. Bridges[2]. [1]Dept. Psychol., Univ. of Richmond, VA 23173 and [2]LHRRB, Harvard Medical School, Boston, MA 02215.

P-S males show reductions in male sexual behavior, medial preoptic area volume and levels of circulating testosterone. We examined the LH response to sexually receptive females, a known index of arousal. Adult male offspring from mothers stressed on days 15-22 of pregnancy (thrice-daily exposures to heat, light and restaint) were implanted with an intra-atrial catheter. 48 hours later they were placed into test chambers divided by a wire mesh partition; the catheter was extended outside the chamber. 30 min later a baseline blood sample (time 0') was taken. Then a sexually-receptive female was placed on the side of the partition opposite the male. Blood was taken at 5, 10, 15, 30, 60 min and 24 h after introduction of the female. With the exception of the 0' sample, P-S males exhibited significantly reduced LH levels in response to the female. These data suggest that attenuations in LH release may account, in part, for the marked reductions in male sexual behavior characteristic of the P-S male.

308.16

CORTISOL MICROINJECTIONS IN THE ANTERIOR HYPOTHALAMUS (AH) EXERT DOSE- AND STEROID-SPECIFIC EFFECTS ON THE AGONISTIC BEHAVIORS OF MALE SYRIAN HAMSTERS. D.M. Hayden-Hixson and C.F. Ferris. Dept. of Physiol., U. Mass. Med. Sch., Worcester, MA 01655.

Previous studies have shown that cortisol (F) implants in AH have site- and context-dependent effects on the agonistic behaviors of male Syrian hamsters. To determine whether these effects were dose- and steroid-specific, male hamsters (N=8/ treatment) received 100nL injections of (a)1 of 6 doses of F (10^{-8}M, 10^{-7}M, 10^{-6}M, 10^{-5}M, 10^{-3}M, 10^{-2}M); or (b)1 of 2 doses (10^{-6}M, 10^{-3}M) of 1 of 6 steroids (testosterone/T; β-estradiol/βE_2; dihydrotestosterone/DHT; progesterone/P_4; deoxycorticosterone; F). Experimentals were tested a)for dominant/subordinate responses (D/S test) 5min postinjection in paired encounters with vehicle-injected Controls; b)for odor-induced flank marking (OI-FM tests) 20min & 24hr postinjection.

Significant ($P<.05$) effects of F, P_4 and DOCA were observed in D/S-tests (10^{-6}M: F induced aggression, P_4 submission; 10^{-3}M: F induced submission, DOCA aggression); of βE_2 and DHT in 20min & 24hr OI-FM tests (10^{-6}M βE_2 stimulated; 10^{-6} & 10^{-3}M DHT inhibited flank marking). These results suggest that in AH, F and P_4 act to promote aggression and submission, respectively; while T-aromatization stimulates and T-reduction inhibits flank marking in response to the odors of conspecifics. (Work supported by NIH grant #NS23557).

308.17

SEXUAL RESPONSIVENESS OF OLD AND YOUNG FEMALE RATS TO ESTRADIOL. K.C. Chambers, D.L. Yuan, and E.A. Brownson. Depts. of Psychology and Neurobiology, Univ. So. Cal., Los Angeles, CA 90089.

This study was designed to determine whether there are age-related changes in behavioral sensitivity to estradiol (E) in gonadectomized female rats. The 18 old (25.5-27.5 mo) and 23 young (2.5 mo) Fischer 344 rats were given tests of sexual behavior after injections of 3 different doses of estradiol benzoate (EB; 0.1, 1.0, or 10.0 ug/250 g of bodyweight) and 1 dose of progesterone (P; 0.5 mg/250 g of body weight). The frequencies of 3 different intensity levels of a receptive behavior (lordosis in response to a mount) and a proceptive behavior (crouch) were measured. There were no differences in the total frequencies (all intensity levels combined) of the lordosis and crouch responses shown by the old and young females after injections of each dose of EB. However, young females exhibited higher frequencies of the most intense level of the lordosis responses after injections of each dose of EB and higher frequencies of the 2 most intense levels of the crouch responses after injections of the 10 ug dose of EB. These data indicate that age-related deficits in the display of these receptive and proceptive behaviors are a function of the intensity of the response rather than the frequency. Factors other than decreased sensitivity to E most likely account for deficits in intensity of response. HD 20970

308.18

SIMILAR BEHAVIOURAL EFFECTS OF ADRENALECTOMY HYPOTHYROIDISM AND FOOD DEPRIVATION IN THE PORSOLT SWIM TEST

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In the Porsolt swimming test intact animals become progressively immobile over a 15 min test period. On retest 24h later, they remain immobile for ~ 70% of the 5 min test period. Adrenalectomy (ADRX) blocks retention of the response, which is restored by dexamethasone (DM) or ketocyclazocine (KCZ), given at test but not retest. Propylthiouracil (PTU) treated, hypothyroid rats neither acquire or retain the response, both of which are reversed by thyroxine (T_4) given at test. The present studies show that (a) the behavioural effect of ADRX (surgical, or with peripheral receptor antagonists MR2266 and RU486) is reversed by T_4; (b) the effect of PTU is reversed by DM, ADRX or to a lesser extent KCZ; (c) rats food-deprived for 24h acquire but do not retain the response, an effect reversed by DM, KCZ, T_4 or ADRX; and (d) rats food-deprived for 48h acquire and retain the response, which is blocked by ADRX. We interpret these counterintuitive findings as suggesting (i) possible physiological equivalents of the commonly described pharmacological U-shaped dose response curves; (ii) and important regulatory role for metabolic state in the acquisition and retention of the immobile response; and (iii) a complex effector interaction between glucocorticoids and thyroid hormones, at either the receptor or post-receptor level.

308.19

AGE-RELATED DEFICITS IN BRAIN ESTROGEN RECEPTORS AND SEXUAL BEHAVIOR OF MALE RATS. C.E.Roselli, J.E.Thornton and K.C. Chambers. Physiology Dept., OHSU, Portland, OR 97201 and Psychology Dept., USC, Los Angeles, CA 90089.

Neural estrogen receptors and sexual behavior were measured in young (5 mo) and old (24.5 mo) Fischer 344 male rats that were gonadally intact (I), gonadectomized (GX),or GX and testosterone-treated (GX-T). After behavioral testing, rats were anesthetized, perfused with 10% DMSO and their brains removed and frozen (4 days). The levels of cytosolic (ERc) and nuclear (ERn) estrogen receptors were measured in the preoptic area (POA), hypothalamus (HYP), and amygdala (AMG). Whereas all of the young I males ejaculated during sexual behavior tests, none of the old I did and their mean ERn levels in POA and AMG were ~50% lower than levels in young I males. Gonadectomy eliminated ejaculatory behavior and reduced ERn levels in both old and young males. After treatment, only 33% of the old GX-T males ejaculated while 86% of the young GX-T males ejaculated. ERn levels in old GX-T males were 30-60% lower than levels in young GX-T males, however, they were comparable to young I levels. These data suggest that there is an association between aging and decreased neural ERn levels. Since testosterone treatment increased ERn levels but not the sexual performance levels of old males to those found in young males, the age-related deficits in behavior are probably not due to changes in ERn. (Supported by HD23293 and HD20970.)

309.1

COCAINE INDUCES RAPID EXPRESSION OF C-FOS AND ZIF/268 IN RAT BRAIN. B. M. Cohen, T. Van Nguyen*, S. M. Babb*, S. E. Hyman, Mailman Research Center, McLean Hospital, 115 Mill St., Belmont, MA 02178 and Molecular Neurobiology Lab., MGH, Boston, MA 02114.

The precise mechanisms contributing to the development of dependence on drugs of abuse remain unknown, but are likely to involve long-term changes in neural functioning. We have been studying early neural events that occur in response to cocaine administration that may contribute to long-term plastic changes in the brain. We have studied the regulation of immediate early gene expression in rat brain after single doses (3 mg or 10 mg) of cocaine given IV. Robust and dose-dependent induction of c-fos mRNA is observed 45 minutes after cocaine administration in frontal and occipital cerebral cortex, cerebellum, amygdala, hippocampus, and striatum. By 75 minutes c-fos mRNA has returned to basal levels. Another immediate early gene, Zif/268 is induced in frontal, parietal, and occipital cortex, amygdala, and hippocampus in dose dependent fashion. Induction of zif/268 is observed 45 minutes after cocaine administration and, in contrast to c-fos, is still observed 75 minutes after administration. Studies of immediate early gene expression permit us to map cells which are activated by cocaine. In addition, since many immediate early genes have been shown to function as transcription factors, their repeated activation with multiple doses of cocaine may lead to alterations in expression of genes involved in neuronal signaling, with important functional consequences. Studies of gene expression following repeated dosing are in progress.

309.2

IDENTIFICATION OF COCAINE-REGULATED PROTEINS IN RAT NUCLEUS ACCUMBENS BY SUBTRACTION HYBRIDIZATION. K.A. Sevarino, J.R. Walker, R.S. Duman, and E.J. Nestler, Laboratory of Molecular Psychiatry, Depts. of Psychiatry and Pharmacology, Yale Univ. School of Medicine, New Haven, CT 06508.

The mesolimbic dopamine pathway from the ventral tegmental area to the nucleus accumbens (NAc) plays a major role in the reinforcement of addictive behavior. In the NAc, behavioral, electrophysiological, and neurochemical studies indicate that chronic exposure to the powerfully addicting drug cocaine results in sensitization to the effects of the drug. To define the biochemical changes that underlie this sensitization we have set out to identify mRNA species regulated in the NAc by chronic cocaine treatment. (Parallel studies on cocaine regulation of second messengers and protein phosphorylation in the NAc are presented herein by Beitner et al. and Nestler et al.)

We isolated mRNA from the NAc of control rats and rats treated chronically with cocaine. In vitro translation experiments detected a number of mRNA species regulated by chronic cocaine treatment. From the control and treated mRNA pools we synthesized cDNA libraries of 10^6 recombinants each. The libraries were screened with subtracted probes generated by hybridizing cDNA from control rats with an excess of biotinylated mRNA from treated rats, and vice versa, to isolate down- and up-regulated mRNAs, respectively. We are using Northern blot analysis and sequencing studies to examine positive clones.

Characterization of proteins in the NAc that are regulated by chronic cocaine exposure will provide important information regarding the molecular basis of drug addiction.

309.3

ENHANCED D_2 RECEPTOR BINDING FOLLOWING CHRONIC COCAINE TREATMENT IS REVERSED BY BROMOCRIPTINE. D.W. Clow and R.P. Hammer, Jr. Dept. Anatomy & Reprod. Biol., Univ. Hawaii Sch. Med., Honolulu, HI 96822.

Cocaine blocks neuronal uptake of dopamine which in turn alters the post-synaptic response to this neurotransmitter. During abstinence following chronic cocaine treatment regional cerebral metabolism is selectively reduced in dopaminergic regions and this can be reversed by bromocriptine (BROM) treatment (Clow and Hammer, *Neuropsychopharmacology*, in press). In the present study, we investigated the effect of BROM during abstinence following chronic cocaine treatment on D_2 receptor binding Male Sprague-Dawley rats were given 14 daily i.p. injections of cocaine HCl (10 mg/kg/ml) or saline followed by 3 daily injections of BROM (10 mg/kg/ml) or vehicle. Animals were then prepared for quantitative 2-[^{14}C]deoxyglucose autoradiography, and the same brains were subsequently prepared for autoradiographic analysis of [^{3}H]spiroperidol binding. D_2 receptor binding was significantly increased relative to control during abstinence following chronic cocaine administration, in the rostral but not the caudal portion of nucleus accumbens (NAc) (+48.76%), and in olfactory tubercle (+51.04%), caudatoputamen (+44.43%), and substantia nigra pars compacta (+125.25%). The effect was significantly reversed in these regions by BROM administration. The increased binding in rostral NAc correlates well with the reduced metabolic activity observed in adjacent sections from the same brain region ($r=-0.8612$, $p\leq 0.05$). These results indicate that chronic cocaine administration results in a sustained increase in D_2 binding which is rapidly reversed by bromocriptine intervention. Cocaine abstinence-induced D_2 alterations in NAc may represent a regional substrate for bromocriptine-induced metabolic recovery in this region. (Supported by USPHS Awards DA04081 and NS01161)

309.4

A RAPID BINDING ASSAY FOR SOLUBLE DOPAMINE TRANSPORTERS. R.A. Vaughan*, R. Simantov, R. Lew, E. Webster and M.J. Kuhar. Neuroscience Branch, NIDA Addiction Research Center, Baltimore, MD 21224.

Presynaptic dopamine transporters have been implicated as sites of cocaine action in membrane binding and synaptasomal uptake experiments. A binding assay for solubilized transporters has previously been developed using ^{3}H-GBR 12935. However, this method is limited by the number of samples that can be processed at one time. We have subsequently developed a charcoal binding assay using the cocaine derivative ^{3}H-WIN 35,428 with which hundreds of samples can be rapidly processed.

Dopamine transporters from dog caudate nucleus were solubilized with 1% digitonin. Binding activity was measured using ^{3}H-WIN 35,428 with mazindol (30 µM) to define non-specific binding. Specific binding was observed in caudate but not in cerebellum and was linear with tissue concentration (20-200 mg/ml original wet weight). Binding was saturable (B_{max} = 0.1 pmole/mg protein) and of high affinity (K_d = 4nM). Soluble binding was inhibited by mazindol, GBR 12909, (-)cocaine, in a manner typical for binding to the dopamine transporter. Binding was also stereospecific with (+)cocaine being 100-fold less potent than (-)cocaine. Finally citalopram and desipramine exhibited low affinity for ^{3}H-WIN 35,428 binding, thus binding was neither to serotonergic nor noradrenergic transport sites.

309.5

IN VIVO BINDING, INTERNALIZATION, AND DOPAMINE-RELEASING EFFECTS OF [H-3]-CFT (WIN 35,428): A POTENTIAL BRAIN IMAGING LIGAND FOR DOPAMINE TRANSPORTER OR COCAINE RECEPTOR SITES. **J.J. Chen*, J.L. Neumeyer, R.A. Milius, R.M. Brown, and C.C. Chiueh,** NIMH & NIDA, Bethesda, MD 20892 and RBI, Natick, MA 01760.

CFT (WIN 35,428) has been shown to exhibit a high affinity for cocaine receptors in vivo and in vitro. In the present study, ^{3}H-CFT (5 µCi) was injected into striatum 10 min prior to the initiation of an intra-striatal microdialysis procedure (1 µl/min) in anesthetized rats. (-)-Cocaine and its tropanyl analogs, CFT, diphenylethylenetropane, and benztropine were added to Ringer solution and perfused for 5 min into the brain through the probe. Dialysates were assayed for tritium overflow or dopamine efflux by HPLC.

The resting efflux of newly bound ^{3}H-CFT from striatum was enhanced 2-3 fold by MPP^+ (a non-tropanyl dopamine uptake blocker and a metabolite of MPTP) or cold CFT. Similar to but not identical to MPP^+, all tested tropanyl compounds produced a calcium-dependent exocytosis of striatal dopamine. ED_{50} of CFT and cocaine for releasing endogenous dopamine were 0.3 and 12 nmol, respectively. It appears that after binding to the dopamine transporter or the cocaine receptor, CFT can be internalized, and be subsequently released from brain dopaminergic terminals in vivo.

309.6

COMPARISON OF [^{3}H]CFT AND [^{3}H]MAZINDOL BINDING IN MOUSE STRIATUM. M.E.A. Reith and G. Selmeci . Center for Neurochemistry, NKI, Ward's Island, New York, NY 10035.

Dopamine uptake sites can be labeled with various tritiated ligands such as GBR 12935, methylphenidate, nomifensin, mazindol (MZ), cocaine and WIN 35428 (CFT), but it still under debate whether a common binding site is involved. In the present study [^{3}H]CFT and [^{3}H]MZ binding was compared in the same preparation (fresh mouse striatal P_2 membranes) under identical conditions (25 mM phosphate, 48 mM Na^+, pH 7.7; 0°C for 2 h). One-site kinetics were observed for both [3H]CFT and [^{3}H]MZ. Three lines of evidence support a common binding site. First, [^{3}H]CFT binding was competitively inhibited by 15 nM MZ (K_d increased from 9 nM in the absence to 28 nM in the presence of MZ, without a change in B_{max}, 4.4 pmol/mg prot.). Vice-versa, [^{3}H]MZ binding was competitively inhibited by 10 nM CFT (K_d from 9 to 23 nM, unchanged Bmax of 5.0 pmol/mg). Second, the dissociation rate of [^{3}H]CFT or [3H]MZ binding (k_{-1} 0.34 and 0.36 min^{-1}) was not affected by the presence of 1 uM MZ or CFT. Third, pretreatment of membranes with N-ethylmaleimide affected [^{3}H]CFT and [^{3}H]MZ binding equally. Conditions used by others for [3H]CFT (frozen tissue, high-speed centrifugation, Tris-Na^+) produced an additional low-affinity binding component. Inhibition of [^{3}H]GBR 12935 binding by CFT under our conditions involved a reduction in Bmax. The present results are consonant with the existence of a common binding site for CFT and MZ, probably distinct from the GBR 12939 site. (Supp.NIDA 03025)

309.7

AUTORADIOGRAPHIC LOCALIZATION OF [^{3}H]CFT BINDING SITES IN SQUIRREL MONKEY BRAIN. M.J. Kaufman, R.D. Spealman and B.K. Madras. Harvard Medical School, New England Regional Primate Research Ctr., Southborough, MA 01772.

The high affinity cocaine analog [^{3}H]CFT (WIN 35,428; 2β-carbomethoxy-3β-(4-fluorophenyl)-tropane) was used to map cocaine binding sites in squirrel monkey brain. Tissue sections (20 μm) were incubated with 5 nM [^{3}H]CFT alone or in the presence of 30 μM (-)-cocaine to define total and nonspecific binding, respectively. The highest densities of [^{3}H]CFT binding were observed in caudate nucleus, putamen, nucleus accumbens, and olfactory tubercle. In these regions, approximately 97% of the total binding was specific. Moderate binding densities were found in the substantia nigra, amygdala, the stria terminalis, zona incerta and hypothalamus. Little or no binding was observed in cortical regions, the globus pallidus, and cerebellum. Previous studies indicate that [^{3}H]CFT binding sites in striatum are associated with the dopamine uptake system and may play a role in the behavioral effects and abuse liability of cocaine. The functional significance of [^{3}H]CFT binding sites in other brain regions and the relevance of these sites to the behavioral effects of cocaine is not yet known. (Supported by USPHS grants DA00088, DA00499, DA06303 and RR00168.)

309.8

^{3}H/^{11}C-WIN-35,428 LABELS THE COCAINE RECEPTOR IN VIVO. U. Scheffel*, R.F. Dannals*, A.A. Wilson*, H.T. Ravert*, J.W. Boja, M. Stathis* and M.J. Kuhar. Department of Radiology, Johns Hopkins Medical Institutions and Neuroscience Branch, NIDA Addiction Research Center, Baltimore, MD.

^{3}H-WIN-35,428 has recently been identified as a useful radioligand for in vivo labeling of the cocaine receptor located on the dopamine transporter (Synapse 4:390, 1989). With a view toward developing a PET ligand for imaging cocaine receptors in the living human brain, we have further evaluated the in vivo binding characteristics of ^{3}H-WIN-35,428 and have synthesized ^{11}C-WIN-35,428 and studied its biodistribution in mice.

For ^{3}H-WIN-35,428 the highest striatal/cerebellar (Str/Cb) ratio in the mouse was 4.0:1 at 60 min after intravenous injection. In vivo ^{3}H-WIN-35,428 binding was saturable and was inhibited by (-)cocaine and dopamine uptake blockers (mazindol, GBR-12909, nomifensine), but not by drugs with different sites of action.

^{11}C-WIN-35,428 was prepared from nor-WIN-35,428. After i.v. injection, ^{11}C-WIN-35,428 accumulated mainly in the striatum. The Str/Cb ratio reached 7.2:1 at 60 min and 9.4:1 at 90 min. ^{11}C-WIN-35,428 in vivo binding was found to be saturable and was blocked by (-)cocaine.

These results indicate that ^{3}H- and ^{11}C-WIN-35,428 are potent and selective in vivo labels of cocaine receptors. ^{11}C-WIN-35,428 should be an excellent PET ligand for studying cocaine receptors in humans.

309.9

NEW PHOTOAFFINITY PROBES FOR THE COCAINE RECEPTOR. R. Lew, J.W. Boja, R. Simantov, F.I. Carroll*, A. Lewin* and M.J. Kuhar. NIDA Addiction Research Ctr, Baltimore, MD 21224 and RTI, Research Triangle Park, NC 27709.

The cocaine binding site located on the dopamine transporter has been studied with a variety of compounds including two photoreactive compounds based upon GBR 12935. However, GBR 12935 has been reported to recognize a single affinity site, whereas cocaine and its congeners recognize both a high and low affinity site. Two novel photoaffinity probes for the cocaine receptor have been developed that are analogs of cocaine and WIN 35,065-2. p-Azidobenzoylecgonine methyl ester tartrate (AZIDO-COC) and 3 -[4-azido-phenyl]-tropan-2 -carboxylic acid methyl ester tartrate (AZIDO-WIN) both displace specifically bound [^{3}H]WIN 35,428 with high affinity (IC_{50} = 748 nM and 1.21 nM). Exposure to UV light resulted in irreversible covalent attachment to a protein located within the dopamine transporter as evidenced by continued inhibition of [^{3}H]WIN 35,428 binding following 3 washes with buffer. Futhermore, AZIDO-WIN significantly inhibited ^{125}I-1-[2-(diphenyl-methoxy)ethyl]-4-[2-(azido-3-iodophenyl)ethyl] piperazine binding to the dopamine transporter.

309.10

HIGH AFFINITY INHIBITION OF THE COCAINE BINDING SITE BY NOVEL COCAINE ANALOGS. J.W. Boja, T. Kopajtic*, F.I. Carroll*, A. Lewin* and M.J. Kuhar. Neuroscience Branch, NIDA Addiction Research Ctr, Baltimore, MD 21224 and Research Triangle Institute, Research Triangle Park, NC 27709.

Several structural congeners of cocaine were prepared and tested for their ability to inhibit specific [^{3}H]WIN 35,428 binding and [^{3}H]dopamine (DA) uptake. Previous studies have demonstrated the addition of fluorine to the para position of the phenyl ring of WIN 35,065-2 (WIN 35,428) increases its potency. Azido-, methyl-, chloro- and bromo-substitutions in the para positions of WIN 35,065-2 resulted in increased potency to inhibit both [^{3}H]WIN 35,428 binding and [^{3}H]DA uptake. These compounds are the most potent cocaine analogs that have been reported to date.

Compound	Binding IC_{50}	DA Uptake IC_{50}
Cocaine	102.0 (nM)	5.05 (μM)
WIN 35,065-2	23.0	0.96
WIN 35,428	15.7	0.51
Azido-WIN	2.1	0.20
Methyl-WIN	1.7	0.11
Chloro-WIN	1.2	0.03
Bromo-WIN	1.2	0.03

309.11

PRELIMINARY EVIDENCE THAT THE HIGH AFFINITY DOPAMINE REUPTAKE INHIBITOR, GBR12909, ANTAGONIZES THE ABILITY OF COCAINE TO ELEVATE EXTRACELLULAR LEVELS OF DOPAMINE. **R. B. Rothman[1], A. Mele[2], A. A. Reid[3], H. C. Akunne[1], N. Greig[4], A. Thurkauf[3], B. R. de Costa[3], K. C. Rice[3] and A. Pert[2].[1]Unit on Receptor Studies, LCS, NIMH;[2]Biological Psychiatry Branch, NIMH;[3]Laboratory of Medicinal Chemistry, NIDDK;[4]Laboratory of Neurosciences, NIA; Bethesda, MD 20892.**

Systemic administration of GBR12909 (10, 20, 40 and 100 mg/kg) to rats resulted in a dose-dependent inhibition of [^{3}H]cocaine, and a dose-dependent decrease in the Bmax of [^{3}H]GBR12935 binding sites in striatal membranes prepared 60 min after drug administration, consistent with a persistent occupation of the dopamine (DA) transporter by this high affinity inhibitor of DA reuptake. In *in vivo* microdialysis studies, anesthetized rats were administered either GBR12909 (25 mg/kg) or saline, and the levels of extracellular DA (ECDA) measured. GBR12909 did not produce a statistically significant increase in ECDA levels. Administration of cocaine (0.1, 1.0 and 10 mM) through the microdialysis probe increased ECDA levels by 467, 686, and 2101 % in saline-treated animals, and 174, 248 and 732 % in GBR12909-treated animals. These data demonstrate that doses of GBR12909 sufficient to persistently occupy the DA transporter also attenuate the ability of cocaine to raise ECDA levels.

309.12

SUBSTITUTED 3-PHENYLCARBAMATE ECGONINE ANALOGUES: SYNTHESIS AND ACTIVITY AS INHIBITORS OF COCAINE BINDING AND DOPAMINE UPTAKE. R.H. Kline, Jr.*, J. Wright*, A.J. Eshleman*, K.M. Fox*, M.E. Eldefrawi* and A.T. Eldefrawi. Dept. of Biomedicinal Chem. and Dept. of Pharmacology and Experimental Therapeutics, Univ. of Maryland, Baltimore, MD 21201.

Substituted 3-phenylcarbamate ecgonine analogues were synthesized and characterized by ^{1}H and ^{13}C NMR, IR and MS. The compounds were synthesized as (-)-stereoisomers from (-)-cocaine. These analogues were assessed for their ability to inhibit [^{3}H]-cocaine binding to rat striatal tissue and to inhibit [^{3}H]-dopamine uptake into synaptosomes prepared from the same tissue. The most potent of the analogues towards inhibition of [^{3}H]- cocaine binding was methyl (1RS-2-exo-3-exo)-3-(3-trifluoromethylphenylcarbamate)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate which showed an IC50 value of approximately 140nM. Methyl (1RS-2-exo-3-exo)-3-(3-nitrophenylcarbamate)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate was most potent in blocking dopamine uptake with an IC50 value of approximately 120nM. The least potent compound was methyl (1RS-2-exo-3-exo)-3-(4-trifluoro-methylphenylcarbamate)-8-methyl-8-azabicyclo[3.2.1]octane-2-carboxylate which showed approximate IC50 values of 6.3μM and 30μM with inhibition of cocaine binding and dopamine uptake, respectively. Funded in part by a grant from NIDA (DA03680).

309.13

REGULATION OF RELEASE-MODULATING TERMINAL DOPAMINE AUTORECEPTORS: EFFECT OF CHRONIC COCAINE TREATMENT. S.-J. Yi and K.M. Johnson. Dept. of Pharmacol., Univ. of Texas, Galveston, TX 77550

The D_2 receptor agonist, N-0437, caused a significant sulpiride reversible inhibition of Ca^{2+}-evoked ^{3}H-DA release from synaptosomes prepared from the striatum and the nucleus accumbens. Chronic cocaine administration abolished the effect of N-0437 in both areas. This suggests a subsensitivity of release-modulating terminal DA autoreceptors as a consequence of repeated cocaine administration. This study is primarily concerned with determining the mechanisms underlying autoregulation of DA release.

The voltage-sensitive Ca^{2+} channel antagonists, verapamil (10 μM), nimodipine (1 μM) and w-conotoxin (60 nM and 1 μM), resulted in a significant reduction of Ca^{2+}-evoked DA release (49 %, 20 %, 47 % and 60 %, respectively). Furthermore, the inhibition of Ca^{2+}-evoked DA release produced by verapamil or nimodipine was not additive with the effect of N-0437, indicating that Ca^{2+} channel antagonists and N-0437 may produce their inhibitory actions through mechanisms in which the same Ca^{2+} channels are involved. However, we also found that the K^+ channel blockers, TEA (5mM) and 4-aminopyridine (0.1 mM), prevented or significantly reduced the inhibitory effect of N-0437. These data are in agreement with Bowyer and Weiner, and indicate that D_2 autoreceptors activate a hyperpolarizing K^+ conductance, resulting in a reduction of Ca^{2+} influx through voltage-sensitive Ca^{2+} channels and DA release. Pertussis toxin (2.5 μg, i.c.v.) did not alter the inhibitory effect of N-0437, suggesting that G-proteins are not involved in D_2 receptor-mediated modulation of Ca^{2+}-evoked ^{3}H-DA release.

Thus, we suggest that chronic cocaine-induced down regulation of DA autoreceptors may invove changes in autoreceptor density or affinity or in transduction mechanisms such as number or fuctionality of K^+ ion channels associated with these receptors. Supported by DA-05159.

309.14

EFFECTS OF CALCIUM AND TETRODOTOXIN ON PHENCYCLIDINE INDUCED DOPAMINE INCREASE IN THE NEOSTRIATUM OF THE RAT D. Chapman* and S. Howard. MRRC, BRI, and Dept. of Pharmacology, UCLA, Los Angeles, CA 90024.

Phencyclidine (PCP) is a drug of abuse which is known to increase extracellular dopamine (DA) levels in the neostriatum of the rat. We examined the effects of reduced calcium (Ca^{++}) or the addition of tetrodotoxin (TTX, 10^{-5}M) on PCP induced DA increase. Rats were given PCP either systemically (20 mg/kg) or locally (5 μl of 100 μM over one minute) through the microdialysis probe. In vivo microdialysis samples were collected every ten minutes and analyzed for DA, DOPAC, and HVA using HPLC-EC. Removal of Ca^{++} from the perfusate decreased baseline extracellular DA, DOPAC, and HVA as well as reduce the PCP induced increase in DA. Addition of TTX to the perfusate also decreased baseline extracellular DA, DOPAC, and HVA as well as reduce the PCP induced DA increase. Ca^{++} and TTX produced similar effects on PCP induced DA increase when PCP was given systemically or locally. PCP increases extracellular DA in the neostriatum by a Ca^{++} dependent and TTX sensitive process. Since Ca^{++} and TTX perturbations were applied to the neostriatum through the microdialysis probe and since systemic and local applications of PCP showed similar results, the effects of PCP on areas outside of the neostriatum did not influence this process. USPHS Grant DA03020.

309.15

EFFECTS OF PERINATAL COCAINE EXPOSURE ON CENTRAL CATECHOLAMINES AND NORADRENERGIC RECEPTORS IN RATS. M.A. Kosek, G.A. Ordway, P.S. Widdowson*, B. Yamamoto and R.M. Kleigman. Dept. of Pediatrics, Rainbow Babies and Childrens Hosp. and Dept. of Psychiatry, MetroHealth Med. Ctr., Case Western Reserve Uni., Cleveland, OH 44106.

Cocaine abuse during pregnancy has reached epidemic proportions and is implicated in neurobehavioral changes in children. The effects of cocaine exposure during development on brain catecholamines are poorly understood. We investigated whether there are changes in brain catecholamines and noradrenergic receptors following perinatal cocaine exposure in rats. Pregnant Sprague Dawley rats were given saline, no treatment, or cocaine HCl (25 mg/kg/twice daily i.p.) from post-conception day 11 to birth. Animals were pair-fed and housed under identical conditions. At birth some pups from each group were sacrificed while the remainder were continued for 30 days on the same regimen as above and sacrificed at post-natal day 31. Catecholamine concentrations were estimated using HPLC-EC while noradrenergic receptors were measured by quantitative autoradiography. Perinatal cocaine exposure did not alter catecholamine concentrations at birth or at age 31 days. There was no change in the binding of [^{125}I]iodopindolol to beta-1 or beta-2 adrenoceptors or in the binding of [^{125}I]iodoclonidine to alpha-2 adrenoceptors in the 31 day old rats.

309.16

LACK OF PERSISTENT EFFECTS FROM POSTNATAL COCAINE EXPOSURE IN THE DEVELOPING RAT. T. B. Sonderegger, E. Y. Fung, H.E. Grotjan Jr.*, R.J. Krueger,* & J.A. Weinberg^, Univ.of Nebraska-Lincoln, Lincoln, NE 68588; ^University of British Columbia, Vancouver, British Columbia.

Litters from 12 Charles Rivers CD albino rats were sized to 9 pups on postnatal day 2; two female and one male pup from each litter were treated with cocaine [45 mg/kg 0.9% saline vehicle once daily] on days 5-12, a brain growth spurt period in the rat; same sex controls received comparable volumes of saline or were merely handled. Body weights of treated animals and controls were comparable at time of weaning on day 21. Three litters served as unhandled controls. Animals were tested in the open field on day 90 for 5-min trials over four consecutive days. Females were significantly more active than males but treatment groups did not differ in activity. Females were bred with nondrug treated males on day 100. (Data are reported elsewhere). When approximately 180 days-old, blood samples were obtained through cardiac puncture immediately upon removal from the home cage and after a 30-min stress period due to placement in a novel cage. Plasma samples were analyzed for levels of b-corticosterone. Neither basal levels nor the significantly elevated 30-min stress levels differed for the treatment groups. Animals were then sacrificed. The corpus striatum from 5 cocaine-exposed males and 5 cocaine-exposed females and a comparable number of saline controls were analyzed for dopamine receptor binding. No significant differences were found. Cells from the adrenal glands from these animals were cultured and dose-response curves of glucocorticoid production obtained; although the rate of production was less in the cocaine-treated males, differences were not significant. Pituitary glands were removed from 15 cocaine-treated females and 10 cocaine-treated males and a comparable number of saline controls and analyzed for Luteinizing Hormone using radioimmunoassay. Comparable levels of LH were found in the treated groups and their controls. Other behavioral data will be presented.

309.17

ISRADIPINE INHIBITION OF COCAINE CONDITIONED PLACE PREFERENCE. L. Pani*, A. Kuzmin*, M.C. Martellotta*, G.L. Gessa* and W. Fratta. "B.B. Brodie" Department of Neuroscience, University of Cagliari, 09124 CAGLIARI, ITALY.

The rewarding effects of cocaine are known to be associated with its ability to block reuptake of dopamine (DA) from nerve terminals. It has been shown that cocaine increases DA release via calcium dependent mechanism (Di Chiara G. and Imperato A., Proc. Natl. Acad. Sci. USA 85, 5274, 1988). Furthermore we showed that only calcium antagonists of dihydropiridine (DHP) class were able to inhibit cocaine-induced DA release and locomotor activity. The aim of the present work was to clarify if blockade of DA release by Isradipine, the most potent among the DHP compounds tested, will also inhibit cocaine induced rewarding effects in rats. The results obtained indicate that Isradipine inhibits cocaine reinforcing properties in a conditioned place preference paradigm at doses ($\leq$ 2.5 mg/Kg s.c.) which did not cause any sedative effects. The present results suggest the possibility that Isradipine might be a useful treatment of cocaine related disordes.

310.1

DIFFERENCES IN THE PRE-REINFORCED BEHAVIOR OF RATS RUNNING AN ALLEY FOR INTRAVENOUS COCAINE & HEROIN REWARD. A.Ettenberg and T.D.Geist. Dept Psychol. Univ. of Calif., Santa Barbara, CA. 93106

Rats were trained to traverse an alley for five I.V. injections of either cocaine (0.75mg/kg/inj) or heroin (0.06 mg/kg/inj). Testing consisted of single daily trials over 15 days during which the following measures were recorded: 1) time to initiate running; 2) time required to traverse the alley; 3) number (and location) of retreats occurring within the alley (a stop in forward locomotion with a return back toward the start box). Cocaine and heroin reinforced rats exhibited extremely different patterns of pre-reinforced (i.e. runway) behavior. While cocaine start latencies remained relatively stable over days, heroin rats initiated slowly at first and then improved as testing continued. Heroin Ss also traversed the alley with progressively faster running speeds while cocaine-reinforced Ss took progressively longer to enter the goal box. These differences in running behavior were directly related to the frequency of retreats observed in the two groups. Cocaine, but not heroin, animals exhibited a greater and greater "reluctance" to enter the goal box over trials. Such results suggest that chronic cocaine self-administration induces a state of conflict that is not observed in animals working for I.V. heroin reward.

310.2

COCAINE-INDUCED PLACE PREFERENCES: EFFECTS OF ALTERATIONS IN LENGTH OF NEUTRAL ZONE ON TEST DAY. T.D. Geist and A. Ettenberg. Dept Psychol. Univ. of Calif., Santa Barbara, CA. 93106

Animals readily exhibit conditioned place preferences (CPP) for environments previously paired with injections of cocaine. However, we have shown using an operant runway paradigm, that in addition to the rewarding properties of cocaine there exists strong anxiogenic effects of the drug. We hypothesized that the runway paradigm is particularly sensitive to such effects since it provides a long "neutral" zone between the start and goal boxes during which "conflict" behavior can be manifested. To determine whether the CPP test might also be sensitive to cocaine's anxiogenic actions, we examined whether CPPs would be reduced in magnitude for tests conducted in a preference apparatus with a long (vs short) neutral zone (between the saline- and cocaine-paired environments). CPPs were induced for one of two distinct environments by pairing it with IV injections of 0.75mg/kg/inj of cocaine. Ss received IV saline injections while in the non-drug environment. On each test day, the Ss were provided a choice between the two conditioned boxes separated by either a long (240cm) or short (40cm) neutral zone (alley). The color of the drug environment and the order of testing was counterbalanced between Ss. Results confirmed our hypothesis that the size of the resulting CPPs would diminish when Ss were tested in the long alley relative to the short alley.

310.3

TOLERANCE OR SENSITIZATION TO COCAINE'S REINFORCING EFFECTS? A STRUCTURAL ANALYSIS OF COCAINE SELF-ADMINISTRATION PATTERNS IN RATS DURING 3-48 HOUR COCAINE BINGES. A. Markou, M.P. Paulus, and G.F. Koob. Dept of Neuropharmacology, Research Institute of Scripps Clinic, La Jolla, CA 92037 and Lab. of Biological Dynamics and Theoretical Medicine, La Jolla CA 92093.

It has been hypothesized that the increase in cocaine dose self-administered by humans during a binge is due to the development of tolerance to cocaine's euphorigenic effects. The present studies investigated this hypothesis in two animal models of cocaine's reinforcing effects: intracranial self-stimulation and cocaine self-administration. Acute cocaine administration (5-10 mg/kg, IP) lowered self-stimulation current thresholds. Chronic cocaine administration (10 mg/kg daily for 10 days) did not produce tolerance or sensitization to cocaine's lowering effects on thresholds. In a third experiment, rats were allowed to self-administer cocaine for 3, 6, 12, 24, 48 hr continuously. Rats consumed a constant dose of cocaine per unit time (5 mg/kg per hr), even during a 24 or 48 hr binge, suggesting a lack of tolerance or sensitization to cocaine's effects. However, a structural analysis of the cocaine interinjection intervals indicated that the patterns of self-administration were changing over time. The first 200 injections occurred at very regular time intervals characteristic for each rat. During subsequent injections, rats started alternating short with long interinjection intervals while still consuming the same amount of cocaine per unit time. The structural changes in the interinjection intervals might reflect an attempt by the animal to avoid the development of tolerance to cocaine's reinforcing effects.

310.4

COCAINE-INDUCED TASTE AVERSIONS: A COMPARISION OF INTRAPERITONEAL AND SUBCUTANEOUS INJECTIONS. C.M. Ferrari, D.A. O'Connor*and A.L. Riley. The American University, Washington, D.C. 20016.

Although a range of pharmacological agents condition taste aversions, aversions induced by cocaine HCl are generally weak. Although the basis for these weak aversions is unclear, one possibility is the route of administration. Reports assessing the ability of cocaine to induce aversions have administered cocaine intraperitoneally (IP). The single paper reporting a robust aversion injected cocaine subcutaneously (SC) (Gale, K., NIDA Research Monograph Series, 54:323-332, 1984). To address the role of route of administration in cocaine-induced aversions, rats were given 20-min access to saccharin followed immediately by an IP or SC injection of 0, 18, 32 or 50 mg/kg cocaine. This procedure was repeated every fourth day for five trials. On intervening days, subjects were given water. Subjects receiving the IP injections displayed weak and non dose-dependent aversions. On the final saccharin exposure, these subjects had decreased consumption by only 35%. Although subjects in Group SC 18 decreased saccharin consumption by only 35%, subjects in Groups SC 32 and SC 50 decreased consumption by approximately 95 and 98%, respectively. It is clear that route may play an important role in the behavioral toxicity of cocaine (Dow-Edwards et al., Pharmac., Biochem. & Behav., 33:167-173, 1989)

310.5

THE EFFECTS OF COCAINE ON DIFFERENT RESPONSE RATES MAINTAINED BY EQUAL RATES OF REINFORCEMENT Frans van Haaren Dept. of Psychology, Univ. of Florida, Gainesville, FL 32611.

Small doses of cocaine increase low response rates and decrease high response rates. The 'rate-dependent' effects of cocaine might be accounted for by differences in reinforcement rates between schedules which generate high and low rates of responding. Three White Carneaux pigeons were trained in a two-component multiple schedule. Keypecking was maintained on a tandem RI 45 s - DRH 0.2 s schedule in one of the components, and on a tandem RI 45 s - DRL 3.0 s schedule in the other. Subjects were then i.m. injected with 0.3, 1.0, 1.7, 3.0, 5.6, and 10.0 mg/kg of cocaine. Small doses of cocaine (0.3 and 1.0) did not affect high rates and increased low rates in 2 of 3 pigeons. Larger doses (3.0 and 5.6) decreased high and low rates, but the decrease was more pronounced on the schedule maintaining high rates. Cocaine's behavioral effects in pigeons thus seem to be a function of the respone rates maintained in the absence of the drug.

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310.6

EFFECTS OF COCAINE ON SLEEP IN RATS. M. Radulovacki, S.D. O'Connor, S. Ticho, C. Vugrincic*, M. Lekovic*. Dept. of Pharmacology, University of Illinois, Chicago, IL 60612

Hill et al (Psychopharm., 51: 125-125, 1977) showed that acute administration of cocaine to rats resulted in decreased sleep time, with suppression of both deep sleep (SWS2) and REM. We examined in rats the effects of chronic administration of cocaine and cocaine withdrawal on sleep. The left carotid artery was cannulated in rats and connected to an Alzet mini-osmotic pump to deliver cocaine for a period of 7 days. Sleep was measured during cocaine administration and the following 7 days of cocaine withdrawal. Continuous infusion of 100mg/kg/day cocaine resulted in a decrease in total sleep ($p<0.05$). Light sleep (SWS1) also decreased ($p<0.05$), while deep sleep (SWS2) and REM initially decreased but returned to control levels by end of the cocaine infusion. During the cocaine withdrawal, wakefulness was reduced for the first 72 hrs ($p<0.05$). SWS2 rebounded at the fifth day of withdrawal ($p<0.05$). These preliminary results ($N=4$) show that chronic administration of cocaine suppresses sleep, but are inconclusive in regard to the total sleep rebound during withdrawal.

310.7

MICROINJECTIONS OF COCAINE, BUPROPION, AND PIPRADROL INTO THE NUCLEUS ACCUMBENS ENHANCE LOCOMOTOR ACTIVITY AND POTENTIATE RESPONDING FOR A CONDITIONED REINFORCER. S. Rosenzweig-Lipson, B. Chu*, J. M. Delfs, and A. E. Kelley. Dept. of Psychology, Harvard University, Cambridge, MA 02138

Many psychomotor stimulant drugs function as indirect dopamine agonists. These drugs can either enhance release of dopamine into the synapse, or they can block the reuptake of dopamine from the synaptic cleft. Psychomotor stimulants have been shown to increase motor activity as well as enhance responding for a conditioned reinforcer. The nucleus accumbens has been implicated as a site of action for the reinforcing and rewarding effects of these drugs. In the studies of motor activity, rats were placed into cages equipped with infrared photocell beams linked to a microprocessor that measured locomotor activity and rearing. In the conditioned reinforcement studies, hungry rats were trained to associate a compound stimulus (light/click) with the delivery of a food pellet. During the test phase, a lever was introduced into the box and lever pressing was reinforced by the stimulus alone. In these studies, the monoamine uptake inhibitors cocaine (1-60 µg) and bupropion (10-60 µg), as well as the monoamine releaser pipradrol (2-20 µg) were infused bilaterally into the nucleus accumbens. All three drugs increased total activity and rearing in a dose-dependent manner. In conditioned reinforcement experiments, all three drugs dose-dependently enhanced responding for a conditioned reinforcer. It is hypothesized that these compounds may affect reward-related processes when infused into the nucleus accumbens.

310.8

A COMPARISON OF COCAINE-INDUCED ACTIVITY PATTERNS FOLLOWING SINGLE OR CUMULATIVE DOSING REGIMENS. P. Terry. Psychobiology Lab., NIDA Addiction Res. Ctr., P. O. Box 5180, Baltimore, MD 21224

Locomotor activity and stereotyped behaviors were measured in male Swiss-Webster mice receiving i.p. cocaine at 5, 10, 20 or 40 mg/kg. Mice in the cumulatively-dosed condition were injected first with saline and then with 5, 5, 10, and 20 mg/kg at 10 minute intervals. In the single-dose/multiple-injection condition, independent groups of mice received a single dose of cocaine at the appropriate time point, with saline injections at other times. Thus all mice received five injections per session. In the single-dose/single-injection condition, the saline injections were omitted and one injection of cocaine was administered at the appropriate time. Scores were taken at each 10 minute interval. All mice were retested six weeks later and one day after that. Dose-response curves were similar for all three conditions on first test, but diverged markedly on subsequent tests. Despite the long inter-test interval, significant locomotor sensitization was evident at the higher doses on second test, but only in the two single-dose conditions. On third test, convulsions occurred at 40 mg/kg, but only in the single-dose conditions. The results demonstrate how certain injection parameters can chronically affect both the behavioral and toxic effects cocaine.

310.9

INDIVIDUAL DIFFERENCES TO SYSTEMIC AND INTRACRANIAL COCAINE. **A.D. Smith, M.S. Hooks, H.O. Pettit, J.B. Justice, Jr. Dept. of Chem., Emory Univ., Atlanta, GA 30322.**

The purpose of this study was to determine if rats show individual differences to cocaine, both peripherally and intracranially. Rats from the same strain have shown different locomotor response to a novel environment (Piazza, *Science*, 245, 1989). This response to a novel enviroment was used to predict how rats would respond to cocaine.

Animals were placed in a photocell cage where their locomotor activity (LA) was monitored. Based on their LA to the novel environment animals were classified into groups. Animals showing LA above the median for the initial hour in the test cages were classified as high responders (HR), while those showing a LA below the median for the initial hour were classified as low responders (LR). Two days after the response to the novel environment animals were placed back into the test cages for a 90 minute habituation period. Animals were given an intraperitoneal (IP) dose of cocaine (0.0, 2.5, 5.0, or 10.0 mg/kg) and their LA was monitored for 1 hour after drug injection. This same procedure was repeated in the animals who received intracranial injections of cocaine. Animals were injected bilaterally into the CA3 region of the hippocampus at a dose of 27.2 ug per side.

There was a significant difference in LA between HR and LR who received IP cocaine ($p<.02$). These animals also showed an interaction between response to novelty, dose of cocaine, and the time course of the LA ($p<.02$). HR showed a significant increase ($p<.01$) in their LA with increasing doses of cocaine, while LR exhibited no significant increase in LA with increasing cocaine dose. A significant difference between HR and LR animals that received intracranial cocaine ($p<.01$) was also observed. Moreover, this interaction was significant over the entire time course ($p<.05$) of LA. The finding that individual differences are observed following both peripheral and central administration indicates that individual differences to cocaine may be mediated by central mechanisms.

310.10

COCAINE PHARMACOKINETICS IN COCAINE EXPERIENCED AND NAIVE RATS AND ITS APPLICATION IN SELF ADMINISTRATION BEHAVIOUR

Stanley Menacherry, Hwai-Tzong Pan, Hugh Pettit and J. B. Justice, Jr. Emory University, Department of Chemistry, Atlanta, GA 30322

Blood and extracellular brain concentrations of cocaine are significantly enhanced in rats that are exposed to repeat administration of cocaine for 10 days prior to an i.p. challenge dose (Pettit et al., J. Neurochem. 1990, in press). The source of this enhancement was traced to a change in the absorption process from the peritoneal cavity into the blood stream. This is evidenced by the result that i.v. administration of cocaine did not produce a significant difference between cocaine experienced subjects and the control group. Nonlinear regression with global optimization was performed on the i.v. and i.p. data using a two compartment open model to determine rate constants for blood/brain transfer, elimination by metabolism and excretion and absorption from the body cavity. The absorption rate constant was found to be higher by 150% in experienced animals over naive animals (0.025 vs 0.016 min^{-1}), whereas the other rate constants were not substantially different. Robust estimates of the rate constants was obtained from the entire data set (rate constants for blood to brain transfer, brain to blood transfer and elimination of drug from the system were 0.35, 0.22 & 0.45 min^{-1} respectively). These rate constants were used to compute the blood and extracellular brain profiles of cocaine that would be obtained for the self adminstration behaviour reported by Pettit et al. 1989 (Pharmacol. Biochem. Behav. 34, 899-904). Individual preferences for drug intake across doses are noticed. Analysis of minimum levels in blood and brain indicate a tighter regulation of brain concentrations.

310.11

COCAETHYLENE IS A PHARMACOLOGICALLY ACTIVE METABOLITE OF COCAINE FORMED AFTER COMBINED INTAKE OF COCAINE AND ETHANOL. J.D. Elsworth, C.W. Bradberry, J.R. Taylor, J.R. Walker*, W.L. Hearn*, P. Jatlow* and R.H. Roth. Depts Pharmacology, Psychiatry and Laboratory Medicine, Yale University of Medicine, New Haven, CT 06510 and Dade County Medical Examiners Dept, Miami, FL.

Cocaethylene (ethylbenzoylecgonine) has been found in high concentrations in plasma (up to 850 ng/ml) of fatalities that were associated with combined use of cocaine and ethanol (1). Since many cocaine users prefer to take ethanol with cocaine, we have examined whether cocaethylene has cocaine-like effects.

Cocaethylene inhibited binding of the dopamine uptake blocker, [^{3}H]GBR (2nM), to rat striatal membranes as effectively as cocaine (IC_{50} 300nM). In addition, cocaethylene and cocaine were equipotent (IC_{50} 250nM) at inhibiting [^{3}H]dopamine uptake (40nM) into synaptosomes prepared from either striatum or nucleus accumbens of rats. Systemic administration of cocaethylene (1 mg/kg i.v.) to rats resulted in a 3 to 5 fold increase in extracellular dopamine concentration in nucleus accumbens, measured by microdialysis; similar effects were observed after injection of the same dose of cocaine. Locomotor activity and rearing in rats treated with 10 mg/kg i.p. of either cocaethylene or cocaine was markedly increased.

Thus, unlike other cocaine metabolites, cocaethylene possesses biochemical and behavioral effects similar to cocaine. This indicates that cocaethylene may play a role in the behavioral effects that occur after concurrent use of cocaine and ethanol. *Support: DA05119 & DA04050.*

1. Jatlow et al. (1990) 52nd Annual Scientific Meeting CPDD.

310.12

GENERALIZATION TESTS OF LOCAL ANESTHETICS AND STIMULANTS IN RATS TRAINED TO DISCRIMINATE COCAINE OR PROCAINE FROM SALINE. **P.B. Silverman. Dept. of Psychiatry and Behavioral Sciences, Univ. of Texas Medical School, Houston, TX 77030.**

Rats were trained to discriminate cocaine (10 mg/kg; 29 umol/kg) or procaine (54 mg/kg; 200 umol/kg) from saline in a two-lever, food-reinforced operant task. The stimulants amfonelic acid, amphetamine and methylphenidate all generalized completely to the cocaine stimulus but not to the procaine stimulus. The local anesthetics, bupivacaine, chloroprocaine and mepivacaine generalized completely to procaine while lidocaine, piperocaine and tetracaine did not. Most of the local anesthetics generalized, at most, only partially to cocaine, but dimethocaine, recently shown to exhibit frank stimulant activity as well as high affinity binding at dopamine uptake sites, generalized completely to cocaine and nearly completely to procaine. (+)-Cocaine did not generalize to either cocaine or procaine. Pseudococaine was tested only in cocaine animals, it did not generalize to cocaine. Apomorphine and caffeine generalized partially to both training compounds. **[Supported by NIDA grant DA04423.]**

310.13

GESTATIONAL EXPOSURE TO COCAINE INDUCED DEFICITS IN SENSORY PRECONDITIONING IN INFANT RAT PUPS. C.J. Heyser, W. Chen*, J. Miller*, N.E. Spear*, and L.P. Spear. Center for Developmental Psychobiology, Dept. of Psychology, SUNY, Binghamton, NY 13901.

Offspring derived from gravid Sprague Dawley dams that received daily subcutaneous injections of 40 mg/kg cocaine hydrochloride (C40) or saline (LC) from gestational days 8-20 were tested for first order Pavlovian conditioning and sensory preconditioning at postnatal days 8 (P8), P12, and P21. Sensory preconditioning consisted of the simultaneous exposure to 2 odors (A and B), followed by the pairing of one odor (A) with footshock. A preference test for location was conducted between the odor previously not paired with footshock (B) and a novel odor. Although the onset cocaine treatment reduced food and water intake for 2-5 days, no significant differences were observed between the prenatal treatment groups in duration of pregnancy, absorption rates, litter size, or pup body weights. C40 pups did not display sensory preconditioning at any of the tested ages, whereas significant conditioning was observed in LC controls at 8 and 12 days of age. In addition, C40 pups exhibited a deficit in first order conditioning at P8. Taken together these results may indicate a more general deficit in cognitive functioning rather than a delay in cognitive development.

[Supported by NIDA Grants R01 DA04478 and K02 DA00140]

310.14

DAILY COCAINE TREATMENT DIFFERENTIALLY AFFECTS ENRICHED- AND ISOLATION-REARED RATS' ACQUISITION AND PERFORMANCE OF AN OPERANT TASK. **J.M. Chase and S.C. Fowler. Depts. of Psychol. and Pharm., Univ. of Miss., University, MS 38677**

Male rats were reared in either enrichment cages (EC) or individual cages (IC) and were then exposed to a nose-poke operant task to assess the effects of cocaine (0.0, 2.5, or 10.0 mg/kg, ip. 10 min pre-session) during 11 15-min sessions of task acquisition. A rat's insertion of its head into a tube produced a light cue; further extension of the snout to break a photobeam resulted in immediate presentation of a dipper of milk below the rat's mouth. In the first session of exposure to the contingencies, 11 of 22 EC rats produced 10 or more reinforced responses, whereas only 4 of 23 ICs did this well. Over the 11 sessions, EC groups spent more of the total session with their head inside the tube, entered/exited fewer times, and maintained individual entries for a greater amount of time than IC rats. 10 mg/kg cocaine retarded task acquisition, and the drug treatment interacted with both rearing condition and training days. Supported by DA 05310.

310.15

THE ONTOGENY OF BEHAVIORAL SENSITIZATION TO PCP OR COCAINE. F.M. Scalzo and R.R. Holson, Div. of Reprod. & Develop. Tox., National Center for Toxicological Research, Jefferson, AR 72079.

Repeated exposure to PCP or cocaine can induce sensitization to later exposures in adults. We assessed developmental exposure effects of these compounds on later psychopharmacological responsiveness to determine the age at which sensitization occurs. Rat pups were injected for 9 days beginning on postnatal days (PNDs) 1, 12, 20 or 37 with saline, 10 mg/kg PCP or 20 mg/kg cocaine. Ten days following the last injection, rats were challenged with either saline, 10 mg/kg PCP or 20 mg/kg cocaine and activity, stereotypies and ataxia were measured. Pups treated on PNDs 1-9 appeared less sensitive to the effects of PCP when challenged on PND 19 compared to PCP-challenged saline-treated controls. In contrast, all other age groups exhibited an increased sensitivity to the PCP challenge. Regardless of age during subchronic treatment, there was no evidence of behavioral sensitization or tolerance to cocaine as measured by activity and stereotypy scores. It thus appears that subchronic PCP exposure has age-dependent effects on PCP sensitivity, while altered behavioral responsiveness to cocaine following subchronic administration does not occur in young animals using this testing paradigm.

310.16

COCAINE IN PREGNANCY AND DEVELOPMENT. E.W. HALLER, R.G.HOFFMAN*, R.M.EISENBERG*, A. VAINIO*. SCH. MED., U. MINN., DULUTH, MN. 55812.

The effect of 20 mg/kg cocaine exposure was investigated on maternal-pup interaction, pup growth rate and pup activity. Eight virgin female Sprague-Dawley-derived rats were synchronously mated with randomly selected males of the same strain. Following the onset of pregnancy, two dams were randomly selected and injected daily with 20 mg/kg cocaine sc from day 0 to day 21 of pregnancy, and two dams were similarly treated from day 0 to day 7 of pregnancy. Cocaine-treated animals were matched with saline controls injected on the same schedule. Following delivery, the litters were reduced to 6 pups each and cross-nurtured. At seven days of age postnatally, serial observations of maternal-pup interaction were made at two-day intervals until post-natal day 17. The pups were weaned from the dams at post-natal day 28 and activity measurements and daily weights were taken on individual pups at two-day intervals for the next 40 days. During each observation period, after a 10-minute separation from the pups, dams treated with cocaine during pregnancy took significantly longer to identify their pups, with a (mean time 90.6 sec vs.16.9 sec for control, paired t-test, $p<.05$). Experimental dams were significantly less likely to nest build or to gather their pups into a nest during the observation period ($p<.01$, Fisher's Exact Test). Although there were no differences in initial litter weights, following weaning female pups born to cocaine-treated dams were significantly heavier as well as significantly less active than female pups born to vehicle treated dams ($p<.05$, ANOVA), both in terms of overall activity and stereotypic activity. The principal differences due to prenatal cocaine occur in growth rate and activity in females rather than in male pups, and the greatest effects appear to be confined to the period between attainment of puberty and adulthood. Supported by NIGMS 5T34GM07680.

310.17

CATECHOLAMINE AND BEHAVIORAL EFFECTS OF PRENATAL COCAINE EXPOSURE IN HUMAN NEONATES. J. Meyer, M. Mirochnick*, J. Cole*, and B. Zuckerman*. Univ. of Massachusetts, Amherst, MA 01003, and Boston City Hospital, Boston, MA 02118.

Although previous studies have reported intrauterine growth retardation and behavioral abnormalities in infants exposed to cocaine prenatally, there has been relatively little research on the physiological consequences of such exposure. We now report initial findings from an ongoing study of catecholamine and behavioral responses in cocaine-exposed neonates. The subjects were 12 infants who were positive for cocaine by maternal history or urine drug screen, and/or infant urine or meconium screen, and 8 infants who were drug negative by maternal history and infant meconium screen. Physical measurements, Neonatal Behavioral Assessment Scale (NBAS) testing, and blood drawing were performed between 24 and 48 h post-partum. Serum samples were assayed for norepinephrine (NE), dopamine (DA), and dihydroxyphenylalanine (DOPA) by HPLC. Compared to controls, cocaine-exposed infants exhibited decreased body weight, head circumference, and height. Serum DOPA levels, but not NE or DA, were elevated in the cocaine infants. However, there was a highly significant negative correlation between NE and NBAS orientation cluster scores within the cocaine group. These results suggest that behavioral abnormalities found in prenatally cocaine-exposed neonates may be accompanied by alterations in peripheral catecholaminergic functioning.

310.18

ADMINISTRATION OF COCAINE TO RATS IN DRINKING WATER. A.B. Kelly, S.K. Overman* and D.G. Morgan, School of Gerontology, Univ. of Southern Cal., Los Angeles, CA 90089-0191

Rats were administered cocaine in their drinking water to develop a method for long term cocaine administration which avoids the stress of multiple daily injections, or surgery. In an obligatory drinking regimen, rats were presented with a single water bottle ad libitum in which cocaine doses were increased at 4 day intervals. At doses of 0.2, 0.3 and 0.5 mg/ml rats consumed the same amount of water as they did prior to receiving cocaine, or control rats receiving water alone. At 0.7, 1.0 and 1.2 mg/ml rats initially decreased their water consumption on the first day, followed by a slight return towards baseline on subsequent days. 0.5 mg/ml produced doses of 45 mg/kg/d while 1.2 mg/ml produced 73 mg/ml due to decreased consumption. No changes in body weight were observed at any dose.

In a two bottle choice paradigm, where the cocaine dose was paired with 0.02% saccharin, rats initially consumed 8-32 mg/kg/d at a dose of 0.77 mg/ml. This decreased to 2-12 mg/kg/d at 1.2 mg/ml. When the cocaine concentration was decreased to 0.2 mg/ml, some rats increased water consumption to maintain 5-10 mg/kg/d, while others continued to drink little of the saccharin+cocaine water. Consumption in the choice paradigm is idiosyncratic with some rats titrating their cocaine dose according to cocaine concentration, and other not drinking much of the cocaine solution at any concentration. Supported by the Greenwall Award from Amer. Fdn. for Aging Res., and an Established Investigator Award from Amer. Heart Assn. (DGM) and NIA training grant AG00093-08.

310.19

ESTRADIOL (E_2) ENHANCES BEHAVIORAL SENSITIZATION TO COCAINE. J.W. Simpkins, J. Peris, N. Decambre* and M. Coleman*, Dept. of Pharmacodynamics, Univ. of FL, Gainesville, FL 32610.

Studies were undertaken to determine the effects of ovarian steroids on sensitization of the locomotor and stereotypic behavioral responses to repeated cocaine injections. Young female adult rats were ovariectomized and 2 weeks later were implanted with one of the following chronic release forms of ovarian steroids in 5 mm silastic tubes: (i) sham (cholesterol); (ii) E_2 (diluted 1:1 with cholesterol); (iii) progesterone (P_4; diluted 3:1 with cholesterol) or (iv) E_2 plus P_4. Behavior was then rated using a 4 point scale (Ernst, Psychopharmacol. 10:316, 1967) after an initial injection of either saline or cocaine (10 mg/kg, i.p.) and after the 8th daily injection of saline or cocaine (n = 6). A significant increase in both locomotor and stereotypic behaviors was seen in all groups after the first cocaine injection compared to saline. Steroid-treatment did not influence the initial cocaine response, although each of the three steroid treatments slightly increased the incidence of locomotor activity after the first saline injection. Repeated injections of cocaine caused sensitization in all treatment groups, i.e. an increase in stereotypic and locomotor behavior over days with no change occurring in saline-treated groups over days. E_2-treatment significantly increased the magnitude of cocaine sensitization compared to the sham-treated controls. When striatal ^{3}H-dopamine release was measured 1 day after the 8th injection, both amphetamine and electrically stimulated release was decreased in E_2-treated animals regardless of saline or cocaine treatment. Collectively these data indicate that E_2 enhances behavioral sensitization to cocaine although more studies on the neurochemical substrate of this effect are warranted (Supported by AG 02021 and AA 08262).

310.20

ENHANCEMENT OF BEHAVIORAL EFFECTS OF COCAINE BY INHIBITORS OF MONOAMINE UPTAKE. R.D. Spealman. Harvard Medical School, N.E.R.P.R.C., Southborough, MA 01772.

The behavioral effects of cocaine were determined alone and after pretreatment with various monoamine uptake inhibitors that differ in selectivity for dopamine, norepinephrine, and serotonin: GBR 12909, talsupram, citalopram, and Lu 19005. When given alone, cocaine (.03-1.0 mg/kg) produced dose-related increases in responding by squirrel monkeys under a fixed-interval schedule of stimulus-shock termination. With the exception of citalopram, all uptake inhibitors produced leftward shifts in the cocaine dose-response curves and sometimes increased the peak response rates produced by cocaine. The order of potency was: Lu 19005> GBR 12909> talsupram. This potency order corresponds to that reported for in vitro inhibition of dopamine uptake and [^{3}H]cocaine or [^{3}H]CFT binding, but not for inhibition of uptake of either norepinephrine or serotonin. The results support the view that cocaine recognition sites associated with the dopamine uptake system play a principal role in mediating the stimulant effects of cocaine on behavior. Although citalopram did not produce an overall leftward shift of the cocaine dose-response curve, it sometimes decreased response rates when combined with cocaine doses $\geq$ 1.0 mg/kg, suggesting that inhibition of serotonin uptake may be involved in some high-dose effects of cocaine. Supported by USPHS Grants DA00088, DA00499, DA06303 and RR00168.

MONOAMINES AND BEHAVIOR IV

311.1

EFFECTS OF DOPAMINE RECEPTOR BLOCKADE IN NUCLEUS ACCUMBENS AND CAUDATE ON FEEDING INDUCED BY INTRA-RAPHE 8-OH-DPAT. P.J. Fletcher. Biopsychology, Clarke Inst. Psychiatry, 250 College St., Toronto, Ontario, Canada M5T 1R8.

The 5-HT_{1A} receptor agonist 8-OH-DPAT (DPAT) elicits a variety of behaviours including feeding. The effect on feeding appears to be due to reduced 5-HT neurotransmission resulting from activation of 5-HT somatodendritic autoreceptors in the raphe nuclei. Some behavioural effects of 5-HT_{1A} agonists are blocked by DA receptor antagonists suggesting that a facilitation of DA activity, secondary to reduced 5-HT function, may mediate the effects of these compounds. Experiments explored the importance of several DA-rich terminal regions in mediating feeding induced by intra-raphe DPAT. Adult male rats were used, and all drugs were injected directly into the brain via chronically dwelling guide cannulae; food intakes were measured 1h after injection. Dose-dependent increases in feeding were observed when DPAT (0.125-2ug) was injected separately into the dorsal raphe (DR) or median raphe (MR). Feeding elicited by DPAT from both DR (1ug) and MR (0.5ug) was attenuated by the DA antagonist alpha-flupenthixol (FLU; 1.25 and 2.5ug) injected into the nucleus accumbens. The effect of DR-injected DPAT was completely blocked by injection of FLU into dorsolateral and ventrolateral regions of the caudate. In contrast FLU injected into these sites failed to modify the effect of MR-injected DPAT. None of the effects of FLU can be explained in terms of non-specific motor impairments. The results confirm that DA is involved in DPAT induced feeding and show that DR and MR induced feeding have separate but overlapping substrates. Thus, while nucleus accumbens DA is involved in both effects, DA in the caudate is involved only in feeding induced by DPAT injected in the DR.

311.2

EFFECTS OF CONDITIONED TASTE AVERSION (CTA) ON SEROTONIN RELEASE IN THE LATERAL HYPOTHALAMUS AND HIPPOCAMPUS. H. L. West, G. P. Mark & B. G. Hoebel. Dept. Psychology, Princeton Univ., Princeton, NJ 08544

Previous data from this laboratory demonstrated that dopamine release in the nucleus accumbens could be attenuated by presentation of a taste stimulus which had previously been paired with LiCl-induced malaise (Mark et al., *Soc. Neurosci. Abstr.*, 1989). Our next question concerned the effect of learned aversion on serotonin (5-HT) output in the perifornical lateral hypothalamus (PLH). Eighteen male, Sprague-Dawley rats were implanted with intraoral catheters and stainless steel guide cannulas ending 5 mm dorsal to the PLH. Prior to testing, subjects were given two conditioning trials in which the taste of Na saccharin (2.5 mM, the CS) was paired with LiCl-induced malaise (the US). Thirty-six hr after the final LiCl injection, microdialysis probes were inserted into the PLH (200 μm diam., 3 mm fiber). Following a 12 hr delay, dialysates were collected every 20 min for 1 hr prior to and 2 hr after oral infusion of the CS. Serotonin concentrations were determined using HPLC-EC. Results showed a 48% increase in 5-HT levels during the 20 min CS infusion ($p<.05$). This effect was absent in unconditioned control subjects and attenuated in pseudo-conditioned (US only) subjects. To explore the possibility that this effect would occur in a 5-HT terminal region not directly implicated in taste aversion learning, the experiment was repeated with 18 animals in which probes (4 mm) were placed in the hippocampus. At this site, 5-HT output was not uniquely affected by oral infusions of saccharin in subjects which had developed a CTA but rather was increased 18-25% in all groups, suggesting that hippocampal 5-HT is related to arousal. These results are consistent with the hypothesis that serotonin transmission in the LH plays a role in the inhibition of ingestion during expression of a CTA that may not be entirely attributable to a global enhancement of the serotonergic system accompanying arousal.

311.3

STUDIES OF THE AFFERENT CONTROL OF THE DORSAL RAPHE NUCLEUS IN THE AWAKE CAT. E. S. Levine, R. N. Holdefer, D. A. Morilak, and B. L. Jacobs. Program in Neuroscience, Dept. of Psychology, Princeton Univ., Princeton, NJ 08544.

These studies investigated the neurochemical control of dorsal raphe nucleus (DRN) serotonergic neurons under physiological conditions by recording neuronal activity in conjunction with microiontophoresis in the awake head-restrained cat. Serotonergic neurons in the DRN were identified on-line by their slow, regular firing rate during quiet waking, long duration action potential, and inhibition by iontophoretically applied 5HT-1_a agonists. When applied iontophoretically, GABA inhibited the activity of DRN serotonergic neurons in the awake cat, and this effect was reversed by the iontophoretic application of bicuculline, a GABA-A receptor antagonist. During quiet waking, the spontaneous activity of DRN cells was not significantly increased by application of bicuculline alone (baseline firing rate = 2.4 $\pm$ 0.4 spikes/sec; firing rate during bicuculline = 2.6 $\pm$ 0.4 spikes/sec), suggesting that these neurons do not receive a tonic GABAergic input during this state. However, preliminary data indicate that iontophoretic application of bicuculline activates these neurons during rapid eye movement (REM) sleep, when serotonergic neurons are otherwise silent. This suggests a role for GABA in mediating the inhibition of DRN serotonergic neurons during REM sleep. Additional studies are examining the roles of other neurotransmitter inputs in the afferent control of DRN neuronal activity. (Supported by AFOSR grant 87-0301).

311.4

A SEROTONIN DEPLETING REGIMEN OF (+/-)-3,4-METHYLENEDIOXYMETHAMPHETAMINE DECREASES THE BEHAVIORAL EFFECTS OF SEROTONERGIC AGONISTS IN RATS PERFORMING ON AN OPERANT SCHEDULE. D. Jolly and L. S. Seiden. Dept. of Pharmacol. and Physiol., University of Chicago, Chicago, Illinois. 60637.

Rats performing on a Differential Reinforcement of Low Rate (DRL) schedule of water reinforcement respond in a characteristic fashion to serotonergic agonists. Administration of either 8-hydroxy-2-(di-n-propylamino)tetralin (DPAT) or 5-hydroxytryptophan (5-HTP) increases reinforcement (Rf) rate and decreases response (Rs) rate. This report describes the decreased DRL effects of DPAT and 5-HTP observed after partial depletion of brain 5-HT in the rat. One group of 9 rats received 20 mg/Kg (+/-)-3,4-methylenedioxymethamphetamine HCl every 12 hours for 4 days (MDMA). A control group of 6 rats received an identical regimen of vehicle (SAL). Fifteen weeks later, the DRL effects of DPAT and of 5-HTP were determined. Both DPAT and 5-HTP increased Rf rate and decreased Rs rate in both groups. These effects were greater in the SAL group than in the MDMA group. Neurotransmitter levels were assayed 22 weeks after the repeated injections of MDMA. These assay results showed that in the MDMA group, there were widespread depletions of 5-HT. These results suggest that an intact 5-HT system is necessary for the expression of characteristic DRL effects of 5-HTP or DPAT.

This work was supported by NIDA DA-00085, MH-1119, and RSA-10562 (L. Seiden).

311.5

THE 5-HT SYNDROME INDUCED BY 5-HTP IN NEONATAL, WEANING AND POSTWEANING RATS. S.A. Sullivan, B.J. Winterson and D.J. Mokler. Depts. Pharmacol. & Physiol., Univ. New England, Biddeford, ME 04005.

There are similarities between the 5-HT behavioral syndrome in adult rats and behaviors in neonates. We observed the 5-HT syndrome in rats at various during various developmental stages. The 5-HT precursor, 5-hydroxytryptophan (5-HTP) was administered ip or sc (dose: 200 mg/kg). Rats were monitored for 2 hours after injection. Behaviors were similar in adult, 4 and 2 wk old rats and consisted of hindlimb abduction, forepaw treading, resting tremors, and circling (5-HT_1 mediated), and headshakes (5-HT_2 mediated). The 5-HT1 mediated behaviors emerged at similar times in these groups, except circling was absent in 4 wk rats. In contrast, headshakes began 40 min post-injection in adults, 26 min in 4 wk rats and 16 min in 2 wk rats. Four wk rats showed fewer of all behaviors and more grooming. Neonates (1-2 d) showed a different pattern of behavior. Although 5-HT_1 mediated behaviors emerged at a time similar to older rats, these were followed by rolling over, limb extension, and ventroflexion. Distress vocalization was present throughout observations. Headshakes were not observed in neonates. These observations suggest that behaviors related to posture and locomotion (5-HT_1 mediated) are present at birth and developmental changes are quantitative. However, headshakes (5-HT_2 mediated) do show developmental differentiation. Supported by AOA grant #88-11-290.

311.6

COMPARISONS OF BEHAVIORAL EFFECTS OF 5-HT1B AGONISTS VERSUS MDMA IN RATS. N. L. Rempel* and M. A. Geyer. Departments of Neurosciences and Psychiatry, Univ. of California at San Diego, La Jolla, CA 92093.

Hyperlocomotion induced by methylenedioxymethamphetamine (MDMA) in rats appears to be due to the serotonin (5-HT) releasing properties of the drug. Unlike amphetamine, the behavioral effects of MDMA are blocked by the 5-HT uptake inhibitor fluoxetine or the 5-HT synthesis inhibitor PCPA. Locomotor hyperactivity induced by 5-HT releasing agents is not consistent with the effects of 5-HT1A or 5-HT2 agonists. In the same behavioral paradigm used in the studies of MDMA, 5-HT1A agonists decrease locomotor activity. 5-HT2 agonists similarly decrease activity, especially in a novel environment. The present experiments were designed to determine if 5-HT1B agonists induce hyperlocomotion qualitatively similar to that induced by MDMA. Activity was examined using the Behavioral Pattern Monitor (BPM) system to provide detailed information regarding the amount and qualitative patterning of locomotor activity and investigatory responses. Six groups of rats were injected with various doses of CGS 12066B (0.003 to 0.3 mg/kg) 10 min prior to placement in the BPM chambers. No differences in locomotion, holepokes, or rearings were evident for any dose compared to the saline-injected control group. The behavioral effects of trifluoromethylphenylpiperazine (TFMPP; 0.625 to 10.0 mg/kg) were examined in the same paradigm. Decreases in locomotion, holepokes, and rearings were observed only at the highest dose. However, CGS 12066B is reportedly less active *in vivo* than *in vitro* and TFMPP has relatively high affinities for the 5-HT1A and 5-HT2 sites, as well as 5-HT1B. The behavioral effects of RU 24969, a more selective 5-HT1B agonist reported to induce hyperlocomotion in other paradigms, remain to be examined in the BPM system. A qualitative comparison of the effects of MDMA and RU 24969 will be important in understanding the stimulant-like effects of increased 5-HT release. (Supported by DA02925)

311.7

LEARNED HELPLESSNESS AND SEROTONIN: *IN VIVO* MICRODIALYSIS. F. Petty, G.L. Kramer*, T.R. Phillips*, L.A. Speece* and D. Dunnam*. DVAMC and Dept. Psychiat., Univ. Texas Southwest. Med. Sch., Dallas, TX

Learned helplessness is a maladaptive behavior caused by exposure to uncontrollable stress. Previous research had shown serotonin to reverse helpless behavior when microinjected into frontal neocortex. Also, serotonin released from tissue slices of frontal cortex and septum was found to be low in helpless rats with this deficit which normalized by chronic administration of tricyclic antidepressants. Other laboratories, however, have shown that serotonin depletion prevents the development of learned helplessness and that serotonin receptor blockade does not influence tricyclic activity in this model. We, therefore, examined serotonin release in frontal neocortex using *in vivo* microdialysis in four experiments. In the first study, basal serotonin release was measured before exposure to inescapable stress and again before testing for helpless behavior and a significant increase was observed in rats which became helpless. In the second experiment, 60 mM K+ was perfused prior to behavioral testing: this resulted in a "cure" of helpless behavior but no correlation was observed between serotonin release and helplessness. In the third experiment, rats were perfused during stress re-exposure and no correlation was found between stress-induced serotonin release and subsequent behavioral activity. Finally, animals were administered diazepam (once), imipramine (7 days)' or training in escape behavior. All three interventions prior to exposure to inescapable stress prevented the development of behavioral helplessness, confirming prior research. None, however, demonstrated a significant correlation between K+-induced serotonin released 24 hours after testing and degree of learned helplessness. Taken together, these results suggest that higher basal release of serotonin prior to stress exposure accompanies the development of learned helplessness, but that learned helpless behavior once established is not related to the functional releasable serotonin pool.

311.8

ZACOPRIDE IN SCHIZOPHRENIA: A SINGLE-BLIND 5-HT3 ANTAGONIST TRIAL. J.W. Newcomer*, W.O. Faustman, B. Roth, R.A. Bierley*, J.A. Moses, Jr.*, J.G. Csernansky. Palo Alto VA Med. Center, Stanford Univ. Sch. of Med., Palo Alto, CA 94304

Zacopride inhibits locomotion associated with mesolimbic dopaminergic hyperactivity in the rat, without rebound hyperlocomotion, CNS depression, or the prolactin elevation seen with neuroleptics. In other animal models and in humans, it is anxiolytic. In animals, it may facilitate learning. It has been hypothesized that zacopride might have efficacy for the treatment of schizophrenic symptoms, without the motor side-effects associated with conventional neuroleptics.

Six severely ill male inpatients with schizophrenia (initial BPRS total score > 50 in 5 subjects) received zacopride in a single-blind trial: no psychotherapeutic medications (except chloral hydrate) for 2 weeks, followed by 1 week of placebo, and 4 successive weeks of zacopride treatment at 0.2 mg, 0.4 mg, 08 mg, and 1.6 mg per day.

Two patients experienced a mild anxiolytic effect without sedation, most pronounced at lower doses of zacopride. A reduction in the distress associated with psychotic symptoms was clinically observed in a third patient. Core positive symptoms showed little change in any patient. Memory function, before and during treatment, revealed no changes.

No adverse motor effects were seen. The only subject who started the trial with a moderate degree of tardive dyskinesia, showed a decrease in abnormal movements in the first week of treatment. Withdrawal of zacopride resulted in a return of abnormal movements. CSF/plasma neurochemistry measures from the subjects will also be presented. Supported by MH30854 from the NIMH.

311.9

AGE-RELATED VARIATIONS OF CENTRAL AMINES FOLLOWING ACUTE STRESS IN MICE. N. Shanks, S. Zalcman, A. Minkiewicz-Janda* and H. Anisman. Dept. of Psychology, Carleton University, Ottawa, Ontario, K1S 5B6, Canada.

Exposure to footshock reduced the levels and increased utilization of norepinephrine (NE), dopamine (DA) and serotonin (5-HT) in some brain regions of CD-1 mice. Transmitter levels and turnover varied with age (3 vs. 9 months) of the organism. Moreover, stressor exposure provoked more pronounced and persistent amine variations in the older animals, at least insofar as some amines were concerned. Hypothalamic NE, for instance, was more readily reduced in 9- than in 3-month old mice, even though the MHPG accumulation was greater in the younger animals. It is likely that in younger animals the increased utilization is more readily met with increased synthesis. In contrast, the accumulation of 5-HIAA was more pronounced in the older animals, and 5-HT levels were still reduced as long as 24 hr after stressor exposure. Finally, mesocorticolimbic DOPAC alterations were initially more marked in older mice, but were more persistent in the younger animals. The data are related to stressor-induced behavioral changes.

311.10

DIFFERENTIAL EFFECTS OF 5HT1A AGONISTS 8OHDPAT, BUSPIRONE, GEPIRONE, IPSAPIRONE AND 5MEODMT ON A DRL 72 SEC SCHEDULE OF REINFORCEMENT. J.B Richards, K.E. Sabol, D.C.Jolly, and L.S. Seiden. Dept. Pharmacol. Physiol. Sci., Univ. Chicago, Chicago, IL 60637

Previous work from our lab has shown that 5HT1A agonists have antidepressant like effects on the DRL 72 sec schedule of reinforcement (increased reinforcement rate). Here we report that although 5HT1A agonists increase reinforcement rate they affect the pattern of DRL 72 responding differently. The DRL 72 sec schedule of reinforcement produces a typical pattern of responding which is reflected in the distribution of interresponse times (IRTs). Rats performing on the DRL 72 sec schedule have a peaked IRT distribution. The peak usually occurs before the 72 sec point. We have previously reported that antidepressant drugs which increase reinforcements shift this peak to the right - toward the 72 second point. If responses on the DRL 72 sec schedule were randomly spaced in time the distribution of IRTs would form a negative exponential curve and would not be peaked. We compared the obtained IRT distributions of drugged animals with the predictions of random performance. At doses which increased reinforcements, buspirone, gepirone and ipsapirone altered the IRT distribution so that it was more similar to the prediction of random performance. Conversely, doses of 8OHDPAT and 5MeODMT which increased reinforcements, did not cause the IRT distribution to become more similar to random performance. In addition, 5MeODMT caused a significant peak shift to the right. This work was supported by: MH-11191; RSA-10562 (L.Seiden).

311.11

SEXUAL BEHAVIOR OF MALE RATS FOLLOWING 5,7-DHT TREATMENT MAY BE RELATED TO PRESENCE OF SEROTONERGIC FIBERS LOCATED IN A SEXUALLY DIMORPHIC NUCLEUS IN THE SPINAL CORD. N.L. Brackett and V.R. Holets. The Miami Project, Dept. of Neurological Surgery, University of Miami, Miami, FL 33136.

Sexually experienced male rats received an intrathecal injection of 5,7-dihydroxytryptamine (5,7-DHT) or its vehicle. Each male was given four mating behavior tests with a receptive female for up to two months after surgery. Males were then sacrificed and their spinal cords were processed for immunohistochemistry.

Measures of mating behavior were not different between the two groups. Cross sections of spinal cord taken from lumbar and sacral segments were examined for 5-hydroxytryptamine (5-HT) immunoreactive fibers. In 5,7-DHT treated males, there was an overall reduction in the number of 5-HT fibers in the gray matter with the exception of the dorsolateral motor nucleus (DLN) located in the ventral horn of lumbar segments. In both vehicle and 5,7-DHT injected males, comparable numbers and densities of 5-HT fibers were observed surrounding motoneurons in the DLN. The DLN is a sexually dimorphic nucleus in adult rats with males exhibiting more motoneurons in the DLN than females (Jordan, et al., Br. Res. 249:309,1982). These motoneurons innervate the ischiocavernosus muscle which lies at the base of the penis (Breedlove and Arnold, Science, 210:564,1980). We hypothesize that serotonergic action on the motoneurons in the DLN is responsible in part for mating behavior in 5,7-DHT treated males. The existence of intrinsic sources of 5-HT within the spinal cord (Newton, et al. Br. Res., 376:155,1986) may account for the 5-HT fibers in the DLN following 5,7-DHT treatment. (Funded by The Miami Project and the Daniel Heumann Fund).

311.12

5,7-DIHYDROXYTRYPTAMINE AND CASTRATION INCREASE DENDRITIC SPINE DENSITY ON VENTROMEDIAL HYPOTHALAMIC NEURONS. Maya Frankfurt. Neuroendocrinology Lab., Rockefeller University, New York, 10021

It has been demonstrated that lordosis facilitation in both female and male rats occurs after intrahypothalamic injection of the serotonin (5-HT) neurotoxin, 5,7-dihydroxytryptamine (5,7-DHT) and that this correlates with 5-HT levels in the ventromedial hypothalamic nucleus (VMN) (Frankfurt, M., Brain Res., 340: 127, 1985). Using Golgi-impregnation we have recently shown that ovariectomy causes a decrease in dendritic spine density on VMN neurons and that this effect is reversed by estrogen (E)(Frankfurt, M., Neuroendocrinology, in press).

In the present study the effect of intrahypothalamic injection of 5,7-DHT in both sexes and castration in the male was studied. In the VMN of intact females, 5,7-DHT injection increased the density of dendritic spines on VMN neurons four-fold as compared to sham. In intact males, 5,7-DHT increased spine density two-fold as compared to sham- injected rats. Castration in males had the opposite effect of ovariectomy in that it increased dendritic spines as compared to intact. E given to castrated males reversed the increase in dendritic spines. These results suggest that 5,7-DHT and E have the same effect in the female; to increase spine density on VMN neurons. Whether this also reflects increased synaptic density must be seen. Supported by NS07080.

311.13

DEPLETION OF NOREPINEPHRINE IN EITHER THE VENTROMEDIAL NUCLEUS OR THE MEDIAL PREOPTIC NUCLEUS FAILS TO ALTER LORDOSIS IN RATS. B.L.Davis[1], J.Manzanares[2], K.J.Lookingland[2], K.E.Moore[2], L.G.Clemens[1]. Neuroscience Prog., Dept. Zoology[1] and Dept. Pharmacol/Toxicol[2], Michigan State Univ., E. Lansing, MI 48824

Norepinephrine (NE) has been implicated as being important in mediating lordosis in estrogen-primed female rats. Lesions in the ventral noradrenergic bundle (VNAB) deplete NE in the whole hypothalamus and inhibit lordosis (Hansen, 1981), but little is known regarding the role of NE in specific hypothalamic nuclei in mediating this behavior. The present study examined the effects of depletion of NE in either the ventromedial nucleus (VMN) or the medial preoptic nucleus (MPN), two areas thought to be important for the control of lordosis. In separate experiments, anesthetized, ovariectomized (OVX), female rats received microinjections of the selective NE neurotoxin, 5-amino-2,4-dehydroxy-α-methylphenylethylamine (5-ADMP), in either the VNAB (8μg/0.3μl, bilateral), the VMN (2μg/0.3μl, bilateral), or the MPN (4μg/0.6μl, bilateral). Females were hormonally-primed and tested for sexual receptivity one week after neurotoxin administration. VNAB lesions depleted NE (VMN: 65%, MPN: 70%), but not dopamine or 5-hydroxytryptamine, and significantly reduced lordosis. In contrast, depletion of NE in either the VMN (83%) or MPN (83%) failed to alter lordosis. These results indicate that the medullary NE input into the VMN or MPN is not required for lordosis in hormonally-primed OVX rats. This work is supported by PHS Grant No. HD 06760.

311.14

NEUROCHEMICAL DIFFERENCES IN STEROID-SENSITIVE AREAS MEDIATING REPRODUCTIVE BEHAVIORS IN QUAIL. A. Foidart, C. Surlemont*, G.F. Ball and J. Balthazart, Lab. Biochemistry, Univ.Liège, B-4020 Liège, Belgium and Dept. Psychology, Boston Coll., Boston MA 02167.

The steroid-sensitive nuclei underlying different reproductive behaviors show a diversity of biochemical mechanisms in generating the steroid-dependent responses. Their comparison could reveal essential principles of brain functioning without the possible confounding effects that are inevitable when comparison across species are attempted. We have analyzed steroid actions on the neurochemistry of two regions in the quail brain: the medial preoptic nucleus (POM) which is critically implicated in the activation by testosterone (T) of copulation and the nucleus intercollicularis (ICo) which presumably controls T-dependent vocalizations. In both nuclei, T metabolism (aromatization, 5α-and 5β-reduction) was quantified by radioenzyme assays, aromatase and estrogen receptors were studied by immunocytochemistry, catecholamines and their turnover were assessed by HPLC and α1 and α2-adrenergic and muscarinic cholinergic receptors were determined by quantitative autoradiography. Although both nuclei control androgen-dependent behaviors and contain androgen and estrogen receptors, they show major neurochemical differences. In particular, aromatase activity and aromatase-immunoreactive neurons are present in POM but not in ICo. Norepinephrine levels are higher in the POM and ICo of females compared to males and this difference does not disappear after gonadectomy followed or not by a treatment with T. Dopamine turnover is higher in male than in female POM but not in Ico. α2-adrenergic and muscarinic cholinergic receptors are also modulated by T specifically in sub-regions of ICo but not in POM. These neurochemical differences may provide insight into a general theory of how T acts biochemically to activate behavior. Supported by NIH HD22064, FNRS and EEC (SC1-0230-C/TT) to JB.

311.15

CONCURRENT BRAIN GLUCOSE UTILIZATION AND BIOGENIC MONOAMINE NEUROTRANSMITTER TURNOVER RATES WITH VTA BRAIN STIMULATION REINFORCEMENT. J.E. Smith, S.I. Dworkin, C. Co* and L.J. Porrino, Dept. Physiol.& Pharmacol., Bowman Gray Sch. Med., Wake Forest Univ., Winston-Salem, NC 27103 and Unit on Brain Imaging, NINDS.

Glucose utilization and biogenic monoamine turnover rates were concurrently measured in discrete brain regions of rats receiving either contingent or non-contingent electrical stimulation of the ventral tegmental area and non-stimulated controls. This combination of procedures was employed to not only identify brain regions with altered glucose utilization, but in addition to determine whether the indicated changes in metabolic activity were also related to biogenic monoamine neurotransmitter turnover. [^{14}C]-2-deoxyglucose and [^{3}H] tyrosine and tryptophan were intravenously administered to 12 rats in each of these three treatment conditions. Significant changes in the turnover rates for dopamine (DA), norepinephrine (NE) and serotonin (5-HT) resulted from non-contingent stimulation in the nucleus accumbens (NA), frontal cortex, medial prefrontal cortex (MPC), substantia nigra, ventral tegmental area, amygdala (AMY) and caudate-putamen (CP). Changes specific to contingent electrical stimulation included an increase in DA and decrease in 5-HT turnover in the contralateral NA and ipsilateral increases in DA and NE in the CP and 5-HT in the AMY. These procedures permit the simultaneous identification of the brain regions showing increased glucose utilization and changes in the activity of specific innervations of these regions in the same animals. The changes in turnover rates are consistent with the alterations in glucose utilization seen in the NA, AMY and MPC in the self-stimulating animals and suggest an important role for 5-HT, NE and DA in the neuronal events underlying electrical brain stimulation reinforcement. (Supported in part by USPHS research grants DA-01999, DA-03628, DA-03631, DA-03832 and DA-00114)

311.16

THE ROLE OF PEDUNCULOPONTINE NUCLEUS IN LOCOMOTOR ACTIVITY. G.J. Mogenson, M. Wu* & S.M. Brudzynski. Dept. of Physiology & Dept. of Clinical Neurological Sciences, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1.

It has been reported that nucleus accumbens-subpallidal-pedunculopontine nucleus (PPN) projections contribute to locomotor activity (Mogenson, Swanson and Wu, Brain Res., 334: 65-76, 1985; Mogenson and Wu, Brain Res. Bull., 20: 241-246, 1988). However, it is not clear whether these projections from accumbens and subpallidal region pass through or terminate in the PPN. Since cobalt chloride is a reversible blocker of synaptic transmission by blocking the release of neurotransmitters (Lee and Malpeli, Brain Res., 364: 396-399, 1986), the present study was undertaken to determine whether the PPN and not just the fibres of passage, mediate locomotor activity.

Locomotor activity measured in an open-field chamber was elicited by unilateral injections of dopamine (DA) into accumbens or by unilateral injections of picrotoxin (PTX) into subpallidal region of rats. The ipsilateral administration of cobalt chloride (4mM, 0.2 μl) into PPN reduced locomotor activity elicited either by DA injections into accumbens or by PTX injections into subpallidal region while the ipsilateral administration of saline into PPN had little or no effect.

These results provide additional evidence that the PPN has a role in initiating locomotor activity.
(Supported by NSERC of Canada)

311.17

DEFICITS IN SENSORIMOTOR GATING OF ACOUSTIC STARTLE FOLLOWING CARBACHOL INFUSION INTO THE HIPPOCAMPUS. N.R. Swerdlow, S.B. Caine and M.A. Geyer. Department of Neuroscience, UCSD, La Jolla, CA 92093.

"Prepulse inhibition" (PPI) occurs when the startle reaction to a startling sensory stimulus is reduced by the prior presentation of a weak stimulus. PPI is a measure of sensorimotor gating and is decreased significantly in rats following dopamine (DA) activation of the nucleus accumbens (NAC). In the present study, we measured PPI after chemical stimulation of NAC afferents originating in the dentate gyrus of the hippocampus (HP). Male Sprague-Dawley rats were surgically equipped with bilateral steel cannulae aimed above the HP, and starting one week later, they were tested during three sessions with four days separating each session. Prior to each session, rats received intra-HP infusions of carbachol (CBC) (0, 0.2 or 0.4 ug/side, n=9; 0, 0.8 or 1.6 ug/side, n=8) and startle amplitude was then measured following 50 presentations of a startle pulse (118 dB [A]) either alone or preceded 60, 120 or 500 msec earlier by a weak prepulse (80 dB [A]). The normal inhibition of startle amplitude by the prepulse was reversed by intra-HP CBC in a dose-dependent manner. Higher doses of CBC significantly depressed pulse-alone startle amplitude. In a separate experiment, the CBC-induced loss of PPI was not completely reversed by systemic pretreatment with the D2 DA antagonist spiperone at a dose (0.1 mg/kg sc) that completely reversed the loss of PPI caused by the DA agonist apomorphine. These findings suggest that HP-NAC circuitry is a neural substrate of PPI; activation of the HP-NAC projection may modify PPI through changes at the NAC that are partly or entirely independent of NAC D2 receptors.

311.18

AN INEXPENSIVE AUTOMATED SYSTEM FOR THE QUANTIFICATION OF ROTATIONAL BEHAVIOR IN SMALL ANIMALS. D.K. McFarlane, B.J. Martonyi*, J.B. Becker, and T.E. Robinson. Dept. Psychology and Neuroscience Program, The University of Michigan, Ann Arbor, MI 48104-1687.

The quantification of turning behavior in small animals is a useful tool for assessing the results of unilateral brain lesions, surgery and drug regimens. We describe here a new inexpensive and reliable system for the automated measurement of rotational behavior.

The system uses a single computer (Commodore 64) to count quarter and full turns from up to ten subjects at once. The essential counting routine is written in machine code. The remaining program is written in BASIC. Input to the computer is memory mapped through a simple, inexpensive and commercially available digital I/O board providing 40 lines. For each subject, two combination LED/photocells and a bisected reflective disk are used to provide a continuous two-bit signal indicating which quadrant the subject is in. The photocells and disk are housed in a small box which can be suspended over any appropriate testing chamber. The subject is attached to the disk by means of an elastic harness and flexible cable.

Multiple counting periods can be designated by the user before the start of an experiment. At the conclusion of an experiment, data that has been gathered can be printed out, stored to disk or transmitted over a standard RS-232 serial line to a Macintosh or other computer for further analysis. The total cost of the system with 10 test sites is approximately $1000.

[Supported by USPHS NS22157 (JBB), NS25662 (JBB) & DA04294 (TER)].

DRUGS OF ABUSE: ALCOHOL V

312.1

POST-TRAINING EFFECTS OF ETHANOL AND ENVIRONMENT ON APPETITIVE TASK MEMORY. M. Babbini*, M. Bejanian, and R.L. Alkana. Alcohol and Brain Research Lab., Sch. of Pharmacy, Univ. of South. Calif, Los Angeles, CA 90033

Colbern et al. found that ethanol's (E) post-training facilitatory effect on aversive memory in mice disappeared if animals were isolated after training suggesting that E and group housing combined to improve retention by adding aversive information to training. To test this hypothesis, we studied the effects of E on appetitive memory. During individual training, C57BL/6 mice were allowed to find a cheese pellet in the corner of an open field. Then, the mice were injected i.p. with E (2.0 g/kg) or saline (S), returned to their home cage and tested 24 hours later for retention [latency to begin eating on day 1 (L1) vs the latency on day 2 (L2)]. L2 was shorter than L1 in both E and S mice grouped 4 per cage before and after training. When mice were isolated the day before training and housed with 5 new mice for 2 hours after training, L2 was significantly longer than L1 in both S and E groups. When put with a single mouse after training, L2 was shorter than L1 in S mice, but was longer than L1 in E mice. The results support Izquierdo's hypothesis that E's post-training facilitory effects on aversive memory may reflect post-training addition to the stimulus complex, rather than or in addition to enhanced consolidation of memory traces. (Supported by NIAAA grant AA03972).

312.2

POST-TRIAL ADMINISTRATION OF ETHANOL FACILITATES RECALL OF PROSE BUT NOT UNRELATED LISTS OF WORDS. G. Lamberty*, B. E. Beckwith, T. V. Petros, and A. R. Ross*. Psychol. Dept., Univ. of North Dakota, Box 7187, Grand Forks, ND 58202.

Although many studies have documented the detrimental effect of pre-trial treatment with ethanol on human memory, few studies (Kalin, 1964; Parker et al., 1980; 1981) have explored the post-trial facilitation of ethanol on human memory. In the present study, either lists of unrelated words or narrative prose passages were presented to young adult males who reported being in good health and reported being moderate users of alcohol. Immediately after encoding these materials, subjects were given either 1.0 ml/kg of absolute ethanol or the masking solution in a double-blind design. After 24 hours, the subjects returned to the lab and were asked to recall the materials they learned earlier. Ethanol significantly enhanced recall of the prose passages, but had no effect on the recall of the lists of words. It appears that the post-trial facilitation effect is specific for some materials, highly organized and elaborated text, but not others, unrelated lists or words that are rote memorized.

312.3

EFFECT OF MORPHINE ON ETHANOL-REINFORCED RESPONDING IN NONDEPRIVED RATS. K.S. Schwarz-Stevens and H.H. Samson. Alcohol and Drug Abuse Institute, University of Washington, Seattle, WA 98118.

It has been reported that morphine increases home cage ethanol (EtOH) drinking. The present studies assessed morphine's effect on oral EtOH-reinforced responding. Nondeprived rats were trained to respond using the sucrose-fading EtOH initiation procedure. In one study, rats responded on a single lever on an FR4 schedule with either 10% EtOH (n=3) or 10% EtOH mixed with 2% sucrose (n=3) reinforcement. In the second study, rats (n=7) responded under a concurrent FR4FR4 schedule with EtOH (10%) and water as the reinforcers. Daily sessions lasted 30 min. Morphine (0.1, 0.3, 1.0 and 3.0 mg/kg, s.c.) was injected 15 min before selected sessions. Each dose was tested twice.

At the three lower doses, morphine slightly decreased responding independent of reinforcer. In the single-lever experiment, the pattern of responding with 10% EtOH reinforcement suggested that morphine may increase the number of drinking bouts per session. Response patterns with the EtOH-sucrose mix were not changed. In the concurrent study, the .3 and 1.0 mg/kg doses slowed response rates for EtOH reinforcement. At the 3.0 mg/kg dose, all rats' responding was suppressed to near zero.

These results suggest that morphine does not enhance drug-seeking behavior nor increase EtOH intake to excessive amounts in this situation. Also, water-reinforced responding was suppressed in a manner similar to EtOH.

312.4

ETHANOL-INDUCED HYPERGLYCEMIA AND CONDITIONED TASTE AVERSION IN C57 AND DBA MICE. F.O. Risinger, C.L. Cunningham and S.I. Lawley. Oregon Health Sciences University, Portland, OR 97201

Acute exposure to ethanol produces an elevation of blood glucose level. In these experiments the genetic basis for the glycemic response to ethanol and its possible role in development of conditioned taste aversion were examined. Adult male C57Bl/6J and DBA/2J were acutely exposed to ethanol (0-6 g/kg, I.P.) and blood glucose levels determined over 4 hours. C57 mice demonstrated profound dose-related elevations in blood glucose levels while DBA mice were relatively unresponsive. Fasting for a 24-h period prior to ethanol exposure reduced the magnitude of the hyperglycemic response in the C57 mice. A water restriction schedule (2 h water/day) resulted in a significant lowering of basal blood glucose level. Water restricted DBA mice showed significant hypoglycemia following ethanol exposure while C57 mice were unresponsive. In a conditioned taste aversion procedure, C57 mice showed less aversion development to an ethanol paired flavor while DBA mice demonstrated greater dose-dependant aversion. Manipulation of blood glucose levels after ethanol exposure using insulin resulted in potentiation of the ethanol-induced conditioned taste aversion in DBA mice, but not in C57 mice. Profound hypoglycemia was noted in both strains. These results indicate a genetic component for the glycemic response to ethanol and provide further evidence for a genetic component determining the hedonic value of ethanol. There appears to be an inverse correlation between glycemic responsiveness to ethanol and the development of ethanol-induced taste aversion, although the mechanisms underlying this relationship have yet to be determined.
(Supported by AA07702 and AA07468 from NIAAA)

312.5

TASTE REACTIVITY FAILS TO PREDICT ALCOHOL CONSUMPTION DURING RESTRICTED FLUID ACCESS TESTS IN RATS. P.J. Bice and S.W. Kiefer. Department of Psychology, Kansas State University, Manhattan, Kansas 66506-5302.

Taste reactivity tests were used to examine the palatability of 0.5%, 3%, 6%, 9%, 12%, and 15% (v/v) alcohol. Rats were then given the same concentrations, one solution per day, during restricted fluid access tests with a single bottle. Results from the reactivity tests showed that rats displayed little aversive responding across the alcohol concentrations; there was a nonsignificant increase in gapes as the alcohol concentration increased. Ingestive responses (tongue protrusions, lateral tongue protrusions) did not vary despite the relatively large range of concentrations. Mouth movements were consistent up to 6% alcohol and then showed an abrupt increase for the 9% and higher concentrations. Consumption during the restricted access tests showed a similar break between 6% and 9% with consumption significantly decreasing at 9% and remaining low. Correlations between reactivity responses and subsequent consumption were consistently nonsignificant. The data indicate that alcohol consumption, even during relatively short periods of access, is dependent on factors beyond those of simply taste.

(Supported by NIAAA grant AA07185)

312.6

ANXIOGENIC BEHAVIOR DURING ETHANOL WITHDRAWAL: REVERSAL BY BUSPIRONE AS EVALUATED IN THE ELEVATED PLUS MAZE. S.M. Rezazadeh, P.L. Prather and H. Lal. Department of Pharmacology, Texas College of Osteopathic Medicine, Ft. Worth, TX 76107

Ethanol withdrawal in rats produces behaviors indicative of an anxiety state (*J. Pharmacol. Exp. Ther., 247:508, 1989*). This study investigated the effects of buspirone, a 5-HT_{1A} receptor agonist with anxiolytic action, in ethanol withdrawn rats. Using the elevated plus-maze, two parameters were measured: percent open-arm entries (%OAE), and percent time spent in the open arms (%OAT). In an anxiety state, rats spend less time and make fewer entries onto the open-arms. The total number of arm entries is an index of locomotor activity. Long-Evans hooded-rats were fed, for 5 days, a liquid diet which was nutritionally complete and contained 4.5% ethanol. Twelve hours after the last ethanol dose, the rats were injected with buspirone (0, 0.32, 0.64, or 1.25 mg/kg). Fifteen min later, the rats were placed in the center of the maze and observed for 5 min. In rats fed with ethanol-free diet, buspirone caused a reduction in both %OAE and %OAT, with no change in the total arm entries. However, in ethanol withdrawn rats, buspirone dose-dependently increased the %OAE and %OAT. In the ethanol withdrawn animals injected with saline, both %OAE and %OAT were significantly reduced. Thus, the re- duction in open-arm activity, suggestive of anxiogenic-like effects, was shown to occur during ethanol withdrawal and this response was reversed by buspirone. The paradoxical anxiogenic-like effect of buspirone observed in rats given ethanol-free diet may be attributed to the partial agonistic action at postsynaptic 5-HT receptors. In conclusion, pharmacotherapy with selective 5-HT_{1A} agonists may be beneficial in alleviation of anxiety occurring during ethanol withdrawal. (Supported by NIAAA grant AA06890).

312.7

ETHANOL-INDUCED CONDITIONED PLACE PREFERENCE IN MICE: ROLE OF CONDITIONING TRIAL DURATION. C.L. Cunningham and L.K. Prather*. Department of Medical Psychology, School of Medicine, Oregon Health Sciences University, Portland, OR 97201.

Recent studies in this lab suggest that the place conditioning paradigm offers a sensitive model for assessing EtOH's rewarding effects in mice. These studies show a conditioned *preference* (CP) for EtOH-paired cues that is influenced by dose and genotype. The purpose of the present experiments was to determine the effect of conditioning trial duration on strength of EtOH-induced CP. In a counterbalanced, differential conditioning procedure, DBA/2J mice received four pairings of a distinctive floor stimulus with injection of EtOH (2 g/kg); a different floor stimulus was paired with saline. Different groups were exposed to the floor stimuli for 5, 15 or 30 min after injection. CP was inversely related to trial duration, with mice in the 5, 15 and 30-min groups spending 83, 74 and 66% of their time, respectively, on the EtOH-paired floor during a choice test. This outcome was replicated in a second study which showed that part of the difference between 5 and 30-min groups is due to a difference in total duration of exposure to the apparatus. In general, these findings support the notion that EtOH produces an initial, short-lived excitatory (rewarding) effect that is replaced by a longer-lasting inhibitory (aversive) effect.

(Supported by NIAAA grants AA07702 and AA07468).

312.8

EARLY HANDLING MODIFIES HYPOTHERMIC RESPONSES OF RATS EXPOSED TO ETHANOL IN UTERO. J. Weinberg, Dept. of Anatomy, Faculty of Medicine, The University of British Columbia, Vancouver, B.C., Canada V6T 1W5

Animals handled daily during the preweaning period are less emotional and show more adaptive responses to stress than nonhandled animals. Previous data have shown that early handling may interact with prenatal ethanol exposure to modulate pituitary-adrenal activity and responsiveness. The present experiment examined effects of early handling on ethanol-induced hypothermia in adult animals exposed to ethanol in utero. Ethanol (A) was administered to pregnant Sprague-Dawley rats in liquid diets, gestation days 1-22; pair-fed (PF) and ad libitum fed (C) control groups were included. From birth to 15 days of age, half the litters in each condition were handled for 3 min daily; the rest were undisturbed until weaning. In adulthood, male and female offspring were tested for hypothermic responses to a challenge dose of ethanol (2 g/kg, ip). For nonhandled males, the hypothermic response to ethanol was greater in A and PF then in C animals; early handling eliminated the differences among groups. Group differences were not significant for females. These data indicate that early handling can modulate physiological responsiveness in animals prenatally exposed to ethanol, and that males and females may be differentially affected.

Supported by grant #AA07789 from NIAAA.

312.9

SPIROXATRINE ANTAGONIZES THE HYPOTHERMIC EFFECT OF ETHANOL IN MICE. M.K. Menon and R.L. Lloyd. Psychopharmacol. Res. Lab., V.A. Med. Ctr., Sepulveda, CA 91343 and Dept. Psychiat. UCLA Sch. Med., Los Angeles, CA 90024.

Male Swiss-Webster mice (Hilltop) were used and, if not stated otherwise, all injections were made i.p. Rectal temperature was measured using a telethermometer. In these animals, spiroxatrine (0.1-1.0 mg/kg), BMY 7378 (1.0-3.0 mg/kg) and pindolol (3-10 mg/kg s.c.) injected 10 min before, blocked the hypothermic effect of the 5-HT_{1A} agonist 8-OHDPAT (1.0 mg/kg., s.c.) in a dose-dependent manner. It was concluded that three drugs act as 5-HT_{1A} antagonists in mice. In the above dose range, only spiroxatrine blocked the hypothermic effect of ethanol (2.4 g/kg, i.p.). It was inferred that the blockade of this response to ethanol by spiroxatrine does not depend upon the 5-HT_{1A} antagonistic effect. Further studies indicated that the interaction between spiroxatrine and ethanol did not take place at the dopamine, opioid or α_2 adrenergic binding sites. Spiroxatrine did not block the hypnotic effect of ethanol (3.9 g/kg). It appears that neurochemical studies between ethanol and spiroxatrine could provide information on the mechanism by which ethanol produces hypothermia. (Supported by the U.S. Veterans Administration).

312.10

FAT-PREFERRING RATS CONSUME MORE ALCOHOL THAN CARBOHYDRATE-PREFERRING RATS. D.D. Krahn, B.A. Gosnell and M.J. Majchrzak, Dept. of Psychiatry, University of Michigan, Ann Arbor, MI 48109.

Rats with a genetic preference for alcohol (ETOH) have been found to consume more dietary fat than ETOH non-preferring rats. We therefore hypothesized that rats selected on the basis of fat and carbohydrate (CHO) preferences would differ in ETOH intake. Patterns of macronutrient self-selection were determined by allowing rats to self-select their diets from separate sources of CHO, fat and protein. From measurements of daily intakes, fat-preferring and CHO-preferring groups were formed (n=8/group). All rats were then returned to a standard lab chow diet, and placed on a schedule in which chow was available for 4 hrs/day (1 hr in testing cages, 3 hrs in home cages). In the 1 hr drinking session, 4% ETOH was available as the only fluid; water was available *ad lib* at all other times. The ETOH concentration was increased by 4%/week to 12%; after 7 sessions with 12% ETOH, the food deprivation schedule was discontinued. Food was then available *ad lib* except during daily 1 hr drinking sessions. For 22 additional days, rats were given daily 1 hr drinking sessions (12% ETOH). After 2 days on which only water was presented in the daily sessions, water and 8% ETOH were alternated daily (for 6 days) as the only available fluid in the session. Intakes were averaged for the final 3 water sessions and the three 8% ETOH sessions and compared with repeated measures *t*-tests. Fat-preferring rats consumed significantly more 8% ETOH than water (3.0±0.5 vs 1.9±0.2 mls, $p<.01$); CHO-preferring rats consumed approximately equal volumes of ETOH and water (1.5±0.4 vs 1.2±0.5 mls, NS). Furthermore, fat-preferring rats consumed more ETOH than CHO-preferring rats ($p<.05$). This study suggests a positive relationship between fat preference and oral intake of ETOH. This effect appears to be due to differences in diet *preference* rather than to actual intake, because both groups were maintained on standard lab chow during testing. (Supported by NIDA Grant DA05471)

312.11

A Comparison of Three Inverse Benzodiazepine Agonists on Ethanol Stimulant Effects. L.H.Hicks,H.L.June,T.O.Moore and M.J.Lewis. Howard Univ.,Washington, DC 20059

A number of reports suggest that GABA-BDZ mechanisms may mediate the behavioral effects of ethanol (E). We previously showed that both the locomotor stimulant and depressant effects of E could be altered by Ro15-4513 (RO) an imidazobenzodiazepine inverse agonist.

The present research compared the actions of RO, BCCE and FG 7142 (two other inverse agonist) and Ro15-1788 (a CNS BDZ antagonist) on the stimulatory effects of E in rats habituated to an open field. To specifically investigate these effects, three components of activity were assessed: horizontal activity, vertical activity and total distance (# of inches travelled). The results showed that E (.12-1.0 g/kg) alone stimulated all components of locomotor activity. All inverse agonists appeared to attenuate E effects on each of the three measures. The antagonistic actions of Ro15-1788 was, however, apparent only in animals pretreated with RO and E. Ro15-1788 given alone, however, enhanced all locomotor activity measures above control levels. These results suggest that some of the antagonistic actions of E may be shared by various inverse agonist compounds.

(Supported in part by NIAAA grants AA06263 and RR08016)

312.12

FEMALE SEX STEROIDS AND ETHANOL: A PROFILE OF ACETALDEHYDE LEVELS, WHEEL RUNNING AND SLEEP IN SPAYED FEMALE RATS. A. Torres and F.A. Holloway, Department of Psychology, University of Alabama at Birmingham, Birmingham, Al 35294, and Department of Psychiatry and Behavioral Sciences, University of Oklahoma Health Sciences Center, Oklahoma City, OK. The effects of the female sex steroids estradiol-17β (E_2) and progesterone (P_4) on responses to acute ethanol administration were evaluated by comparing responses in four groups of spayed female rats: OVX, E2, P4, E2P4. Estrogen administration resulted in higher acetaldehyde levels (e.g., 8.6-9.3 ng/ml versus 4.9-6.0 ng/ml) although no differences in peak ethanol level were found for 0.4, 1.2, 3.2 g/kg EtOH. Running wheel activity was monitored for one hour in 15-minute sessions at 0.4, 0.8 and 1.2 g/kg EtOH. A significant depression in cumulative running activity was produced by ethanol in groups with P_4 implants. When evaluating wheel running for the 15-minute sesssions, increases above saline rates were found in groups with E_2 implants. Finally, progesterone was associated with an increase in sleep onset time to 4.0 g/kg EtOH. This research deomonstrates that ethanol produces differential effects in rats with different steroid backgrounds.

312.13

PERFORMANCE ON THE PLUS-MAZE TEST OF ANXIETY IN WISTAR, ALCOHOL-PREFERRING (P) AND -NONPREFERRING (NP) LINES OF RATS: EFFECTS OF ETHANOL AND CHLORDIAZEPOXIDE (CDP) RB Stewart, JM Murphy, L Lumeng* and T-K Li* Pyschiat Res & Regenstrief Insts, Indiana Univ Sch Med, & Psy Dept, Purdue Sch Science, Indianapolis, IN 46202

The plus-maze test of anxiety was used to assess the effects of selective breeding for ethanol preference in alcohol-naive P, NP and Wistar rats. In this test, the degree of anxiety is assumed to be inversely proportional to length of time spent on the open arms of the maze. Number of arm entries is used to measure activity. In Expt 1, Wistar rats (n = 7-9/dose) received ip injections of saline, 0.25, 0.5 or 1.0 g ethanol/kg BW. Time spent in open arms and activity were unchanged by all doses of ethanol. In Expt 2, P and NP rats (n = 8-11/line/dose) received saline, 0.5 or 1.0 g ethanol/kg BW or 7.5 mg CDP/kg BW. CDP produced a similar increase in time spent on the open arms for both lines ($p<0.001$), *while ethanol had no effect. However, compared with NP* rats, P rats spent less time on the open arms under all conditions ($p<0.003$). No line differences in activity were observed, except that 1.0 g ethanol/kg BW reduced activity only in NP rats ($p<0.001$). The findings indicate that selective breeding for ethanol preference is associated with increased levels of anxiety in rats of the P line, but ethanol is an ineffective anxiolytic compared with CDP. (AA03243, AA07611)

312.14

COCAINE POTENTIATES ETHANOL'S THRESHOLD LOWERING EFFECT ON BRAIN-STIMULATION REWARD. M. Moolten and C. Kornetsky. Laboratory of Behavioral Pharmacology, Boston Univ. Sch. of Med., Boston, MA 02118.

We have found that ethanol will, as do other abused substances, lower the threshold for rewarding brain stimulation. In addition, abused drugs that increase an animal's sensitivity to brain stimulation reward (BSR), such as morphine or cocaine, will have synergistic effects on BSR, when co-administered. Because cocaine and ethanol are often used together, the present experiment was designed to determine whether cocaine would potentiate the threshold lowering effects of ethanol. Dose effect curves for ethanol's action on BSR were determined in Lewis rats with stimulating electrodes in the medial forebrain bundle, with and without the co-administration of an ineffective dose of cocaine (0.5 mg/kg ip, or less). The addition of the cocaine significantly increased the potency of the ethanol, shifting the dose-effect curve to the left. These results support the hypothesis that ethanol's rewarding effects are mediated, in part, by similar neuronal systems as other abused substances.
(Supported by NIAAA grant AA05950 and Research Scientist Award *DA00099* to CK).

312.15

ALCOHOL AND RELAXATION EFFECTS ON LOW FREQUENCY EEG VARIABILITY. L. T. Crow, Y. G. Quevedo*, and P. J. McKinley*, Dept. of Psychology, Western Washington University, Bellingham, WA 98225.

Parietal electroencephalograms (P3 and P4) were taken from college students after a 30-min. ingestion period of 0.4 g/kg ethanol in table wine. FFTs of AD samples were taken at three intervals during a post-drinking relaxation period in which subjects reclined in a dark room.

While variance measures of frequency magnitudes between 4 and 16 Hz indicated significant alcohol effects for the first [$F(7,12)=7.31$] and third [$F(7,12)=3.59$] readings, coefficients of variation (100 x S.D./mean) did not. There were no significant differences in either mean magnitudes [$F(1,19)=0.31$] or coefficients of variation [$F(1,19)=.028$] between alcohol and control groups.

Coefficients of variation did, however, indicate significant increases in EEG variability over relaxation time [$F(2,42)=6.68$]. The results are believed to support a general theory of "environmental tolerance" and behavioral variability (Crow, L. T., Psych. Rec., 27:783, 1977) and to bear on the heuristic aspects of drug effects on EEG variability.

312.16

WITHDRAWN

312.17

BRADLEY BIOLOGICAL ALCOHOL SCREENING TEST (BBAST)
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MI. 48824-1316.
The use, abuse and dependency of psychoactive drugs (as DSM-III-R defined) in the U.S.A. is an at an all time high. The psychoactive drugs are seriously affecting the working population between the ages of 20-40 years of age. In this study, we have examined 40 psychoactive drug using adults [PDUA](mean age for both=30) and 20 aged matched sex control adults. The majority of the reports on alcoholism indicate that it is a behavioral problem. This study shows that 50% of the PDUA have a clear genetic linkage via the peripheral immune system via conservation of the genome. We have examined their psychosocial, psychiatric, SMAC-21, HbA/HbB&non HbA/B, HTL-IIIAb and a series of immunological functions(all examined at the same time of day 8-11 a.m.). Exclusion criteria were strictly upheld. This study revealed that the PDUA had no Axis I diagnosis of any major psychiatric magnitude but we did find 50% of the PDUA to possess an Axis II diagnosis: cluster B. The CD-8 genetic marker (U.S.A. Patent Pending-Test Kit for inherited substance abuse dependency) is 2 (two) standard deviations from the control CD-8 (T-Suppressor/Cytotoxic) absolute total number Human Lymphocyte. Profound meanings can be derived from PDUA CD-8 deviation.

NEUROETHOLOGY: INVERTEBRATES

313.1

THE CRAWLING BEHAVIOR OF THE LEECH, *Hirudo medicinalis*: PROPERTIES AND CONNECTIONS OF CIRCULAR MOTOR NEURONS. A. Baader and W. B. Kristan, Jr., Deptartment of Biology, University of California at San Diego, La Jolla, CA 92093-0322.

A starving leech can either swim or walk to a prey to satisfy its appetite. How does it decide between the one or the other behavior? In contrast to swimming, little is known about crawling. As a first step, we have analyzed candidate neurons for crawling behavior on three different levels:

1) In behaving animals. A leech walks on a ball and a movable stage in a water filled tank with two segments of its midbody suspended in a holder and a ventral ganglion was exposed to allow intracellular recordings. 2) In ganglia with an attached piece of body wall. Interneurons and motor neurons of circular muscles were stimulated to determine their output effects. 3) In an isolated chain of ganglia. We recorded from motor neurons of the circular muscles which are active during crawling but not during swimming.

Dorsal circular muscles are innervated by at least three pairs of motor neurons: CV, 112 and 152. CV neurons are excited by cell 152 and inhibited by cell 157 stimulation. Cell 208, a swim interneuron, produces circular contraction of the skin when stimulated, and excites cell 152. Other identified interneurons (e.g. 204 or S) have no effect on the CV cells. CV cells receive strong mechanosensory inputs from P cells and weaker input from T and N cells. The results provide the basis for studying the circular motor output in a tethered walking animal and finding interneurons involved in either swimming and/or walking.

Supported by a USPHS research grant, MH 43396 to WBK.

313.2

NEURONAL PATHWAYS INVOLVED IN THE EXPRESSION OF EGG LAYING BEHAVIORS IN THE POND SNAIL *LYMNAEA STAGNALIS*. R.F. Jansen, G.P. Ferguson+, A.W. Pieneman* and A. Ter Maat, Dept of Biology, Vrije Universiteit, De Boelelaan 1087, 1081HV Amsterdam, The Netherlands, +Stazione Zoologica, Naples, Italy.
The egg laying behavior of the pond snail consists of three consecutive phases: Resting, Turning and Oviposition, each named after the activity of the animal during these phases. The patterns of motor activity characteristic of overt egg laying behaviors (locomotion, turning, and rasping) are caused by the coordinated action of sensory feedback from the ovotestis and several neuropeptides. These neuropeptides are released during a synchronous burst of spiking activity in the neurosecretory caudodorsal cells (CDCs) that precedes egg laying.
The pathways via which sensory feedback from the genital tract reaches the central ganglia ring to activate motor circuits of egg laying behavior were identified in a lesion study. Of the visceral nerves, the intestinal nerve is both necessary and sufficient for the full expression of the high rasping activity by the buccal mass during Turning and Oviposition. For the activation of shell turning the intestinal nerve is necessary, but not sufficient. In vivo recordings were made from the intestinal nerve in freely behaving animals during egg laying. Using a spike separating algorithm we were able to show that during egg laying a specific change occurs in most of the individual units contained in this nerve. The ways in which the individual firing patterns are modulated during egg laying and how they account for the observed effects of the sensory feedback on the overt behaviors is currently being investigated.
One question that remains to be answered is whether the CDC peptides only serve to release eggs from the ovotestis. Preliminary experiments show that the CDC ovulation hormone suppresses normal feeding responses of the buccal system. This suggests that CDC peptides are involved in the suppression of behaviors that are not compatible with egg laying.

313.3

ORIENTATION TO WATER-FLOW IN *TRITONIA*: NEURAL BASIS OF RHEOTAXIS. J.A. Murray* and A.O.D. Willows, Friday Harbor Laboratories, Friday Harbor, WA 98250.

The nudibranch gastropod *Tritonia diomedea* orients to and moves toward water-currents in the lab and in nature. Field observations indicate that tidal flow threatens to pull the animal off the substrate and away from food and potential mates. Additionally, flow-tank experiments show that orientation to current reduces hydrodynamic drag. We examined the neural basis of this rheotaxis behavior by recording intracellularly from the brain, in a semi-intact animal preparation. Neurons of the pedal and cerebral ganglia respond to water-flow directed to the oral veil and rhinophore sheaths with bursts of EPSPs and action-potentials.

We identify 14 neuron pairs of the pedal ganglia that respond tonically and/or phasically to water flow directed at the rhinophore sheaths and oral veil tips. Most respond equally to inputs from either side of the body, but 5 respond with greater intensity to stimuli originating ipsilaterally. When stimulated intracellularly, 5 pairs of these neurons drive ipsilateral movements that turn the animal in the direction of the stimulus. Further, 6 of these pairs of neurons make polysynaptic, excitatory connections to another pedal neuron (Pd5), previously shown to be sensitive to changes in earth-strength magnetic fields. Pedal neurons like those we found that respond more strongly to ipsilateral water-flow, affect ipsilateral turns, and don't fully adapt to the water-flow stimulus (eg Pd3) after a minute are likely to play a key role in rheotaxis. Pd3 is also thought to contribute to escape swimming (Willows *et al.*, J. Neurobiol. 4:255, 1973). These observations are consistent with the hypothesis that current-sensitive neurons contribute to previously described behavioral orientation to water currents and to the lunar-modulated magnetic heading preference of *T. diomedea*.

Supported by N.I.H. grant NS22974 to A.O.D.W..

313.4

ODOR-MEDIATED UPWIND FLIGHT BEHAVIOR OF THE TOBACCO HORNWORM MOTH, *Manduca sexta*. M.A. Willis and E.A. Arbas, ARL Div. of Neurobiol., Univ. of Arizona, Tucson, AZ 85721.

The upwind flight response of male moths to sex-pheromone released by conspecific females has long been used as a model for study of long-distance orientation to odors by insects. A primary mechanism mediating this response is an odor-triggered, visually guided anemotaxis.

We studied the odor-mediated upwind flight response of freely flying males and females of *M. sexta* in a laboratory wind tunnel. We presented appropriate odors to males and females in flight, removed the odor while the moths were still in flight, and varied the wind velocities while video-recording the flight responses. The track of each moth was digitized, and velocity and steering manoeuvers were measured every 0.03 seconds.

Males and females responded to their appropriate odors (females, host-plant odor; males, pheromone) with zigzagging upwind flight. The loss of odor causes a rapid change in steering and flight speed, resulting in the arrestment of upwind progress and widening of the cross-wind component of the flight path. When confronted with varying wind velocities, males flying toward sources of female pheromone make compensatory changes in their visually guided steering and flight speed. Of particular interest is the temporal regularity of the cross-wind counterturns. Both males and females exhibited turns at similar frequencies, ca. 2 Hz. This turning frequency remained similar at all wind velocities tested, even though flight speed and steering changed significantly. Upon loss of odor, the frequency of counterturning decreased significantly until it eventually ceased. This temporal regularity suggests the presence of a stereotyped program of counterturning, triggered by olfactory stimuli, that combines with the motor program for wing beating to enable successful odor source location. [Supported by NIH grants NS-23405 and NS-07309.]

313.5

OLFACTORY AND VISUAL INFLUENCES ON FICTIVE FLIGHT IN *MANDUCA SEXTA*. R. Kanzaki and E.A. Arbas ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

Male sphinx moths *Manduca sexta* respond with oriented flight when they are stimulated with the sex-attractant pheromone blend released by a receptive female. Multimodal inputs (olfactory, visual, mechanosensory etc.) coordinate this flight and guide the male to the female. To study the influences of these inputs, we have developed techniques to elicit fictive flight (herein called "flight") from intact or dissected *Manduca*, and apparatus to provide multimodal stimuli. To elicit flight we injected chlordimeform [N-(2-methyl-4-chlorophenyl)-N',N'-dimethylformamidine, 10^{-9} - 10^{-7} moles/insect dissolved in acetone and diluted in saline] and provided sensory stimuli of different modalities. A continuous current of filtered air was delivered via a small wind tunnel equipped with ports into which pulsed odor stimuli were injected. The mouth of the wind tunnel was surrounded by an array of >125 green LEDs controlled by a computer to simulate the visual flow fields for forward, backward, or turning movements. All tethered moths (n>20) so stimulated flew robustly with wingbeat frequencies (measured with EMG) of 15-25 Hz and attempted to steer when turns were simulated. Stimulation with pheromone blend alone elicited flight in fewer preparations than did visual stimuli alone. After all of their peripheral nerves had been cut, and during presentation of visual stimuli, thoracic ganglia produced antiphasic activity in depressor and elevator motorneurons (Mns) at burst frequencies of 8-10 Hz. If neck connectives were cut during ongoing activity, bursting continued for 15-30 sec before stopping. In a small number of preparations tested with connectives intact, stimulation with pheromone blend alone caused increased motor outflow in elevator and depressor Mns but not, as yet, in the pattern of flight. [Supported by NIH grants NS-23405 & NS-07309].

313.6

STATE-DEPENDENT ACTIVITY CHANGES IN DESCENDING NEURONS IN OLFACTORY PATHWAYS OF *Manduca sexta*. E.A. Arbas, R. Kanzaki, and J.G. Hildebrand ARL Div. of Neurobiology, Univ of Arizona, Tucson, AZ 85721.

Our aim is to understand how the sex-attractant pheromones released by female moths activate and influence the oriented nuptial flight of male moths. We have used intracellular recording and staining with Lucifer Yellow followed by reconstruction from serial sections to characterize higher-order pheromone-responsive olfactory interneurons in the brains of male spinx moths, *M. sexta* including neurons whose axons descend in the ventral nerve cord [Soc. Neurosci. Abstr. 14: 379 (1988)]. Descending neurons that were excited by pheromonal stimuli were usually also excited by visual stimuli (lights on/off; movement) and sometimes by non-pheromonal olfactory stimuli as well. Excitatory responses to pheromone blends were of two general types: (a) brief increases in firing that recovered to background in <1 sec after the stimulus, and (b) long-lasting excitation (LLE) that outlasted the stimulus by >1 sec and, in some cases, as long as minutes. Neurons showing LLE also exhibited additional, state-dependent responses to sensory stimuli. For example, neurons firing at rates near 8 Hz responded to pheromone blend with acceleration of firing to persistent levels near 20 Hz (i.e. LLE was elicited). When similar stimuli were reapplied, firing was reduced to 8-12 Hz for up to 5 sec and then gradually increased to the initial high level. Thus, identical pheromone blend stimuli: (a) accelerated firing when the neurons were in a state of low background firing, and (b) decelerated firing when the neurons were in a state of high background firing. Individual pheromone components were ineffective. Visual stimuli (lights on/off) interacted with olfactory stimuli to produce other state-dependent responses. [Supported by NIH grants NS-23405 & NS-07309].

313.7

THE GIANT FIBER PATHWAY IN HAWAIIAN *DROSOPHILA* (INSECTA: DIPTERA). D. G. King, Dept. of Anatomy and Dept. of Zoology, Southern Illinois Univ., Carbondale, IL 62901.

Evolution in the Hawaiian islands has produced many diverse species of *Drosophila*. Axons of the giant fiber pathway were recognizable in each of six such species, using the same criteria that identify homologous axons in *D. melanogaster* and other flies (King & Valentino, J. Comp. Neurol. 219:1, 1983). Body size of each species was larger than that of *D. melanogaster*, and axons in the pathway were correspondingly more conspicuous. These observations are unsurprising since such closely related species might be expected to share similar structures, even though giant axons are known to vary tremendously among flies generally (King, J. Morph. 175:27, 1983; Benson & King, Soc. Neurosci. Abstr. 10:51, 1984).

However, the putative escape behavior controlled by the giant fiber pathway appears weaker in these Hawaiian drosophilids than in *D. melanogaster*. Unlike many more familiar species, these flies can be caught easily by hand, suggesting that evolution may have altered neuronal functions associated with the pathway. Comparative physiology of this pathway might prove interesting.

(Specimens were provided by K. Kaneshiro, Hawaiian Evolutionary Biology Program, University of Hawaii.)

313.8

PLASTICITY OF THE CRICKET RIVALRY SONG DURING AGGRESSIVE ENCOUNTERS. T.G. Nolen and C. Lam, Dept. of Biology, University of Miami, P.O. Box 249118, Coral Gables, FL 33124.

Male crickets employ acoustic signals to establish and defend their territories. As a prelude to neuroethological studies of aggressive behavior we have found that the probability of singing the RIVALRY SONG depends on the experience of the combatants; and that the RIVALRY SONG generally is necessary (but not sufficient) for territorial success. Adult males (*Acheta domesticus*), matched for size, were introduced into an arena and their aggressive interactions and acoustic behavior recorded over a ten minute observation period (sufficient for one male to gain control of the territory).

The territory holder at the end of ten minutes had won >90%, and initiated >70% of the encounters, and was the first to sing (88% of encounters); [all $p<0.005$; 25 pairs, 340 encounters]. Moreover, while the probability that either male would sing was about 50% initially, by the end of the observation period, the winner sang in >80% of the encounters (vs <20% for the losser). Muted males were less likely to establish control of the territory in encounters with a vocal male [$p<0.01$, compared to vocal controls, n=17 & 12 pairs respectively]; and were less likely to maintain control of the territory [85% won BEFORE, vs 35% AFTER muting; $p<<0.0001$, compared to sham control groups]. Nevertheless, some muted males retained their territory and some vocal males could not establish control in encounters with a muted male.

Pilot studies suggest that the RIVALRY SONG could play two roles in aggressive interactions: (1) a conditioning role in establishing male-male relationships, and, (2) a conventional communication (signalling) role involved in maintenance of the territory. Current experiments involve manipulation of the individuals' acoustic experience, the analysis of changes in the song during encounters and the plasticity of auditory neurons in response to RIVALRY SONG. [Supported by NSF & the UM Biol. Dept. Alumni Fund.]

313.9

REGULATION OF PHONOTAXIS BY FEMALE CRICKETS THROUGH JUVENILE HORMONE III CONTROL OF AN IDENTIFIED AUDITORY INTERNEURON. J. Stout, D. Zacharias*, V. Rochette*, and G. Atkins, Dept. of Biol., Andrews Univ., Berrien Springs, MI 49104.

Removal of the corpora allata from sexually responsive female crickets (*Acheta domestica*) eliminates their phonotactic response to the calling song of conspecific males. Treatment of these allatectomized females with Juvenile Hormone III (JHIII) restores phonotaxis. Females normally become phonotactically responsive on the third or fourth day following the imaginal molt, at a time when production of JHIII is maximal. However females do not suddenly become phonotactically responsive on days 3 or 4. Rather the behavioral threshold for the female's response to the intensity of the male's call drops progressively between days 2 and 4, while JHIII production is increasing.

The L1 auditory interneuron is tuned to the carrier frequency of the male's calling song and encodes its temporal pattern. Activity of this neuron is necessary for the female's phonotactic response to model calling songs with an intensity below 70 dB. The threshold for the L1 neuron's response to model calling songs drops in females 2 to 4 days of age in parallel with the decrease in the female's behavioral threshold for phonotaxis. Treatment of a one day old female with JHIII causes the female's phonotactic threshold to drop 20 to 30 dB over the next 12 hours. The threshold of the L1 neuron also drops 25 to 35 dB, 12 hours following treatment with JHIII.

The effect of JHIII on the female's phonotactic threshold can be blocked by simultaneous injection of α-amanatin (a transcription blocker) or emetine (a translation blocker). These results suggest that JHIII might regulate the sensitivity of the female to the male's call by genetically regulating the sensitivity of the L1 neuron to the calling song.

313.10

A NEURAL CORRELATE FOR CONTROL OF SYLLABLE PERIOD SELECTIVE PHONOTAXIS IN THE CRICKET *ACHETA DOMESTICA* BY JUVENILE HORMONE III. G. Atkins, J. Henley* and J. Stout. Dept. of Biol., Andrews Univ., Berrien Springs, MI 49104.

Phonotaxis of female *Acheta domestica* is dependent on the syllable period (SP) of the calling song. This SP selective response is age-related. Young females (4-5 days following the imaginal molt) respond to a narrow range of SPs (50-70 ms), while old females (14 - 21 days) respond to a wide range of SPs (30-100 ms). This decreasing selectivity is inversely correlated with Juvenile Hormone III (JH3) synthesis which peaks in 3 to 5-day-old adult females and is relatively lower in older females. Addition of JH3 to old, unselective females resulted in these females becoming more selective 4 days following application. They became as selective as 5-day-old crickets.

A pair of auditory interneurons in the prothoracic ganglion of females known to be involved in phonotaxis (L3), has been shown to exhibit SP specific responses to calling songs (Atkins et al. J. Comp Physiol 165:827. 1989). In intracellular recordings L3 shows a decrementing response to successive syllables of a chirp having ideal SPs (50-70 ms) which is less apparent in reponse to increasing or decreasing SPs. We now report that this response in L3 is related to the age of the females and parallels the changing behavioral selectivity to SP. In 4-day-old females, L3 shows maximal decrement to ideal SPs whereas in 21-day-old females, L3 does not decrement at any SP and thus is unselective. Application of JH3 to old females (21 days) results in L3s which exhibit a strong decrementing response to ideal SPs similar to that of L3s in young females. Further, the effect of JH3 on L3 is maximal 3 - 4 days following application which parallels the time course of the behavioral effect of JH3.

313.11

THE "PRECEDENCE EFFECT" AND "ECHO SUPPRESSION" IN NEGATIVE PHONOTAXIS OF THE CRICKET *TELEOGRYLLUS OCEANICUS*.
R.A. Wyttenbach and R.R. Hoy. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

Tethered flying crickets make steering movements away from pulses of ultrasound. These movements include forewing tilt, abdomen swing, and metathoracic leg swing, all of which are lateralized relative to the sound source. A bilateral pair of ascending prothoracic auditory interneurons (int-1) has been shown necessary and sufficient to elicit these responses: stimulation of an int-1 (electrically or by ipsilateral ultrasound) causes steering to the contralateral side, while hyperpolarization prevents steering in response to sound. To correctly localize a sound source in the presence of echoes, directional information must be taken only from the first-arriving wavefront (precedence effect) and subsequent wavefronts cannot be treated as coming from additional sources (echo suppression).

We simulated a sound and its echo by presenting equal-intensity pulses from the right and left of a cricket with a variable delay between pulses. For delays of 4 to 75 ms, a single turn away from the first pulse is made. With smaller delays, a single turn in an arbitrary direction is made, while greater delays give rise to separate turns in response to each pulse. Thus the precedence effect occurs at delays greater than 4 ms and echo suppression occurs at delays less than 75 ms. In these experiments, abdomen swing, forewing tilt, or EMG of metathoracic leg abductors was monitored as an indicator of turning.

Investigation of the neural basis of this response has begun with extracellular recordings from both int-1s. When two pulses are presented as above, there is no difference between the two int-1s in spike number and only a small difference in maximum spike frequency at delays of 4 to75 ms. This difference probably cannot account for the clear single turns, and there are no int-1 differences that could account for the arbitrary turns at 0 to 2 ms delay. Work on suppression of the response to the delayed pulse is continuing with intracellular recording from int-1 and consideration of other auditory cells.

313.12

KINEMATICS AND PHYSIOLOGY OF THE ULTRASOUND-INDUCED LEG SWING OF FLYING CRICKETS.
C.I. Miles, M.L. May and R.R. Hoy. Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

In tethered flying crickets, *Teleogryllus oceanicus*, ultrasound (20kHz) induces a rapid swing of the metathoracic leg into the sweep of the hindwing contralateral to the sound source. This movement directly impedes the downstroke, and exerts a pronounced aerodynamic effect, assisting in a rapid turn away from the sound. This reaction is believed to be mechanism for evading hunting bats. We analyzed the kinematics of the leg's movement and recorded electromyograms from a muscle involved in producing it.

The leg movement involves both abduction and elevation, and the extent of these movements is linearly related to the intensity of the sound. Abduction and elevation increase from about 4° and 3°, respectively, at threshold intensity (72dB) to 18° and 10°, respectively at 97dB. The duration of the leg's swing is also linearly related to stimulus intensity, ranging from 136ms at 72dB to 204ms at 97dB, in either case being long enough to impede the wing for several wingbeats. The latency of the leg swing decreases exponentially with increasing stimulus intensity, from about 72ms at 72dB to 35ms at 97dB. The shortest latency we recorded was 29ms.

The metathoracic leg abductor (muscle 126) responds to ultrasound with a burst of spikes followed by activity that is in phase with the flight motor pattern. The response also showed linear increases in response and duration, and a linear decrease in latency with increasing stimulus strength. The mean response increases from about 1 spike/stimulus at 68dB to 16 spikes/stimulus at 98dB, while the mean duration increases from 103ms at 72dB to 218ms at 98dB, similar to the durations measured for the leg's swing. Latency to the first muscle spike declines from 63ms at 68dB to 36ms at 98dB. The shortest latency measured was 24ms.

The metathoracic leg swing has the shortest latency of any aspect of negative phonotaxis described so far. It is a simple yet effective component of this behavior, and provides a favorable system for further neurophysiological studies. We have identified the motoneuron for 126, and its role in the behavior is under investigation.

313.13

NEURAL ACTIVITIES OF HIGHER BRAIN CENTERS OF FREELY-MOVING COCKROACHES.
M. Mizunami and N. J. Strausfeld. Arizona Research Labs, Div. of Neurobiology, Univ. of Arizona, Tucson AZ 85712

Two areas of the insect brain, the mushroom bodies and the central complex, have been implicated in memory and behavioral control (Huber F., Z. Vergl. Physiol. 44:60, 1960), but their respective functional organization is less understood. We have established methods for recording units in higher brain centers from freely behaving cockroaches. Enamel-coated copper or nichrome wires (20 μm in diameter) were implanted chronically into the brain of cockroaches. Unit activity was recorded while the cockroaches move freely in an arena. The site of recording was detected by copper or nickel sulfide precipitation followed by silver intensification. This procedure also revealed the profile of single identifiable neurons which could be matched to the morphology of cobalt-silver or Golgi impregnated elements. Units of higher centers could be classified into several types from their sensory and behavioral correlates. Possible participation of the mushroom bodies in cognition of environmental space and possible participation of the central complex in the control of behavior will be discussed.
Supported by NIH Grant No. F05 TWO4390-01.

313.14

VIDEOTAPE MOTION ANALYSIS OF LEG JOINT ANGLES DURING ESCAPE TURNS OF THE COCKROACH.
S.W. Nye and R.E. Ritzmann. Dept. of Biology, Case Western Reserve University, Cleveland, OH 44106.

The cockroach, Periplaneta americana, escapes from wind puffs by making a rapid and directional turn away from the wind source followed by a more random run. To achieve our goal of relating neural activity to the turning movements, we require a detailed understanding of the changes in each joint angle for all of the six legs. We have employed a high speed videotape recording system (200 frames/second) and motion analysis software to analyze each joint angle of tethered cockroaches responding to wind stimulation presented at 45, 60, 90, and 120 degrees relative to the rear of the insect.

Our data indicate that the legs of each segment play very different roles during the turn. For example, the mesothoracic legs provide direction for the turn, whereas the metathoracic legs provide more power than direction. Directionality arises in the mesothoracic legs by changes in the femoro-tibial (F-T) joint angles. In the mesothoracic leg ipsilateral to the wind, the F-T joint angle increased, while in the contralateral mesothoracic leg the F-T joint angle decreased. This caused both mesothoracic legs to move toward the wind source providing a force that would turn the animal away from the wind. In contrast, winds from the rear caused the C-F joints of both metathoracic legs to increase in angle driving the legs backward. Unlike the mesothoracic legs, the changes in the F-T joints of the metathoracic legs were approximately equal to changes at the C-F joint of the same leg. As a result, the force vector produced by these legs remained parallel to the long axis of the animal's body.

Presently we are obtaining electromyographic recordings that will relate changes in leg joint angle to the activity of individual muscles.
This work was supported by NIH grant NS 17411 to R.E.R.

313.15

A COMPUTER MODEL FOR ESCAPE IN THE COCKROACH.
R.D. Beer, G.J. Kacmarcik, R.E. Ritzmann and H.J. Chiel. Dept. of Comp. Eng. and Sci. and Dept. of Biology, Case Western Reserve University, Cleveland, OH 44106.

We have begun a long term project designed to simulate the cockroach escape system. This system represents a relatively complex distributed decision making and motor control circuit that is amenable to detailed cellular analysis. The model is based upon biological data that has been collected over several years both in our laboratory and in those of others. In response to gentle wind puffs, the cockroach rapidly turns away from the wind source and runs. The circuit that controls this behavior involves sensory neurons located on the cerci (two antennae-like appendages located on the rear of the animal's abdomen) which excite a population of 4 pairs of ventral giant interneurons (the vGIs). The vGIs in turn excite a population of approximately 100 interneurons in the three thoracic ganglia. Finally, the thoracic interneurons connect to motor neurons and local interneurons which control movements of each individual leg.

A three-dimensional kinematic model of the cockroach was constructed which accurately represents all leg segments and joint angles, and we have begun to incorporate actual neural circuitry into that model. We have simulated the wind responses of the nine most prominent columns of cercal hairs and their connections to vGIs. We have reproduced the wind fields for each vGI. Moreover, we found that the specific shape used for the sensory response has significant effects on the simulated wind fields of the individual vGIs. We are currently in the process of simulating the connections from the vGIs to the thoracic interneurons. The model will allow for experimental interaction to assist in predicting and planning future neurobiological experimentation, which will in turn be used to improve the model.
[Supported by ONR grant N00014-90-J-1545 to RDB, NIH grant NS 17411 to RER and NSF grant BNS-8810757 to HJC]

313.16

FUNCTION OF FEEDBACK LOOPS BETWEEN THE GIANT INTERNEURONS AND THE FLIGHT CUIRCUITRY OF THE COCKROACH.
F. Libersat, M. Kiflawi* and J.M. Camhi. Dept. of Zoology, Hebrew University, Jerusalem, Israel.

In the flying cockroach, a small population of neurons located in the thoracic locomotory centers produces a copy of the central flight rhythm (an efference copy) and conducts this to the last abdominal ganglion. There, this rhythm modulates two groups of giant interneurons (GIs), the dorsals (dGIs) and the ventral (vGIs) (Libersat et al, J. Comp. Phys. 165: 651-668). The dGIs are a command system for flying behavior and the vGIs for running escape behavior of the insect on the ground. In this report, we examine the behavioral functions of the efference copy and of its influences on the GIs.

Intracellular rhythmic stimulation of a single dGI (burst of 25ms at 400/sec, burst repetition rate: 25/sec) during flight resulted in a prolongation of flight sequences and an increase of the wingbeat frequency. A single short train (20 to 50ms, 400/sec) in a dGI has complex effects including a change of the next wingbeat cycles duration. Cutting one abdominal connective, thereby removing 50% of the dGI rhythm, reduces both the flight frequency and duration. Cutting both connectives further reduces both these parameters. Theses results suggest that the rhythmic activation of the dGIs during flight participates at least in the control of the wingbeat frequency and the flight duration.

In contrast to the dGIs, stimulation (400/sec for 50ms) of one of the vGIs, GI1, evokes immediate cessation of flight. So far, stimulations of GIs 2 and 3 do not have this effect on flight.

In summary, the dGIs and vGIs have antagonists effects on flight, the former excitatory and the latter inhibitory.
(Supported by NINDS grant RO1 NS20923)

313.17

GETTING IT TOGETHER: COORDINATING A MULTI-GANGLIONIC DECISION. J. M. Camhi, P. Levi*, A. Neville* and H. Sassoon*. Dept. Zoology, Hebrew U., Jerusalem, Israel.

Cockroaches turn away from wind puffs. Posterior wind receptors activate ventral giant interneurons (vGI's). These excite, in all 3 thoracic ganglia, thoracic interneurons (TI's) that activate local interneurons (LN's) and leg motoneurons (LMN's) of their own ganglion. TI's also send axons to the other thoracic ganglia.

How does the thoracic network read the vGI code for wind direction? (1) A midline saggital cut of any one thoracic ganglion severs the decussating axons of all of this ganglion's TI's known to receive vGI input, but does not sever the vGI's, LI's, or LMN's. After one week, the TI axons in the connectives have degenerated, yet turn directions are normal. (2) After cutting the left abdominal connective (and thus all left vGI's), left winds result in 100% left ("wrong") turns. (3) As a control, cutting the left neck connective did not bias turn direction. (4) Cutting the left connective between thoracic ganglia 2 and 3 (T2-3) severs the left vGI's and the left ascending axons of TI's of ganglion T3. These vGI's all respond most strongly to left winds, whereas these TI's are of mixed directional response. This cut resulted in 100% left (wrong) turns to left wind. (The front and middle legs dominated in effecting the turn.) (5) Cutting the left T1-2 connective causes the T1 (front) legs to turn the body toward the left in response to left winds. The above results suggest that the vGI's, and not TI's, instruct each ganglion which way to turn, and that each ganglion separately reads out the vGI code. (6) Following the left T1-2 cut, in response to left wind, the vGI's should instruct ganglia T2 and T3 (behind the cut) to effect a right turn with their mid- and hindlegs, but should instruct ganglion T1 (beyond the cut) to effect a left turn with the front legs. Surprisingly, the front, middle (and possibly hind) legs all turned left. This suggests that although each ganglion separately reads the vGI code, if the ganglia disagree, T1 decides for all. (7) Fluorescence photoablation of single vGI's and TI's so far confirms the above results

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LEARNING AND MEMORY: PHYSIOLOGY IV

314.1

UNIT ACTIVITY IN CINGULATE CORTICAL LAYERS AND ANTERIOR THALAMIC SUBNUCLEI DURING LEARNING IN RABBITS. M. Gabriel, A. Poremba, Y. Kubota, E. Kang and B.A. Vogt. Dept. of Psychol. and Beckman Institute, Univ. of Illinois, Urbana IL 61801; Dept. Pharmachol, Boston Univ. Sch. of Med., Boston MA.

Past studies implicate cingulate cortex and limbic thalamus in mediation of disciminative avoidance learning, wherein stepping in an activity wheel to a tone CS+ prevents a footshock US 5 seconds after CS+ onset. This experiment documents learning-relevant neurophysiological plasticity (NP) related to recently demonstrated training-induced up-regulation of M2 cholinergic receptors in posterior cingulate area 29 and the AV thalamic nucleus (AVN, Vogt, B.A., et al., Neurosci. Abst., 1990). The NP took the form of greater multi-unit discharges in response to the CS+ than to a CS-, a tone that did not predict the US. The present results showed: a) NP acquisition in early stages of training in layers I-III, V and in parvocellular AVN; b) NP acquisition in late stages of training in layers IV, VI and magnocellular AVN. These distinct patterns of NP development were paralled by similar patterns of M2 receptor changes in these areas, suggesting a causal relationship. These data provide the first localized correlation of NP with receptor regulation in mammalian learning-relevant brain circuitry. (Supported by AFOSR grant 89-0046 and NIH grant NS26736 to MG).

314.2

TEMPORAL FIRING PATTERNS OF INFEROTEMPORAL NEURONS IN A VISUAL MEMORY TASK. A.E.P. Villa and J.M. Fuster , Dept. of Psychiatry and Brain Research Institute, UCLA School of Medicine, Los Angeles, CA 90024

Single units from the inferotemporal (IT) cortex of monkeys performing a color delayed-matching task (Fuster and Jervey, J. Neurosci. 2:361, 1982) were studied by means of time series analyses of the spike train (Abeles, J. Neurosci. Meth. 5:317, 1982). A task-trial consisted of the following: (1) sample color; (2) 10-20 seconds of delay (retention period); and (3) choice of the color (among 2 or 4) that matched the sample. Eighty-eight units that were activated during the delay were selected for the analysis; in 19 of them the firing activation was significantly higher after one particular sample color (preferred color of the unit). Discharge during delay periods was compared with discharge during intertrial periods. The spontaneous (intertrial) activity of 20 units was tonic and that of the 68 others exhibited bursts with intermingled and isolated spikes. The autocorrelogram of 32% of the units was significantly changed during the delay, independently of level of spontaneous firing. Most of these cells (20/28) showed during the delay a tendency to decreased bursting activity; furthermore, the average intraburst frequency stayed unchanged or decreased. Retention of the cue affected the firing pattern of a much larger proportion of color-selective units (63%) than of non-selective units (23%). In 5 color-selective units the autocorrelogram was only modified after the preferred color. These results show that some IT units that are selectively activated during the retention of the visual cue also exhibit a change in their temporal pattern of discharge (mostly in the form of decreased bursting). This indicates that the temporal structure of the spike train is an important feature of the changes that IT neurons undergo in visual short-term memory.

314.3

LOW FREQUENCY OSCILLATORY ACTIVITY OF SINGLE NEURONS IN THE TEMPORAL POLE OF THE RHESUS MONKEY. K. Nakamura*, A. Mikami and K. Kubota. Dept. of Neurophysiol., Primate Res. Inst., Inuyama, Aichi, 484, Japan.

To examine roles of the temporal pole (TP) in visual recognition and short-term memory, we recorded the single neuron activity from the TP of the monkey (Macaca mulatta) during a sequential visual discrimination task with a time delay. On pressing a lever, a stimulus selected from 600 colored photographs (human face, monkey, primate chair, etc.) was presented on TV monitor several times for 0.5-1.0 s with 1.0-5.0 s delay interval. When a new stimulus was presented after a varied number of presentation, the monkey released the lever and was rewarded. Thirteen neurons showed a regular oscillatory activity of 5-8 Hz with average peak discharge rate of 98 sp/s. Twelve neurons showed a sustained activity during delay (average, 14 sp/s). Mean latency from the stimulus onset was 157 ms. Most of these neurons showed statistically significant responses only to particular stimuli. The oscillatory activity might be co-active with the hippocampus known for its theta activity and connectivity to sharpen responses to some aspects of a particular visual stimulus. Sustained activity during delay might be storing visual information during delay period.

314.4

NEURONAL ACTIVITY IN HUMAN TEMPORAL LOBE WITH MULTIPLE MEMORY RETRIEVALS. MM Haglund, G Ojemann, D Cawthon, and E Lettich. Dept. Neurological Surgery, Univ. Washington,Seattle,WA 98195

Extracellular microelectrode (me) recordings were obtained from left lateral neocortex that was subsequently resected in patients undergoing craniotomies for treatment of medically intractable epilepsy. During me recordings, the patient performed four trials of a visually presented short term verbal memory task that entailed multiple(3) memory retrievals of an initially overtly named memory input. The multiple memory retrievals were separated by overtly named distractors and blank slides which served as controls. Previous studies from our laboratory showed lateral temporal lobe neurons with sustained increases in activity during both memory input and the first retrieval compared to controls (Brain 111:1393,1988). The present study was to determine how this activity changed with later retrievals of the same item, i.e. as part of the process of learning.

Technically satisfactory me recordings were obtained in 12 patients, 8 of which performed the 4 trials of the multiple memory retrieval tasks with no or one mistake. From these patients 19 neuronal populations(1-few units) were recorded. 16 of 19 neuronal populations showed significant changes in activity between controls and retrieval #1. Of these 16 populations, 5 showed an overall inhibition between retrieval #1 and 3, while 10 demonstrated "sharpening". "Sharpening" or fine tuning of neuronal activity was defined as a reduction in the number of 500 msec bins showing increases in avg. firing frequency. These increased periods of neuronal activity flanked by decreases did not result in an overall change in the firing frequency during the 4 sec task.

In addition to providing further evidence of a lateral temporal neocortical role in recent verbal memory, these findings suggest that neural activity becomes more compact with multiple retrievals of an item. Some neurons presumably in the periphery become inhibited while those more central in the pool become sharpened. (supptd. by AES fellowship-MH;NIH NS21724,17111 20482-GO)

314.5

A PHYSIOLOGICAL CORRELATE OF SITUATIONALLY DEPENDENT LTP IN PIRIFORM CORTEX. U. Staubli, T. Le* and G. Lynch. CNLM, University of California, Irvine, CA 92717.

Previous work in freely moving rats has established that induction of LTP in the lateral olfactory tract synapses in piriform cortex is dependent on the past experience of the animal and the use of electrical stimulation as a discriminative cue in a two-odor discrimination task (Roman et al., Brain Res., 1987, 418). In the present experiments we tested if the post-synaptic responses to bursts of stimulation pulses differed according to the behavioral situation. A single high-frequency burst (4 pulses at 100 Hz) is followed by a positive afterpotential lasting about 60msec in naive animals (n=4) and in rats (n=7) tested in an olfactory discrimination task in which they had been extensively trained. This afterpositivity was also present on subsequent bursts in a theta pattern (i.e., successive bursts delivered at 200msec intervals) in naive rats but was significantly reduced on subsequent bursts when the stimulation served as a discriminative cue to the experienced animals. The suppression of the afterpositivity was obtained when 150-200msec between burst intervals were used but not with 1-2 sec intervals. These findings suggest that the effect in piriform cortex may represent a situationally dependent variant of the IPSP blocking effect known to occur in hippocampus when LTP is induced with theta burst stimulation (Larson & Lynch, Brain Res., 1988, 441). Work in progress will examine if these observations in piriform cortex and the phenomenon of IPSP suppression ('priming') in hippocampus have mechanisms in common. The results could provide new insights in how attention and past experience influence synaptic plasticity. (Supported by NSF grant BNS 8809316 to U.S. and ONR grant N00014-89-J-1255 to G.L.)

314.6

CODING OF SPACE IN THE DORSOLATERAL PREFRONTAL CORTEX OF MONKEY PERFORMING A DELAYED ALTERNATION TASK. S.Carlson*, H.Tanila*, A.Pertovaara, I.Linnankoski* and A.Lähteenmäki* Dept.Physiology,Univ. Helsinki, Helsinki, Finland.

The aim was to study neuronal activity in and around sulcus principalis during the upward performance of a delayed alternation task (DA) and compare it with the firing pattern of the right and left directed performances. Electrophysiological response properties of single neurons were recorded conventionally in a monkey performing a DA task in three directions; to the right, left and upwards. Of the 154 neurons recorded in all three directions 72 % were task related neurons. 68 % of the task related neurons were spatially selective. Of the task related neurons 14 % were selective upwards, 14 % either to the right or to the left, 59 % in two directions and 13 % in all three directions. Functional properties of part of the neurons are reported, too. The results support the earlier suggestion (by Funahashi et al., J.Neurophysiol.1989.61.331) that there is a spatial memory map in the prefrontal cortex and indicate that spatial selectivity is a common feature in the task related neurons.

314.7

INTRACELLULAR RECORDINGS FROM THE FRONTAL CORTEX OF CONSCIOUS, LICKING RATS. M.P. Kristensen and C.D. Woody. UCLA Med.Ctr., Los Angeles, CA 90024.

Intracellular records were obtained from 67 neurons of the frontal cortex of conscious rats, so that their properties might be compared with those observed by other investigators in vitro. Spontaneous activity and responses to a 1 nA depolarizing current pulse were analyzed in 54 units, of which five showed bursting (spiking activity > 100 hz) in response to depolarization. The majority fired at a slower rate spontaneously as well as during depolarization. Input resistances were determined for 21 cells and found to be 11 MΩ, with a resting membrane potential of -56 mV.

Cells exhibiting afterhyperpolarizations, delayed spike repolarizations, low threshold spikes, multiple spikes, adaptation, bursting with inactivation, and spontaneous, oscillatory membrane-depolarizations supporting spiking were seen.

The setup enabled intracellular recording of electrical activity for as long as 5 minutes while the animal was licking. Intracellular records were obtained from 6 cells, of which 2 showed increased spike discharge correlated with the behavior, as did 1 extracellularly studied unit. (Supported by HD05958)

314.8

BASAL FOREBRAIN NEURON RESPONSES DURING RIGHT EYE VS. LEFT EYE LIGHT DISCRIMINATION. J.H. Pirch. Department of Pharmacology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Extracellular recordings were obtained from single units in the basal forebrain of rats during associative conditioning using light stimuli. Medial forebrain bundle (MFB) stimulation was paired with a 2-second light stimulus (CS+) to one eye, or a similar light stimulus (CS-) was presented to the other eye without MFB stimulation. Event-related slow potentials were recorded from the frontal cortex to monitor the development and maintenance of discrimination between CS+ and CS-. Recordings were obtained while the animals were anesthetized with urethane (1.5 g/kg with supplementation). Previous studies demonstrated that differential slow potential and cortical single unit responses to reinforced and non-reinforced tone stimuli can be recorded in urethane-anesthetized rats (Rucker, Corbus and Pirch, Brain Res. 376: 368, 1986). Some animals had permanently implanted stimulating electrodes and were trained to associate light with MFB stimulation prior to the recording session; others had no prior training.

Recordings were obtained from 21 basal forebrain units which demonstrated discrimination between CS+ and CS-. Six of the units were located in the globus pallidus and 15 were in the substantia innominata area. Of the 10 units that responded to right-eye CS+, 7 were excited and 3 were inhibited. Left-eye CS+ excited 9 units and inhibited 2 units. A few additional units responded with excitation or inhibition, but failed to discriminate between CS+ and CS-. Single units in the frontal cortex demonstrated differential responses in this light discrimination paradigm, as well as during tone discrimination. These results provide further evidence that basal forebrain neurons respond selectively to conditioned stimuli, and that these neurons may play a role in modulation of cortical cell activity during associative conditioning. Supported by NINDS NS22408.

314.9

DIFFERENTIAL EFFECTS OF ASSOCIATIVE LEARNING ON RECEPTIVE FIELDS IN THE SUBDIVISIONS OF THE GUINEA PIG MEDIAL GENICULATE BODY. Jean-Marc Edeline[1] and Norman M. Weinberger[1,2] Cntr. Neurobiol. Learn. Mem.[1] & Dept. Psychobio. Univ. California[1,2] Irvine, Ca. 92717.

CS-specific frequency receptive field (RF) plasticity was previously found in several areas of the auditory cortex (Diamond & Weinberger, Br.Res., 372:357, 1986; Bakin et al. Soc. for Neurosci. 14:862, 1988), and in the dorsal division (MGd) of the medial geniculate body (MGB) (Edeline & Weinberger, Soc. for Neurosci., 15:81, 1989). We investigated the degree of generality of this phenomenon at the thalamic level by determining the RF of clusters or single neurons in the ventral (MGv) and the medial (MGm) divisions of the MGB in the adult guinea pig before, after, and 1 hour after classical conditioning (CS=a non-best frequency tone, 6 sec.; US=250 msec footshock at CS offset; CR=conditioned bradycardia, which developed in all subjects). RF were determined by presenting ranges of pure tone frequencies (50 ms,1/sec) at different intensities (20-80dB) via a calibrated system to the contralateral ear. General changes in the RF (increases or decreases), with no shift of best frequency, were commonly found in the MGv (11/15, 73%) both immediately and 1 hour post-conditioning. General changes were also observed in 6/13 cases (46%) of recordings in the MGm. These effects contrast with the lower proportion (31%) of general effects and the high proportion of CS-specific effects found in the MGd (21/38, 55%), including maximal increased response to the CS frequency and shift of tuning to this frequency. The present findings suggest different functional roles for the MGB subdivisions, and possibly, a different involvement in information processing during learning. Supported by Fyssen fellowship (JME) and ONR #00014-87-K-0433 and The Monsanto Company (NMW).

314.10

SENSITIZATION PRODUCES NON-SPECIFIC INCREASED RESPONSES OF RECEPTIVE FIELDS IN GUINEA PIG AUDITORY CORTEX. Jonathan S. Bakin and Norman M. Weinberger, Centr. Neurobiol. Learning and Memory and Dept. Psychobiol., Univ. of California, Irvine, Ca. 92717

Previously we reported that classical conditioning produces frequency-specific receptive field (RF) plasticity in the auditory cortex of the guinea pig (Bakin, Condon and Weinberger, Soc. Neurosci., 14:862,1988). Following tone-shock pairing until development of behavioral conditioned responses (CR), 70% of single neurons or neuronal clusters showed maximal increased responses to the CS frequency, while responses to the pre-training best-frequency (BF) and other frequencies exhibited decreased responses. These effects were observed immediately and at the longest post-conditioning interval tested, 24 hours. The present experiment determined the effects of sensitization training on receptive fields. Training consisted of unpaired presentation of 30 trials in which a non-best frequency tone (10 sec) and footshock (2 sec) were presented with the same density as during conditioning. RF were obtained prior to training and immediately, 1 hour and 24 hours following training. Sensitization training produced no behavioral CRs and a general increased response across the RF, with no specificity to the CS frequency. Such non-specific RF plasticity was present immediately and for as long as 24 hours post-training. These results indicate that sensitization can produce a long-lasting general facilitation of acoustic processing in distinction to classical conditioning which produces a highly CS-specific modification of frequency receptive fields. Supported by Chancellor's Fellowship (JSB) and ONR #00014-87-K-0433 and the Monsanto Co. (NMW)

314.11

CLASSICAL CONDITIONING SELECTIVELY ALTERS THRESHOLDS IN THE AUDITORY CORTEX OF THE GUINEA PIG. David A. South, Scott J. Cruickshank, Duc Q. Pham and Norman M. Weinberger, Centr. Neurobiol. Learning and Memory and Dept. Psychobiol., Univ. California, Irvine, Ca. 92717

Classical conditioning produces frequency-specific plasticity in the auditory cortex. Specifically, responses to the frequency of the conditioned stimulus (CS) are increased while responses to other frequencies, including that of the pre-training best frequency (BF), are reduced (Bakin, Condon and Weinberger, Soc. Neurosci., 14:862, 1988). The purpose of this experiment was to determine whether classical conditioning alters thresholds. Conditioning consisted of presentation of a tone (6 sec.) and footshock (0.3 sec.) unpaired (sensitization control) or paired (conditioning) during the development of the cardiac deceleration conditioned response. Thresholds were determined before and after training (immediate, 1 hour and 24 hours) at the frequency of the conditioned stimulus and the frequency of the pre-training characteristic frequency (frequency with lowest threshold, CF). Following training, thresholds were changed, with the dominant effect consisting of a relative change between the frequency of the CS and the CF such that thresholds to the former were lowered relative to the latter. These findings indicate that classical conditioning produces a relative decrease in threshold for a stimulus that has acquired behavioral significance and produces a relative increase for a potent stimulus that is not involved in training. Thus, learning alters thresholds as well as response magnitudes of neurons within the auditory cortex. Supported by ONR #00014-87-K-0433 and the Monsanto Co. (NMW)

314.12

FREQUENCY-SPECIFIC RECEPTIVE FIELD PLASTICITY IN AUDITORY CORTEX DURING HABITUATION. C.D. Condon[1] and N.M. Weinberger[2], [1]Neuroscience Program, Univ. Illinois, Champaign-Urbana, IL. [2]Dept. Psychobiology and Center Neurobiology Learning & Memory, Univ. Calif., Irvine, CA.

Associative processes during classical conditioning induce CS-specific receptive field (RF) plasticity in the auditory cortex (Diamond & Weinberger, Br. Res., 372:357, 1986: Bakin, Condon and Weinberger, Soc. Neurosci. Abst., 14:862, 1988). This experiment asked whether frequency-specific RF plasticity also characterizes a non-associative form of learning, habituation. Neuronal discharges were recorded in the auditory cortex of waking guinea pigs. First, at least two frequency RF were determined (15min. interval) followed by repetition of a single non-best frequency (REP: 1.25/sec.), followed by one or more additional RF. Discharges analyzed were typically onset responses (10-30 ms latency). RF were stable prior to REP. Thereafter they exhibited a specific decrease at the REP frequency (72% of sessions), which averaged 74% below control values. This decrease was highly selective as responses to frequencies +/- 1/8 octave showed only a 10-15% reduction. During REP, responses declined, reaching asymptote after 108 stimuli, and either remained depressed (n=14) or recovered to near control levels despite continued presentation of tones (n=8). Both types of REP effect were associated with the same magnitude and degree of selectivity of RF decreased responses. Therefore, a depressed response at the end of the REP is not necessary for the RF effect. Further, the RF decrease at the REP frequency could develop over time (n=8), not reaching maximal depression for several minutes. These characteristics rule out sensory adaptation or fatigue and indicate that habituation induces stimulus-specific RF plasticity. Accordingly, frequency-specific RF plasticity in the auditory cortex characterizes habituation as well as classical conditioning. Supported by NIMH Training Grant 1 T32 MH18412, ONR #N00014-87-K-0433 and Monsanto Co.

314.13

EVIDENCE FOR DIFFERENCES IN AUDITORY TRANSIENT AND SUSTAINED STIMULUS PROCESSING USING THE STARTLE PARADIGM. E.J. Schicatano and T.D. Blumenthal. Dept. of Psychology, Wake Forest University, Winston-Salem, NC. 27109.

Two distinct neurophysiological systems underlie acoustic reflex behavior, a transient system which possesses neurons sensitive to brief acoustic stimuli, and a sustained system in which neurons are sensitive to stimuli with prolonged durations. The startle reflex provides a noninvasive means for separately assessing auditory transient and sustained system activity. The present study examined startle reflex habituation in the transient and sustained systems. Thirty-six undergraduate students were exposed to 30 presentations of 85 dB broadband noise stimuli (.1 ms rise time) with the ISI randomly varied from 25-35 s. Two types of stimuli were presented: single acoustic pulses with durations of 6, 20, and 50 ms, or a pair of 3 ms acoustic pulses with pulse onsets separated by 6, 20, or 50 ms. While females exhibited habituation only to stimuli activating the sustained system (single pulse), males habituated to stimuli activating both the transient (pulse pairs) and sustained systems. Furthermore, sustained system activity habituated more and faster in females than in males. Habituation was more pronounced for single than for paired stimuli, possibly due to larger initial responses to single stimuli, for both sexes. These results suggest that neuronal plasticity (startle habituation) in auditory sensory processing differs in males and females based on the type of stimulus presented. Also, sustained systems may be more appropriate than transient systems in habituation research.

314.14

COMPARISON OF CROSS-MODAL TRANSFER EFFECTS ON BEHAVIORAL RECOVERY AFTER EITHER AUDITORY OR VISUAL CORTEX LESIONS IN RATS. E. R. Delay and T. M. Rudolph*. Department of Psychology, Regis College, Denver, CO 80221.

Cross-modality training (CMT) with noise intensity cues enhances recovery of a brightness discrimination more than within-modality training (WMT) after visual decortication in rats (Delay, E.R., Neuropsychologia, 26:667, 1988). To extend these findings, this study examined the effects of CMT and WMT on behavioral recovery after either auditory or visual cortex lesions. One group of rats was trained with a high intensity light cue 24 hours before visual cortex ablations were made under Nembutal anesthesia. Another group was trained with a high intensity noise cue 24 hours before auditory cortex lesions. Six days later, 6 rats from each lesion group were given brief training in one of five conditions: 1) WMT with the preop cue, 2) reversal WMT with a low intensity cue, 3) CMT with a high intensity cue, 4) reversal CMT with a low intensity cue, or 5) no training. The next day all rats were retrained on the preop task. Both lesions produced relearning deficits. CMT reduced behavioral deficits more than WMT after both lesions. Transfer of CMT appears to occur through common multisensory structures and to have general facilitative effects on behavioral recovery after brain injury. [Supported by NSF Grants BNS-8909803 & USE-8951043]

314.15

CEREBELLAR MOTOR LEARNING: QUANTITATION OF MOVEMENT ADAPTATION AND PERFORMANCE IN RHESUS MONKEYS AND HUMANS IMPLICATES CORTEX AS THE SITE OF ADAPTATION. J.G. Keating, and W.T. Thach. Dept. of Anatomy and IWJ Rehab. Institute, Washington Univ. Sch. Med., St. Louis, MO 63110.

Rhesus monkeys and humans placed their hand in a manipulandum which controlled the position of a cursor on a screen in front of them. The subject held in a target zone and stepped to a new target zone by flexing or extending the wrist in one quick movement. After a series of control trials, the gain was altered such that the hand/cursor relationship appeared unchanged during the initial hold, but the previously performed movement was no longer of the right magnitude to place the cursor in the target zone. Over several trials the movement magnitude changed to produce the proper ranging. The endpoints of the initial ballistic movement from a series of trials were fit by regression to an equation of the form: $f(x) = A + B*e^{(-X/C)}$. Residuals were trial-invariant and had normal distrubutions. C, analogous to a time constant, was used as a measure of the rate of adaptation. Performance was measured as the standard deviation of regression of the curve fit, and was independant of adaptation.

Purkinje cells recorded from a Rhesus monkey during adaptation showed a increase in peri-movement complex spike activity during the period of adaptation and a slow decrease in simple spike activity which was maintained after adaptation.

A right handed patient of 61 yrs. who suffered a right cerebellar cortex infarct, adapted behavior with his right hand one third as fast as with his left. Both hands of a control subject showed the same rate of adaptation as the patient's left hand.

Changes in Purkinje cell discharge similar to that seen by Gilbert and Thach (1977) and Watanabe (1984) and a specific loss in adaptation on damage to cerebellar cortex support the Marr-Albus theory of motor learning. (NIH grants NS12777 and NS15070).

314.16

SINGLE UNIT RESPONSES FROM THE CEREBELLAR CORTEX OF NAIVE RABBITS. D.J. Krupa, J. Tracy, C. Weiss and R.F. Thompson, Neurosciences Program, Univ. Southern Cal., Los Angeles, Ca. 90089-2520.

The cerebellum has been identified as the most likely site to contain the associative elements which mediate eyeblink conditioning in the intact rabbit. To further investigate the underlying mechanisms we have been recording the activity of single units in response to unpaired stimuli typically used in conditioning paradigms i.e., tones, white noise & airpuffs. Thus far we have recorded 61 single units from seven New Zealand white rabbits. Of these, 29 were identified as Purkinje cells (based on the presence of complex spikes). Most were from the HVI lobule. We found that the responsive cells (n=15) were often grouped together and responded preferentially to the airpuff (14/15) with both complex and simple spike activity. Airpuff responsive cells showed various patterns of simple spike excitation and inhibition, but almost always responded by firing a complex spike as well. Interestingly, most of the responsive non-Purkinje cells were excited about equally by airpuff (n=8) and auditory stimuli (n=5). These cells tended to be preferentially located nearby the responsive Purkinje cells. Nonresponsive cells (Purkinje and non-Purkinje cells) also tended to be located nearby each other. These data will form a foundation for comparisons with data that are to be collected from rabbits acquiring a classically conditioned eyeblink. Supported by: ONR N00014-88-K-0112, BNS 8718300 and McKnight Foundation.

314.17

CEREBELLAR INTERPOSITUS NUCLEUS LESIONS AND LIMBIC SYSTEM ACTIVITY DURING CLASSICAL EYELID CONDITIONING IN RABBITS. J.L. Stotler, D.P. Miller, and J.E. Steinmetz. Program in Neural Science & Department of Psychology, Indiana University, Bloomington, IN 47405

Recent evidence showed that the interpositus nucleus was necessary for acquisition and maintenance of classically conditioned responses and training-related activity in the hippocampus (Clark et al, Brain Research, 326, 1984; Sears & Steinmetz, Behav. Neurosci., in press). It has been suggested that the hippocampus is important when conditioning with relatively long interstimulus intervals (ISIs) or when trace procedures are used (Port et al, Behav. Neurosci., 99, 1985; Port et al, Behav. Neurosci., 100, 1986). The present experiments were conducted to determine the effects of interpositus lesions on hippocampal activity during conditioning with long ISIs and trace intervals and also on activity in the septal region, a region closely associated with the hippocampus.

In the first experiment, the nictitating membrane (NM)/eyelid responses of rabbits with unilateral interpositus lesions were either trained with a relatively long ISI (700 ms) or trace conditioned (250 ms CS, 450 ms trace period) using a tone CS and an air puff US. Multiple-unit activity was recorded bilaterally from the hippocampus throughout training. The interpositus nucleus lesions disrupted conditioning on the ipsilateral eye and bilaterally disrupted hippocampal plasticity during training at long ISI's. Similar results were found for trace conditioning. In the second experiment, multiple unit activity was recorded from the medial and lateral septal nuclei during NM conditioning that followed a unilateral lesion of the interpositus nucleus (tone CS, air puff US, 250 ms ISI). Interpositus lesions abolished conditioned responses during training on the ipsilateral eye but did not abolish stimulus-related activity in the medial septum. Training-related activity in the lateral septum was blocked by the cerebellar lesion. These data suggest that the hippocampus continues to receive stimulus-related information from the medial septum following interpositus nucleus lesions but that the interpositus nucleus is necessary for the formation of training-related activity in the hippocampus and one of its efferents, the lateral septum.

This research was supported by NIMH Grant MH44052 to JES.

314.18

RABBIT CEREBELLAR ACTIVITY EVOKED BY PONTINE NUCLEI OR INFERIOR OLIVE STIMULATION -- POTENTIAL REGIONS OF CS-US CONVERGENCE DURING EYELID CONDITIONING. T.J. Gould, L.L. Sears, and J.E. Steinmetz. Program in Neural Science and Department of Psychology, Indiana University, Bloomington, IN 47405.

Neural responses recorded in the cerebellar and deep nuclei are related to the learning of a conditioned response (McCormick & Thompson, J. Neurosci. 4:2811, 1984). These recording data suggest that the cerebellar cortex and deep nuclei may be areas that code plasticity associated with performance of the learned response. Also, some studies indicate that CS information may be relayed from the pontine nuclei to the cerebellum via mossy fibers and US information may be relayed from the inferior olive to the cerebellum via climbing fibers (Steinmetz et al., Synapse, 3:225, 1989).

In an initial attempt to locate and characterize cerebellar neuronal responses associated with activating the pontine nuclei and inferior olive, both single units and population potentials evoked by electrical stimulation were recorded. In acute, untrained, anesthetized rabbits, stimulating electrodes were placed in the pontine nuclei or inferior olive. Recording electrodes were then systematically lowered through the cerebellar cortex, dentate nucleus, and interpositus nucleus and activity evoked by single pulse stimulation was recorded. Both pontine-evoked single unit activity and pontine-evoked population potentials recorded in cerebellar cortex (e.g., Larsell's HVI) demonstrated activation of granule cells and Purkinje cells. Pontine-evoked single unit activity and population potentials recorded in the interpositus and dentate nuclei showed an initial phase of excitation (1 msec latency, presumably reflecting direct mossy fiber activation) followed by a later phase of inhibition (presumably from cortical inputs). Similar patters of cerebellar activation (in the same regions of cortex and deep nuclei that were mapped for pontine inputs) were observed when olivary stimulation was used. These data support anatomical evidence that inputs from the pontine nuclei and the inferior olive converge in the cerebellar cortex and deep nuclei (Steinmetz & Sengelaub, Neurosci Abstr., 14, 782, 1988) and suggest possible cerebellar regions that may receive both CS and US information.

This research was supported by NIMH Grant MH44052 to JES.

314.19

UNCONDITIONED STIMULUS-EVOKED ACTIVITY IN THE DORSAL ACCESSORY INFERIOR OLIVE DIMINISHES WITH ACQUISITION OF THE CLASSICALLY CONDITIONED EYELID RESPONSE. L. L. Sears and J.E. Steinmetz. Program in Neural Science and Department of Psychology, Indiana University, Bloomington, IN 47405.

The cerebellum exhibits learning-related plasticity and is essential for execution of the rabbit classically conditioned eyelid/nictitationg membrane (NM) response. Evidence suggests that information about the unconditioned stimulus (US) is projected to the cerebellum along climbing fibers that arise from the dorsal accessory inferior olive (DAO) (e.g., Steinmetz et al., Synapse, 3:225, 1989).

In the present study, multiple-unit DAO recordings from eight rabbits revealed significant US-evoked neural activity during initial paired presentations of a tone CS and air puff US. Across five training sessions, however, as percent conditioned responses (CR) increased, there was a significant decrease in DAO activity on paired CS-US trials. In contrast, no across-session decrease in DAO activity was observed on US-alone trials given at the end of each paired training session. These results are similar to findings of diminished inferior olive activity in response to "expected" cutaneous stimuli (Gellman et al., J. Neurophysiol., 54:40, 1985) and demonstrate that the "reinforcement" nature of DAO input to the cerebellum may diminish as CRs develop.

This research supported by NIMH Grant MH44052 to JES.

314.20

CONDITIONED RESPONSE LEARNING IN THE *IN VITRO* TURTLE BRAINSTEM-CEREBELLUM. J. Keifer and J.C. Houk. Dept. of Physiology, Northwestern Univ. Medical Center, Chicago, IL 60611.

The eye-blink reflex has been widely adopted for physiological studies of the mechanisms of conditioned response learning. In behaving animals, a typical approach is to pair an auditory conditioned stimulus (CS) with a corneal air puff unconditioned stimulus (US), which results in a conditioned reflex response (CR) after a few training sessions. Recently, we developed an *in vitro* preparation from the turtle to study mechanisms of motor program generation in the cerebellorubral circuit (Neurosci. Letters 97: 123, 1989). Studies indicate an essential role of the cerebellorubral circuit in the conditioned eye blink response. To examine how motor programs produced by this circuit are modified by learning, we have obtained the neural correlate of the conditioned eye blink response generated entirely in the *in vitro* preparation.

Neural activity characteristic of the blink reflex is evoked by electrical stimulation applied to nV (US), which contains afferents from the eye, while recording from nVI, which contains efferent fibers that normally project to retractor muscles of the eyelid. The reflex consists of a nVI burst discharge having a latency of 10-15 ms and a duration of several tens of ms to many seconds. The CS is applied electrically to the posterior root of nVIII, which contains predominantly auditory afferents. Paired CS-US presentations of a 100 Hz, 1 s train stimulus to nVIII (CS) immediately preceded a single shock to nV (US) and both stimuli terminated simultaneously. Intertrial intervals were 30 s. Fifty paired stimuli were interspersed by a 30 min rest period. The results show that after about the third training session, nVI activity is recorded during the end of the CS and preceding the US. With further training, the response to the CS shifts into earlier stages of the CS presentation until a nVI burst discharge occurs at ~150 ms following the CS onset. This conditioned response can be recorded without further pairing for at least 5 hours but not longer than 12 hours. These data show that it is possible to produce conditioned response learning in vertebrate reflex pathways *in vitro*. This will allow studies of the mechanisms for generating conditioned responses at both cellular and circuit levels.

HORMONAL CONTROL OF BEHAVIOR III

315.1

COMPARISON OF [^{125}I]ESTROGEN UPTAKE IN MALE AND FEMALE MOUSE BRAIN. M. J. Walters, R. B. Hochberg* and N. J. MacLusky. Div. of Reproductive Science, Toronto General Hospital, Toronto, Ontario M5G 1L7, and Dept. Obstetrics and Gynecology, Yale University Sch. of Med., New Haven, CT 06510.

Sex differences in gonadotropin secretion and sexual behavior are presumed to result from functional differences within the central nervous system. In the present study, quantitative autoradiography was used to assess sex differences in estrogen uptake in the adult mouse brain. Gonadectomized male and female mice were injected i.v. with a saturating dose (2 μg/kg b.w.) of ^{125}I-labelled 11β-methoxy-16α-iodoestradiol (MIE_2), a synthetic analog of estradiol. Control animals were injected with 5 μg DES 5 min prior to [^{125}I]MIE_2 injection. Thin frozen sections (10 μm) were then cut through the hypothalamus and preoptic area and exposed to high-resolution Hyperfilm for 8 days. No sex difference in silver grain density was observed in the arcuate-median eminance region, the bed nucleus of the stria terminalis, or amygdala. However, [^{125}I]estrogen uptake in the medial preoptic area and ventromedial hypothalamic nucleus was significantly greater in females than in males. These findings suggest that sexually dimorphic patterns of sexual behavior and gonadotropin secretion in this species may be due to sex differences in estrogen-binding capacity in specific regions of the hypothalamus-preoptic area.

Supported by MRC Canada

315.2

BEHAVIORALLY-EFFECTIVE ESTRADIOL PULSES INDUCE PROGESTIN RECEPTORS SELECTIVELY IN SUBSTANCE P-CONTAINING CELLS IN THE VENTROLATERAL HYPOTHALAMUS OF FEMALE GUINEA PIGS. D.H. Olster and J.D. Blaustein. Psychology Department and Neuroscience and Behavior Program, University of Massachusetts, Amherst, MA 01003.

Substance P (SP), which may mediate steroid-induced lordosis in rodents, is found in the ventrolateral hypothalamus (VLH), a site at which estradiol (E_2) primes ovariectomized (OVX) guinea pigs to display progesterone-facilitated lordosis. In OVX guinea pigs implanted with E_2 capsules, many progestin receptor-immunoreactive (PR-IR) VLH cells also contain SP-IR (Nielsen and Blaustein, 1990, in press). Low doses of E_2, administered as pulses, prime OVX females to respond behaviorally to progesterone as effectively as much higher doses of E_2. In the current experiment we compared the degree of cellular colocalization of SP-IR and PR-IR following E_2 capsules (20 mm for 40 h) and pulses (2 injections of 2 μg, 28 h apart) in the VLH of OVX guinea pigs (after intracerebroventricular colchicine injection). The total number of PR-IR cells in the VLH and SP-IR cells in the VLH/ventromedial nucleus did not differ between treatment groups. However, in the VLH of E_2 pulse-treated animals, 50% more PR-IR cells contained SP-IR than in the E_2 capsule-treated animals (52% ± 1 vs. 36% ± 4, respectively). Thus, behaviorally-effective E_2 pulses induce PR-IR selectively in SP-IR cells in the VLH. These data are consistent with the hypothesis that SP cells in this region play a role in steroid induction of lordosis in guinea pigs. (Supported by NIH grants HD 23483, NS 00970 and NS 19327.)

315.3

PROGESTERONE IMMOBILIZED ON BSA IMPLANTED IN THE VTA BUT NOT THE HYPOTHALAMUS FACILITATES SEXUAL RECEPTIVITY IN HAMSTERS. C.A. Frye, P.G. Mermelstein* and J.F. DeBold. Dept. Psychology, Tufts University, Medford, MA 02155.

Progestogenic stimulation of **both** the ventromedial nucleus of the hypothalamus (VMH) and the ventral tegmental area (VTA) is critical for normal receptivity in estrogen-primed hamsters. However, anatomical and biochemical studies have identified very few estrogen-induced progestin receptors in rodent ventral midbrain. To determine whether progesterone might be working on the membrane of neurons in the VTA, progesterone 3-CMO BSA (P-3-BSA) was applied intracranially. The size of P-3-BSA makes it relatively impermeable to the cell membrane.

Ovariectomized hamsters were implanted with 2 chronic cannulae, one aimed at the VMH and the other at the contralateral VTA. These animals were then estrogen-primed and tested for sexual receptivity after progesterone containing tubes were inserted just dorsal to the VMH and P-3-BSA inserts were applied above the VTA. The following week the hamsters were again tested with the contents of the inserts reversed. Animals with progestogenic stimulation to the VMH and P-3-BSA to the VTA were significantly more receptive than those with P-3-BSA to the hypothalamus and progesterone to the VTA. Facilitation of receptivity also occurred when progesterone was applied to other sites within the hypothalamus concurrent with P-3-BSA stimulation to the VTA. When P-3-BSA was applied to the VTA alone or in conjunction with extra-hypothalamic progesterone stimulation the animals were less receptive. These data suggest that progesterone is capable of facilitating sexual receptivity within the VTA by actions on the cell membrane. The non-genomic effects in the VTA may require concurrent genomic activation by progesterone within the hypothalamus.

315.4

LOCALIZATION OF CELLS CONTAINING ESTROGEN RECEPTOR-LIKE IMMUNOREACTIVITY IN THE BRAZILIAN OPOSSUM FOREBRAIN. C. A. Fox, L. R. Ross*, and C. D. Jacobson. Department of Veterinary Anatomy, Iowa State University, Ames, IA 50011

The Brazilian opossum (Monodelphis domestica) is a small, pouchless marsupial whose young are born in an immature, sexually undifferentiated state. Etgen and Fadem (Gen. Comp. Endo. 66: 441), and Handa et al. (Soc. Neurosci. Abst. 15: 1099) have biochemically detected and characterized estrogen receptors in the forebrain of the Brazilian opossum. In this study, we have examined the distribution of estrogen receptor-like immunoreactive (ER-LI) cells in the forebrain of six gonadectomized (three male and three female) Brazilian opossums using Abbott H222 rat monoclonal estrogen receptor antibody (H222 is a gift of Abbott labs). An indirect immunohistochemical procedure employing the Vectastain Elite system and a nickel-enhanced DAB chromogen was used. A large number of ER-LI cell nuclei were seen in the medial preoptic area, ventral septal nucleus and intermediate septal nucleus, lateral part of the ventromedial hypothalamus, arcuate nucleus, and the periventricular hypothalamic nucleus. Lower numbers of ER-LI cell nuclei were observed in bed nucleus of the stria termimalis and the anterior and medial amygdaloid nuclei. The anatomical distribution of ER-LI in the Brazilian opossum brain is similar to that which has been reported for estrogen binding sites following biochemical analysis. The results presented here provide evidence for the cellular localization of estrogen receptors in the Brazilian opossum brain. This data indicates that specific regions of the forebrain may serve as a substratum for the action of estrogen in the adult opossum brain. In addition, these results support the use of this animal model in studies on the organizational effects of estrogen on the developing central nervous system. Supported by Iowa State University, NSF grant BNS 8909751 and USDA Formula Funds Section 1433.

315.5

MALE ACTIVATION OF SEXUAL RECEPTIVITY IN OVARIECTOMIZED ESTROGEN-PRIMED RATS: ROLE OF THE VENTROMEDIAL NUCLEUS OF HYPOTHALAMUS. G.Rajendren*, C.A.Dudley and R.L.Moss. Dept. of Physiology, Univ. of Texas Southwestern Med. Ctr., Dallas, TX 75235-9040.

The role of the ventromedial nucleus of hypothalamus (VMH) on the mating-induced enhancement of sexual receptivity in ovariectomized estrogen-primed rats was investigated. In the first experiment, the females were subjected to bilateral VMH lesion or sham-operation. Forty eight hours after priming with 2µg of estradiol benzoate, they were tested repeatedly for sexual receptivity. The sham-operated females exhibited a gradual increase in lordosis quotient (LQ) during the 5 hr testing period. However, the VMH-lesioned rats did not show any sign of lordosis.

In the second experiment, ovariectomized rats were bilaterally implanted with estradiol (E) at various sites in the hypothalamus. Females with bilateral E implant in the VMH exhibited a gradual increase of LQ over time. Those with bilateral E implant in other hypothalamic areas lying close to the VMH did not exhibit lordosis. To further exclude the possibility of the spread of E to areas lying outside the VMH, plasma luteinizing hormone (LH) was measured in the females. Castration-induced elevation of LH was not suppressed in females bearing bilateral E implant in the VMH. Since the median eminence, which lies close to the VMH, is one of the important neural sites where estrogen exerts its negative feedback on gonadotrophin release, the results further support the conclusion that in the present studies the effects of E on sexual receptivity are due to its local action within the VMH.

Together, the experiments described above suggest that an intact VMH is necessary for the male-induced enhancement of LQ in ovariectomized rats and that selective priming of the VMH with E is sufficient for the activation of sexual receptivity following mating. Supported by NIH grants HD 09988 and MH 41784.

315.6

SEX DIFFERENCE IN ESTRADIOL(E2)-CONCENTRATING CELLS WITHIN SPECIFIC COMPONENTS OF THE RAT MEDIAL PREOPTIC AREA (MPOA). S. Kurth[1], T.R. Akesson[2], P.E Micevych[3], and G. J. Bloch.[1] [1] Brigham Young U., Provo, UT; [2]VCAPP, Wash State U Pullman; [3]Anatomy, UCLA Sch Medicine.

Several studies show that MPOA cells accumulate and bind more estrogen (E) in the adult female than in the male, but other studies do not document these differences. A reason for the discrepancy may be differences in methods used to assess E binding (see Breedlove, Trends in Neurosci 6,'83; Brown et al, Endoc 123,'88). Using autoradiography as outlined separately by Pfaff and Stumpf (Science 161, 162, '68) and cytoarchitecture as described by Simerly et al and Bloch et al (J. Comp. Neurol 225, 272, '84, '88), the density (# cells/mm2X10-2) of E-concentrating cells was determined within the medial preoptic nucleus (MPN) and its medial lateral, and central components (MPNm, MPNl, & MPNc), as well as the anteroventral and periventricular preoptic nuclei (AVPV and PVPO). All of these areas had a significantly greater density of E2-concentrating cells in the female than in the male (range: 3.28 to 5.73X greater). These data suggest a large sex difference in E cells within discrete components of the MPOA.

315.7

CHRONIC FREE TESTOSTERONE (T) IN FEMALE RATS DURING 15-30 DAYS OF AGE: MASCULINIZATION OF OVARIAN AND BEHAVIORAL FUNCTION IN ADULTHOOD. S.D. Gale, S. Kurth, and G.J. Bloch, Dep't of Psychology, Brigham Young U, Provo, UT, 84602.

Lordosis behavior is markedly stimulated in adult-gonadectomized (Gxd), estrogen(E)-primed male rats given medial preoptic CCK (Bloch et al, Physiol Behav 46, '89), and adult-Gxd males can be made highly sensitive to the lordosis-stimulating effects of progesterone (P) after pulse administration of E (Sodersten et al, Endoc 112 '83 Olster et al, Horm.Behav 6, '88). To further study sex reversal during periods beyond the perinatal period, Silastic capsules containing 7 mm T or nothing (blank) were implanted s.c. in 15-day-old females that were either Gxd or not Gxd (intact) at this time, and the capsules were removed 15 days later (30 days old). Blank-implanted rats showed vaginal cycles, normal ovaries, and a high level of proceptive behavior after sequential E and P treatment at 100 days of age. In contrast, T-implanted rats showed persistent vaginal estrus starting at vaginal opening, polyfollicular and smaller ovaries, and virtually no proceptive behavior. Thus, chronic androgen during the prepubertal period can markedly masculinize reproductive function. Supported by BYU research funds.

315.8

GALANIN (GAL) MICROINJECTED INTO THE MEDIAL PREOPTIC AREA (MPOA) FACILITATES COPULATORY BEHAVIOR IN THE MALE. P. Butler*, R. Hammond*, S. Kurth, B. Padgett*, C. Lowry*, J. G. Kohlert, R. Mills*, and G. J. Bloch, Dep't of Psychology, Brigham Young U, Provo, UT, 84602.

Because steroid-sensitive, sexually dimorphic regions of the male rat MPOA appear to contain relatively large amounts of GAL (Bloch et al, Neurosci Abst 15,'89 and unpublished), copulatory behavior was assessed in sexually experienced animals that were gonadectomized in adulthood, then implanted with 2mm testosterone capsules in order to maintain behavior at low levels. 0.3 ul microinjection of 50ng or 500ng GAL into the MPOA of these males significantly increased the percentage of animals that mounted or ejaculated when compared to vehicle-injected controls, and also significantly increased the percentage of animals showing intromission behavior within the first 2 minutes (i.e., a short intrommission latency). General body movement measured in an open-field apparatus (number of line crossings, rears) was unaffected by GAL microinjection. These data suggest that GAL within the MPOA may play a specific role in the maintenance of copulatory behavior in the male. Supported by BYU research funds.

315.9

BRAIN ANTIESTROGEN BINDING SITES. J.M.Gray and L.Ziemian* Program in Biopsychology, Vassar College, Poughkeepsie, NY 12601.

Antiestrogens (AE; e.g., tamoxifen, TAM) are synthetic substances which antagonize many of the reproductive actions of estradiol (E) while mimicking many of the anorectic and metabolic effects of E. AE may exert many of their effects by competing with E for the estrogen binding site (EBS) in peripheral reproductive and metabolic, as well as central neural tissues. Recent studies have also demonstrated the presence of microsomal sites in reproductive tissues which bind AE, but not E (antiestrogen binding sites, AEBS). We are reporting the presence and characteristics of AEBS in several areas of the brain and pituitary in ovariectomized rats.

Single point binding assays with 2 nM ^{3}H-TAM (+ 1 uM TAM) in the presence of saturating amounts (2 uM) E indicated the existence of specific binding to AEBS throughout the brain and pituitary. In most areas of the brain and pituitary, Scatchard analyses revealed the presence of a single AEBS with a dissociation constant (K_d=1-4 x 10^{-9}M) similar to that previously reported for other tissues. However, in both hypothalamus and preoptic area, an additional, higher affinity site (K_d=6-9 x 10^{-11}M) was found.

Ongoing competetive inhibition studies using a variety of neurotransmitter agonists and antagonists are exploring the specificity of ^{3}H-TAM binding to the two AEBS.

315.10

COMPARISON OF THE DISTRIBUTIONS OF ESTROGEN TARGET NEURONS IN THE BRAINS OF ADULT MALE AND FEMALE MACAQUES. R.P. Michael, H.D. Rees and R.W. Bonsall. Department of Psychiatry, Emory University School of Medicine, Atlanta, Georgia 30322 and the Georgia Mental Health Institute, Atlanta, Georgia 30306.

To compare the uptake of estradiol by the brains of male and female monkeys, 2 adult female and 4 adult male *Macaca mulatta* were gonadectomized and 3-5 days later administered ^{3}H-estradiol i.v. (males 0.47 mCi/kg; females 0.15 mCi/kg). After 60 min, brains were rapidly removed and frozen for autoradioagraphy. Sections were cut in a cryostat at 4 μm and thaw-mounted on emulsion-coated slides. After 140-days exposure, slides were developed and stained. A total of 6,600 neurons in 11 regions of the hypothalamus, preoptic area, amygdala and septum were examined using a rigorous procedure based on the Poisson distribution. There were significant differences in the percentages of labeled neurons ($P<0.001$) in the 11 regions. In both males and females, highest percentages (59%-84%) occurred in the ventromedial and arcuate hypothalamic nuclei and in the cortical and basal accessory amygdaloid nuclei. Moderate percentages of labeled neurons (45%-59%) occurred in the anterior hypothalamic area, bed nucleus of stria terminalis and medial preoptic nucleus. Lower percentages (20-29%) occurred in the lateral septal and medial amygdaloid nuclei and lowest percentages (<15%) occurred in the premammillary and intercalated mammillary nuclei. No significant differences between males and females were found either in the regional distribution of labeled neurons or in the intensity with which the neurons were labeled. Results suggested that the distribution of estrogen-target neurons in the adult primate brain is not sexually dimorphic.

Work supported by USPHS grant MH 19506 and by the Georgia Department of Human Resources.

315.11

DISTRIBUTION OF ESTROGEN RECEPTORS IN THE BRAIN OF THE FEMALE MUSK SHREW: AN IMMUNOCYTOCHEMICAL STUDY. T.L. Dellovade, J.D.Blaustein [1] and E.F. Rissman. Dept. of Psychology, Univ. of Virginia, Charlottesville, VA 22901 and [1]Neuroscience & Behavior Program and Dept. of Psychology, Univ. of Mass., Amherst, MA 01003

Neural aromatization of testosterone to estradiol is essential for the regulation of sexual behavior in the female musk shrew (Rissman, et al. Neuroendo. 51:468, 1990). One step toward better understanding the neural mechanisms involved with this process, is to localize the distribution of the cells containing estrogen receptors (ER). In this study, we used immunocytochemistry for the localization of ER. We used a multi-bridging peroxidase-anti-peroxidase technique with a monoclonal antibody, H222 (a gift of Abbott Laboratories, Blaustein and Turcotte, Brain Research 49:75,1989).

ER-immunoreactive cells were found in the preoptic-hypothalamic and limbic regions of the brain. Relatively dense staining was seen in the anterior medial, arcuate and ventromedial nuclei of the hypothalamus. Cells in the medial and lateral preoptic,lateral hypothalamus, and the ventral premammillary nuclei also contained ER staining. Limbic regions containing ER-immunoreactivity included the bed nucleus, lateral septum and the central and cortical nuclei of the amygdala. ER-immunoreactivity was primarily nuclear in appearance, with an occasional process lightly stained. As compared with similar studies in other species, these results suggest that the location of ER in the brain is highly conserved.

This work is supported by NSF grant BNS 8706770 (EFR) and NIH grants NS 19327 and NS 00970 (JDB).

315.12

NOVEL STEROID-BINDING SITE ON SYNAPTIC MEMBRANES MAY MEDIATE STRESS-INDUCED INHIBITION OF SEXUAL BEHAVIORS. M. Orchinik, T.F. Murray and F.L. Moore. Oregon State Univ., Corvallis, OR 97331.

Short-term stress inhibits sexual behaviors of male amphibians (*Taricha granulosa*) through a mechanism that involves corticosterone (CS), and injection of low doses of CS inhibits the behavior within 10 min (Moore and Miller, *Horm.Beh.*, 18:400, 1984). This response is too rapid to be mediated through classical intracellular receptors. To determine if there are high-affinity membrane-bound steroid receptors that could mediate this rapid response, we have partially characterized the binding of ^{3}H-CS to brain membranes from *Taricha*. ^{3}H-CS binding to a crude synaptosomal preparation was specific, saturable and reversible. The binding site had high affinity ($Kd=5.06x10^{-10}M$) for CS and the density of binding sites was 143 fmol/mg protein. We examined ^{3}H-CS binding to more purified subcellular fractions obtained from sucrose gradient centrifugation and found specific binding of ^{3}H-CS most enriched (>11x) in the synaptosomal fraction. The enrichment of ^{3}H-CS binding in the synaptosomal fraction precisely paralleled the enrichment of ^{3}H-quinuclidinyl benzilate binding (a muscarinic cholinergic antagonist), used as a positive control for neuronal membranes.

Competition experiments indicated that the membrane-bound binding site was highly specific for corticosteroids, and the specificity differed from both Type I and Type II intracellular receptors. In behavior experiments, the potency of steroids to inhibit male sexual behaviors correlated with their potency to inhibit ^{3}H-CS binding. Our results suggest that rapid behavioral responses to corticosteroids may be mediated by this previously undescribed high-affinity, steroid-binding site on amphibian brain synaptic membranes. Supported by NSF (BNS-8901500) to FLM and MO and NSF Graduate Fellowship to MO.

315.13

BEHAVIORAL EFFECTS OF ANDROGENS AND ESTROGENS IN THE QUAIL PREOPTIC AREA C. Surlemont*, A. Vockel, A. Foidart and J. Balthazart, Laboratory of General and Comparative Biochemistry, University of Liège (BAT L1), B-4020 Liège, Belgium.

Stereotaxic implantation of hormones, antihormones and metabolism inhibitors was used to study the sites of androgen and estrogen action on sexual behavior in the preoptic area of castrated male Japanese quail. Bilateral implantation of the aromatase inhibitor, androstatrienedione (ATD) in the sexually dimorphic nucleus (POM) of the preoptic area completely suppressed the behavioral activation produced by a systemic treatment with testosterone (T). The effects of ATD were only observed if the implants were located in the POM. In a second experiment, implants of the synthetic estrogen, diethylstilbestrol in the POM restored copulatory behavior in castrated males while implants of the synthetic non-aromatizable androgen, methyltrienolone (R1881) were almost ineffective. During a third experiment, the activating effects of a systemic treatment with T were blocked by stereotaxic implants in the POM of the antiestrogen, tamoxifen or the antiandrogen, flutamide. The effects of tamoxifen were more pronounced than those of flutamide. In addition, tamoxifen was active in all parts of the POM while a behavioral inhibition was observed only for flutamide implants which were located in the caudal part of the nucleus. Taken together, these results demonstrate that the sexually dimorphic POM is the area where the behaviorally active estrogenic metabolites of T have to be produced. The estradiol derived from T aromatization in the POM presumably acts within the nucleus to activate copulation as demonstrated by the effectiveness of DES implanted in this region. Androgens might also have a direct action on sexual behavior as suggested by the partial inhibition observed in flutamide-treated birds. It is however possible that androgens and estrogens do not act in the same brain area to activate behavior. Supported by NIH HD22064, FNRS and EEC (SC1-0230-C/TT)

315.14

EFFECTS OF ANDROGEN ON SEXUAL DIFFERENTIATION OF THE ZEBRA FINCH SONG SYSTEM. B.A. Schlinger and A.P. Arnold. Laboratory of Neuroendocrinology, Brain Research Institute and Department of Psychology, University of California, Los Angeles, CA 90024-1563.

Although estradiol is generally considered the hormone responsible for masculinization of the zebra finch song system, studies of Gurney (1981, 1982) suggest that the non-aromatizable androgen 5α-dihydrotestosterone (DHT) has limited effects, most notably masculinizing cell number in nucleus RA. To replicate and extend this work, hatchling (day 1) male and female zebra finches were treated with Silastic pellets containing 0, 50 or 200 ug crystalline DHT. Birds were sacrificed as adults (day 90) and their brains were sectioned at 30 um and stained with thionin. Treatment of hatchlings with DHT had small effects on volumes of several song control nuclei and the direction of these effects was opposite in males and females. In females, 50 or 200ug DHT increased RA volume by 35% and 37% and in males decreased RA volume by 4% and 22%. Similar changes were observed in volumes of female and male HVc, but these were not significant. These divergent effects were most obvious in Area X where 200ug DHT made Area X recognizable in 4 of 9 females and significantly reduced the volume of Area X in males by 34%. Although treatment of females with either 50 or 200ug DHT increased neuron number in RA 1.9- and 1.3-fold, these increases were not significant. In addition, 200ug DHT significantly increased soma size in RA by 1.6-fold. These data suggest that androgens have diverse effects on development of the zebra finch song system. Supported by NIH grants DC00217, HD07228 and NS08649.

315.15

ANALYSIS OF THE CANARY ANDROGEN RECEPTOR. K.L. Nastiuk and D.F. Clayton. Lab. of Animal Behavior, Rockefeller Univ., New York, NY 10021.

In order to investigate the mechanisms by which gonadal steroids regulate the highly plastic song circuit in the brains of canaries, we have cloned the canary androgen receptor and have begun experiments to examine its regulation. We used the polymerase chain reaction to amplify canary androgen receptor mRNA from testes in sufficient quantities to clone and sequence. Analysis reveals that the DNA binding domain is almost identical to the rat and human domains, whereas the hinge has fifty percent identity and many conservative substitutions. Preliminary analysis indicates that the steroid binding region has an intermediate level of homology. This suggests that, unlike currently available antibodies, cDNA probes will make possible the analysis of seasonal regulation and localization of the receptor in the canary and other songbirds.

315.16

EFFECTS OF AMBIENT TEMPERATURE ON BAG-CELL ACTIVITY AND EGG LAYING IN *APLYSIA CALIFORNICA*. N.L. Wayne and G.D. Block. Dept. of Biology, Univ. of Virginia, Charlottesville, VA 22901.

Aplysia are seasonal breeders, and previous studies suggest that water temperature is an important environmental signal which regulates the timing of reproduction in this marine mollusc. Specifically, cold water inhibits while warm water can stimulate egg laying. The purpose of the present studies was to investigate the effects of temperature on activity of the neuroendocrine bag cells controlling egg laying. Reproductively mature animals were maintained either in warm (20 °C; n=8) or cold (15 °C; n=7) water. *Aplysia* were fitted with a stimulating/recording electrode at the bag-cell neurites. Following electrical stimulation of the bag cells, 8 of 8 warm *Aplysia* layed eggs but only 1 of 7 cold *Aplysia* layed eggs (subsequently, all animals layed eggs in response to hormonal stimulation of the ovotestis documenting that they were capable of showing this behavior following surgery). This difference in electrically-stimulated egg laying could not be accounted for by differences in duration of bag-cell afterdischarge, in number of impulses during afterdischarge, or in bag-cell content of bioactive egg laying hormone (ELH). Further, there was no significant difference between cold and warm animals in the sensitivity of the ovotestis (egg laying response) to varying doses of atrial-gland extract. These results suggest that cold temperatures inhibit some process between bag-cell afterdischarge and ovotesticular response to ELH. We are currently investigating whether cold temperature inhibits egg laying by suppressing bag-cell secretion of ELH. Supported by NIH grants NS-08725 to NLW and NS-15264 to GDB.

315.17

EFFECTS OF FLUID DEPRIVATION ON TESTOSTERONE SENSITIVITY AND EXTINCTION OF A CONDITIONED TASTE AVERSION. E.A. Brownson, C.B.Sengstake* and K.C. Chambers. Depts. of Neurobiology and Psychology, Univ. of So. Cal., Los Angeles, CA 90089 and Dept. of Psychology, Portland State Univ., Portland, OR 97207.

Fluid deprivation reduces behavioral sensitivity to testosterone (T). The amount of T required to prolong extinction of a conditioned taste aversion (CTA) is greater in fluid deprived than nondeprived male rats. In the first experiment, we observed that the minimum dose of T required to prolong extinction in fluid deprived Sprague-Dawley (SD) male rats is not sufficient in Fischer 344 (F344) males. To determine whether this is due to a greater effect of fluid deprivation on F344 males, 40 F344 and 40 SD male rats were either fluid deprived (1 hr/day access to fluid) or nondeprived (24 hr access to fluid). Following the first presentation of a 10% sucrose solution, a CTA was induced by injection of 0.15 M LiCl (10 ml/kg). Daily extinction trials began 2 days later and continued until criterion for extinction (100% of first day consumption) was reached. The fluid deprived F344 and SD rats extinguished faster than the nondeprived rats. However, the percentage increase in the extinction rates of the deprived F344 was 2-fold greater than that of the SD. These results suggest that F344 are affected more strongly by fluid deprivation and have a greater reduction in sensitivity to T. ONR N0014-J-1296

LEARNING AND MEMORY—PHARMACOLOGY: EXCITATORY AMINO ACIDS

316.1

MK-801 IMPAIRS LEARNING IN AVERSIVELY-MOTIVATED COMPLEX MAZE TASKS IN RATS. *E. Spangler*, P. Garofalo*, E. Bresnahan, N. Muth*, J. Long, D. Ingram.* Gerontol. Res. Ctr., NIA, NIH, Baltimore, MD 21224; Essex Community College, Baltimore, MD 21237.

We evaluated NMDA receptor blockade by MK-801 in male F-344 rats in a 14-unit T-maze (Ingram D., *Neurobiol. Aging*, 9:475, 1988) and in a 3-unit detour (D) maze (Bresnahan E., *et al., Soc. Neurosci. Abst.*, 15:1382, 1989). For the T-maze, 3-mo old rats were pretrained in 1-way active avoidance to criterion (8 avoidances/10 trials), and 24 hr later received s.c. injections of either saline (SAL, n=15) or MK-801 (ns=8) in doses of 0.025, 0.05, or 0.1 mg/kg 20 min before T-maze training (15 trials). Rats had to locomote toward goalbox via 5 segments each within 10 sec to avoid footshock (0.8 mA). SAL-treated rats received 1 wk later either 0.05 mg/kg MK-801 (n=5) or SAL (n=5) s.c. 20 min before a 10-trial retention test. During acquisition significant ($ps<.01$) drug effects appeared in all measures (errors, alternation errors, runtime, shock frequency and duration) with 0.1 mg/kg MK-801, but 0.05 mg/kg MK-801 impaired cognitive performance (errors, alternation errors) only. No MK-801 effect was observed in retention. After extensive D-maze pretraining, 1-yr old rats (n=7) received 4 sessions with 4 problems each in which they ran to a goal through a path containing pairs of U-shaped Ds deviating bilaterally at 3 locations. Each problem involved 2 forced runs with entrance to 1 side of a D pair blocked at 2 locations followed by choice runs with D entrances opened. To avoid footshock (0.4 mA), the rat had to run to goal without stopping. During counterbalanced sessions 2-3, rats received either 0.025 or 0.05 mg/kg MK-801 s.c. 20 min before testing, and SAL during sessions 1,4. Relative to SAL sessions, 0.05 but not 0.025 mg/kg MK-801 significantly ($p<0.05$) increased errors. Thus, alterations in NMDA receptors might underlie age-related performance declines in these tasks.

316.2

REPEATED ACQUISITION OF THREE-LEVER SEQUENCES: DIFFERENT BEHAVIORAL MECHANISMS OF SCOPOLAMINE AND MK-801. **J. Cohn* and D.A. Cory-Slechta. Environmental Health Sciences Center, Univ. Rochester School of Medicine & Dentistry, Rochester, NY 14642.**

Several different neurotransmitter systems have been implicated as biological substrates for learning processes, particularly the cholinergic and glutaminergic (NMDA) systems. Analyses of behavioral mechanisms by which such drugs impact learning should result in a greater understanding of the differential roles of these neurotransmitter systems. In this study, a simplified repeated acquisition procedure was used to compare the effects of scopolamine, a cholinergic antagonist, and MK801, an NMDA-receptor antagonist, on response sequence learning. Rats were required to learn a different 3-response sequence each session (e.g. Left (L), Right (R), Center (C)) for food reinforcement. Perseverative sequences, e.g. LLR were excluded. Presentation of sequences was ordered such that each member of the response sequence always differed in its position from the previous session's sequence. Errors produced a 2 sec timeout period, and an increase in the ratio of correct sequences for food presentation (FR1 to FR2). An error also required the rats to begin the sequence anew. A tone stimulus followed each correct response.

Both scopolamine and MK-801 significantly increased error rates in a dose-dependent manner. However, microanalysis of error patterns revealed that this effect was produced through different behavioral mechanisms. Following administration of scopolamine, rats tended to proceed directly from the first to the third member of the sequence, suggesting a loss of control of the reinforcement contingencies over behavior. MK-801 produced perseverative responses on the first sequence member, an effect similar to that observed following administration of d-amphetamine. Both scopolamine and MK-801 produced a slight increase in overall response rate at lower doses which then fell at the highest doses of each compound. The results of these studies suggest the involvement of both cholinergic and NMDA systems in learning, albeit through quite different behavioral processes. **Supported by Grant R01 ES05017.**

316.3

EFFECTS OF MK801 ON LEARNING AND MEMORY AS ASSESSED USING A NOVEL WATER MAZE. G.J. Kant, C.P. D'Angelo*, T. N. Robinson III*, W. L. Wright*, C.P. Wingfield* and D.D. Rigamonti. Dept. of Medical Neurosciences, Walter Reed Army Institute of Research, Washington DC 20307.

We have recently reported on the usefulness of a novel water maze for the assessment of learning and memory [Kant et al., Pharmacol. Biochem. Behav., 31: 487,1988; Przybelski et al., Biomat. Art. Cells Art. Org., 17: 583-596, 1989; Neurosci. Abs.15: 1170]. In the present study we investigated the effects of MK801, an NMDA receptor antagonist reported to have beneficial effects both as an anticonvulsant and neural protectant as well as adverse effects on cognition and neuronal morphology. Groups of rats (n=12/group) were injected with saline or MK801 (0.1 mg/kg) 15 min prior to daily water maze testing. Rats learned to swim a path through an alley maze set inside a 5 ft diameter pool to an exit platform. Both time to completion (300 sec maximum) and errors (entries through incorrect doors) were recorded. Testing was conducted daily (1 trial/day) in 3 phases. In phase 1 (24 trials), rats were tested on one maze configuration. After rats had learned this first maze, six of each group continued to get saline or MK801 prior to daily testing and the other six rats in each group received the alternate treatment (phase 2: memory testing, 9 trials). Drug treatment remained as in phase 2 testing for phase 3 which consisted of 8 trials on a new maze configuration. MK801 retarded learning in both phase 1 and 3 as shown by increased swim times and increased numbers of errors made, but did not significantly impair memory in phase 2.

316.4

EFFECTS OF POSTNATAL NMDA ANTAGONISTS AND ENVIRONMENTAL STIMULATION ON SPATIAL MEMORY IN THE ADULT RAT. N.Venable*, P.H.Kelly. Preclinical Research, Sandoz AG, CH-4002 Basel, Switzerland.

Blockade of NMDA receptors in adult animals disrupts memory formation, whereas environmental enrichment increases learning abilities. The present study examined the separate and combined effects of postnatal NMDA antagonists and environmental enrichment on adult learning. At 3 days of age Sprague-Dawley littermates were redistributed into 8 groups (n=8) to form a 3-factor design (drug x environment x sex). From days 10 to 24 pups received 300μg/kg MK-801 or saline s.c. $\geq$1 hr after the last of 4 daily 30-min enrichment sessions. Environmental controls remained in social conditions. A similar experiment was carried out using (±)-CPP (10-20 mg/kg s.c.). At 6-8 months, drug-treated animals showed significant deficits in several indicators of learning in a water maze and in a delayed alternation task. Enrichment significantly facilitated the alternation task in the MK-801 and CPP experiments and the water maze task in the CPP experiment, and partially reversed some drug effects. Sex differences were also observed. It is concluded that 15 days of MK-801 or CPP treatment during early development produces a long-term deficit in spatial memory.

316.5

LONG-TERM CHANGES IN RESPONSIVENESS OF ACOUSTIC STARTLE CAN DEVELOP UNDER KETAMINE ANESTHESIA. S. A. Rabchenuk*, B. J. Young, and R. N. Leaton. Dept. of Psychology, Dartmouth College, Hanover, NH 03755

Edeline and Neuenschwander-El Massioui recently showed (Brain Research, 1988, 457, 274-280) that Pavlovian conditioning can occur under ketamine anesthesia. Since ketamine anesthesia may allow the processing of sensory information, we asked if rats can develop long-term habituation of acoustic startle under ketamine anesthesia. Male hooded rats, 70 days old, were trained under ketamine anesthesia and tested undrugged. Animals in Group E (n = 7), received 20, 115-dB, 100-ms, white noise startle stimuli on a 30-sec interstimulus interval in one 10-min session. Animals in Group C (n = 8), were placed in the startle chamber for 10 min but without stimulus presentations. Anesthesia was induced in both groups with an initial injection of 170 mg/kg ketamine hydrochloride, i.p., supplemented as necessary to eliminate any response to tail pinch before training. Responsiveness of both groups to the initial startle stimulus was tested without drug injections 48 hours later and again 24 hours later. On the first trial of Test Day 1 Group E was more responsive than Group C. This pattern reversed for the first trial of Test Day 2. Statistical analysis yielded a significant group x trial interaction ($p < .05$). There were no detectable responses to the startle stimuli during the training session. The results indicate that long-term changes in responsiveness can develop under ketamine anesthesia which is adequate to suppress detectable responses. However, the outcome was complex, suggesting that both long-term sensitization and habituation can occur under ketamine anesthesia.

316.6

EXCITATORY AMINO ACID ANTAGONISTS INFUSED INTO THE AMYGDALA BLOCK EXTINCTION OF FEAR-POTENTIATED STARTLE. W.A. Falls, M.J.D. Miserendino & M. Davis. Psychology & Psychiatry Dept., Yale Univ. Med Sch., 34 Park st., New Haven, CT. 06508.

Paired presentation of a neutral stimulus, such as a light, and an aversive stimulus, such as a shock, allows the light to now potentiate the startle response. Presentation of the same light, in the absence of shock, leads to a disappearance of this potentiation. These results are generally attributed to the acquisition and extinction, respectively, of conditioned fear of the light. The amygdala is known to be important for the acquisition of conditioned fear, however, relatively little is known about its role in extinction. Recently it has been shown that activation of N-methyl-D-aspartate (NMDA) receptors in the amygdala is necessary for the acquisition of potentiated startle (Miserendino, M.J.D., et al., Nature, in press). We therefore examined whether blockade of NMDA receptors in the amygdala would affect extinction of fear-potentiated startle.

Rats were implanted with bilateral cannulae aimed at the basolateral nucleus of the amygdala. One week later, the rats underwent training in which a light was paired with footshock (0.6 mA). Five days after training, the initial level of fear-potentiated startle was assessed by measuring the amplitude of noise-elicited startle in the presence or absence of the light. One day later, the rats were infused with either the non-selective excitatory amino acid (EAA) receptor antagonist γ-D-Glutamylglycine (DGG; 10μg), the NMDA receptor antagonist DL-2-amino-5-phosphono-valeric acid (AP5; 10μg) or vehicle immediately prior to receiving 30 light-alone extinction trials. One day later, the level of fear-potentiated startle was again assessed but without drug infusion.

Rats that received vehicle during light-alone trials did not exhibit potentiated startle relative to their initial levels (i.e., extinction). Rats receiving DGG or AP5 exhibited potentiated startle equivalant to their initial levels (i.e., no extinction).

These data suggest that activation of NMDA receptors in the amygdala are importantly involved in extinction of conditioned fear.

316.7

INTRA-AMYGDALA INJECTION OF N-METHYL-D-ASPARTATE RECEPTOR ANTAGONISTS IMPAIRS MEMORY IN AN INHIBITORY AVOIDANCE TASK. K.C. Liang & M. Davis, Dept. of Psychology, Natl' Taiwan Univ., Taipei, Taiwan 10764, R.O.C. & Dept. of Psychiatry , Yale Univ., Sch. of Med., New Haven, CT 06508, U.S.A.

N-methyl-D-aspartate (NMDA) receptors have been implicated in neural plasticity. Previous findings indicate that central administration of NMDA antagonists such as dl-2-amino-5-phosphonopentanoic acid (AP5) affects acquisition and/or retention in several learning tasks. A recent study reports that pretraining injections of AP5 into the basolateral nucleus of the amygdala impair acquisition of fear conditioning in a fear-potentiation of startle paradigm. The present study examined the generality of this effect by investigating the influences of pre- and posttraining intra-amygdala injections of AP5 on retention in an inhibitory avoidance task.

Male Sprague-Dawley rats were implanted with cannulae aiming at the basolateral amygdala. In the first experiment, four groups of rats received bilateral intra-amygdala injections of buffered vehicle, 1.25, 2.5 or 5.0 ug of AP5 (0.5 ul/side). Five minutes after the injection, rats were trained on a one-trial step-through inhibitory avoidance task with 1.6 mA/1 s footshock. Retention tested 24 hrs later indicated that pretraining intra-amygdala injections of AP5 produced a dose-dependent retention deficit: 5.0 ug inuced a profound effect but 1.25 ug had no significant effect. The second experiment examined the effect of posttraining injections of AP5 on retention to determine whether the retention deficit could be due to AP5 influencing performance factors other than memory processing. Three groups of rats were trained and tested on the task described above. Immediately following training, two groups received intra-amygdala injections of vehicle or 5.0 ug of AP5, while the third group received a delayed injection of AP5 5 hrs after training. Results indicated that while immediate posttraining intra-amygdala injections of AP5 significantly impaired retention performance, delayed injections of AP5 did not produce a significant effect.

These findings suggest that amygdala NMDA receptors may be normally activated by certain learning experiences and involved in memory formation processing.

316.8

MICROINFUSION OF AN N-METHYL-D-ASPARTATE ANTAGONIST INTO THE AMYGDALA IMPAIRS AVOIDANCE LEARNING IN RATS. M.Kim* and J.L.McGaugh. Center for the Neurobiology of Learning and Memory and Department of Psychobiology, University of California, Irvine, CA 92717.

Extensive evidence indicates the importance of the amygdala (AM) in avoidance learning. However, whether memory storage occurs in the AM is not clear. Recently, long-term potentiation (LTP) was demonstrated in the AM (Clugnet et al., 1989). Assuming that LTP serves as a basis of learning, interfering with LTP may impair learning and retention if the AM is a locus of neuronal change underlying avoidance learning. Since LTP induction depends on activation of the NMDA receptors, we examined the effects on learning and memory, of an NMDA antagonist D,L-2-amino-5-phosphono-valeric acid (AP5) injected into the AM.

Sprague-Dawley rats were implanted with bilateral cannulae aimed at the AM, and trained with continuous multiple-trial inhibitory avoidance (CMIA) and one-way active avoidance (AA) tasks. In CMIA, whenever a rat entered dark compartment, a footshock was given until the rat escaped to the starting compartment. The number of trials required before the rat remained in the starting compartment for 100 sec in one trial was recorded. Step-through latency was measured in a retention test 48 hours later. Vehicle or AP5 (1, 3, or 10 ug/0.5 ul) was injected into the amygdala 3 min before training in both tasks.

10 ug AP5 impaired acquisition and retention in CMIA and AA as compared to vehicle. 3 ug AP5 did not affect acquisition but impaired retention in CMIA, and impaired both in AA. The effects of AP5 were not due to state-dependency or altered sensitivity to footshock. These findings are consistent with other evidence (Miserendino et al., 1989), suggesting that learning and retention of aversively-motivated tasks may be mediated by changes involving activation of NMDA receptors. MH12526 from NIMH/NIDA and ONR N00014-87- K-0518.

316.9

BLOCKING NMDA RECEPTORS IN TURTLES BY MK-801 PREVENTS MAZE ACQUISITION. M. Avigan* and A. S. Powers. Dept. of Psychology, St. John's University, Jamaica, NY 11439.

The dorsal cortex of turtles has been shown to be involved in learning and memory. This cortex contains NMDA receptors and, in lizards, it shows long-term potentiation. The present study investigated whether blockade of NMDA receptors by systemic injections of the NMDA antagonist MK-801 would affect maze learning.

Fifteen turtles were first trained on a T-maze for food reward to determine whether they were capable of learning a maze. They were then divided into three groups: Group I was given sham lesions or injected with saline daily (n=5), Group 2 was given lesions of the dorsal cortex (n=5) and Group 3 was given daily subcutaneous injections of MK-801 (dose: .05 mg/kg) 20 min before running. All three groups were trained on an X-maze for food reward. Three trials per day were given, and the turtles were run until they had reached a criterion of learning or until they had had 40 days of training.

The results showed that acquisition of the maze was significantly impaired both for the subjects that were given dorsal cortex lesions and for those that were given MK-801. This finding is consistent with the possibility that NMDA receptors in the dorsal cortex may be involved in learning in turtles.

316.10

NMDA RECEPTORS MEDIATE ACQUISITION BUT NOT EXPRESSION OF PAVLOVIAN FEAR CONDITIONING. J. J. Kim, J. Landeira-Fernandez*, J. P. DeCola* and M. S. Fanselow. Dept. of Psychology, UCLA, Los Angeles, CA 90024.

The role of N-methyl-D-aspartate (NMDA) receptors in Pavlovian fear conditioning was examined using a specific NMDA antagonist DL-2-amino-5-phosphonovaleric acid (APV). A 2 x 2 factorial design was employed in which APV (5ug) or saline was administered icv before the training and/or testing phases. APV completely blocked acquisition but not expression of fear conditioning. To separate encoding from consolidation process, APV was administered either before or immediately after the footshock unconditional stimulus during the training phase. The results indicate that APV must be present during the unconditional stimulus to produce its effect on fear conditioning. In addition, our findings suggest that there may be both long-term and short-term associative memories for learned fear.

These effects on fear conditioning offer a striking parallel with APV's *in vitro* effects on the acquisition but not expression of long-term potentiation (LTP) and suggest that endogenously generated NMDA-dependent LTP participates in the neural plasticity underlying fear conditioning.

316.11

EFFECT OF THE NMDA ANTAGONIST MK-801 ON CLASSICAL CONDITIONING OF THE RABBIT NICTITATING MEMBRANE RESPONSE. J.H. Stillwell* and G. B. Robinson. University of New Brunswick, Fredericton, New Brunswick, Canada, E3B 6E4.

Several lines of investigation suggest that hippocampal long-term potentiation (LTP) or potentiation-like effects are involved in acquisition of the classically conditioned rabbit nictitating membrane (NM) response (Berger et al., 1986; Robinson et al., 1989). If LTP is a neural substrate of associative learning then manipulations which reduce or abolish LTP should have similar effects on learning. The noncompetitive NMDA receptor antagonist MK-801 is known to reduce or abolish hippocampal LTP. The present study examined the effects of MK-801 on acquisition of the conditioned NM response of the rabbit.

Male New Zealand rabbits were administered MK-801 1.5 hrs. prior to each daily conditioning session. Dosages ranged from 0.0 - 0.5 mg/kg. Each session consisted of 12 blocks of 9 trials (8 CS-US pairings and 1 CS alone test trial). A tone (1 KHz, 82 db, 400 ms) served as the CS and coterminated with a corneal airpuff US (5 psi, 100 ms duration). Administration of MK-801 prior to each session resulted in a dose-dependent decrease in acquisition rates that did not appear to result from changes in CS or US sensitivity. We are currently examining the effects of MK-801 on the development of enhanced hippocampal neural activity that occurs during acquisition of the NM CR. Supported by MRC and NSERC.

316.12

NETWORK LEARNING: PHARMACOLOGICALLY-INDUCED LONG-LASTING CHANGES OF ACTIVITY STATES IN CULTURED MONOLAYER NETWORKS. M.B. Gordon and G.W. Gross. Dept. of Biological Sciences and Center for Network Neuroscience, Univ. of North Texas, Denton, TX 76203

This study was undertaken to explore a possible role of NMDA receptor-dependent long-term potentiaion (LTP) mechanisms in the development of enduring changes in network activity states. The network states are determined by the long-term monitoring of spatio-temporal patterns within a 1mm diameter monolayer network cultured on 64 photoetched microelectrodes. Both mouse spinal cord and olfactory bulb cultures are being investigated. The cultures are pretreated with norepinephrine (NE) in 5 separate 5 min pulses one hr before addition of NMDA and glutamate. The pretreatment with NE elevates c-AMP levels which cause transient increases in spike and burst frequencies but with a return to the original activity 1 min after washout. After pretreatment the networks are exposed to a 2 min pulse of NMDA to which glutamate is added during the last min. This regimen is meant to activate the NMDA receptor-dependent LTP mechanisms.

Our preliminary results show long-lasting changes in network activity states (spike and burst patterns) which persist for 60-90 minutes and decay slowly over 3-4 hrs to the original activity. We suggest that this behavior could be a reflection of LTP-dependent storage mechanisms expressed on the network level.

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316.13

EFFECT OF PHOSPHATIDYLSERINE TREATMENT ON SPATIAL MEMORY AND HIPPOCAMPAL SYNAPTIC PLASTICITY IN AGED RATS. **D. Guidolin*, L. Petrelli*, A. Zanotti*, M.G. Nunzi and G. Toffano. Fidia Research Laboratories, 35031 Abano Terme (PD), Italy.**

It has been shown that chronic oral administration of phosphatidylserine, significantly improves spatial memory in aged rats with a severe place-navigation deficit (Zanotti et al., 1989, Psychopharmacol., 99:316). In aged rats, loss of axospinous perforated synapses in the dentate gyrus is regarded as one of the structural substrates of this memory deficit (Geinisman et al., 1986, Brain Res. 398:266). We evaluated the number of perforated and nonperforated axospinous synapses per granule cell in the middle molecular layer of the dentate gyrus from behaviorally characterized rats: young adults (5 mo); and aged-(27 mo) nonimpaired and impaired animals, the latter untreated or treated with BC-PS 50mg/kg/d for 12 weeks. The total number of axospinous synapses per neuron decreased significantly in control aged-impaired and nonimpaired rats relative to young adults. However, the percentage of perforated synapses was significantly lower only in the aged-impaired rats. BC-PS restored both the number of axospinous synapses and the incidence of perforated synapses to normal values. Enhancement by BC-PS of synaptic plasticity may well relate to its effects on neurotransmission and cognitive function in the aged.

316.14

IMPLICATION OF HIPPOCAMPAL PROTEIN KINASE C (PKC) IN MOLECULAR MECHANISMS INVOLVED IN LEARNING AND MEMORY PROCESSES : PHARMACOLOGICAL AND BIOCHEMICAL DATA. X. Nogues*, J. Micheau* and R. Jaffard., Laboratoire de Psychophysiologie, URA CNRS 339, Univ. Bordeaux I, avenue des Facultés 33405 Talence Cedex France.

Recent evidences have suggested that PKC may be a critical intracellular second messenger in learning and memory processes. 1) The involvement of PKC in long term potentiation (LTP) that is proposed as a cellular model of learning and memory, is suggested by studies showing correlations between PKC activation and LTP. 2) Classical conditioning of the rabbit nictitating membrane produces translocation of PKC activity from the cytosolic to the membrane compartments in the CA1 hippocampal region (BANK et al., Proc. Natl Acad. Sci., 1988, 85, 1988-1992). In the following experiments we have investigated the contribution of PKC activity to learning and memory processes. The first set of experiments was aimed at studying the effects of intrahippocampal administration of phorbol-12-myristate-13 acetate (TPA: a PKC activator; 1.6 mM in 0.2 µl/hippocampus) on the acquisition and the 24 hr delayed retention of a bar pressing task on a CRF schedule. TPA administration 5 min before the retention session produced an improvement of learning at the beginning of the session while the other treatment conditions had no effect on this task. In the second set of experiments, we measured PKC activity in membrane and cytosol compartments of the hippocampus at different delays (5 min, 1 hr, 24 hr) after a spatial concurrent discrimination task performed in a 8-arm radial maze. The results showed a decrease of PKC activity in the cytosolic fraction at the 3 delays ($p < 0,01$; Dunnett t-test) and an increase at the 1 hr delay of the percentage of PKC activity in the membrane fraction on the total PKC activity ($p < 0.05$). Together, these data support the hypothesis of the implication of PKC in the molecular mechanisms by which the hippocampus may exert its influence on information processing. Moreover, since we observed changes in PKC activity 5 min after the learning task, these results may suggest an early involvement of PKC in the biochemical changes induced by learning processes.

316.15

SELECTED PKC INHIBITORS DISRUPT LONG-TERM MEMORY FORMATION IN THE 2-DAY-OLD CHICK FOR A PECK-AVERSION TASK; FORSKOLIN ATTENUATES THE AMNESIA. P.A.Serrano, M.G. Oxonian*, E.L.Bennett and M.R. Rosenzweig. Dept. of Psychology, University of California, Berkeley, CA 94720.

Dose response functions were determined for melittin (MT), H-7, H-8, H-9, H-A and polymyxin-B. All agents were injected 5 min pre-training into the area of the medial hyperstriatum ventrale (MHV) (10ul/hemisphere). All agents except polymyxin-B were effective in producing significant amnesia as compared to saline injected controls ($p<.01$). MT (.04 mg/ml) and H-7 (4.0 mg/ml) were found to produce amnesia beginning 45 min and 60 min posttraining, respectively. These time courses for the appearance of amnesia suggest that these agents inhibit formation of long-term memory. There was no evidence of inhibition for short-term and intermediate-term stages of memory formation.

At a 24 hr test, chicks injected with both MT (.04mg/ml) and forskolin (.25mg/ml), an adenylate cyclase activator, showed attenuation of the memory impairment induced by MT. These results suggest that PKC and adenylate cyclase activation are involved in long-term memory formation. Supported by NSF grant BNS-88-10528.

316.16

EFFECTS OF GLUTAMATE, OUABAIN & ANISOMYCIN ON MEMORY FOR WEAK TRAINING IN CHICKS. D.W.Lee, G.G.Murphy*, E.L.Bennett & M.R.Rosenzweig. Dept. of Psychology, Univ. of CA, Berkeley, CA 94720.

Memory for 1-trial peck avoidance using strong training (100% methylanthranilate (MeA, a strong liquid aversant) is impaired by glutamate (GLUT, an STM inhibitor), ouabain (OUAB, an ITM inhibitor) and anisomycin (ANI, an LTM inhibitor). The present study investigated the effect of these agents using weak training (10% MeA).

Bilateral i.c. injections (10 µl/hemisphere) were made into the region of the intermediate-medial hyperstriatum ventrale 5 min pretraining using one of the following: saline; 25, 50 or 75 mM GLUT; .01, .03 or .05 mM OUAB; or 7.5, 11.3 or 15.0 mM ANI. When tested at 24 hr, memory for weak training is impaired by GLUT and ANI. The effective doses for GLUT (50 & 75 mM) and ANI (11.3 mM) are the same as those that impair memory for strong training. OUAB did not significantly impair weak memory formation. Further studies will extend the dose range for OUAB and determine if other ITM inhibitors impair weak memory formation.

To determine the time courses of amnesia following weak training, chicks were tested at times from 10 s up to 24 hr after training. Using strong training, GLUT results in amnesia between 5 & 10 min posttraining. With weak training, GLUT also results in amnesia between 5 & 10 min posttraining and, therefore, inhibits STM for weak training as well as strong training. Work is in progress with ANI and other drugs.

Supported by NSF grant BNS-88-10528 & NIDA grant DA04795.

316.17

SEASONAL VARIATION IN THE PERFORMANCE OF CHICKS IN A 1-TRIAL PECK AVOIDANCE TASK. M.R. Rosenzweig, D.W. Lee, G.G. Murphy*, & E.L. Bennett. Dept. of Psychology, Univ. of CA, Berkeley, CA 94720.

Upon presentation of a small metal bead dipped in methylanthranilate (MeA, an aversive liquid) chicks will peck the bead, show disgust, then subsequently avoid a similar but dry bead presented at test.

Five minutes pretraining, neonate chicks were given bilateral i.c. injections of 10 µl saline into the region of the intermediate-medial hyperstriatum ventrale. They were then trained with a target bead dipped in either 5%, 10%, or 100% MeA. Testing occurred at times from 10 s up to 24 hr posttraining. Runs were conducted either during the summer (Jun-Aug) or winter (Nov-Jan). At every test time, groups of chicks trained with 100% MeA showed greater avoidance than those trained with 10% or 5% MeA indicating that diluted MeA results in weaker training and retention.

Winter runs resulted in lower % avoidance scores, shorter test latencies, greater numbers of pecks at test, and longer training latencies than summer runs. Measures of variability -- standard deviations & ranges -- were greater for almost all winter data. In the winter, chicks were more difficult to train (longer training latencies & greater number of discarded data due to lack of training) and showed less retention (lower % avoidance scores & shorter test latencies). Data obtained from chicks given weak training (5% & 10% MeA) showed particularly strong seasonal differences. Data from chicks receiving strong training (100% MeA) showed differences due to season but not to the same extent as those seen with weak training.

Supported by NSF grant BNS-88-10528 & NIDA grant DA04795.

316.18

HALOTHANE ANESTHESIA INDUCES STATE DEPENDENT ANTEROGRADE BUT NOT RETROGRADE AMNESIA FOR INHIBITORY AVOIDANCE LEARNING Edwin Rosman MD, David Quartermain PhD, Herman Turndorf MD Departments of Anesthesiology and Neurology, New York University Medical Center, New York, NY 10016

Memory for a single brief foot shock administered for entering the dark side of a two compartment chamber was tested by measuring latency to enter the dark compartment 24 hours after training. Mice were exposed to 2% halothane in oxygen with a flow rate of 6 L/min in a 5 liter acrylic chamber. Retrograde amnesia was studied by exposing mice to halothane immediately after the training trial and anterograde amnesia was investigated by training animals following recovery from halothane. Animals trained 15 mins after exposure exhibited normal activity levels and had a normal response to foot shock. Results showed (1) exposing mice to halothane for 15, 30 or 60 min immediately after training failed to produce retrograde amnesia. (2) Mice trained 15 mins after recovery from 30 mins of anesthesia exhibited significant memory loss when tested 24 hrs after training. (3) The anterograde amnesia was temporally graded, i.e. mice were trained up to 2 hrs after recovery were amnestic, but mice trained 4 hrs or longer after recovery were not. (4) The anterograde amnesia resulted from state dependent retrieval failure since mice re-exposed to halothane before testing showed restoration of memory while mice untreated before training but exposed to halothane prior to testing exhibited memory loss.

BIOLOGICAL RHYTHMS AND SLEEP III

317.1

CIRCADIAN RHYTHMS RESTORED USING GRAFTS OF CULTURED SUPRACHIASMATIC CELLS. M. R. Ralph and E. R. Torre. Dept. of Psychology, Univ. Toronto, Toronto, Canada, M5S 1A1; Dept. of Neuroscience, Univ. Virginia, Charlottesville, VA 22908.

Circadian rhythmicity can be restored to arrhythmic hamsters with suprachiasmatic lesions using neural grafts from the embryonic suprachiasmatic region. The rhythm produced is specific to the donor cells. In an attempt to isolate the types of cells required for successful restoration, we have asked whether cultured cells retain the ability to produce overt rhythms following implantation, and if particular populations are required.

Tissue from wild type litters was harvested on embryonic day 13, dissociated and maintained in cell culture conditions that restricted survival and growth to defined subpopulations of cells. Under these conditions, we can produce from the same harvest, cultures of neurons only, glia only and a neuron-glial mixed population. Identification of basic cell types was based upon morphology and immunocytochemical characteristics.

After 8-days in culture, the cells were collected and implanted into the 3rd ventricle of arrhythmic hosts. Animals that were heterozygous for a period mutation (*tau*) were used as hosts to help distinguish restored rhythms. Circadian rhythmicity in the wild type range was restored to animals given implants of the neuron-glia culture. As yet, we have not seen an effect of the neuron only or glia only implants.

The results indicate that suprachiasmatic cells can be maintained in culture while retaining their pacemaker capability. By reducing the number of cell types in the cultures, it may be possible to identify those that are required for the production and expression of rhythmicity. Supported by an NSERC operating grant to M. Ralph.

317.2

AGE OF DONOR INFLUENCES SURVIVAL OF SCN GRAFTS IN HAMSTERS. M.-T. Romero[1], M. N. Lehman[2], A. Philpot-DeBernardo[1] and R. Silver[1]. Barnard College of Columbia University[1], N.Y., N.Y. 10027 and Dept. of Anat. & Cell Bio., U. of Cincinnati College of Medicine[2], Cincinnati, OH 45267.

Hypothalamic grafts of embryonic day 15 (E15) fetuses develop fully and promote behavioral recovery when implanted in the 3rd ventricle of SCN-lesioned (SCN-X), arrhythmic hamsters (Lehman et al., J Neurosci., 7:1626, 1987).

In the present experiment hypothalamic tissue, taken from donors at ages E13 to postnatal day 10 (P10) was implanted into the 3rd ventricle of SCN-X hamsters. Immunocytochemical staining for vasoactive intestinal polypeptide (VIP) and neurophysin II (NP) of serial sections of the implanted brains was performed at least 40 days following transplantation. Results indicate that hypothalami taken from E13 to P1 readily develop and express nonoverlapping clusters of VIP and NP cells in a pattern characteristic of the SCN. In grafts taken from older donors, P3 to P10, necrotic tissue is often observed and the size of the graft is decreased. Clusters of VIP and NP cells were less frequent in older tissue, although immunoreactive fiber plexi were still observed in some grafts. Recovery of circadian locomotor rhythms in SCN-X arrhythmic hosts bearing grafts of ages E13-P10 will be analysed. (Supported by NIH grant NS 08518 to MTR and NS 24292 to RS & ML)

317.3

TRIAZOLAM FAILS TO PHASE ADVANCE CIRCADIAN LOCOMOTOR ACTIVITY IN SCN-LESIONED HAMSTERS WITH FETAL SCN TRANSPLANTS. R. Silver, M.-T.Romero and R. S. Canbeyli. Barnard College of Columbia University, NY, NY 10027.

A single ip injection of the short-acting benzodiazepine, triazolam (Tz), at CT6 produces a permanent phase shift of about 1.5 hrs in the circadian locomotor activity of the golden hamster (Turek and Losee-Olson, Nature,1986). It is not known whether Tz acts directly on the SCN or on other target sites that communicate with the SCN.

The present study investigated the effect of Tz in SCN-lesioned (SCN-X) hamsters bearing fetal SCN grafts in the 3rd ventricle, on the assumption that the grafted SCN lacks normal afferent connections. Tz (2.5 mg/0.1 or 0.5 ml DMSO ip) was administered to hamsters housed in DD once they had regained circadian locomotor rhythmicity. While 5/8 intact controls (8 injections) phase advanced when injected with Tz at CT6, 0/7 SCN-X grafted animals (11 injections) showed a permanent phase shift. Immunohistochemical staining for VIP, NP and NPY indicated that 4 of the grafted animals (6 injections) had complete SCN-X. The results suggest that Tz does not act directly on the SCN. This is consistent with reports that lesions of the intergeniculate leaflet blocked Tz effects (Johnson et al., PNAS, 1988), though it is possible that the phase response curve or the dose-response curve of the SCN-grafted animals may differ from that of intact animals. (Supported by NS 24292 to RS).

317.4

TRACING SCN GRAFT EFFERENTS WITH THE CARBOCYANINE DYE DiI. R. S. Canbeyli[1], M. Lehman[2] and R. Silver[1]. Barnard College of Columbia University[1], NY, NY.10027; Univ. Cincinnati[2], Med. Coll., Cincinnati, OH 45267.

SCN lesioned hamsters recover circadian locomotor rhythmicity following implantation of embryonic day 15 donor SCN irrespective of attachment site of whole tissue grafts in 3rd V. (Lehman et al., J. Neurosci. 7:1626, 1987). Furthermore, adult hamsters bearing an SCN island retain locomotor rhythmicity (Hakim and Silver, Soc. Neurosci. Abstr.14: 51,1988), thus raising the question of functional role of SCN efferents.

This study was undertaken to evaluate efferent connections of SCN grafts using DiI. Hamsters were sacrificed 40 days after implantation of fetal SCN into the 3rd V. DiI crystals were placed on implants in paraformaldehyde fixed brains. Following incubation for 4 weeks, brains were sectioned, photographed and stained for VIP- and NP-like immunoreactivity (to locate the SCN). Histological analysis revealed that ventricular wall surrounding the graft was largely intact. Graft efferents entered the host brain at sites where the ependymal cell boundary of the 3rd V was absent and projected only a small distance into the host brain. Even at sites where the graft and host seemed to merge, most labelled fibers remained within the graft. The few fibers that entered host tissue did not travel far regardless of whether the SCN was present in the graft or not. The observation of limited connectivity between the transplanted SCN and the host is consistent with immunocytochemical evidence in SCN implanted NP-deficient Brattleboro rats (Wiegand, Restor. Neurol. Neurosci. Suppl. 1: 17,1989). (NIH Grant 24292 to RS & ML).

317.5

CHARACTERIZATION OF PROTEIN KINASE A SUBSTRATES IN THE RAT SUPRACHIASMATIC NUCLEUS. L. Faiman* and M.U. Gillette. Dept. of Physiol. & Biophys. and Neuroscience Program, Univ. of Illinois, Urbana, IL 61801.

Specific stimulation of cAMP-dependent pathways induces a 4-5 hr advance in the phase of the circadian rhythm of neuronal activity of the suprachiasmatic nucleus (SCN) *in vitro*. Sensitivity is limited to the subjective day of the SCN circadian pacemaker. This suggests that stimulation of protein kinase A (PKA)-mediated phosphorylation is important to the mechanism underlying phase-shifting. Therefore, we are characterizing PKA substrates in rat SCN.

Protein phosphorylation was examined in an *in vitro* system consisting of tissue punches of SCN collected from brain slices at CT 5.0 $\pm$0.5, a time in the circadian cycle most sensitive to cAMP stimulation. Phosphorylatable substrates in single SCN (~6 μg protein) were identified by SDS-PAGE. We have found that 1) 10-100 μM exogenous cAMP uniformly increases the phosphorylation level of specific protein bands, 2) the PKA catalytic subunit phosphorylates specific substrates in a sonicated extract subjected to heat, and 3) a specific inhibitor of PKA prevents phosphorylation. We are comparing the pattern of PKA substrates at CT 5.0 with that at points in the circadian cycle when the SCN is insensitive to cAMP stimulation. (Supported by NINDS Grant NS22155.)

317.6

ELECTRICAL CHARACTERIZATION OF VENTROLATERAL AND DORSOMEDIAL REGIONS OF THE SUPRACHIASMATIC NUCLEUS. **T.K. Tcheng and M.U. Gillette. Neuroscience Program and Department of Physiology & Biophysics, University of Illinois, Urbana, IL 61801.**

The rat suprachiasmatic nuclei (SCN) contain a circadian pacemaker that is expressed in the brain slice as a 24-hr oscillation in ensemble neuronal firing rate. We are studying neuronal firing patterns within the dorsomedial (DM) and ventrolateral (VL) SCN, the two major anatomical subdivisions, to further characterize the circadian pacemaker.

We have shown previously that hemisection of the SCN into DM and VL halves results in an unperturbed electrical circadian rhythm (ECR) in the VL-SCN and the possible loss of an ECR in the DM-SCN. Our current work elaborates upon this finding. In this study, the SCN are *unequally divided*, leaving less SCN in either the DM-SCN (DM-biased) or VL-SCN (VL-biased). Preliminary results suggest that after DM-biased hemisection the ECR in the DM-SCN is completely abolished (N=5). The effect of VL-biased hemisection on the ECR in the DM and VL regions is presently being examined. Additionally, we have observed that firing patterns of individual neurons change after hemisection. Firing patterns from control SCN and isolated VL and DM regions will be compared in order to identify firing patterns contributing to the ECR.

317.7

PERTUSSIS TOXIN BLOCKS MELATONIN'S ABILITY TO RESET THE SUPRACHIASMATIC CIRCADIAN CLOCK IN VITRO. **A.J. McArthur and M.U. Gillette. Dept. of Physiol. & Biophys., Univ. of Illinois, Urbana-Champaign, IL 61801.**

Melatonin (MEL) acts directly on the SCN *in vitro*, phase advancing (ϕ_A) the time-of-peak neuronal activity nearly 4hr when applied at the subjective day-night transition. Other systems have indicated that MEL can act through a G_i protein. To further elucidate the transduction mechanism by which MEL affects the SCN clock, a long-term pertussis toxin (PTX) incubation prior to a short MEL+ PTX treatment at circadian time (CT) 10 was used.

Coronal hypothalamic slices containing the SCN were prepared from 8 wk old male Long Evans rats housed in a 12L:12D cycle. The media in the brain slice chamber was replaced with fresh media containing PTX (1.0μg/ ml) for 6hr. At CT 10 this treatment was replaced with media containing both PTX and 10^{-9}M MEL for 10min. All measurements of phase were made on day 2 by monitoring firing rates and determining the time-of-peak in the ensemble neuronal activity *in vitro*.

Whereas 10 min exposure to MEL alone induces ϕ_A > 3hr, long-term exposure to PTX blocks the MEL-induced phase advance. The time-of-peak in these experiments (CT 6.6 $\pm$ .1, n=4) does not deviate significantly from that seen in PTX controls (CT 6.8 $\pm$ .2, n=3). These results suggest that MEL may regulate the SCN pacemaker during late subjective day through PTX-sensitive pathways. (Supported by NINDS grant NS22155).

317.8

ENDOGENOUS CIRCADIAN CHANGES IN cGMP LEVELS IN THE RAT SUPRACHIASMATIC NUCLEUS. E.T. Weber and M.U. Gillette. Dept. of Physiology & Biophysics, Univ. of Illinois, Urbana, IL 61801.

Stimulating the SCN with cGMP analogs induces a nearly immediate, large advance in the phase of the circadian rhythm of SCN neuronal activity. The sensitive period is limited to subjective night and is in antiphase to SCN sensitivity to cAMP analogs. We questioned whether changes in endogenous cGMP levels might occur within the SCN over the circadian cycle.

Brain slices were made from 8-wk-old Long-Evans rats reared on a 12L:12D cycle. Tissue punches of SCN and of hypothalamus were obtained from 500 μm brain slices quick-frozen at the desired time-of-day. Six time points at 4-hr intervals were sampled. Levels of endogenous cGMP were measured by radioimmunoassay (NEN).

Preliminary results suggest that cGMP levels in the SCN change over the course of the day, possibly with a 24-hr period. Levels in the SCN ranged from 0.02 to 0.60 pmol/mg protein, peaking late in the subjective night. Levels in the hypothalamus ranged from 0.02 to 0.35 pmol/mg protein, consistent with other reports. This cyclicity, in addition to the phase-shifting effects of cGMP, suggests a central role for cGMP in regulating the SCN circadian system during the night. (Supported by NINDS Grant NS22155.)

317.9

SEROTONIN PHASE SHIFTS THE CIRCADIAN RHYTHM OF ELECTRICAL ACTIVITY IN THE RAT SCN IN VITRO.

M. Medanic and M.U. Gillette. Dept. of Physiol. & Biophys. and Neuroscience Program, Univ. of Illinois, Urbana, IL 61801.

Ventrolateral (VL) regions of the suprachiasmatic nuclei (SCN) receive serotonergic projections from the raphe nuclei. We investigated the possible role of serotonin (5-HT) in the mammalian circadian system by examining its effect on the rhythm of electrical activity in the rat SCN *in vitro*.

Eight week old, male Long-Evans rats from our inbred colony, raised on 12L:12D schedule, were used. Hypothalamic brain slices containing the paired SCN were made during the day, and maintained *in vitro* for two days. A 30 *fl* drop of 10^{-6}M 5-HT was applied for 5 minutes to the VL region of one of the SCN at CT 7 (n=3), 13 (n=3) or 19 (n=4). The time of peak in the rhythm of neuronal activity was determined on the following day.

Exposure of the VL-SCN to 5-HT treatment during the subjective night (CT 13 and 19) did not significantly alter time-of-peak compared to untreated slices. However, treatment during the subjective day (CT 7) resulted in a 6.9±0.1 hr advance in the time-of-peak of neuronal activity in the next cycle. This suggests that the SCN are sensitive to 5-HT during the subjective daytime and respond with a phase advance in the circadian clock. (Supported by AFORS grant 90-0205.)

317.10

TIME-BASED CHANGES IN THE PHOTOINDUCTION OF FOS EXPRESSION IN THE SUPRACHIASMATIC NUCLEUS.

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Induction of the c-*fos* proto-oncogene in neurons within the brain occurs in response to various extracellular stimuli. Consistent with this observation, we have reported that light exposure causes an increase in the immunohistochemical expression of c-*fos* protein (Fos) within the ventrolateral subfield of the suprachiasmatic nucleus (SCN). Since the light-entrainable circadian pacemaker located in the SCN exhibits rhythmic changes in its sensitivity to light, the present study was conducted to determine whether the photoinduction of Fos expression within the ventrolateral SCN is similarly time-dependent.

During exposure to constant light, Long-Evans rats (N=13) were sacrificed and perfused at 4-hour intervals over a period of 24-hours. Brain sections were processed for the immunohistochemical localization of Fos protein(s) along with neuropeptide Y (NPY) in the SCN.

In all animals, most of the cells in the SCN with Fos-positive nuclei were segregated in an area that was coextensive with the NPY-immunopositive fibers found in the ventrolateral subfield. Moreover, temporal variation in the expression of Fos was evident within the ventrolateral SCN, such that the density of immunopositive cells during the subjective night was 2-3 times greater than that observed during the subjective day. These results demonstrate that the inductive effect of light on Fos expression in the ventrolateral SCN oscillates over time. Furthermore, the observation that the photoinduction of Fos expression in the SCN was greatest at times when light is known to phase shift circadian rhythms serves to further implicate the c-*fos* proto-oncogene in the processing of photoentrainment information within the mammalian circadian system. Supported by AFSC Grant #90-0182 (D.E.).

317.11

CIRCADIAN RHYTHM IN C-FOS-LIKE IMMUNOREACTIVITY IN THE RAT BRAIN.

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The proto-oncogene c-fos encodes a nuclear phosphoprotein (Fos), which has been suggested to act as a transcriptional regulatory protein for a specific set of genes. It has been demonstrated that various types of stimulation induces activation of c-fos gene expression in specific neuron populations. However, little attention has been paid to the possible diurnal variation of c-fos expression in the brain. Therefore we studied immunohistochemically the basal c-fos expression in the rat brain at different times of the day.

Sprague-Dawley rats were maintained on a 12/12 h light/dark schedule. The animals were killed at every four hours starting at 24.00 h. Sections were stained for Fos with standard ABC-immunohistochemical technique. We observed that in certain brain areas, such as the cortex, hippocampus and amygdala, the number of c-fos immunoreactive nuclei was considerabl higher at 24.00 h than that at 12.00. In many other areas, such as in periventricular, paraventricular, septal and supraoptic nuclei no changes were observed, between the same time points. The variation of Fos protein induction at different times of the day indicates diurnal variation of Fos activation and should be considered when planning Fos related studies.

317.12

PHASE-SPECIFIC DISTRIBUTION OF LIGHT-INDUCED FOS IMMUNOREACTIVITY IN THE SCN

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Male, Syrian hamsters were housed individually in activity cages and maintained under constant darkness for at least 10 days. Hamsters were exposed to 15 min of white light (33 lux) at either circadian time (CT)6, CT13 or CT18. Light exposure at these times resulted in phase shifts of the free running activity rhythm of -0.12 ± 0.12 hrs (no shift), -1.0 ± 0.2 hrs (phase delay) and +1.9 ± 0.8 hrs (phase advance), respectively. Two hours after stimulation, groups of hamsters (n=8) were anesthetized and perfused with 4% paraformaldehyde. Serial 70 micron-thick sections containing the SCN were immunostained for c-fos protein (FOS) using antiserum provided by Dr M. Iadarola (NIDR).

Light exposure at both CT13 and CT18 resulted in the appearance of FOS-immunoreactive (FOS-ir) cell nuclei in the suprachiasmatic hypothalamus. In CT13 animals, FOS-ir cells were confined to the SCN and concentrated within the ventrolateral aspect of the nucleus. In contrast, FOS immunostaining was more widespread in CT18 animals, with FOS-ir cells present throughout the SCN and extending dorsally into the surrounding hypothalamus. Stimulation at CT6 did not increase FOS-ir in the SCN. These data indicate that (1) c-fos expression in the SCN occurs in association with light-induced alterations in pacemaker activity, and (2) different populations of cells in the suprachiasmatic hypothalamus are activated by retinal illumination at phase delay and phase advance times. Supported by AFOSR 2312W6 (MAR)

317.13

EFFECT OF OPTIC NERVE STIMULATION ON PEPTIDE RELEASE AND mRNA LEVELS EXPRESSED BY CULTURED SUPRACHIASMATIC EXPLANTS.

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Since visual inputs to the light-entrainable circadian pacemaker in the suprachiasmatic nucleus (SCN) presumably trigger a cascade of cellular events locally, the present study was conducted to examine the photic regulation of cellular activity within the SCN. Experiments examined the effect of optic nerve stimulation on peptide release and mRNA levels expressed by perifused SCN explants. Preliminary emphasis was placed on studying the distinct populations of SCN neurons containing vasoactive intestinal polypeptide (VIP) or vasopressin (VP) since these neurons receive visual input and/or maintain local connections.

Individual rat SCN explants were maintained in a perifusion culture system and after equilibration, electrical stimuli were delivered to the optic nerves via cuff electrodes. Peptide levels in the perifusate and mRNA levels in the explants were analyzed in parallel at regular intervals before and after stimulation.

Stimulation of the optic nerves during the early subjective night evoked acute increases in the release of VP, but not VIP. VP output during optic nerve stimulation was 2-5 times greater than that observed during the preceding control interval. Immediately after stimulation, VP release declined to pre-treatment levels. In accord with its acute effect on VP release, optic nerve stimulation also induced a 2-fold increase in the levels of VP mRNA in SCN explants. The present results suggest that VP neurons in the SCN, although devoid of retinal innervation, may be responsive to photic stimulation. Further analysis of the effect optic nerve stimulation at different circadian times and using different parameters is necessary to complement these observations, but the present approach may provide a basis for examining the light responsiveness of different cell types within the SCN. Supported by AFSC Grant #90-0182 (D.E.).

317.14

CHANGE IN CIRCADIAN PATTERNS OF RUNNING ACTIVITY IN RATS AS A FUNCTION OF VASOPRESSIN ANTAGONISM IN THE SUPRACHIASMATIC NUCLEUS.

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Vasopressin (VP) has been implicated as a possible CNS neuromodulator for circadian cycles. The major locus of control for circadian rhythms is believed to be the Suprachiasmatic Nucleus (SCN) in the hypothalamus . VP in the SCN has been shown to be depleted in aged rats, and as rats age, their circadian cycles tend to be disrupted, becoming shorter and occurring with higher frequency. In this study, the relationship between the blockade of VP and circadian cycles was examined. Vasopressin antagonist (VPA) was administered chronically over an eight day period from a mini osmotic pump into the SCN of three month old rats via a cannula tube. This infusion should produce daily cycles similar to the cycles found in older rats with no change in the mean level of activity. Results indicate that the chronic administration of VPA produces disrupted circadian cycles which are higher in frequency than controls without changing the total level of activity, a pattern which resembles those found in old rats .

317.15

VASOACTIVE INTESTINAL PEPTIDE (VIP), PEPTIDE HISTIDINE ISOLEUCINE (PHI) AND GASTRIN RELEASING PEPTIDE (GRP) INTERACT WITHIN THE SUPRACHIASMATIC NUCLEUS (SCN) IN A DOSE-DEPENDENT MANNER TO MIMIC THE PHASE DELAYING EFFECTS OF LIGHT. H.E. Albers, E.G. Stopa & R.T. Zoeller. Lab. Neuroendocrinol. & Behav., Depts. Biol. & Psychol., Georgia State Univ., Atlanta, GA 30303; SUNY Health Sci. Ctr., Syracuse, NY 13210; Dept. Anat., Univ. Missouri Med. Sch., Columbia, MO 65212.

VIP, PHI and GRP are co-localized in neurons found in the terminal field of SCN photic afferents. To determine whether VIP, PHI and GRP interact within the SCN in a dose-dependent manner male hamsters were implanted with guide cannula aimed at the SCN and microinjected with a cocktail containing equimolar concentrations of VIP, PHI and GRP at either 0.3 (N=6), 3 (N=7), 30 (N=5), 300 (N=10) or 3000 (N=7) μM during the 3 hrs following activity onset. Phase delays were observed at all doses, but the magnitude of the delay differed significantly ($P<0.05$). The smallest delays (i.e. 0.57±0.21) were produced by 0.3 μM and the largest (i.e. 1.92±0.22) by 3000 μM. These data support the hypothesis that the interaction of VIP, PHI and GRP within the SCN is necessary for light-dark cycle entrainment.

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317.16

APPLICATION OF MK-801 INTO THE SUPRACHIASMATIC REGION ATTENUATES THE PHASE-SHIFTING EFFECTS OF LIGHT ON THE CIRCADIAN ACTIVITY RHYTHM OF THE HAMSTER. ALFRED B. LORD, FRED W. TUREK AND JOSEPH S. TAKAHASHI. Department of Neurobiology and Physiology, Northwestern University, Evanston, IL 60208.

Entrainment of mammalian circadian rhythms to environmental light cycles is dependent on a branch of the optic nerve known as the retino-hypothalamic tract (RHT). Data implicating an excitatory amino acid neurotransmitter (EAA) in mediating the effects of light on the circadian clock in the suprachiasmatic nucleus (SCN) of the hamster have been presented (Colwell et.al., Society for Research on Biological Rhythms Abstract #22, 1990). Although systemically applied antagonists of EAAs can block light-induced phase-shifting in hamsters, the site of action of MK-801 is not clear. For example, this effect could have its basis in the retina, known to utilize EAAs, or in a polysynaptic effect at or near the SCN. In order to test whether MK-801 acts in the SCN region, microinjections of the NMDA blocker were applied to the SCN region. Hamsters were implanted with a guide cannula in the SCN region and allowed to free-run in constant darkness. Light pulses were given at circadian time (CT) 19 at an intensity and duration to yield 70-80% of maximal phase shifts in the activity rhythm. MK-801 or vehicle were injected 10 minutes before the light pulse was presented. A second control group consisted of animals receiving only MK-801 at the appropriate CT. Each animal ($n=18$) received all three treatments, but in different orders. Hamsters receiving the vehicle showed the expected phase advances of approximately 120 min. in response to the light pulse. Those animals that received MK-801 before the light pulse showed a significantly decreased phase shift ($p< .05$) with a mean of about 60 min. MK-801 alone caused no significant phase shift. These results show that MK-801 application into the SCN region can attenuate light-induced phase shifts and are consistent with the hypothesis that an EAA transmitter in the region of the SCN is involved in light-induced phase shifts in the circadian clock.

317.17

EFFECTS OF MELATONIN ON THE NEUROCHEMISTRY OF THE SUPRACHIASMATIC HYPOTHALAMUS. S.A. Ferreira*, W.W. Randolph*, M.A. Rea[1] and J.D. Glass. Dept. Biological Sciences, Kent State Univ., Kent, OH 44242 [1]U.S.A.F. School of Aerospace Med., Brooks A.F.B., TX 78235.

In vivo microdialysis was used to determine the effect of melatonin on serotonergic and amino acid activities in the area of the SCN. Adult male Djungarian hamsters under L:D 16:8 (n=4) received a microdialysis probe (1.0 X 0.2 mm tip size) stereotaxically placed in or near the SCN. Samples were collected every 20 min for 120 min prior to and 120 min following s.c. injection of 100 ug melatonin or vehicle at 1900 h (4 h prior to lights-off). 5-hydroxyindoleacetic acid (5-HIAA) and amino acids were measured by HPLC. The nature of release of neurotransmitters and metabolites to the dialysis probe has been validated in previous experiments. Melatonin increased the concentrations of 5-HIAA in dialysate by 26.6±5.9% compared to preinjection levels ($p<0.05$); vehicle did not increase 5-HIAA. Glutamate was increased by 70.8±22.1% ($p<0.05$). The maximal effect of melatonin on 5-HIAA occurred 40-80 min post-injection, but the rise in glutamate was more prolonged with peak levels occurring ≥100 min. Supported by AFOSR 440785 (J.D.G.) and AFOSR 2312W6 (M.A.R.).

317.18

EFFECTS OF EXOGENOUS MELATONIN ON CIRCADIAN TIMING IN A NON-HUMAN PRIMATE. T. M. Hoban-Higgins* and C. A. Fuller. Dept. of Animal Physiology and California Primate Research Center, University of California, Davis, CA 95616.

Melatonin administration has been shown to affect the circadian timing system of a variety of species. This study was designed to examine the effect of daily melatonin administration on the circadian system of a non-human primate. Six individually housed, adult male squirrel monkeys were maintained in a constant environment (LL=30 lx). Drinking data were collected in half-hour bins. After a 50 day free-run to establish a baseline circadian pattern, a daily timed dose of melatonin was administered for 140 days (5 mg/kg D 51-77; 10 mg/kg D 78-191). As a control, the animals were given vehicle alone from day 191 to 214. Administration at this dose level was unable to produce consistent entrainment to the 24-h period of melatonin presentation. However, a beating pattern in the rhythm period, analogous to relative coordination, is evident in the data. All six animals increased free-running period when melatonin was administered during their late subjective day-early subjective night and five of six shortened free-running period when administration coincided with late subjective night. This would occur if the melatonin produced phase delays in the early subjective night and phase advances late in the subjective night. It could not be determined if this beating pattern continued during the control portion of the experiment. Our results suggest that melatonin does have an effect on the circadian timing system of this diurnal primate. (Supported by Smokeless Tobacco Research Council Grant 0167, NIH Grants MH41477 and NSRA MH09464 to TMH.)

317.19

CHARACTERIZATION AND DEVELOPMENT OF [125I]IGF-I BINDING SITES IN THE SUPRACHIASMATIC NUCLEUS OF THE RAT HYPOTHALAMUS. K.M. Michels and J.M. Saavedra. LCS, NIMH, Bethesda, MD 20892.

Although insulin-like growth factor-I (IGF-I) is involved in the growth and differentiation of a variety of neural and non-neural tissues specific receptors for IGF-I have also been demonstrated in the brain of the adult rat. One of the brain regions shown to exhibit [^{125}I]IGF-I binding is the suprachiasmatic nucleus (SCN) of the rat hypothalamus. In the present study we used quantitative autoradiography to characterize the specific binding of [^{125}I]IGF-I within the SCN *in vitro* at equilibrium conditions. Saturation binding of [^{125}I]IGF-I at 0^0 C revealed a single class of sites with a K_d of 0.21 nM and a B_{max} of 82 fmoles/mg protein.

Binding sites for [^{125}I]IGF-I were already present at near-adult levels in the rat SCN at embryonic day 18. The receptor density doubled by postnatal day 1 then decreased precipitously to day 7, attaining near-adult levels by day 12 which remained constant thereafter. These results suggest that IGF-I may play a role particularly in the postnatal development of the SCN but also may have some role in regulating function of the adult SCN.

317.20

EVIDENCE FOR CENTRAL SITES OF MELATONIN ACTION. L.L. Badura & B.D. Goldman, Dept. Physiol. & Neurobiol., Univ. of Connecticut, Storrs, CT 06268.

The suprachiasmatic nuclei (SCN) of the hypothalamus are putative sites for central melatonin (MEL) action. In the Siberian hamster, the SCN are MEL binding sites, and lesions of this region render the animals unresponsive to the effects of exogenous MEL on the reproductive system. In this study, the effects of central MEL administration on testicular maturation were assessed in juvenile Siberian hamsters.

Hamsters were implanted with central microdialysis probes and were infused with one of two doses of MEL (0.75 ng or 0.075 ng/infusion) or saline for 12 days under long days (16L), with 5 hr infusions commencing 3 hr before lights out, or constant light (LL) with either 5 or 10 hr infusions. On day 13, all animals were anesthetized, perfused transcardially, the testes weighed, and the brains prepared for histological evaluation of the infusion site.

Animals housed in LL receiving either dose of MEL for 10 hr in the SCN region had smaller testes weights than animals infused for only 5 hr, or animals infused with saline. The 5 hr infusions in 16L also delayed gonadal maturation at both doses. However, for the lower dose, infusions were effective only if centered in the SCN area, whereas the higher dose delayed maturation at slightly more dorsal levels if the third ventricle was also involved. Infusions in other periventricular regions were not effective unless there was substantial damage to the ventricle. Probes centered in the lateral hypothalamus had no effect on gonadal size. These results suggest that the SCN may play an important role in the central mediation of MEL-dependent physiological responses. However, it will be important to evaluate the effects of MEL administered at other sites near the SCN and other sites reported to exhibit uptake of labeled MEL.

317.21

AUTORADIOGRAPHIC LOCALIZATION OF 2-[125] IODOMELATONIN BINDING SITES IN THE BRAIN OF GROUND SQUIRRELS (CITELLUS LATERALIS). Stanton, T.L., J.A. Siuciak[1], D.N. Krause[2] and M.L. Dubocovich[1]. Dept. of Physiology, CSU, Long Beach, CA 90840; Depts. of Pharmacol., [1]Northwestern Univ. Med. Sch., Chicago, IL 60611 and [2]UCI, Sch. of Med., Irvine, CA 92717.

Melatonin (mel) may play a role in the modulation of brain arousal level. In earlier studies, using the hibernating ground squirrel as a model system, we demonstrated significant variations in pineal mel level throughout the hibernation bout cycle. A direct correlation between mel level and the duration of individual bouts of hibernation suggested that mel availability might be one of the factors that determines bout duration. Subsequent work showed that ICV infusion of mel during hibernation could prolong the bout. The present study was undertaken to investigate possible CNS sites of mel action by determining the distribution of specific 2-iodomelatonin binding sites in the brain of the golden-mantled ground squirrel.

Quantitative autoradiography was employed using 72-123pM 2-[125] iodomelatonin and 20u coronal brain sections of hibernating and awake animals. Non-specific binding was defined using 3uM mel. Initial results indicate discrete localization of specific binding in the region of the pars tuberalis that is significantly greater in awake compared to hibernating brain. The results are consistent with mel's ability to induce gonadal regression, a prerequisite for successful hibernation in ground squirrels. Supported by CSULB and USPHS grants NS07140 to JAS and MH42922 to MLD.

317.22

DAILY AND CIRCADIAN PATTERNS IN 2-[^{125}I]-IODOMELATONIN BINDING IN SPECIFIC SITES OF AVIAN BRAIN. David S. Brooks, Lu Jun* and Vincent M. Cassone. Department of Biology, Texas A&M University, College Station, TX 77843.

Melatonin binding sites have been localized in avian brains using image analysis of 2-[^{125}I]-iodomelatonin (IMEL) autoradiography (Rivkees et al. Endocrinology 125:363-368, 1989, Cassone et al., in press.). We characterized the daily and circadian patterns of IMEL binding in avian brain. Newly hatched chicks and adult house sparrows were obtained locally. The daily and circadian pattern of binding was investigated over 48 hours in a 12:36 LD cycle. Five birds were sacrificed every 4 hours beginning on Day 1, Zeitgeber time (ZT), and continuing Day 2, circadian time (CT). Anesthetized birds were perfused and brains were removed and immediately frozen. The brains were sectioned coronally at 20 μm on a cryostat. Brain sections were incubated with IMEL at varying concentrations from 5 to 500 pM for the binding characteristic study and at 75 pM for the daily binding study. Non-specific binding was determined in the presence of 1 μM melatonin. The slides were exposed to X-ray film for 7 days at -20°C. Autoradiographs were analyzed with a computer visual analysis system and the binding densities quantified to ^{125}I-microscale standards. As previously shown, specific binding sites are located in several structures of the telencephalon, thalamus, hypothalamus, and midbrain of the chick including structures associated with the visual and auditory systems. Scatchard analysis of the binding indicates a low affinity and high affinity binding site, with a change in B-Max occurring in both sites from (subjective) day to (subjective) night. Analysis of data collected every four hours reveal a daily rhythm in binding sites with a peak occurring at ZT 10. This rhythm persisted in DD with a peak occurring at CT 10. The source(s) of this endogenous rhythm and its consequences to avian circadian organization are currently under investigation.(Supported by NSF Grants BNS 85-19660 and BNS 88-96225)

317.23

DEVELOPMENTAL APPEARANCE OF SPECIFIC BINDING SITES FOR 2-[125I]-IODOMELATONIN IN THE SYRIAN HAMSTER BRAIN AND PITUITARY. M.J. Duncan, K. Jaeck*, and F.C. Davis. Dept. of Anatomy & Neurobiology, Univ. of Missouri Medical School, Columbia, MO 65212; Dept. of Biology, Northeastern Univ., Boston, MA 02115.

In Syrian hamsters, melatonin administration exerts both circadian and reproductive effects which are prevalent at different developmental stages and have different anatomical substrates. The circadian effects are most prominent in the prenatal period and most likely involve the suprachiasmatic nuclei (SCN); the reproductive effects occur during adulthood and do not require the SCN. In order to elucidated the basis for these effects of melatonin, the present studies investigated the regional distribution of specific 2-[125I]-iodomelatonin (IMEL) binding sites in the brain and pituitary of hamsters sacrificed at different ages (days relative to birth): fetal (-2), neonatal (+2), prepubertal (+25) or adult (+119). Slide-mounted coronal sections through the diencephalon were incubated with IMEL (400 pM), washed and processed for autoradiography (Duncan et al, Endocrinol. 125:1011, 1989). Specific IMEL labelling was identified in the pars tuberalis and lateral habenular nucleus at all ages and in the SCN of fetuses and neonates but not older hamsters. These results suggest that specific IMEL binding sites are present by late gestation and that their density, at least in the SCN, may decrease during development.

INGESTIVE BEHAVIOR: PEPTIDES I

318.1

PATTERNS OF EATING BEHAVIOR ELICITED BY NEUROPEPTIDE Y INJECTED INTO THE MEDIAL PERIFORNICAL HYPOTHALAMUS. B. G. Stanley and W. J. Thomas*. Dept. of Psychology, Univ. of California, Riverside, CA 92521.

Neuropeptide Y (NPY) is a powerful stimulant of eating behavior that appears to be most effective when injected directly into the medial perifornical region of the hypothalamus (mPFH). Little, however, is known about the patterns of food intake elicited by this peptide. To elucidate these patterns, we injected NPY (78 pM/10 nl) or vehicle into the mPFH of 10 satiated rats and measured intake of the mash diet every 60 sec for 24 hrs. These NPY-elicited intake patterns were compared to naturally-occurring nocturnal feeding patterns. The results indicate that NPY produced a marked increase in food intake, predominantly within 2 hrs of injection (9.8 g for NPY versus 1.9 g for vehicle). The feeding behavior was not fragmented, but rather occurred within discrete clearly defined meals, during which eating was relatively continuous. Compared to the mean nocturnal-phase meal size of 2.2±0.3 g, meal duration of 12.8±1.2 min, and intermeal interval of 98.4±9.4, the NPY-elicited meals were both larger and more frequent. Specifically, the animals began to eat 14.1±1.5 min postinjection, ate a 5.9±1.3 g meal lasting 21.7 min, paused for 30±8.6 min, and then ate another meal of 3.2±0.8 g. These results indicate that NPY may produce eating through mechanisms that influence both meal size and intermeal interval.

318.2

COLOCALIZATION OF NEUROPEPTIDE Y (NPY) AND TYROSINE HYDROXYLASE (TH) IMMUNOREACTIVITY IN CAUDAL MEDULLARY NEURONS PROJECTING TO THE REGION OF THE ANTEROVENTRAL THIRD VENTRICLE (AV3V). G.L. Edwards and A.K. Johnson. Dept. of Physiol. and Pharmacol., Univ. of Georgia, Athens, GA 30602 and Depts. of Psychol. and Pharmacol. and the CV Center, Univ. of Iowa, Iowa City, IA 52242.

We have previously demonstrated that neurons containing NPY-immunoreactivity (NPY-IR) project from the caudal ventral lateral medulla (CVLM) and caudal nucleus of the solitary tract (NTS) to the AV3V region (Neurosci. Lett. 105: 19, 1989). Since catecholamines in the AV3V are reportedly important in the control of drinking behavior, we have investigated the possibility that NPY-IR and TH-IR are colocalized in neurons of the caudal medulla. We have utilized rhodamine labelled beads injected into the AV3V region to retrogradely label neurons in the caudal medulla. Sections from the medulla were then immunostained for NPY-IR and TH-IR using rabbit anti-NPY and mouse anti-TH antibodies to localize NPY-IR and TH-IR in the same cell. We observed that many cells that were retrogradely filled from injections in the AV3V region were immunoreactive for both NPY and TH, particularly in the CVLM and NTS. These data suggest that many of the catecholamine projections from the caudal medulla may also contain NPY. Thus, NPY may interact with catecholamines at the level of the AV3V to modulate drinking behavior.

318.3

DISCRIMINATIVE CHARACTERISTICS OF NEUROPEPTIDE Y (NPY) IN RATS. D. C. Jewett, A. S. Levine, J. Cleary* and T. Thompson*. University of Minnesota, Minneapolis, MN 55455 and VA Medical Center, Minneapolis, MN 55417

The ability of rats to discriminate certain agents following intracerebroventricular (ICV) injection has been demonstrated in previous studies. We asked if the subjective stimuli associated with NPY can be discriminated from those stimuli associated with an injection of vehicle. Rats were maintained at 80% of their free feeding weight and trained to press one of two levers depending upon whether they had received NPY or vehicle. Rats were injected ICV with either 5.0 µg of NPY or saline 30 minutes before the session. Responses on one lever following injection with NPY were reinforced, while responses on the other lever following injection of saline were reinforced. Responses prior to the first reinforcer or timeout were a measure of the discriminative stimulus properties of the agent. Our findings suggest that NPY can be rapidly discriminated from saline. The NPY-saline discrimination was established within 11 sessions for all subjects (n=4). In addition, rats were injected with 1, 3, 5 and 10 µg of NPY (1µg/1µl). A 1µg dose of NPY generalized to saline (i.e. rats responded as if they were injected with saline). A 10 µg dose of NPY completely generalized to the training dose (5µg), suggesting that the higher dose of NPY feels similar to an injection of 5 µg of NPY. An intermediate dose of NPY (3µg) partially generalized to the training dose. Approximately 60% of the responses following the 3 µl injection were made on the NPY appropriate lever. Thus, 5 µg NPY can be discriminated from saline and other doses of NPY may generalize to the training dose.

318.4

NEUROPEPTIDE Y (NPY) INDUCED FEEDING: CALORIES OR VOLUME? M. Grace*, C.J. Billington* and A.S. Levine. Neuroendocrine Research Laboratory, VA Medical Center, Minneapolis, MN. and the University of Minnesota, Minneapolis and St. Paul, MN. 55108

Central administration of NPY is a potent stimulator of feeding in a variety of species. We asked whether rats given NPY intracerebroventricularly would differentiate the energy content from volume of food. NPY-injected rats were divided into two groups (n=14-15); one receiving high fiber chow (50% celufil mixed with chow) and the other low fiber chow (10% celufil mixed with chow). During a four hour period there was no difference in the volume of chow ingested by these groups (high fiber: 5.2 ± 1.1; low fiber: 4.7 ± 1.1 g/4 hr); even though the low fiber group contained almost twice as many calories. These two groups were then given a high fat (68% of calories) or a low fat diet (12% of calories). During a four hour period the NPY-injected rats in the high fat group ate the same amount of volume (4.0 ± 1.0 g) as those in the low fat group (3.9 ± 0.9 g), although the high fat group contained about 1.5 times the calories. Finally, the rats were divided into four groups and were given either water or a 10%, 20% or 40% sucrose solution. During the first two hours of the study the NPY-injected rats drank the same volume of all of the sucrose containing solutions (water: 2.7 ± 0.8; 10% sucrose: 11.4 ± 4.9; 20% sucrose: 9.8 ± 3.4; 40% sucrose: 9.6 ± 2.0 g/2 hr). Our results suggest that NPY-injected rats eat for volume without relation to calories.

318.5

INVESTIGATION OF PITUITARY-ADRENAL AXIS IN NEUROPEPTIDE Y-INDUCED FEEDING. S. Sheriff*, A. Balasubramaniam*, F. Zhang*, M. Kalmonpunpour*, T. Foley-Nelson, J.E. Fischer* and W.T. Chance. Dept. of Surgery, Univ. Cincinnati Med. Ctr. and V.A. Med. Ctr., Cincinnati, OH 45267.

Although neuropeptide Y (NPY) is a potent orexigenic agent, mediation of this effect is not well understood. Recent results (Inoue et al., Life Sci. 44:1043, 1989) suggests that intraventricular NPY stimulates the pituitary-adrenal axis. To assess pituitary-adrenal involvement in NPY-induced feeding, 24 ga cannulae were implanted into the paraventricular hypothalamic area of 28 adult, male S.D. rats. Two weeks later half of these rats were treated with saline (SAL) or dexamethasone (DEX: 50 & 200 µg/kg, sc) for 5 days. Feeding was assessed for 4 hrs following the iht injection of 2 µg NPY or artificial CSF 3 days after 50 µg/kg DEX and 1 day after 200 µg/kg DEX. Although the intake of rat chow was increased ($p<0.01$) by NPY in both groups, intake by the DEX-NPY group was reduced ($p<0.05$) by 38% as compared to NPY controls. Ad lib. intake was also reduced ($p<0.01$) by 30% in DEX-treated rats. Assessment of immunoreactive CRF in the hypothalamus 30 and 60 min after injection of NPY or CSF revealed decreased ($p<0.05$) CRF in DEX-CSF rats, while CRF was increased ($p<0.05$) in the SAL-NPY group. In the DEX-NPY rats, however, no elevation of CRF was observed. These results indicate an interaction of NPY with the pituitary-adrenal axis in the control of NPY feeding and ad lib. feeding, possibly involving release of CRF. [Supported by NIH grant CA48057 and a V.A. grant to W.T.C.]

318.6

REDUCTION OF PLASMA AND HYPOTHALAMIC NEUROPEPTIDE Y IN ANORECTIC TUMOR-BEARING RATS. W.T. Chance, S. Sheriff*, F. Zhang*, M. Kalmonpunpour*, J.E. Fischer* and A. Balasubramaniam*. Dept. of Surgery, Univ. Cincinnati Med. Ctr. and V.A. Med. Ctr., Cincinnati, OH 45267.

The potent orexigenic action of neuropeptide Y (NPY) suggests that it might be involved in the normal control of food intake. We reported (Ann. N.Y. Acad. Sci., in press, 1990) that tumor-bearing (TB) rats exhibit decreased feeding to NPY. To determine if endogenous concentrations of NPY were altered as TB rats became anorectic, we measured immunoreactive NPY in plasma and hypothalamus of control rats and 32 days after transplantation of methylcholanthrene sarcomas. Food intake by TB rats was significantly reduced (1.3±0.7 vs 8.5±0.3 g/100 g BW on day of sacrifice) and nontumor body weight was reduced by 40%. To approximate this cachectic response, a 16-day period of food restriction resulted in the reduction of body weight of one control group by 33%. Immunoreactive NPY level was decreased significantly in both the plasma (4.9±0.3 vs 9.2±0.3 ng/ml) and hypothalamus (0.33±0.03 vs 1.64±0.08 ng/mg wet wt.) of TB rats as compared to ad lib.-fed rats. Food-restricted rats exhibited significant increases in NPY in both plasma (16.3±0.4 ng/ml) and hypothalamus (2.08±0.11 ng/mg). These results indicate that NPY is altered in anorectic TB rats and that this change is not secondary to the anorexia. Therefore, aberration in NPY function may be of importance in the mediation of experimental cancer anorexia. [Supported by NIH grant CA48057 and a V.A. grant to W.T.C.]

318.7

FOOD DEPRIVATION EPISODES CHANGE THE RESPONSE TO OPIATE AGONISTS AND EATING BEHAVIOR IN AN ANIMAL MODEL OF BULIMIA AND ANOREXIA NERVOSA. M.M. Hagan* and D.E. Moss, Univ. of Texas at El Paso, Texas 79968.

Fasting patterns in eating disorders may trigger changes in opiate systems that control eating (Marrazzi, M.A. and Luby, E.D., Int. J. Eat. Disord. 5: 191-208, 1986). Little is known, however, about the interaction between a history of fasting episodes (i.e., an animal model of eating disorders) and the effect of opiate agonists. Therefore, one group of female rats was deprived (DEP) to 75% of body weight three times (at 25, 90, and 140 days of age). Littermates were maintained as controls and to establish the "normal" body weight. Food intake was measured with and without butorphanol tartrate (BUTR, a kappa-sigma agonist, 8 mg/kg SC) following recovery to normal weight from the second and third episodes. DEP history produced an increase in post-recovery eating (at normal body weight and satiated at testing) ($p<0.01$). BUTR affected eating ($p<0.05$) and its effect changed with DEP ($p<0.05$). BUTR prolonged intake in the 3 h eating test ($p<0.001$). DEP rats consumed 11.3 and 6.9 g in 3 h with and without BUTR, respectively, whereas controls consumed only 5.7 g under both conditions. Fasting episodes, therefore, may be a cause of bulimia or anorexia nervosa by changing opiate sensitivity.

[BUTR was from Bristol Myers. Supported by NIMH (MBRS)]

318.8

EFFECT OF VENTRAL TEGMENTAL AREA (VTA) MORPHINE ON FEEDING. M.B. Noel and R.A. Wise. Ctr. Stud. Behav. Neurobiol., Dept. Psychol., Concordia U., Montreal, Canada.

VTA morphine injections facilitate brain stimulation reward and are rewarding in their own right; they also facilitate feeding induced by lateral hypothalamic stimulation. We determined the effects of VTA morphine on deprivation-induced feeding. Rats were implanted with stainless steel cannulae aimed at or dorsal to the VTA. Feeding behavior was determined daily after 18 hours of food deprivation; food was presented in 18 meal segments consisting of five 45-mg food pellets per segment. VTA morphine (0.1, 1.0 and 10 nmol) had no effect on the latency to initiate feeding, but caused a dose-dependent acceleration of feeding. VTA saline and morphine injected dorsal to the VTA were ineffective. VTA morphine had motivational rather than motoric effects, since its effect on speed of feeding was to reduce the response-slowing effects of satiation rather than to accelerate uniformly across trials.

318.9

PARABRACHIAL OPIOID ANTAGONISM BLOCKS STIMULATION-INDUCED FEEDING. K.D. Carr and D. Aleman*. Dept. of Psychiatry, NYU Med. Ctr., New York, NY 10016.

The pontine parabrachial area contains cell bodies of third order gustatory relay neurons (Norgren and Leonard, 1973) and a high density of mu and a moderate density of kappa opioid binding sites (Mansour et al., 1987). In the present study, bilateral parabrachial microinjection of the universal opioid antagonist, naloxone (10 ug), produced a 49.1% (±12.4) elevation in lateral hypothalamic electrical stimulation threshold for eliciting feeding behavior in rats. This differed significantly from the 12.7% (±3.7) elevation produced by microinjection of the distilled water vehicle (n=8; p<.01). Injection of the selective kappa antagonist, nor-binaltorphimine (10 ug), produced an 18.6% (±8.3) elevation which did not differ from the vehicle effect. Microinjection of naloxone and nor-binaltorphimine into extra-parabrachial pontine sites produced nonsignificant elevations of 15.8% (±9.6) and 20.9% (±5.7), respectively (n=8). Thus, injection of naloxone within the parabrachial complex elevates feeding threshold while nor-binaltorphimine does not. This effect is not likely due to naloxone's diffusion to the fourth ventricle because direct i.c.v. injection of the same dose of naloxone elevated threshold by only 9.6% (±3.9) (n=6). These findings suggest that mu opioid activity within the parabrachial area is involved in the mediation of stimulation-induced feeding. Supported by DA03956 from NIDA.

318.10

THE EFFECT OF MORPHINE ON DIET SELECTION IN RATS IS DEPENDENT UPON BASELINE DIET PREFERENCES. B.A. Gosnell, D.D. Krahn and M.J. Majchrzak. Dept. of Psychiatry, University of Michigan, Ann Arbor, MI 48109.

It has been reported that morphine increases fat intake in dietary self-selection regimes (Marks-Kaufman, *Pharmacol. Biochem. Behav.* 16:949-955, 1982). Because we have noted considerable variability among rats in their preferences for carbohydrate and fat, we reasoned that the effect of morphine on diet selection may differ in fat-preferring vs. carbohydrate-preferring rats. Male Sprague-Dawley rats (n=25) were given *ad lib* access to 2 diets: a carbohydrate diet (corn starch, dextrin and sucrose) and a fat diet (vegetable shortening and safflower oil). Both diets contained equal amounts of protein (20% of total energy), vitamins, minerals, choline chloride and fiber. After adaptation to the diets, daily intake was measured for 2 days. Percent of total calories consumed from the carbohydrate diet ranged from 5 to 98%. On the basis of these percentages, rats were divided into 3 groups: a carbohydrate-preferring group (n=8), a fat-preferring group (n=8), and an intermediate group (n=9). The effect of morphine sulfate (0, 2 and 10 mg/kg, s.c.) on intake was then tested in all rats. Each rat was tested with all doses, and all trials were 2 days apart. Both doses of morphine significantly increased 4 hr total intake in all 3 groups. There was, however, a significant group x diet x morphine interaction: morphine significantly increased intake of the fat diet only in the fat-preferring group; the 2 mg/kg dose increased carbohydrate intake in all groups, but the increase was smaller in the fat-preferring group than in the other groups. The 10 mg/kg dose increased carbohydrate intake only in the carbohydrate-preferring group. These results indicate that the effect of morphine is to increase intake of the preferred diet rather than to increase intake of a specific macronutrient. (Supported by NIDA Grant DA05471)

318.11

METHADONE AND FEEDING: SPACED VERSUS DAILY INJECTIONS. J.M. Rudski, T. Thompson*, J. Cleary*, and A.S. Levine University of Minnesota, Minneapolis, MN 55455 and VA Medical Center, Minneapolis, MN 55417.

Peripheral administration of opiates stimulates feeding. In Experiment 1, food intake was measured 1,2,4, and 6 hours following 0, 1.5, 3.0, 5.0, and 10.0 mg/kg SC methadone for three successive days. Methadone increased feeding in a dose-dependent manner, and feeding occurred earlier in the session following each subsequent injection. Both the overall increase and earlier onset of food intake might be due to the development of tolerance to the sedative properties of methadone. Tolerance may be inhibited by the spacing of opiate injections. Experiment 2 compared food intake following 0 or 5.0 mg/kg SC methadone presented daily or once every fifth day. Both schedules of methadone presentation increased food intake 4 and 6 hours post-injection, with increased intake occurring following subsequent injections. Two hours post-injection feeding was significantly increased following repeated daily but not spaced methadone presentation.

TABLE (Mean 2 Hour Food Intake in grams per injection)

	1st	2nd	3rd	4th	5th	6th	7th	8th
daily	0.2*	0.4	1.8	2.2*	3.1*	3.5*	3.8	3.3*
spaced	0.1	0.7	1.6	0.5	1.5	1.6	2.6	1.0

*p<0.05 compared to spaced

Thus, spaced injections of methadone are not as effective as daily injections in stimulating feeding, possibly due to an inhibition of tolerance to methadone's sedative effect.

318.12

INTERACTIONS BETWEEN OPIOID AND SEROTONIN RECEPTOR ANTAGONISTS UPON FOOD INTAKE IN RATS. I.W. Beczkowska and R.J. Bodnar. Dept. of Psychology, Queens Col., CUNY, Flushing, NY 11367.

Ingestive interactions between endogenous opioid and serotonergic (5HT) systems were examined by pairing systemic naloxone with antagonists of different 5HT receptor subtypes in rats deprived of food for 24 h. Naloxone (NAL: 1-5 mg/kg) decreased intake by 29-45%. The nonspecific 5HT antagonist, methysergide (1-5 mg/kg) reduced intake by 25% and augmented NAL hypophagia (20-34%). A highly selective 5HT2 antagonist, ritanserin, increased intake by 20% and reversed NAL hypophagia by 33%. A less selective 5HT2 antagonist, ketanserin decreased intake by 25% and augmented NAL hypophagia by 25%. The selective 5HT3 antagonist, ICS205930 decreased intake by 16% and dose-dependently augmented NAL hypophagia (30-70%) with higher NAL and ICS205930 doses also producing parasympathetic activation and hypoactivity. The data indicate heterogeneity among 5HT receptor subtypes in their ingestive effects upon both deprivation-induced feeding itself and NAL hypophagia. Supported by DA04194.

318.13

DECREASES IN SPONTANEOUS AND GLUCOPRIVIC FEEDING FOLLOWING SELECTIVE MU AND KAPPA OPIOID ANTAGONISTS IN RATS. D. Arjune and R.J. Bodnar. Dept. of Psychology, Queens Col., CUNY, Flushing, NY11367.

While central administration of selective mu, kappa and delta opioid agonists stimulate food intake in rats, general antagonists, like naloxone decrease intake. The present study examined the central hypophagic properties of beta-funaltrexamine (BFNA: 1-20 ug, ICV), a reversible kappa agonist and irreversible mu antagonist, and nor-binaltorphamine (Nor-BNI: 1-20 ug, ICV), a reversible kappa antagonist, under different spontaneous and regulatory challenge conditions. BFNA stimulated free feeding for up to 6 h which was blocked by Nor-BNI, but not BFNA pretreatment, indicating a kappa action. BFNA decreased free feeding at 24, 48 and 72 h, and BFNA pretreatment (24 h) decreased intake following either food deprivation (24 h) or glucoprivation with 2-deoxy-D-glucose (2DG), indicating mu-mediated actions. Nor-BNI decreased 2DG hyperphagia, nocturnal free feeding and hyperphagia stimulated by a high fat diet, indicating kappa-mediated actions in these responses. These data indicate the importance of both mu and kappa receptors in the opioid control of ingestive behavior. Supported by DA 04194.

318.14

BOMBESIN-LIKE PEPTIDE EFFECTS ON INGESTIVE BEHAVIOR AND PLASMA GLUCOSE IN DECEREBRATE RATS. F.W. Flynn, Dept. of Psychology, Univ. of Wyoming, Laramie, WY 82071

Bombesin (BN)-like peptides are implicated in the suppression of food intake. These peptides are present in mammalian brain stem nuclei that often have reciprocal connections with forebrain sites. To determine if forebrain input to brain stem nuclei is involved, sucrose intake and plasma glucose were measured in decerebrate rats following a range of doses of BN and gastrin releasing peptide (GRP). Ten min following an ip injection (saline; BN and GRP in doses of 1 µg/kg, 2.5 µg/kg, 5 µg/kg, and 10 µg/kg) intraoral sucrose (0.1 M) intake was measured in intact (N=25) and supracollicular decerebrate rats (N=18). All doses of BN suppressed intake in both groups, p's <.05. Injections of 5 µg/kg BN produced a significantly greater percent suppression of intake in decerebrate rats than in intact rats, p<.05. All doses of GRP suppressed sucrose intake by intact rats, p's<.05. In decerebrates, 1 µg/kg GRP was ineffective but higher doses produced a suppression in intake similar to that observed in intact rats. Thus, while the caudal brain stem contains the requisite neural systems to mediate the effects of BN-like peptides, forebrain systems appear to contribute to the expression of the responses elicited by these peptides. (Supported by R01-NS24879 awarded to F.W.F.)

318.15

CENTRAL EFFECTS OF VASOACTIVE INTESTINAL POLYPEPTIDE (VIP) ON FOOD INTAKE IN FASTED RATS. R. R. Schick, V. Schusdziarra*, C. Nussbaumer*, M. Classen*, 2nd Dept. of Internal Medicine, Techn. Univ. of Munich, D-8000, Germany

VIP is present in brain areas concerned with the regulation of feeding. Therefore, the aim of this study was to determine the central effects of VIP on food intake. 24-hr fasted rats (N=16) were injected (10 µl) with saline or VIP at 1, 5, 10 and 25 µg into the lateral cerebral ventricles. In some animals (N=7), the putative VIP receptor antagonist Ac-Tyr^1-D-Phe^2-GRF-(1-29)-amide (ANTAG; 25 µg) was given with or without 25 µg VIP. After injection, the rats received food pellets and latency to feed (L) and food intake after initiation of feeding (F) were recorded. L was dose-dependently augmented by VIP (4; 9; 15; 29 min) vs. saline (4 min; $p < 0.05$) and F 0-20 min was significantly reduced to 1.5 g (5 µg) or 1.4 g (25 µg) vs. 2.1 g (saline). The VIP-induced overall reduction of food consumption after 2 hours was mainly due to the robust increase in L. Peripherally injected VIP did not affect feeding behavior. Following injection of VIP + ANTAG, L was reduced to 17 min vs. 29 min (VIP alone; $p < 0.05$) and F 0-20 min was 2.2 g vs. 1.4 g (VIP alone; $p < 0.05$), thus restoring the saline value (2.1 g). These data suggest that VIP may act as a central inhibitor of food intake. However, further studies are required to determine the brain site(s) acted upon by ventricularly injected VIP. (Supported by DFG Schi 237/2-2)

318.16

CENTRAL VASOPRESSIN CAN MODULATE THE FEEDING BEHAVIOR OF THE RAT. A. BURLET, J. PERANI*, J.P. MAX*, J.P. NICOLAS* and C. BURLET *. INSERM U.308, Nancy, France.

Vasopressin (VP) has been shown to decrease food intake after peripheral injection in the goat. The selection between two contrasted diets, containing carbohydrate (80 % CHO) or protein (80 % P), was studied in the rat genetically unable to synthesize central VP (Brattleboro strain, DI). The effects of the VP infusion in this rat strain were compared to the disturbances induced by specific immunotoxins injected into the brain of Long Evans (LE) rats.

When they were fed during the dark period, DI rats consumed more CHO than LE rats whereas P intake was equal. The chronic subcutaneous infusion of dDAVP restored a normal drinking behavior but failed to modify the diet selection of DI rats. Conversely, the central infusion of AVP did not decline the water intake but significantly decreased P intake (17.07 ± 1.22 before AVP versus 17.88 ± 1.27 g/24h, $p < 0.01$) ; CHO intake was unchanged. One microinjection of a monoclonal antibody to VP (IgG2a), with ricin A and monensine into the paraventricular nuclei of LE rats disturbed the local synthesis of VP (Burlet et al., Ann. Endocr. 50 : 20N, 1989). In the same time, it increased the total food intake (10 %) of LE rats, especially during the light period (35 %). These data confirmed that the central release of AVP could play a role in the regulation of food intake.

318.17

CCK CONCENTRATION IN SPECIFIC BRAIN AREAS OF SHAM (SF) AND REAL FED (RF) RATS. M.A. Della-Fera, B. Coleman*, R.L. Gingerich* and C.A. Baile. Washington U. Sch. Med. St. Louis, MO 63104.

Feeding has been found to alter CCK concentrations ([CCK]) in specific brain areas of rats, but the site(s) of origin of the signals which trigger these changes are not known. To determine whether food stimulation of the oropharyngeal region might cause activation of CCK-containing brain areas, the effect of SF on [CCK] in specific brain areas was studied. Three groups of rats (N=12) with gastric cannulas were used. All were fasted 6 hr. One group consumed liquid diet with cannulas open (SF), one group with cannulas closed (RF), and one group consumed no food (NF). After 30 min of eating, rats were euthanized and brain tissues collected. [CCK] was measured in tissue extracts by RIA. [CCK] was increased ($p<.05$) by both SF and RF, compared to NF, in three areas: paraventricular nucleus, area postrema-nucleus of the solitary tract and dorsal parabrachial nucleus. In no area studied did either SF or RF alone cause changes in [CCK]. These findings indicate that food stimulation of the oropharyngeal region is sufficient to result in activation of CCK-containing neurons in these areas. They also suggest that CCK-containing pathways transmit gustatory information to an area of the hypothalamus which has been implicated in its centrally-mediated satiety effect. Supported by NIH grant NS-20000.

318.18

FPL 14294: A NOVEL CCK-8 AGONIST WITH POTENT INTRANASAL ANORECTIC ACTIVITY IN THE RAT. RD Simmons*, SA McCreedy*, JA Zongrone*, JD Rosamond*& JC Blosser. Depts. Biology & Chemistry, Fisons Pharmaceuticals, Rochester, NY 14603

FPL 14294 {4-(sulfooxy)phenylacetyl-[MePhe^6]CCK-6} is a CCK analog with enhanced metabolic stability as assessed using in vitro kidney protease preparations . In vitro, FPL 14294 was comparable to CCK-8 in affinity at the pancreatic CCK-A receptor (K_i=0.3 vs 0.3 nM) and at the cortical CCK-B receptor (K_i=0.3 vs 1.0 nM). Potency to contract isolated gallbladder was also equivalent to CCK-8 (ED_{50}=1.09 vs 1.05 nM), although the time to peak contraction was longer for FPL 14294 (10 vs 1.0 min). However, FPL 14294 was >50 times more potent than CCK-8 in inhibiting 30 min feeding in 21 hr fasted rats (RD_{50}=0.04 vs 2.6 µg/kg i.p.). With 3 hr feeding periods, FPL 14294 was >200 times more potent (RD_{50}=0.56 vs 101 µg/kg i.p.), suggesting a greater duration of action. FPL 14294 also possessed intranasal anorectic activity at doses as low as 5µg/kg, while CCK-8 was inactive at doses up to 500 µg/kg. Anorectic activity was inhibited by pretreatment with a CCK-A antagonist (MK-329, 100 µg/kg i.p.), but not by a CCK-B antagonist (L365,260, 300 µg/kg i.p.).

In summary, these results indicate that FPL 14294 is a highly potent, intranasally active anorectic agent whose enhanced potency over that of CCK-8 may reflect differences in stability, bioavailability or receptor kinetics, but not affinity for CCK receptors.

318.19

ENDOGENOUS CCK DELAYS GASTRIC EMPTYING IN FASTED RATS. A.J. Strohmayer, D. Greenberg, G.P. Smith and J. Gibbs. Dept. of Psychiatry, Cornell Univ. Med. Coll.: North Shore Univ. Hosp. Manhasset, NY 11030 and The New York Hosp., White Plains, NY 10605.

One proposed role for endogenous CCK is to slow gastric emptying. Green et al. (Am. J. Physiol., 1988) demonstrated that the protease inhibitor FOY-305 would slow gastric emptying of saline through the release of endogenous CCK. Previously, we (Smith et al. Am. J. Physiol., 1989) were unable to show this effect with soybean trypsin inhibitor (STI) using a phenol red dilution marker technique. We now report that STI can be shown to slow gastric emptying when a more direct technique was used.

Adult male Sprague-Dawley rats (n = 10; body weight approximately 300 g) were maintained on a liquid diet (BioServ) for 2 weeks prior to testing to insure that stomachs would be free of solids. Rats were 17h food deprived then gavaged with 5 ml 0.15M NaCl alone or STI (200 mg/rat) dissolved in 5 ml 0.15M NaCl. Ten minutes later rats were sacrificed, their stomachs were removed and weighed. Gastric contents were then expressed and measured, and the stomachs were weighed empty. The volume of gastric contents after STI (1.98 ± .33 ml) was significantly more than the volume after 0.15M NaCl (0.61 ± .24 ml; $t=3.3(9)$, $p<.02$).

Since this dose of STI releases CCK from the small intestine in the rat, these results are additional evidence that endogenous, small intestinal CCK decreases the rate of gastric emptying in the rat.

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318.20

EFFECT OF AREA POSTREMA ABLATION ON GASTRIC EMPTYING. M. Kathleen Gruver*, M. M. Heitkemper* and Nancy J. Kenney, Dept.of Psychology and Physiological Nursing, University of Washington, Seattle, WA 98195.

Vagal afferents, including those from the stomach, terminate within the area postrema and subjacent caudal-medial aspect of the nucleus of the solitary tract (AP/cmNTS). Vagal efferents arise mainly from the dorsal motor nucleus of the vagus which is interconnected with the AP/cmNTS region. This study examines the effect of AP/cmNTS ablation on gastric-emptying rate of rats.

Charcoal suspension (200 ul of a 20% solution) was injected into the fundus of the stomach of Urethane-anesthetized (1.25 g/kg) AP/cmNTS-lesioned and sham-lesioned rats 6 days after neural surgery. Two hours after charcoal injection, the suspension had traversed only 5% of the total intestinal length of the lesioned rats as compared with 26% of controls.

These data suggest that the AP/cmNTS region is involved in the control of gastric emptying. Further research is needed to determine whether decreased gastric emptying may underlie the decrease of food intake and/or the development of food aversions which also result from such ablation.

318.21

FEEDING RESPONSES TO BACTERIAL LIPOPOLYSACCHARIDE AND MURAMYL DIPEPTIDE. W. Langhans and G. Balkowski*. Institute of Animal Science, Swiss Federal Institute of Technology, 8092-Zurich, Switzerland.

Lipopolysaccharide (LPS) derived from gram negative bacteria and muramyl dipeptide (MDP), the minimal immunologically active structure of gram positive bacterial cell walls, may be involved in the anorexia during severe bacterial infection in man and animals. We have recently shown that the anorectic effects of LPS (from *E. coli)* or MDP in rats both are due to increases in meal frequency and are similarly attenuated by the antiphlogistic and antipyretic drug indomethacin. To further test whether LPS and MDP inhibit feeding through the same mechanism, rats were given MDP (1.6 mg/kg b.wt., IP) after repeated injections of LPS (3 x 100μg/kg b.wt. IP, within 5 days) or LPS after repeated injections of MDP and food intake was measured. MDP reduced food intake after repeated injections of LPS, despite the tachyphylaxis that developed to the anorectic effect of LPS. With repeated injections of MDP, a tachyphylaxis to its anorectic effect did not develop and LPS also still reduced food intake. In further experiments, a potentiation of the food intake suppression was observed after combined injection of lower doses of LPS and MDP (50μg and 0.8mg/kg b.wt., respectively), which had only a weak effect on food intake when given alone. The results indicate that LPS and MDP inhibit feeding through activation of separate mechanisms. These mechanisms, however, seem to interact.

TRAUMA: BRAIN INJURY

319.1

BEHAVIORAL CONSEQUENCES OF MODERATE AND SEVERE CORTICAL CONTUSION IN THE RAT. R.L. Sutton, L. Lescaudron and D.G. Stein. Brain Research Laboratory, Rutgers University, Newark, NJ 07102.

To evaluate a pneumatically-controlled device that allows independent control of cortical compression and impact velocity, adult, male rats were assessed for neurologic outcome following moderate (MTBI) or severe (STBI) traumatic brain injury. Rats with STBI to the right sensorimotor cortex had significant (8 d) impairments of beam-walking ability compared to MTBI rats who showed a mild, transient (2-4 d) deficit relative to sham operates. All TBI rats showed deficits in retraction of the left hindlimb after lateral displacement (at 6 h, 24 h and 8 d postinjury). STBI, but not MTBI, rats had greater L-R forepaw griptimes from a suspended 1mm wire (vs shams) at 6 and 24 h, suggesting pathologic grasp in the left forepaw after severe injury. Extent and recovery from initial deficits in tactile and chin placing with the left forelimb was found to be time and injury dependent. TBI rats placed the left forelimb after the right in tests of visual placing, in contrast to simultaneous limb placements exhibited by sham operates. These results indicate this novel device reliably induces deficits correlated with TBI severity. Supported by FIDIA and 9 RO1 NS25685-05.

319.2

BLOCKADE OF ACUTE HYPERTENSIVE RESPONSE DOES NOT PREVENT CHANGES IN BEHAVIOR OR IN CSF ACETYLCHOLINE (ACH) CONTENT FOLLOWING TRAUMATIC BRAIN INJURY (TBI). E.K. Enters, J.R. Pascua*, K.P. McDowell*, C.A. Stamford*, J.T. Povlishock, and S.E. Robinson. Dept. of Pharmacology & Toxicology and Dept. of Anatomy, Medical College of Virginia, Virginia Commonwealth University, Richmond, VA 23298.

Depletion of circulating ACh by A-5 reduces behavioral deficits and increased CSF ACh content following TBI (Brain Res. 509: 41, 1990). As hypertension has been associated with opening of the blood-brain barrier (bbb), we studied the effect of hexamethonium (H) on behavioral and CSF neurochemical changes following TBI. Male Sprague-Dawley rats (294-322 g) were surgically prepared under Equithesin anesthesia 24 h prior to injury. Fifteen min after H (30 mg/kg, i.p.) or saline, rats received fluid percussion injury (2.1-2.2 atm) while under methoxyflurane anesthesia. This dose of H had been demonstrated to block the hypertensive response to TBI. The duration of suppression of several reflexes and responses was measured up to 60 min after TBI and performance in measures of motor and vestibular function was recorded daily up to 10 days after TBI. Pretreatment with H prevented neither the acute nor the more enduring behavioral deficits observed after TBI. H did not prevent TBI-associated increases in CSF ACh content in separate groups of rats sampled 12 min following TBI. Histological inspection of rats treated with horseradish peroxidase indicated that H did not prevent TBI-induced disruption of the bbb. Thus, blockade of the hypertensive response to TBI does not afford behavioral protection nor does it prevent changes in the bbb or CSF ACh content following TBI. (Supported by NS#24413 and NS#7288).

319.3

IMMEDIATE HYPERTENSIVE RESPONSE TO GRADED FLUID PERCUSSION BRAIN INJURY IS CLOSELY CORRELATED WITH PERCUSSION MAGNITUDE AND MAY BE RELATED TO INTRACEREBRAL HEMORRHAGE AND HYPOTHALAMIC DAMAGE. X-Q. Yuan, C. E. Wade, C. B. Clifford*, G. Hanson*, M. Bozzo* and W. G. Rodkey*. Letterman Army Institute of Research, San Francisco, CA 94129

Fluid percussion brain injury is associated with an immediate rise in systemic mean arterial pressure (MAP). However, the cerebral morphological basis for this response is still not clear. Thirty-four anesthetized rats were injured through a lateral craniotomy preparation to examine the postinjury hypertensive response. The impact levels ranged from 1.3 to 3.5 atmospheres (atm), and the impact duration was kept at 25 msec. MAP was monitored for two hours following injury. Fluid percussion injury produced a remarkable increase in MAP from 93±2 to 140±4 mmHg ($p<0.001$) with an increment range of -2 to 108 mmHg (47±5 mmHg) or 0 to 144% increase. The MAP peak appeared at 15±2 seconds and then rapidly returned to the preinjury level. There was a linear relationship between impact magnitude (X, atm) and increment in MAP (Y, mmHg) (Y=28.1*X-14.0, $r=0.62$, $p<0.001$). Nineteen brains underwent histopathological examination. Histologic findings, principally hemorrhage, were graded and ranked from 1 to 19 based on severity. In ranking the lesions, hypothalamic damage was given relatively greater weight than cerebral cortical or ventricular hemorrhage. MAP rise was also related to the injury ranking ($r=0.66$, $p<0.05$), and there was a trend of correlation between impact magnitude and brain lesion score ($r=0.43$, $p=0.058$). Our study indicates that the immediate post injury hypertensive response is closely correlated with the magnitude of fluid percussion implemented and may be related to intracerebral hemorrhage and hypothalamic damage.

319.4

THE EFFECTS OF EXPERIMENTAL BRAIN INJURY ON REGIONAL CATION CONCENTRATIONS. M.J. Thomas, D. Breault, B. Nolan, D. Smith and T.K. McIntosh. Department of Surgery, Univ. of Connecticut Health Ctr., Farmington, CT 06032.

Recently it has been demonstrated that tissue cation concentrations change markedly following traumatic spinal cord and brain injury. The specific time course of cation changes following brain injury have not been elucidated. In the present study, anesthetized rats (300-350gm) were subjected to lateral fluid percussion (FP) brain injury (over the left parietal cortex) of moderate severity (2.3 - 2.5 atms). Sham-operated control and injured animals were sacrificed at 10 min., 1, 6 and 24 hrs post-injury, brains removed and dissected into injured left parietal cortex, cortex adjacent to the injury site, contralateral right parietal cortex, bilateral hippocampi and brainstem. Dissected samples were weighed, extracted and analyzed for Mg++ and Ca++ concentrations using flame atomic absorption spectrophotometry (Perkin-Elmer 403 Spectrophotometer). At 10 min. postinjury, Mg++ increased in the injured cortex ($p=0.03$), while Ca+ remained at control levels. At 1 hr postinjury, Mg++ decreased in the cortex adjacent to the injury site ($p=0.001$) and the brainstem ($p=0.002$), while Ca++ increased in the injured cortex ($p<0.0001$). Mg++ remained decreased in the injured cortex at 6 and 24 hours ($p=0.005$ and $p=0.009$,respectively). Ca++ concentrations increased in the ipsilateral hippocampus at 6 hrs ($p=0.005$) and in the contralateral hippocampus ($p=0.05$), contralateral cortex ($p=0.03$) and brainstem ($p=0.03$) by 24 hrs. These changes in ion homeostasis after injury may play a role in the pathophysiology of irreversible posttraumatic tissue damage. Supported, in part, by NIH NS26818, a VA Merit Review grant 74R and a grant from the Sunny Von Bulow Coma and Head Trauma Foundation.

319.5

EFFECTS OF CALCIUM ENTRY BLOCKER (S)-EMOPAMIL ON EXPERIMENTAL BRAIN INJURY.

K Okiyama*, DH Smith, MJ Thomas and TK McIntosh. Surg. Res. Ctr., Dept. of Surg., Univ. of Connecticut Health Center, Farmington CT 06032

Calcium may play a pivotal role in the pathogenesis of traumatic central nervous system injury. In this study, we investigated the effect of (S)-emopamil, a calcium channel blocker and serotonin-2 antagonist, on post-traumatic brain edema. Sprague-Dawley rats (330-400g, n=36) were anesthetized (sodium pentobarbital 60 mg/kg ip) and subjected to lateral fluid percussion (FP) injury of moderate severity (2.4-2.5 atm). Fifteen minutes post-injury, the animals randomly received 1 ml of either (S)-emopamil (10 mg/kg ip, n=12), (S)-emopamil (20 mg/kg ip, n=12), or saline (ip, n=12). Mean arterial blood pressure (MAP), arterial blood gasses, core body and brain (temporalis muscle) temperature were measured during a 1 hour post-injury period. At 48 hours post-injury, all animals were sacrificed, brains removed and sectioned into : injured left parietal cortex, cortex adjacent to the injury site, contralateral right parietal cortex, bilateral hippocampi and thalami. Brain water content was evaluated by the wet weight/dry weight technique. Although a transient decline in MAP was seen after injection of (S)-emopamil, there were no significant differences in arterial blood gasses or in temperature compared to the control group. FP brain injury caused significant cerebral edema in saline-treated animals 48 hours post-injury. Administration of (S)-emopamil significantly attenuated edema in both right (p=.004) and left (p=.001) thalami compared to saline-treated controls. These results suggest that (S)-emopamil might have a beneficial effect on post-traumatic brain edema. Supported, in part, by a grant from Knoll AG Pharmaceuticals.

319.6

CALCIUM ACCUMULATES FOR AT LEAST 48 HOURS FOLLOWING FLUID PERCUSSION BRAIN INJURY IN THE RAT.

I. Fineman, D.A. Hovda, T. Kawamata, A. Yoshino, and D.P. Becker. Dept. of Surg./Neurosurg., UCLA Sch. Med., Los Angeles, CA 90024, USA.

Calcium is known to accumulate following cerebral ischemia and spinal cord contusion. To determine if a similar accumulation exists following a concussive brain injury, we gave male rats (250-300 g) a 2-4 atm. fluid percussion epidurally over the left frontoparietal region. Five animals served as sham controls. Injured rats were administered a bolus injection of $^{45}Ca^{++}$ (1 uCi/g, i.v.) at 0 (n=5), 5 (n=3), 24 (n=3), and 48 h (n=3) following injury. Five hours after the injection animals were sacrificed, and 20 um frozen coronal sections were processed for autoradiography and thionin staining. Histological evaluation revealed no remarkable cellular disruption at the injury site. Digitized autoradiographs showed markedly higher concentrations of $^{45}Ca^{++}$ in the cortex and hippocampus of the injured hemisphere (200-400% of sham and injured contralateral) at all injection time points. These results suggest an elevated flux of calcium into affected regions for at least 2 days following concussion.

319.7

TREATMENTS WITH $ACTH_{4-10}$ ANALOGUE (BIM-22015) REDUCES COGNITIVE AND ANATOMICAL ALTERATIONS FOLLOWING MEDIAL FRONTAL CORTEX ABLATION.

S. W. Hoffman, R. L. Sutton, and D. G. Stein. Brain Research Lab., Rutgers University, Newark, NJ 07102.

Adult rats with bilateral medial frontal cortex lesions were given s.c. injections of either sterile water or 1, 10, 100, or 1000µg/kg of $ACTH_{4-10}$ analogue (BIM-22015) every other day for 28 days after surgery. Seven days after surgery the rats were tested on a water maze task for ten days (2 trials/day). Repeated measures ANOVA revealed a significant difference between the groups on time ($p < 0.01$) and distance ($p < 0.05$) to locate the escape platform over the 10 days of acquisition. Post-hoc analyses indicated that only the 1µg/kg dosage improved water maze acquisition as compared to lesion controls ($p < 0.05$). No significant effects of lesion or treatment were found for retention tests conducted 10 days following acquisition. Fifty-six days after surgery the rats were given one week of pretraining, followed by acquisition testing, in a T-maze test of delayed spatial alternation learning. No group differences for number of days to criterion, errors, or perseverations were found on this task. Histological analyses showed the lesions were restricted to the anterior portions of the medial frontal cortex. These overall findings are in agreement with those Silva et al. (Exp. Brain Res.,1986), who reported that rats with small medial frontal lesions lack spatial alternation deficits. An ANOVA revealed that there were significant differences between the treatment groups with respect to the number of AChE-positive neurons in the nucleus basalis magnocellularis (NBM) ($p < 0.001$). Post hoc analyses showed that rats with the 1µg/kg treatments had significantly more AChE-positive neurons in the NBM than lesion controls.

Supported by Biomeasure Inc., Hopkinton, MA., who also provided the analogue.

319.8

DSP-4 SIGNIFICANTLY RETARDS RECOVERY FROM SENSORIMOTOR CORTEX INJURY.

M.G.Boyeson and T.R.Callister*. Dept. of Rehab.Medicine,Univ.of Wisconsin, Madison,WI 53706.

Rats were trained to walk on a narrow, elevated beam. When animals reached criterion on the task, they were injected with 50 mg/kg ip of DSP-4 (a NE neurotoxin) or saline and tested on the beam every other day for 15 days, at which time both groups received a unilateral right sensorimotor cortex (SMCX) injury. Beam walking ability was assessed every other day for an additional 17 days. At 24 days postinjury, when all groups had reached recovery on the beam, they were administered ip 10 mg/kg phenoxybenzamine (PBZ; an α_1 receptor blocker). The results indicated that DSP-4 caused a transient deficit in beam walking ability and significantly retarded functional recovery after the SMCX injury compared to saline injected SMCX injured controls. Additionally, PBZ profoundly reinstated a unilateral beam walking deficit (hemiplegia) in DSP-4 treated animals compared to saline injected SMCX injured controls, indicating an upregulation of alpha receptors. The results are discussed in terms of the important role the NE system plays in behavioral recovery from cortical injury.

319.9

ADMINISTRATION OF EXCITATORY AMINO ACID ANTAGONISTS VIA MICRODIALYSIS PREVENTS THE INCREASE IN GLUCOSE UTILIZATION SEEN IMMEDIATELY FOLLOWING CONCUSSIVE BRAIN INJURY.

T.Kawamata, D.A.Hovda, A.Yoshino, Y.Katayama and D.P.Becker. Dept. Surg./Neurosurg., UCLA Sch. Med., Los Angeles, CA, 90024 USA.

In previous work we demonstrated that glucose utilization is increased immediately following a fluid percussion (FP) injury in rat using [^{14}C]-2-deoxy-D-glucose (2DG) autoradiography. In order to determine the possible reasons for this high metabolic demand, excitatory amino acid (EAA) antagonists (kynurenic acid (KYN): 10 mM, CNQX: 0.3, 1.0, 10 mM, AP5: 0.1, 1.0, 10 mM) and the calcium channel blocker cobalt (Co^{2+}: 10 mM) which prevents neurotransmitter release were perfused (5 µl/min) through a microdialysis probe positioned in the parietal cortex of rats. Following 30 min of dialysis the probe was removed, 2DG administered (i.v) and the FP injury applied. Animals who did not receive dialysis showed an increase (up to 94%) in cortical glucose utilization following injury. In contrast, this high demand for glucose was prevented in areas infiltrated with KYN, CNQX (high dose), AP5 and Co^{2+}. These results suggest that the EAAs are involved in the increase glucose utilization seen immediately after the concussive injury reflecting the energy demand of cells required to reestablish normal ionic balance disrupted by ionic shifts through EAA activated ion channels.

319.10

DYNAMIC CHANGES IN LOCAL CEREBRAL GLUCOSE UTILIXATION FOLLOWING FLUID PERCUSSION INJURY: EVIDENCE OF A HYPER- AND SUBSEQUENT HYPOMETABOLIC STATE.

A.Yoshino, D.A.Hovda, T.Kawamata, Y.Katayama and D.P.Becker. Dept. Surg./ Neurosurg., UCLA Sch. Med., Los Angeles, CA, 90024.

We have previously determined that following a fluid percussion (F-P) brain injury in rat, cells are subjected to ionic shifts across their membrane. In order to determine the extent of metabolic demand required to reestablished normal ionic balance, the present study measured local cerebral metabolic rates for glucose (lCMRGlc; µmol/100g/min) utilizing [^{14}C]-2-deoxy-D-glucose autoradiography in rats (N=72, 200-250 g) immediately, 30 min, 6 h, 1, 2, 3, 5 and 10 days following unilateral frontoparietal F-P injury (3.7-4.3 atm). Compared to sham controls injured animals showed a marked increase in lCMRGlc primarily in the ipsilateral cortex (134-153 %) and hippocampus (142-187 %) during the first few minutes following injury. Whereas by 6 h lCMRGlc in these same regions went into a state of metabolic depression (77 %) lasting for several days. We conclude that following concussion, cells must increase their energy demands in order to return to ionic equilibrium. After this short period of hypermetabolism they go into a state of metabolic depression which may reflect a period of vulnerability.

319.11

EFFECTS OF TRAUMATIC BRAIN INJURY IN RATS ON BINDING TO OPIATE RECEPTOR SUBTYPES. D.C. Perry[1], B.G. Lyeth[3], L.P. Miller[2], R.L. Getz[1], L.W. Jenkins[3,4] & R.L. Hayes[3]. [1]Dept. of Pharmacology, The George Washington University Medical Center, Washington, DC 20037, [2]Veterans Administration Medical Center, Washington, DC 20422, [3]Division of Neurosurgery & [4]Dept. of Anatomy, Medical College of Virginia, Richmond, VA 23298.

Sprague-Dawley rats were subjected to a moderate level (2.2 Atm) of traumatic brain injury (TBI) using fluid percussion. Injured animals were allowed to survive post-trauma for periods of 5 minutes, 3 hours and 24 hours. The effect of TBI on binding to forebrain opiate receptors was assessed using quantitative receptor autoradiography, and compared to a sham control group. Binding of [^{3}H]DAGO to mu receptors in neocortex was significantly lower in the 24 hour group ($p<0.05$). [^{3}H]Bremazocine binding to kappa receptors was unchanged at 5 min and 24 hour, but showed large decreases 3 hour after TBI in the CA1 pyramidal layer (65%, $p<0.05$) and dentate gyrus (43%, $p<0.05$). Delta binding (using [^{3}H]DSLET) was very low throughout the brain; no changes were detected. Lambda binding, measured by [^{3}H]naloxone, was high in hippocampus, cortex, and amygdala; no changes were detected at any time points. These data support previous suggestions of a role for endogenous opioids in TBI, and furthermore indicate that mu and kappa opioid receptor subtypes may have different functions in TBI.

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319.12

GFAP IMMUNOREACTIVITY FOLLOWING CORTICAL DEVASCULARIZATION. D.G. Herrera, and A.C. Cuello. Dept. of Pharmacology and Therapeutics. McGill University, Montreal, P.Q., H3G 1Y6, Canada.

Disruption of a restricted area of pia-arachnoid compromises vascular irrigation of the underlying cortex. This form of brain injury is accompanied by both short- and long-term effects including c-fos activation in neurons and putative neuroglia cells, and a decrease of cholinergic markers in the ipsilateral nucleus basalis magnocellularis. The latter effect can be attenuated by GM1 and NGF treatment. The present study was undertaken to examine the effects of such a brain injury on glial cells. Adult male Wistar rats with unilateral cortical devascularizing lesioning were intracardially perfused with 4% paraformaldehyde at 1, 7, 15 and 30 days post-operatively and processed for glial fibrillary acidic protein (GFAP) immunoreactivity. One day post-lesioning, reactive glia containing intense GFAP immunostaining were localized to the affected cortex and subjacent corpus callosum. At longer survival times (7 to 30 days) reactive glia were also present in ipsilateral striatum and thalamus. Administration of NGF and/or the glycosphingolipid GM1 did not appear to affect the glial reaction. Supported by M.R.C. (Canada) and Canadian Centers of Excellence Network for Neural Repair and Functional Recovery.

319.13

EFFECT OF DELAYED AMPHETAMINE TREATMENT ON PRIMARY AND SECONDARY NEUROPATHOLOGY AFTER CORTICAL CONTUSION. D.M. Feeney, R.L. Sutton, M.P. Weisend, and K.P.Eagan* Dept. of Psychology, Univ. of New Mexico, Albuquerque, NM 87131 & I.A.B. Rutgers Univ., Newark, NJ 07102.

Delayed, amphetamine (AMPH) treatment reduces the extent of cortical necrosis, morbidity and mortality produced by embolic stroke in rat (Neurosci. Abst. 13:1268, 1987) and promotes locomotor recovery in other models of brain injury. This study compared the effect of a single i.p. treatment of AMPH (2 mg/kg) or saline (SAL), given 24h postinjury, on cortical necrosis and subcortical neuronal pathology and gliosis 90 days after unilateral cortical contusion in the rat (Brain Res., 211, 1981). Focal impact (400 gm/cm) injury was centered over bregma in one group (ANT) and 2.25 mm posterior to bregma in another (POST). Reconstructions from serial, thionin sections were used to calculate the volume of cortical necrosis. Subcortical pathology and gliosis (Neurosci. Abst., 15:69, 1989) in the hippocampal CA3, ventral basal complex, dorsolateral striatum, substantia nigra, medial geniculate, arcuate and red nuclei was evaluated by raters uninformed of the treatment. Compared to SAL, AMPH significantly reduced cortical necrosis following ANT but not POST cortical contusion. No effects of AMPH on subcortical pathology were found after ANT or POST injury. The reduction of cortical necrosis by AMPH after ANT contusion injury may be due to the dense noradrenergic innervation to this region. Druq therapy for reducing neuropathology must consider the differing neurochemistry and connectivity of the lesion sites. Supported by DHHS Grant RO1 NS 20220 and #3 SO6 RR08134.

319.14

FETAL CORTICAL TRANSPLANTS PROMOTE NEURONAL SURVIVAL IN HIPPOCAMPUS OF ADULT RATS SUBJECTED TO EXPERIMENTAL FLUID PERCUSSION BRAIN INJURY. T.K. McIntosh and H. Soares. Surgical Res. Ctr., Univ of Connecticut Health Ctr, Farmington, CT 06032.

Fluid percussion (FP) traumatic brain injury induces contralateral motor deficits associated with neuronal death and cavity development in the left parietal motor cortex. Pronounced damage is also evident in the hippocampus ipsilateral to the injured cortex. In the present study, anesthetized rats (sodium pentobarbital, 60 mg/kg ip) were subjected to FP brain injury of moderate severity (2.2-2.4 atmospheres). Fetal cortical transplants (E16) were then injected into the injured motor cortex at 2 days (n=6), 1 week (n=6), 2 weeks (n=6) or 4 weeks (n=6) postinjury. Histological assessment of transplant survival and integration was based upon Nissl staining, glial fibrillary acidic protein (GFAP) immunocytochemistry and staining for acetylcholin esterase. Fetal cortical tissue injected at 2 days, 1 week or 2 weeks postinjury survived, incorporated with host brain, exhibited little GFAP immunoreactivity and successfully attenuated glial scaring. Neuronal sparing of CA2/CA3 hippocampal regions was dramatically evident in host brains receiving transplants at 2 days postinjury. Fetal cortical tissue injected into injured cortex 4 weeks postinjury failed to incorporate with host brain, exhibited extensive GFAP immunoreactivity and did not affect glial scar formation. These results suggest that there exists a temporal window in which fetal cortical transplants can attenuate glial scarring and spare neuronal populations within injured host brain. Attenuation of hippocampal neuronal death after fetal cortical transplants suggests a possible trophic role for CNS transplants in brain injury. Supported, in part, by NIH NS26818, the Sunny von Bulow Coma and Head Injury Foundation and a NIDCD training grant T32 DC00025.

319.15

MEMORY IMPAIRMENT FOLLOWING EXPERIMENTAL TRAUMATIC BRAIN INJURY IN THE RODENT. M. Ray, D. White, R.J. Hamm, R.L. Hayes. Depts. of Psychology and Neurosurgery, Virginia Commonwealth U., Med. Sch. of Va., Richmond, VA 23298.

Memory deficits are generally regarded as one of the most important sequelae of human traumatic brain injury (TBI). While cognitive function following TBI is of great importance, tests of motor performance have exclusively been used to evaluate outcome following experimental TBI. The development and validation of an animal model of cognitive function following TBI will allow the examination of putative therapeutic interventions in promoting cognitive recovery.

We used the Morris water maze, a task known to be dependent on hippocampal function, to assess memory performance following TBI. Rats were injured at moderate (n=5) or mild (n=5) levels of fluid percussion TBI. Additional rats served as uninjured control animals (n=6). Following injury or control treatment rats were assessed on weight, beam balance, and beam walking for 6 days until motor deficits were absent. Morris water maze performance was assessed on days 6-10 after injury. Results revealed that the moderately injured animals exhibited significant memory deficits over all test days [$F(2,13)= 4.49$, $p<.05$].

Previous research in our laboratory has shown that the hippocampus, a critical brain area for cognitive function in humans and rats, is selectively vulnerable after TBI. Since the Morris water maze is sensitive to hippocampal damage, our current observations support the hypothesis that memory deficits following TBI are the result of hippocampal dysfunction. Future therapeutic interventions directed at preventing hippocampal dysfunction may attenuate the occurrence of memory deficits after TBI.

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319.16

AN NMDA RECEPTOR-ASSOCIATED GLYCINE SITE ANTAGONIST ATTENUATES MEMORY LOSS AFTER EXPERIMENTAL BRAIN INJURY.

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The N-methyl-D-aspartate (NMDA) receptor has been shown to mediate hippocampal damage in central nervous system injury. Memory function has also been shown to be mediated by the NMDA receptor with the glycine site playing an integral role. In the present study, we examined the ability of the glycine site antagonist indole-2-carboxylic acid (I2CA) to attenuate post-traumatic memory deficits. Male Sprague-Dawley rats (350-400g) were trained in the Morris water maze (MWM) to find a submerged platform (20 trials over 2 days) using external visual cues. Four hours after the final trial, animals were anesthetized (sodium pentobarbital 60mg/kg ip) and subjected to lateral fluid percussion (FP) brain injury of moderate severity (2.2-2.3atm). Fifteen minutes post-injury, the animals randomly received 1ml of either I2CA (20mg/kg IV n=12), I2CA (50mg/kg IV n=12) or buffer (IV n=13). 42 hours post-injury, animals were tested for memory retention in the MWM using a video/computer recording unit. FP brain injury induces bilateral hippocampal cell loss as well as significant memory loss. Animals treated with either 20mg or 50mg I2CA showed highly significant memory preservation ($p=.02$ and $p=.009$ respectively) compared to buffer treated control animals. These results suggest that the NMDA receptor may play a role in post-traumatic memory dysfunction and that glycine antagonists may be potentially beneficial in the treatment of this memory dysfunction. Supported, in part, by NS2681-8, a VA Merit Review grant 74R and a grant from the Sunny von Bulow Coma and Head Trauma Foundation.

319.17

MEMORY DEFICITS FOLLOWING TRAUMATIC BRAIN INJURY PRODUCED BY CONTROLLED CORTICAL CONTUSION. D. White, A. Singha*, R.J. Hamm, C.E. Dixon, L.W. Jenkins, B.G. Lyeth, and R.L. Hayes. Depts. Neurosurgery, Rehab. Medicine, and Psychology, Virginia Commonwealth U., Medical College of Virginia, Richmond, VA 23298.

Memory deficits are a prominent and significant feature following human traumatic brain injury (TBI). While cognitive function following TBI is of great importance, tests of motor performance are the most common outcome measure used following experimental TBI. The development and validation of an animal model of cognitive function following TBI will allow the testing of the therapeutic efficacy of interventions designed to promote cognitive recovery following TBI.

The Morris water maze is a spatial memory task that has been shown to be dependent on hippocampal function. Therefore, we used the Morris maze to assess memory performance following contusional TBI. Rats were injured at moderate level (6 m/sec, 2.0 mm penetration, n=8) or received a sham-injury procedure (n=8). Morris water maze performance was assessed on days 11-16 after injury. Results revealed that the injured animals exhibited significant memory deficits over all test days.

The hippocampus is known to be a critical brain area for cognitive function in humans and rats. The observation of Morris maze deficits following TBI supports the hypothesis that memory deficits following TBI are the result of hippocampal dysfunction. Future therapeutic interventions directed at preventing hippocampal dysfunction may attenuate the occurrence of memory deficits after TBI.

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319.18

THE SYSTEMIC PHYSIOLOGICAL RESPONSE OF ADULT AND AGED RATS TO TRAUMATIC BRAIN INJURY. R.J. Hamm, D. White, and A. Singha*. Depts. of Psychology and Neurosurgery, Virginia Commonwealth U., Medical School of Virginia, Richmond, VA 23298.

Age has been shown to be associated with an increase in mortality and morbidity following traumatic brain injury (TBI) in humans (J. Neurosurg., 68:409-416, 1988) and rats (Soc. Neurosci. Abstr., 14:1151, 1988). The mechanisms that are responsible for the age-related increase in vulnerability to TBI are unknown. As a first step in the investigation of this problem, we anesthetized (1.5% Halothane, 70% N_2O, 30% O_2) 3-month-old Fischer 344 (adult, n=5) and 20-month-old (aged, n=4) rats. Rats were then subjected to a mild level (1.8 atm) of fluid percussion brain injury (J. Neurosurg., 67:110-119, 1987). For 60 min following injury we recorded blood gases, pH, glucose, blood pressure, heart rate, and body temperature.

Results revealed that there were no significant age-related differences in the systemic physiological response to mild TBI. As has been reported earlier (J. Neurosurg., 67:110-119, 1987), both age groups demonstrated mild hypertension for approximately 10 min following TBI. Similarly, all rats also exhibited increased plasma glucose levels at all time points following fluid percussion brain injury. These results indicate that the aged animal's higher mortality and morbidity following TBI are not the simple consequence of age-associated differences in the systemic physiological response to injury. The role of age-related changes in receptor-mediated neurotoxicity following TBI remains to be examined.

Supported by NINDS grant NS 12587

EPILEPSY: ANIMAL GENETIC MODELS

320.1

DECREASE IN SEIZURE SEVERITY IN THE GENETICALLY EPILEPSY-PRONE RAT (GEPR) FOLLOWING YOHIMBINE. M.L. Maring[1], R.W. Clough[1], P.K. Mishra[2], P.C. Jobe[2] and R.A. Browning[1]. [1]Sch. Med., So. Ill. Univ-Carbondale, IL 62901 and [2]Univ. of Ill. Coll. of Med., Peoria, IL 61656

GEPRs have an innate deficit in norepinephrine (NE) and display enhanced seizure susceptibility to a variety of seizure-evoking stimuli. While elevation in brain NE provides seizure protection and depletion produces seizure facilitation, little is known about the types of adrenoceptors involved. We have examined the effect of yohimbine, an alpha2 antagonist on audiogenic seizures (AGS) and intracerebral NE release in GEPRs. Fourteen male severe-seizure GEPRs (GEPR-9s) received either yohimbine (5mg/kg, i.p.) or vehicle one hour before AGS testing. Seizure severity was scored according to the scale of Jobe et al. (*J. Pharmacol. Exp. Ther.* **184**: 1-10, 1973). A reduction in seizure severity was observed in 13 of 14 yohimbine-treated rats, while none of these animals displayed reduced seizure severity following treatment with vehicle. Moreover, in a separate study, one GEPR-9 rat whose seizure score was reduced from 9 to 5 following a graft of fetal locus coeruleus into the third ventricle, was completely protected from AGS (score = 0) following yohimbine (5mg/kg, i.p.) treatment. Although the mechanism by which yohimbine attenuates AGS is unknown, we have found that administration of a 50mM yohimbine solution through a microdialysis probe into the thalamus of GEPR-9s produced an elevation (approximately 2-fold) of NE in the extracellular fluid as compared to basal concentration. The present findings are at variance with the seizure-facilitating effects of yohimbine observed by others using seizure models that display facial & forelimb clonic convulsions (e.g. pentylenetetrazol and kindling), but are in agreement with the anticonvulsant effect observed using a tonic seizure model (e.g. maximal electroshock). Such findings suggest that yohimbine facilitates facial and forelimb clonic convulsions (forebrain, or limbic seizures) while inhibiting tonic convulsions (brainstem seizures). Supported in part by NIH grant NS22672.

320.2

NOREPINEPHRINE-STIMULATED INOSITOL PHOSPHATE ACCUMULATION IN CORTEX, AMYGDALA/PYRIFORM CORTEX AND HIPPOCAMPUS OF GENETICALLY EPILEPSY-PRONE AND KINDLED RATS. D.L. Yourick, M. LaPlaca* and J.L. Meyerhoff. Dept. Med. Neurosci., Walter Reed Army Inst. Res., Washington, D.C. 20307-5100.

Genetically epilepsy-prone rats (GEPR-9) have a reduced number of noradrenergic nerve endings, and α_1-adrenergic receptors, in cortex, amygdala and hippocampus and reductions in noradrenergic function may also exist in kindling. GEPR-9 respond to anticonvulsant therapy with norepinephrine (NE), NE uptake inhibitors or α-adrenergic receptor agonists. It was the purpose of the present study to evaluate NE-stimulated inositol phosphate (IP) accumulation (method of Maier and Rutledge, JPET 240:729-736, 1987) in genetic and kindled models of epilepsy. NE-stimulated IP accumulation was reduced ($P < 0.05$) in cortex of GEPR-9 at 30 and 100 μM when compared to control cortex. IP_2 accumulation was also reduced at 30, 100 and 300 μM NE in the GEPR-9 when compared to epilepsy-resistant controls. Kindled rat cortical responses (28-30 days after last Stage 5 seizure) were similarly reduced when compared to unstimulated controls. No differences were noted in hippocampus or amygdala/pyriform cortex of either GEPR-9 or kindled rats when compared to their controls. These results support the hypothesis that cortical noradrenergic systems may represent endogenous anticonvulsant mechanisms and that these are impaired in epilepsy. Functional subregions of cortex are currently being examined.

320.3

DIMINISHED IN VIVO POTASSIUM (K^+)-STIMULATED GABA RELEASE IN GENETICALLY EPILEPSY-PRONE RATS (GEPR). S.M. Lasley, Q-S. Yan* and R.L. Burger*. Dept. of Basic Sci., U. of Ill. Coll. of Med., Peoria, IL 61656.

Previous studies in severe seizure GEPR (G-9) have found decreased regional brain concentrations of GABA and increased levels of aspartic acid (ASP) compared to non-epileptic controls (Lasley et al., Soc. Neurosci. Abstr. 15:1215, 1989). The present work initiated the functional assessment of these changes by the use of microdialysis. Five G-9 and 6 non-epileptic controls were implanted with guide cannulae under anesthesia, and for test sessions 7-10 days later dialysis probes were inserted unilaterally in rostral caudate. Each animal was perfused in the awake state with 100 and 150 mM K^+ for 80 min in separate counterbalanced sessions spaced 5-10 days apart. 20-min fractions were collected, derivatized and analyzed for amino acids. Solutions containing high K^+ increased dialyzable ASP and glutamic acid (GLU) by 3-7 times and GABA by 10-15 fold over the 80-min interval. At 150 mM K^+ GABA release in G-9 was significantly less than in controls ($p < 0.01$) throughout the stimulation period. In contrast, the increase of ASP and GLU in extracellular fluid after high K^+ could not be distinguished statistically between G-9 and controls. These results suggest an important role for presynaptic GABAergic mechanisms in the seizure susceptibility observed in G-9.

320.4

MANGANESE-DEPENDENT ENZYMES IN GENETICALLY EPILEPSY PRONE AND CHRONICALLY SEIZURED RATS. G.F.Carl, J.W.Critchfield,* F.Barnett,* L.Blackwell,* B.B.Gallagher, C.L.Keen*. Dept. Neurol., Med. Coll. Ga. and Med. Res. VA Med. Ctr., Augusta, GA 30910 and Dept. Nutr., Univ. Calif., Davis CA. 95616

It has been suggested that seizures cause a tissue redistribution of Manganese(Mn). It has also been suggested that a genetic predisposition to seizures may be marked by low blood Mn concentrations. It has been shown that seizures cause an increase in liver Mn levels but have no significant effect on brain Mn levels. Brain Mn, on the other hand, is lower in genetically epilepsy prone rats (GEPR) than in the parent strain but liver Mn is not different between these strains. Since Mn concentration differences are observed in both genetically prone and chronically seizured animals, it is possible that Mn levels are affected by both seizure mechanisms and genetic mechanisms. In brain, where genetic associations are seen, the primary Mn binding protein is glutamine synthase. In liver, where the seizure effects are observed, the primary Mn binding protein is arginase. To examine the relationship between tissue levels of Mn and the activities of these enzymes we compared the arginase activity in livers and the glutamine synthase activity in the brains of chronically seizured animals and found that arginase activity was higher in the seizured animals but seizures did not have a significant effect on brain glutamine synthase. We also compared the activities of these enzymes in the same tissues from GEPR and age matched parent strain controls and found that glutamine synthase activity in brain was lower in the GEPR but arginase activity in liver showed no difference. We conclude that the differences in Mn levels in the tissues of chronically seizured and GEPR animals are a reflection of the amount of Mn bound to the primary binding protein in that tissue and in liver and brain that binding is reflected in differences in the activities of arginase and glutamine synthase, respectively.

320.5

EFFECT OF 6-OHDA-INDUCED LESIONS OF THE MEDIAL FOREBRAIN BUNDLE (MFB) ON AUDIOGENIC SEIZURES IN GENETICALLY EPILEPSY-PRONE RATS (GEPR-3s). C. Wang*, P.C. Iobe and R. A. Browning. Southern IL Univ. Sch. Med., Carbondale, IL 62901, and Univ. IL. Coll. Med., Peoria, IL 61656.

Widespread depletion of CNS norepinephrine (NE) produced by infusion of 6-hydroxydopamine (6-OHDA) into the *locus coeruleus* (LC) has been shown to increase the severity of audiogenic seizures (AGS) in moderate seizure GEPRs (GEPR-3s) (Browning *et al., Epilepsia* 30: 651, 1989). The present study was designed to determine whether depletions of NE restricted to the forebrain could alter the severity of AGS. GEPR-3s (which display clonic convulsions only) were infused with either 6-OHDA (4 µg/2 µl) or vehicle in the MFB bilaterally. Ten to 14 days after 6-OHDA treatment rats were subjected to the AGS test and seizures were scored according to the scale of Jobe *et al.* (*J. Pharmacol. Exp. Ther.* 184:1-10, 1973). Subsequently, brains were assayed for NE. No increase in seizure severity was observed in the treated group (i.e. no increase in the incidence of tonic seizures) despite significant forebrain NE depletion (expressed as % of control: cortex, 26%; hippocampus, 21%; amygdala, 33%; thalamus, 54%, and hypothalamus, 47%). No significant depletion of brainstem or spinal cord NE was observed. Of interest, 3 of 10 GEPRs depleted of forebrain NE displayed facial and forelimb clonus with rearing (prior to generalized clonus) in response to audiogenic stimulation, whereas no rats in the vehicle group exhibited facial and forelimb clonus. These findings suggest that depletion of forebrain NE is not responsible for the increase in seizure severity following LC lesions and are consistent with the hypothesis that brainstem NE is more important in regulating AGS severity.

320.6

EVIDENCE OF TRANSIENT NEONATAL HYPOTHYROIDISM IN THE MODERATE SEIZURE GENETICALLY EPILEPSY-PRONE (GEPR-3) RAT. **S Razani-Boroujerdi and DD Savage, Department of Pharmacology, University of New Mexico School of Medicine, Albuquerque, NM, 87131.**

Previous studies from our laboratory have shown that severe seizure Genetically-Epilepsy Prone (GEPR-9) rats exhibit a moderate degree of hypothyroidism throughout life. Among the consequences of neonatal hypothyroidism (NH) in rats is the development of audiogenic seizure susceptibility (AGS). Given the morphological and neurobiological similarities among the moderate seizure GEPR-3, GEPR-9 and experimentally-induced NH rats, we hypothesized that GEPR-3 rats are functionally hypothyroid.

Serum thyroxine (T4), triiodothyronine (T3) and thyrotropin (TSH) levels were measured in GEPR-3 and euthyroid non-epileptic Sprague-Dawley control rats at eleven different ages from day 5 up to one year. Like GEPR-9 rats, GEPR-3 rats had decreased serum total and free T4 levels and elevated TSH levels from day 5 through day 22 of age. However, unlike GEPR-9 rats, GEPR-3 rats had increased T4 levels and persistently high TSH levels as adults and normal serum T3 levels throughout life. The GEPR-3 rat thyroid hormone data suggest that: 1) the thyroid gland is not responding appropriately to elevations in TSH during the neonatal period, 2) feedback regulation of TSH production by T4 is impaired and 3) feedback regulation of peripheral 5'-monodeiodinase activity is intact, since normal serum T3 levels are maintained irrespective of low or high serum T4 levels.

These data indicate that GEPR-3 rats experience a transient T4 deficit during the neonatal period. The critical impact of neonatal T4 deficiency on brain function coupled with the development of AGS by GEPR-3 rats during the neonatal period suggests that neonatal T4 deficiency may be one contributing factor in the development of the seizure-prone state in GEPR-3 rats. Further, the differences in GEPR-3 and GEPR-9 rat thyroid hormone status after 22 days of age provide one explanation for differences in weight gain, serum growth hormone and audiogenic seizure responses between GEPR-3 and GEPR-9 rats after 22 days of age.

320.7

THE WAG/RIJ STRAIN: A RAT MODEL FOR ABSENCE EPILEPSY WITH PREDICTIVE VALIDITY. E.L.J.M. van Luijtelaar*, J.M.H. Vossen*, A.A. Ellenbroek and A.M.L. Coenen*, Dept. of Psychol., [1]Dept. of Pharmacol., Nijmegen Univ., P.O. Box 9104, 6500 HE Nijmegen The Netherlands.

During the last years genetic animal models in which the seizures are not induced by sensory stimulation have become the focus of interest. It appeared that several types of rats, e.g. the WAG/Rij strain, exhibit spontaneous EEG and behavioral manifestations: chronically recurring 7-10 Hz spike-wave discharges (SWD's) accompanied by accelerated breathing and facial myoclonic movements closely resembling features of human absences. In addition, there is agreement between the states of vigilance in which the SWD's in man and rat occur, mainly at unstable vigilance periods. Also similar are the effects of the anticonvulsant actions of ethosuximide and trimethadion and of the proconvulsants carbamazepine and hydantoin, the involvement of GABA-ergic and glutaminergic agents and the dominant mode of inheritance. A final and most important characteristic of absence epilepsy in man are the cognitive impairments during the presence of SWD's. Until now, cognitive impairments during SWD's have not been described in this model. The length of the post-reinforcement pause in a fixed interval (FI) task was used as an index for the accuracy of estimation of a time interval.

Eleven rats, previously trained on a FI 60 schedule of reinforcement, were provided with chronic EEG electrodes. During five sessions and in addition pre- and post training, the EEG was continuously recorded. It was found that during trials with SWD's, the post-reinforcement pause was enhanced compared to trials without SWD's suggesting that the capability of the animals to estimate a time period was disrupted. Furthermore, there were less SWD's during the task compared to the preceding and following rest hour. Based on these results, it was predicted that children with absence epilepsy should show similar impairments on FI responding. Under continuous EEG registration a FI task of one hour was presented to five children with absences. It appeared that after short (<3 sec.) SWD's the post-reinforcement pause was longer compared to trials without aberrant EEG signs of epilepsy. Considering the similarity of the influence of SWD's on FI responding between rat and man it is concluded that the proposed animal model has predictive validity.

320.8

SEIZURE SUSCEPTIBILITY IN MICE GENETICALLY SELECTED TO BE ETHANOL WITHDRAWAL SEIZURE-PRONE (WSP) OR -RESISTANT (WSR). J.C. Crabbe, T.J. Phillips, J.K. Belknap, and C.D. Merrill*. VA Med. Ctr. and Dept. Med. Psychol., Ore. Hlth. Sci. U., Portland, OR 97201

WSP mice have severe, and WSR mice mild, handling induced convulsions (HIC) after withdrawal from chronic ethanol (EtOH). Previous studies had found no differences between WSP and WSR mice in threshold sensitivity to seizures elicited by a number of convulsants. We examined the effects of low doses of several convulsants on basal HIC, reasoning that this would be a more sensitive assay. All convulsant drugs exacerbated HIC in WSP mice, but only three affected WSR mice. These were picrotoxin, pentylenetetrazole, and TBPS, agonists at the picrotoxin site of the GABA-gated chloride ion channel. Thus, the GABA system does not modulate the genetic difference between WSP and WSR mice. Acute EtOH injection is followed by elevated HIC 8-10 hr later in WSP mice, a state of acute withdrawal. NMDA had no effect on HIC in naive WSP mice, but exacerbated acute ethanol withdrawal HIC. NMDA had no effects in WSR mice, and kainic acid exacerbated both basal and acute withdrawal HIC in WSP mice. Thus, NMDA receptor function may underlie the EtOH HIC severity differences between WSP and WSR mice. Studies supported by Grants AA05828, AA06243, AA06498, and DA05228.

320.9

SINGLE UNIT ACTIVITY IN THE INFERIOR COLLICULUS (IC) OF RATS DURING ETHANOL (ET) INTOXICATION AND WITHDRAWAL ASSOCIATED WITH AUDIOGENIC SEIZURES (AGS). **A.Riaz and C.L.Faingold. Dept. Pharmacol. So. IL. Univ. Sch. Med. Springfield, IL.62794.**

Normal rats undergoing ET withdrawal (ETX) become susceptible to AGS. The IC plays a crucial role in initiation in many AGS models. The present study examined if changes in IC neuronal responses occur during ET administration and ETX. Under anesthesia microwire electrodes were implanted into IC of normal rats. To produce dependence, ET was given intragastrically (9-15g/kg) daily and was withdrawn at the end of day 4. Single-unit responses and behavior to AGS-inducing stimulus (12 kHz tone) were recorded in control, during ethanol treatment (ET+) and during ETX. At high stimulus intensities, which precipitate AGS, the rate-intensity functions of evoked unit firing in 61% of IC neurons (N=13) in the ET+ group showed a plateau or a decrease. All of the ETX group showed increased IC neuronal firing. A significant decrease in evoked unit firing occurred in the ET+ group as compared to control at highest but not at lower intensities. The evoked unit firing in ETX was significantly greater than control at all intensities. Spontaneous unit firing was decreased in ET+ (23.6% of control) and greatly increased (552.5% of control) during ETX. GABA is implicated in the effects of ET and in acoustically-evoked inhibition in normal rats. Evidence supporting a reduced efficacy of GABA-mediated inhibition in the IC has been observed in genetically epilepsy-prone rats (GEPRs) that exhibit AGS. Microinjections of GABAergic agents into IC block AGS during ethanol withdrawal. CONCLUSION: In light of previous findings the response pattern alterations observed in the present study might be mediated through ET-induced increases in GABAergic transmission during intoxication and rebound hyper-excitability involving reduced GABAergic function during ETX. This suggests that decreased GABA-mediated inhibition in IC could be a common factor in AGS initiation both in GEPRs and during ETX in normal rats.

320.10

KANAMYCIN-INDUCED AUDIOGENIC SEIZURE (AGS) SUSCEPTIBILITY REQUIRES MONOAMINE DEPLETION IN SPRAGUE-DAWLEY (SD) RATS. **C.E. Reigel and W.M. Aldrich*. Dept. of Basic Sci., Univ. of Ill. Col. of Med. Peoria, IL 61656.**

Numerous studies in adult and developing GEPRs suggest AGS susceptibility is due to a combination of developmental hearing impairment and deficits in central noradrenergic and serotonergic inhibitory function. The necessity of both hearing impairment and monoaminergic deficits for the expression of AGS is supported by a recent observation of endogenously hearing impaired SD rats becoming AGS susceptible following monoamine (MA) depletion [Reigel et al. FASEB J. 2: A1069]. This report is in contrast to that of Pierson and Swann [Hearing Res. 32: 1-10, 1988] in which a kanamycin-induced hearing loss alone was capable of inducing a 100 percent incidence of AGS in Wistar rats. The purpose of the present study was to determine if it is possible to induce AGS susceptibility in SD rats through the production of a kanamycin-induced hearing loss alone or if monoaminergic depletion was required in addition to the kanamycin-induced hearing loss (as hypothesized to be the case in the GEPR). SD pups received 100 mg/kg/day kanamycin monosulfate or saline, given in one daily IP injection on postnatal days 9-12, the optimal parameters of the Wistar study. Kanamycin and saline groups were split, with half of each group receiving a SC injection of 10 mg/kg Ro4-1284 or saline at 30 days of age. Forty-five minutes following this injection, the time of maximal Ro4-1284 induced MA depletion, each subject was tested for AGS susceptibility. Kanamycin treatment alone or MA depletion alone (Ro4-1284) failed to induce AGS. The combination of kanamycin on postpartum days 9-12 and MA depletion at the age of AGS testing induced a 66.7 percent incidence of AGS. These results demonstrate that both hearing impairment and monoaminergic deficits are necessary for the induction of AGS susceptibility in SD rats.

320.11

A QUANTITATIVE COMPARISON OF THE LATERAL CEREBELLUM BETWEEN NORMAL AND GENETICALLY EPILEPTIC MICE (tg/tg and tg/la). K. Isaacs and L. Abbott. Neuroscience Program and Dept. of Vet. BioSci. U. of Illinois, Urbana, IL 61801.

The lateral folia (L. paramedianus) of the posterior cerebellum of the mutant mouse tottering (tg/tg, n=3, 115 days old) and compound heterozygous mutants (tg/la, n=3, 137 days old) were compared to age-matched control mice (+/+, n=5) for changes in size of Purkinje cell somas and nuclei, and molecular layer thickness. tg/tg mice first exhibit signs of neurological disorder at 3-4 weeks, beginning with an ataxic gait followed by development of stereospecific, intermittent seizure activity. tg/la mice develop the same symptoms, but at an accelerated rate and with more severity and longer seizure duration. Semi-thin plastic sections were sectioned and stained with toluidine blue. Somas in tg/tg and tg/la mice were 8% and 9% smaller in diameter than controls ($p<0.02$). tg/tg nuclei were the same size as control mice, but tg/la were 8.6% smaller than tg/tg mice ($p<0.01$). The molecular layer was drawn with a camera lucida at 20x and perpendicular lines were made from the pia to the middle of the Purkinje cell layer. When the tg/tg and tg/la mice were compared to wild type mice, there was a noticeable 15% drop in thickness of the molecular layer ($p<0.03$).

320.12

GENETIC DIFFERENCES IN NEOCORTICAL HIGH VOLTAGE SPIKE AND WAVE SPINDLE (HVS) PATTERNS: PARALLEL VARIATIONS IN BRAIN DOPAMINE (DA) SYSTEMS. I.Laszlovszky, G.Buzsaki[1], A.Lajtha, C.Vadasz. Nathan S. Kline Inst., Orangeburg, NY 10962 & [1]Dept. Neurosci. Univ. California at San Diego, La Jolla, CA 92093

Neocortical HVS episodes in the rat are similar to EEG patterns characteristic of the human petit mal epilepsy. Thus, neocortical HVS in the rat is regarded as an animal model for the latter. Recent EEG studies (Buzsaki, G. et al., Neurosci., in press, 1990) demonstrated age- and DA antagonist-dependent differences in the expression of HVS episodes. Here we report genetically based associations between brain DA systems and the expression of cortical HVS in the Fischer 344 (F344) and Buffalo (BUF) inbred rat strains. Activity of tyrosine hydroxylase (TH) was 15%-59% higher in the F344 strain in the corpus striatum (CS), tuberculum olfactorium (TUO), substantia nigra A8-A10 area and hypothalamus (HT), but not in pons-medulla. The 3H-spiperone receptor-binding studies evinced significantly lower (17%) D-2 DA receptor binding in CS and TUO of the BUF strain. Moreover, marked sexual dimorphism (with 50% higher TH activity in females) was found in the HT, in contrast to other brain regions. The findings that the HVS phenotype is expressed far more frequently in F344 than in the BUF and that there is a several-fold increase in the incidence of HVS after DA antagonist treatment in F344, but not in BUF, suggest that HVS in rats is under genetic control and that the genetic variations in the DA system might significantly affect the expression of HVS.

320.13

ALTERATIONS IN CENTRAL BENZODIAZEPINE RECEPTOR SUBTYPES IN THE CEREBRAL CORTEX OF THE MUTANT MOUSE TOTTERING. E.M. Barnes, Jr. and M.H. Jalilian Tehrani. Baylor Col. of Med., Houston, TX 77030.

The single locus mutant mouse tottering (tg/tg) displays spontaneous cortical discharges which resemble those found in petit mal epilepsy. In order to study possible alterations in GABAergic transmission in tg/tg mice, we measured the clonazepam-displaceable binding of [^{3}H]flunitrazepam to GABA/benzodiazepine receptors on cortical membranes. Scatchard analysis of [^{3}H]flu binding to membranes from tg/tg and co-isogenic controls (+/+) revealed a single binding site with similar densities but different affinities (tg/tg, Kd = 1.8 nM; +/+, Kd = 4.7 nM). The pharmacological profile of benzodiazepine receptors was determined by displacement of [^{3}H]flu by clonazepam, CL 218872, and ethyl β-carboline-3-carboxylate (β-CCE). Clonazepam competition produced Hill numbers near unity for +/+ and tg/tg, while the Hill values for CL 218872 and β-CCE were below one in both strains, suggesting receptor multiplicity. The net IC_{50} values for CL 218872 were 170 nM in +/+ and 530 nM in tg/tg cortex. The displacement curves indicate that nearly equal numbers of type I and type II benzodiazepine receptors are present in +/+, while tg/tg cortex has a higher proportion of type II receptors. The results suggest that the tottering mutation leads to a switching of GABA/benzodiazepine receptor isoforms. (Supported by NIH grant NS 11535.)

EPILEPSY: ANTI-CONVULSANT DRUGS

321.1

BLOCKADE OF 4-AP-INDUCED AFTERDISCHARGES BY U-54494A, A NOVEL ANTICONVULSANT, IN THE HIPPOCAMPAL SLICE OF RAT. M.Camacho-Ochoa, P.F. VonVoigtlander, W.E.Hoffmann and M.F.Piercey. CNS Diseases Research, The Upjohn Company, Kalamazoo, MI 49001.

U-54494A, a 1,2-diamine structurally related to the opiate kappa receptor agonist U-50488H, antagonized convulsions induced by excitatory amino acid agonists (EAAA: kainic, N-methyl-aspartic, and quisqualic acids) and the Ca^{++} channel agonist BAY K 8644 (*J. Pharmacol. Exp. Ther.* 243:542, 1987) and antagonized glutamate excitation of cortical neurons (*Soc. Neurosci. Abst.* 14:1034, 1988). The anticonvulsant activity of U-54494A was studied on a 4-aminopyridine (4-AP) epilepsy model using extracellular recordings in *in vitro* hippocampal slices in the rat brain. Field potentials were evoked by stimulation of Schaffer collaterals and recorded from the CA1 region of the hippocampus after perfusion of 4-AP in the absence and presence of U-54494A. The number of afterdischarges was significantly decreased by increasing doses of U-54494A. In contrast, U-54494A did not significantly change the latency, duration, and area of the evoked PS, either in the presence or absence of 4-AP. Thus, by itself, U-54494A does not affect normal neuronal transmission in the hippocampal slice. This effect is in agreement with the results seen *in vivo* in the rat hippocampus where only mild depression of firing rates of pyramidal cells was observed, and *in vitro* where no effect on axonal conduction was found for U-54494A on an isolated sciatic nerve preparation (*Soc. Neurosci. Abst.* 14:1034, 1988). By contrast, U-54494A effects are observed on altered physiological systems such as the *in vivo* models of epilepsy (excitatory amino acids and Ca^{++} agonist epilepsy models). These results provide more evidence that U-54494A is an effective anticonvulsant that may be useful in the treatment of paroxysmal neuronal activity, without having generalized depressant effects.

321.2

THE NEUROPROTECTIVE ANTICONVULSANT U-54494A BLOCKS KAINIC ACID INDUCED GLUTAMATE RELEASE. M. M. Payson, B. A. Donzanti and P. F. VonVoigtlander, NEOUCOM, Rootstown, OH 44272 and The Upjohn Company, Kalamazoo, MI 49001.

U-54494A, an analogue of the kappa agonist U-50488H, is a unique anticonvulsant in that it lacks the sedative, analgesic, and diuretic effects typical of selective kappa opioid agonists (VonVoigtlander et al, J. Pharmacol. Exp. Ther. 243:542, 1987). In an attempt to understand its mechanism of action, U-54494A was tested *in vivo* for its ability to alter spontaneous and evoked glutamate release from the CA_3 region of the rat hippocampus. Studies were performed in the urethane anesthetized animal by placing a 0.5 mm exposed dialysis membrane (210 μ o.d.) into the left hippocampal CA_3 region and perfusing the probe with artificial CSF at a flow rate of 1.5 μl/min. Twenty minute dialysate samples were collected and analyzed for glutamate content using HPLC-ECD following precolumn derivatization with o-pthalaldehyde and B-mercaptoethanol. U-54494A (50 mg/kg, s.c.) did not alter spontaneous glutamate efflux. Similarly, high K^+ (80 mM) perfusion evoked glutamate release was not altered following a 1 hr pretreatment with U-54494A. In contrast, kainic acid (3 mM) perfusion-induced glutamate release was significantly inhibited by 1 hr pretreatment with U-54494A (1-15 mg/kg, s.c.). These data suggest that U-54494A selectively inhibits kainic acid-induced glutamate release at non-sedating, anticonvulsant doses. Furthermore, these data may be mechanistically related to previous studies which showed U-54494A to protect against kainic acid-induced convulsions and CA_3 neurotoxicity.

321.3

EFFECTS OF INTRANIGRAL INFUSION OF AP7 AND KETAMINE ON PENTYLENETETRAZOLE SEIZURES IN ADULT AND IMMATURE RATS. John ND Wurpel and Luisa M Milevoj*. Department of Pharmaceutical Sciences, St. John's University, Jamaica, NY 11439

AP7 and ketamine are reported to suppress seizures induced by a variety of models, our data support the hypothesis that the anticonvulsant effects of AP7 and ketamine may be mediated, in part, by the substantia nigra (SN). Further, these effects appear to be age-related. Animals were anesthetized with a mixture of ketamine and xylazine, and bilateral guide cannulae were implanted into the SN of adult & immature (16 day old) rats. After 2 days recovery, rats received AP7, ketamine or saline in a volume of 0.2 ul infused into both SN. AP7 (22.5-2250pg) and ketamine (25-250ng) reduced the incidence of pentylenetetrazole (PTZ; 80mg/kg, s.c.)-induced seizures of adult rats in a dose-dependent manner, without reducing seizure severity of those animals which demonstrated a clonic convulsion. AP7 infused into the SN of immature rats did not reduce the incidence of PTZ-induced seizures, while ketamine infusions into the SN increased the occurrence of tonic seizures following PTZ.

Both AP7 and ketamine appear to exert an age-specific effect on seizure activity when infused into the SN. AP7 or ketamine are anticonvulsant when infused into the SN of adult rats whereas, AP7 or ketamine do not exert anticonvulsant effects when infused into the SN of immature rats.

321.4

SELECTIVE ANTICONVULSANT PROPERTIES OF GLYCINE RECEPTOR ANTAGONIST, 7-CHLORO-KYNURENIC ACID (7CK). S. Hoshino, D.W. Bonhaus and J.O. McNamara. Duke and V.A. Med. Ctr., Durham, N.C. 27705

NMDA receptor antagonists inhibit seizures in a diversity of models. Activation of NMDA receptors requires the presence of agonists for a glycine binding site. We therefore hypothesized that glycine antagonists would exhibit anticonvulsant properties. To test this idea, we examined the effects of glycine antagonists in two seizure models, electroshock (ES) and electrically-induced hippocampal afterdischarge (AD). Injection of 7CK (3.35 μg or 10.06 μg in 5 μl saline) into the right lateral ventricle (ICV) produced ataxia and sedation reversible with D- but not L-serine. Surprisingly, ICV 7CK failed to reduce the duration of ES evoked tonic hindlimb extension (THE) (Vehicle 12.5±1.3, 3.35 μg 13.4±1.4, 10.06 μg 10.1±1.4 sec). ICV injection a second glycine receptor antagonist, 5F-indole-2-carboxylic acid (179 or 537 μg in 5 μl), also failed to inhibit ES whereas ICV injection of the uncompetitive NMDA antagonist, MK-801 (45 μg in 5 μl) abolished THE. The effects of ICV 7CK on AD and wet dog shakes (WDS) induced by left hippocampal stimulation were also examined. 7CK reduced the duration of recurrent discharge (RD) in the right hippocampus (Vehicle 23.1±2.9, 7CK 7.3±4.6 sec; $p<0.02$) and the number of WDS (Vehicle 17.6±2.1, 7CK 7.4±2.8; $p<0.03$), but not the duration of AD (Vehicle 41.7±6.3, 7CK 39.3±9.7 sec). Thus ICV injection of 7CK produced behavioral evidence of glycine receptor antagonism and inhibited hippocampal epileptiform activity but not ES seizures. The effects of 7CK on hippocampal RD and WDS suggest that glycine receptor antagonists may provide a new class of anticonvulsants. The lack of effect against ES seizures may be due to inadequate access of 7CK to the responsible pathways. Alternatively, the effectiveness of MK-801 but not 7CK raises the interesting possibility that not all NMDA receptors are coupled to glycine receptors.

321.5

D-SERINE POTENTIATES PHENYTOIN, PHENOBARBITAL, DIAZEPAM AND CARBAMAZEPINE IN MAXIMAL ELECTROSHOCK SEIZURES OF RATS. S.L. Peterson Dept. of Medical Pharmacology and Toxicology, Texas A & M University, College Station, Texas 77843.

Previous studies in this laboratory have shown glycine to potentiate the activity of anticonvulsant drugs in maximal electroshock seizures (MES). The purpose of this study was to determine if D-serine, an agonist of N-methyl-D-aspartic acid linked glycine receptors, also potentiates anticonvulsant drugs in MES.

MES was induced in male Wistar rats by passing a 60 Hz, 150 mA and 0.2 second duration current through saline soaked corneal electrodes. Seizure severity was quantified by tonic hindlimb extension. Statistical significance between groups of anticonvulsant treated rats with or without D-serine were determined by maximum likelihood logistic regression analysis.

D-serine (20 mM/kg,p.o.) administered 1, 2 or 4 hours before the seizure test did not prevent tonic hindlimb extension in any rat tested. However, 20 mM/kg D-serine (4 hours before seizure test) significantly enhanced the anticonvulsant effect induced by 20 mg/kg phenytoin, 7.5 mg/kg phenobarbital, 7.5 mg/kg diazepam and 7.5 mg/kg carbamazepine. As D-serine is metabolically inactive, this data suggests a select action of glycine agonists to potentiate anticonvulsant drugs in MES. (Supported by NIH Grant 24566)

321.6

THE ANTICONVULSANT ACTION OF GABAPENTIN INVOLVES THE GLYCINE/NMDA RECEPTOR.
R J Oles*, L Singh*, JHughes and G N Woodruff
Parke-Davis Research Unit, Cambridge, England

Recent clinical trials have shown that gabapentin is effective in medically refractory patients with partial or generalised epilepsy. However, its mechanism of action is unknown. We have examined the involvement of the glycine/NMDA receptor.

Intraperitoneal administration of gabapentin or the competitive NMDA receptor antagonist CPP in mice, 30 min before pentylenetetrazol (120mg/kg, s.c.) antagonised tonice seizures with ED_{50} values of 139.3 and 7.9mg/kg respectively. The anticonvulsant effect of a submaximal dose of gabapentin (200mg/kg) was dose-dependently antagonised by i.c.v. administration of the glycine receptor agonist, D-serine (30μg completely antagonised the effect of gabapentin). In contrast, up to 30μg of D-serine, did not antagonise a similar anticonvulsant effect of a submaximal dose of CPP (10mg/kg).

These results may suggest an involvement of the glycine/NMDA receptor in the anti-convulsant action of gabapentin.

321.7

MK-801 AND MK-801+DIAZEPAM IN STATUS EPILEPTICUS: MOTOR SEIZURES, EEG AND CORTICAL GABA CONCENTRATION. N.Y. Walton, S. Gunawan* and D.M. Treiman. Neurology and Research Services, Wadsworth VAMC and Department of Neurology, UCLA School of Medicine, Los Angeles, CA 90024.

Status epilepticus (SE) induced by lithium and pilocarpine produces a progressive increase in cortical GABA concentrations which are first apparent at the time continuous, high-amplitude, rapid spiking is established on EEG (Exp. Neurol. 1990, 108:61-70). In this study SE induced by lithium and pilocarpine was treated with one of the following regimens when continuous spiking had been present on EEG for 10 minutes: 2 mg/kg MK-801; an equal volume of vehicle only; or 2 mg/kg MK-801 followed in 10 minutes by 20 mg/kg diazepam (DZP).

MK-801 alone stopped overt motor seizures but not electrographic seizure activity. MK-801+DZP stopped all manifestations of SE within 5 minutes of DZP injection. Cortical GABA concentrations were less elevated 30 minutes after treatment in the rats receiving MK-801+DZP than either of the other treatments. By two hours after treatment, GABA levels were lower in rats treated with MK-801 alone than vehicle-treated rats, and lower still in MK-801+DZP rats.

321.8

DEXTROMETHORPHAN(DM) AND CARBETAPENTANE (CBP) ANALOGS BIND TO DM AND SIGMA SITES IN THE GUINEA PIG BRAIN. M. Klein*, J.M. Musacchio, A.H. Newman[1], S.N. Calderon*[1] and F.C. Tortella[1]. Dept. Pharmacol., NYU Med. Ctr., New York, NY, 10016 and [1]Walter Reed Army Inst. of Res., Washington D.C. 20307-5100.

DM and (+)-3-PPP bind to a common high-affinity site that is allosterically modulated by phenytoin (PHT) and ropizine (Musacchio et al., Mol. Pharmacol. 35:1, 1989). DM, CBP and caramiphen, which displace [^{3}H]DM in the nM range, protect rats and mice against MES seizures and potentiate the effect of PHT. However, several sigma ligands, which bind to the common DM/sigma site with nM affinities have no anticonvulsant activity (Tortella et al., TIPS 10:501, 1989). Since DM binds to an additional high- and to a low-affinity site, for which (+)-3-PPP has respectively low and intermediate affinity, it is not clear which site mediates the anticonvulsant activity. To elucidate this question, several DM- and CBP- analogs with/without anticonvulsant activity were tested in competition studies against [^{3}H]DM and (+)-[^{3}H]3-PPP. Most analogs tested bind to the common high-affinity DM/(+)-3-PPP site with K_i values from 2 to 500 nM. Several CBP analogs bind also to the low-affinity DM/medium-affinity (+)-3-PPP site. Preliminary results have not shown a clear correlation between binding and anticonvulsant activity. Supported in part by USPHS grants DA-02013, MH-17785 and NS-23926.

321.9

SYNTHESIS AND ANTICONVULSANT ACTIVITY OF DEXTROMETHORPHAN AND CARBETAPENTANE ANALOGS. S.N. Calderon[1]*, A.H.Newman[1], M. Klein[2]*, J.M.Musacchio[2], F.C. Tortella[1]. [1]Walter Reed Army Institute of Research, Washington D.C., 20307-5100 [2]NYU Medical Center, NY, NY, 10016.

The non-opioid cough suppressants, dextromethorphan (DM) and carbetapentane (CBP), show anticonvulsant activity. The mechanism of anticonvulsant action of these drugs is not known. High and low affinity binding sites labeled with [^{3}H]DM have been described for DM and CBP in brain, leading to the speculation that the anticonvulsant action of these drugs may be mediated at DM binding sites. Analogs of each of these parent compounds have been synthesized to determine structural requirements for anticonvulsant activity as well as to attempt to correlate anticonvulsant action with receptor binding affinity. To eliminate the possibilty of metabolism to dextrorphan, the primary metabolite of DM, which is anticonvulsant but also produces undesirable behavioral side effects, a series of analogs with bioisosteric replacement of the 3-position methoxy group were prepared. Chemical modification at the ester function in CBP was explored to determine whether this group was necessary for anticonvulsant activity and to determine how these changes effected receptor binding. Preliminary testing in the supramaximal electroshock test in rats showed at least one compound in each series to be anticonvulsant.

321.10

ANTICONVULSANT PROPERTIES OF DIFFERENT CLASSES OF Ca^{++}-CHANNEL ANTAGONISTS ON AMYGDALA KINDLED SEIZURES. CM Mack ME GILBERT & DB PEELE, NSI Technology Services, RTP, NC, and N.C. State Univ., Raleigh, NC.

N-methyl-D-aspartate (NMDA) channel activation permits an influx of extracellular Ca^{++} and can produce epileptiform activity. NMDA antagonists protect against seizures induced by electrical kindling of the amygdala in rats. However, other voltage sensitive Ca^{++}-channels exist and may also contribute to seizure induction. The anticonvulsant efficacy of drugs from 3 classes of Ca^{++}-channel antagonists (phenylalkylamines, diphenylalkylamines, and dihydropyridines) was assessed in the kindled amygdala model. Male rats (n=12) were kindled to stage 5 seizures (GS), then a threshold intensity required to evoke a GS was determined. The Ca^{++}-channel antagonists (nimodipine 0,5,25,50 mg/kg; nitrendipine 0,25,50,100 mg/kg; verapamil 0,10,20,40 mg/kg and flunarizine 0,20,40,80 mg/kg) were administered po 60-90 min prior to amygdala stimulation at the established threshold. One to two weeks intervened between drugs, and thresholds were redetermined during that time. The phenylalkylamine verapamil, and the dihydropyridines nimodipine and nitrendipine, did not protect against amygdala kindled seizures. Although not statistically significant, the high dose of nitrendipine did appear to suppress the afterdischarge (AD) duration and the duration of clonic activity. The diphenylalkylamine flunarizine (80 mg/kg) did significantly reduce seizure severity (25%), AD duration (46%), and duration of clonic activity (50%), relative to vehicle control. Thus voltage sensitive, non-NMDA, Ca^{++}-channels, may contribute to epileptiform activity induced by kindling.

321.11

SEROTONIN (5-HT) DEPLETION PREVENTS THE ANTICONVULSANT EFFECT OF CARBAMAZEPINE IN SEVERE SEIZURE GENETICALLY EPILEPSY-PRONE RATS (GEPRs). J.W. Dailey, A.F. Bettendorf*, R. Burger*, and P.C. Jobe. Dept of Basic Sci, Univ of IL Col of Med, Peoria, IL 61656.

Considerable evidence suggests that previously documented widespread deficits in CNS norepinephrine and serotonin (5-HT) are integral components of the seizure predisposition characteristic of GEPRs. Other results are compatible with the concept that carbamazepine (CBZ) produces its anticonvulsant effects in GEPRs at least in part through release of CNS 5-HT (Neurosci Abs 15: 24.8, 1989). To further evaluate this concept, 29 severe seizure GEPRs (GEPR-9s) had their phenotypic seizure expression (fore and hind limb tonic extension) confirmed by three separate tests of audiogenic seizure severity at weekly intervals. The 29 GEPR-9s then received a challenge dose of CBZ (6 mg/kg i.p.- 2 x ED_{50} dose) to confirm its anticonvulsant effect. All 29 rats experienced the anticonvulsant effect (i.e., prevention of hindlimb tonic extension in audiogenic seizure test). The next week, 14 rats received 3 daily injections (150 mg/kg/day) of the 5-HT depleter, p-chlorophenylalanine (PCPA) methyl ester. The remaining 15 rats received 3 daily vehicle injections. On the day after the last injection, each animal was again challenged with 6 mg/kg of CBZ. Hindlimb extension was prevented in 12/15 vehicle treated animals and in 3/14 PCPA treated animals (p=0.003, Fisher's Exact Probability Test). Thus, 5-HT depletion prevented the anticonvulsant effect of CBZ in GEPR-9s. This finding further supports a role for 5-HT neurons in the CBZ anticonvulsant effect.

321.12

PARENTERAL CARBAMAZEPINE: EFFECT ON CONVULSIONS AND ON DIALYZABLE HIPPOCAMPAL SEROTONIN (5-HT) IN GENETICALLY EPILEPSY-PRONE RATS (GEPRs). Q-S. Yan*, P.C. Jobe and J.W. Dailey, Dept. of Basic Sci, Univ of Illinois Col of Med, Peoria, IL 61656.

Carbamazepine (CBZ) is a widely used antiepileptic drug which is virtually insoluble in traditional parenteral vehicles. For experimental and perhaps medical reasons, an intravenous (i.v.) use of this drug is desirable. CBZ can be solubilized for i.v. use through complexing with beta-cyclodextrin (Molecusol^{tm}, Pharmatec, Inc.). This solubilized CBZ (provided by K. Estes, Pharmatec) was used in studies of the anticonvulsant mechanism of CBZ in GEPRs. Tail vein injections of a range of CBZ doses (up to 30 mg/kg) produced rapid (as little as 2 min.) and significant anticonvulsant effects in both moderate (GEPR-3s) and severe seizure animals (GEPR-9s), animals in which seizures are regulated, at least in part, by 5-HT. To determine the effect of i.v. CBZ on dialyzable serotonin, GEPR-3s were implanted with a jugular cannula and had a guide cannula stereotaxically placed in the skull over the hippocampus. After 10 days, the microdialysis probe was inserted and the hippocampus was dialyzed with artificial CSF for 3 hrs after which vehicle and CBZ were given i.v. Within minutes after drug, dialyzable 5-HT increased about 3 fold. Vehicle had no effect. Dialyzable 5-Hydroxyindoleacetic acid, the major 5-HT metabolite, was not affected by vehicle or CBZ. This result confirms and extends our earlier observation (Neurosci. Abs. 15: 24.8, 1989) that the peak anticonvulsant effect with CBZ and the peak increase in dialyzable 5-HT occur simultaneously. These results are consistent with a role for 5-HT in the anticonvulsant effect of CBZ (Supported in part by a grant from Tsumura Juntendo Fdn).

321.13

ETHOSUXIMIDE BLOCKADE OF THE EXCITATORY EFFECT OF GAMMA HYDROXYBUTYRATE ON MOUSE CENTRAL NEURONS IN CELL CULTURE. A.W. Wamil* and M.J. McLean. Dept. of Neurology, Vanderbilt Univ. Med. Ctr., Nashville, TN 37212.

Gamma hydroxybutyrate (GHB) is a naturally occurring metabolite of GABA in the mammalian brain which produces a trance-like state and EEG changes in animals which resemble generalized absence seizures in man. The effects of GHB were reversed by ethosuximide (ES; O.C. Snead, Neurology 28:636-642 and 1173-1178, 1978). We used conventional intracellular electrophysiological recording techniques to study effects of GHB on mouse spinal cord neurons in monolayer cell culture. Experiments were performed at 37°C during superfusion with protein-free phosphate buffer (pH 7.4) containing 7 mM Mg^{++} to suppress spontaneous activity. Application of 10^{-4} M GHB by pressure ejection from a blunt-tipped micropipette positioned nearby resulted in depolarization and burst firing of action potentials. Co-application of a therapeutically relevant concentration of ES (7 x 10^{-4} M, 100 ug/ml) resulted in use-dependent block of the response to GHB. These results suggest that the therapeutic efficacy of ES may depend on post-synaptic blockade of GHB-mediated excitation. (Supported by a Mallinckrodt Scholarship and grant NS 00817 from the U.S. National Institutes of Health to MJM and a post-doctoral fellowship from the Cissy Patterson Trust to AWW.)

321.14

DIFFERENTIAL EFFECTS OF HALOPERIDOL AND SCH 23390 IN A MODEL OF EPILEPSY IN THE RABBIT. P. Bo*[1], E. Ongini[2] and F. Savoldi*[1]. [1]Inst. of Neurology "Mondino", Univ. Pavia, I-27100 Pavia, and [2]Research Labs, Schering-Plough S.p.A., Comazzo (Milan), Italy

Dopamine antagonist neuroleptics can induce EEG discharge patterns and increase susceptibility to seizures in epileptic patients. Using a model of epilepsy in the rabbit, we examined the effects of two neuroleptics which differ in selectivity for DA receptors, haloperidol (D-2) and SCH 23390 (D-1). The EEG pattern of afterdischarge (AD) induced by electrical stimulation of hippocampus (Cornu Ammonis dorsale) or amygdala was studied before and after drug administration. Haloperidol (1 mg/kg iv) increased significantly duration of AD upon stimulation of either hippocampus or amygdala. The drug also lowered threshold of the AD. SCH 23390 (0.3 mg/kg iv) produced no change upon stimulation of amygdala and reduced duration of AD when the hippocampus was stimulated. Unlike haloperidol, SCH 23390 induced either no effect or protective action in a model of experimental epilepsy. The data suggest that the D-1 and D-2 receptor subtypes have different function in mechanisms underlying convulsions.

321.15

IN VITRO HIGH INTENSITY TETANUS (HIT) INCREASES KINDLING THRESHOLD IN HIPPOCAMPAL SLICES. L.S.Jones. Dept. of Anat., USC Sch. of Med., Columbia, SC 29208.

Electroconvulsive seizures (ECS) interfere with kindling in animals (Exp. Neur. 78:483, 492; Epilepsia 25:234) and elevate seizure thresholds in humans; the effect may be related to antidepressant properties (Biol. Psych. 18:1301). The anticonvulsant mechanism is unknown, but opioids have been implicated (Science 228:1106; Brn. Res. 177:273; Mol. Brn. Res. 6:11).

Hippocampal slices from young rats were used to develop a model to allow in vitro monitoring of effects that may accompany ECS and the early seizure-resistant period following ECS in whole animal. 625 μM slices received a HIT either through the Schaffer collaterals (SCs) or the mossy fiber (MF) pathway prior to being "kindled" 45 min later through the same or the untreated pathway using the stimulus train induced bursting paradigm of interictal-like burst induction (Brn. Res. 344:296). A HIT via the SCs delayed kindling via the same fibers as measured by the appearance of after-discharges (ADs) in CA1 and spontaneous bursts (SBs) in CA3, and also delayed kindling via the MFs. A HIT via the MFs substantially delayed kindling via either the MFs or the SCs as measured by the appearance of spontaneous bursts in CA3, but had no effect on CA1 ADs. A HIT was not effective in arresting SBs established by earlier kindling or removing Mg^{++}.

321.16

INFLUENCE OF ACTH AND ACTH FRAGMENTS ON (^{3}H)MK-801 BINDING TO RAT HIPPOCAMPAL NMDA RECEPTORS. R.R. Trifiletti and M.R. Pranzatelli. Dept. of Pediatrics and Neurology, Columbia-Presbyterian College of Physicians and Surgeons, New York, NY 10032.

Adrenocorticotropic hormone (ACTH) is widely used in the treatment of infantile spasms. The anticonvulsant mechanism of action of ACTH remains unknown. Its mechanism of action is felt to be independent of cortisol release in that it is equally efficacious in adrenally-surpressed infants. We explored the possibility of a direct influence of ACTH upon rat brain N-methyl D-aspartate (NMDA) receptors labelled with the synthetic NMDA-antagonist (^{3}H)MK-801.

We found that ACTH(1-39) competitively inhibits binding of (^{3}H)MK-801 to rat hippocampal membranes with a K_i of 2.7 uM. The potencies of ACTH fragments at inhibiting (^{3}H)MK-801 binding varies in the order ACTH(1-39) > ACTH(1-24) > ACTH(1-17) > ACTH(4-10). The known potencies of ACTH fragments at treating infantile spasms also varies in this order. This suggests that glutamatergic mechanisms might play a role in the anticonvulsant action of ACTH.

321.17

EFFECTS OF NMDA ANTAGONISTS ON INFERIOR COLLICULUS (IC) NEURONAL RESPONSES AND AUDIOGENIC SEIZURES (AGS) IN BEHAVING NORMAL AND GENETICALLY EPILEPSY-PRONE RATS (GEPRs). C.A. Boersma Anderson and C.L. Faingold, Dept. Pharmacol., So. IL. Univ. Sch. Med., Springfield, IL. 62794.

Systemic administration of the competitive NMDA antagonist, CPP, or the noncompetitive NMDA antagonist, MK-801, blocks AGS in GEPRs. The IC is vital for initiation of AGS in the GEPR, and abnormal acoustically-evoked IC neuronal firing occurs in GEPRs, leading to AGS. The present study examined the effects of systemic administration of CPP and MK-801 on IC neuronal firing of behaving GEPR-9s and normal rats. Microwire electrodes were implanted into IC of normal rats and GEPRs anesthetized with ketamine/xylazine. Unit activity and behavior were examined in freely moving animals. CPP (5 mg/kg i.p.) or MK-801 (.05 mg/kg i.p.) was administered, and acoustic stimuli (12 kHz tone bursts, 100 msec duration) at 2/sec were presented. Neuronal responses were evaluated to stepwise increases in sound level up to 105 dB SPL. Responses were recorded before and 30-240 min after drug administration. CPP greatly inhibited IC neuronal firing (mean inhibition, 67.5% $\pm$ 9.1 SEM, N=13), while MK-801 inhibited firing to a small extent (mean, 29.8% $\pm$ 10.0, N=6) in normal rats and GEPRs (SPL 20-30 dB above 12 kHz tone threshold). With each drug the reduction in IC neuronal firing was significant at a time (60 min post injection) when both agents were effectively anticonvulsant. However, the degree of firing reduction was significantly greater with CPP than with MK-801. Previous studies indicate that CPP bilaterally microinjected into the IC is very potent and effective in blocking AGS, but microinjection of MK-801 is considerably less effective. However, MK-801 is the most potent agent systemically. **CONCLUSION:** CPP appears to be producing its anticonvulsant effect, in large part, on the brainstem auditory pathway. However, actions of MK-801 on additional and more rostral parts of the AGS network may be required for the complete anticonvulsant effects of this agent. (Support NIH NS 21281; 13849).

321.18

SENSITIVITY DIFFERENCES TO BLOCKADE OF AUDIOGENIC SEIZURES (AGS) IN THYROID DEFICIENT (THX) OR GENETICALLY EPILEPSY-PRONE RATS (GEPRs): MICROINJECTION INTO BRAINSTEM AUDITORY NUCLEI. D.L. Patrick and C.L. Faingold. Dept. Pharmacol. So. IL. Univ. Sch. Med., Springfield, IL 62794.

Neonatally THX rats and GEPRs exhibit cochlear damage and are susceptible to AGS. GEPRs are also THX during development. Excitant amino acids (EEAs) and GABA in the inferior colliculus (IC) are critically involved in initiation of AGS in GEPRs, while EAA action in cochlear nucleus (CN) appears to be relatively less important to epileptogenesis. This study examined the effects of microinjection into IC or CN of the EEA (NMDA) antagonists, AP7 and MK 801, or the GABA agonists, THIP (GABA-A) and baclofen (BAC, GABA-B). Comparisons were made between effects in THX rats and GEPRs. THX rats were depleted of thyroid hormone using propylthiouracil (PTU) (0.0075% postnatal day 0-19). AGS severity was tested using a bell (122 dB SPL, for 60 sec or until AGS onset). Bilateral infusion of MK-801 (in 0.5 μl, 2 min) into IC completely blocked AGS at 50 (THX) vs. 40 nmol (GEPR). Microinjection of THIP completely blocked AGS at 68 (THX) vs. 13.6 nmol (GEPR). Microinjection of BAC completely blocked AGS at 28 (THX) vs. 1 nmol (GEPR). Relative doses of AP7 in CN were 50 (THX) vs. 5 nmol (GEPR) for reduction of AGS severity. **CONCLUSION:** These data suggest the sensitivity for blockade of AGS in IC and CN is considerably less in THX rats than in GEPRs. Cochlear damage may be more important in AGS of THX rats as compared to GEPRs wherein the IC may be relatively more critical in AGS control. The reduced sensitivity in THX rats may be due to consistent changes in receptor properties in CN and IC induced by the extensive neonatal deficiency of thyroid hormone in PTU-treated rats. Although GEPRs exhibit some degree of thyroid deficiency, the deficiency in PTU-treated rats may be more extensive than that observed in GEPRs. (Support: NIH NS 21281, NS 13849)

321.19

EFFECTS OF GLYCINE AND MILACEMIDE, A GLYCINE PRODRUG, ON AUDIOGENIC SEIZURES IN THE GENETICALLY EPILEPSY-PRONE RAT (GEPR-9). D.K. Naritoku, L.B. Mecozzi*, M.E. Randall*, C.L. Faingold. Div. of Neurol. and Dept. of Pharmacol., So. IL . Univ. Sch. Med., Springfield, IL.

Glycine (GLY) is a putative inhibitory neurotransmitter in the brainstem auditory system, but may also enhance excitation via the NMDA receptor complex. Its role in audiogenic seizures (AGS) is unknown. Studies have implicated the inferior colliculus (IC) as important during AGS genesis, and we observed the effects of increasing brain glycine by systemic administration of milacemide (MIL), a glycine prodrug, and microinfusions of GLY or MIL into bilateral IC. In systemic studies, MIL (200 or 400 mg/kg) was given i.p. At various intervals the animals were exposed to bell stimulus (120 dB SPL) for 1 min or until AGS onset. The resulting seizures were scored using the scale of Jobe (1981). Systemic administration of MIL at 200 mg/kg did not affect AGS, whereas a dose of 400 mg/kg reduced the median AGS severity score to 3 at 30 min and 1 hr ($p < .001$). For microinfusion studies, cannula guides were placed over IC. After one week of recuperation, GLY (0.5 - 1 μg/side) or MIL (200 or 400 μg/side) were infused into bilateral IC (0.5 μl, 2 min) and tested at various intervals. Microinfusions of GLY (1 μg/side) reduced the mean severity score to 1 at 2 hrs ($p < .001$). No significant reduction occurred with a dose of 200 μg/side of MIL, and 400 μg/side infusions reduced the median severity score to 7 at 4 hrs ($p < .01$). There was a prolonged reduction of the severity score ($p \leq .05$) from 1 to 5 hrs after the MIL infusions. **CONCLUSION:** The data suggest GLY plays a role in the neuronal network which subserves AGS in the GEPR-9. The relatively large amounts of GLY or MIL required at IC to reduce AGS severity suggest that GLY may exert its antiepileptic effect at a site different from IC during AGS, or alternatively, the ability of GLY to facilitate excitatory transmission may partially offset its inhibitory effects. Support: Epilepsy Foundation of America, NIH NS-21281, and SIU School of Medicine.

322.1

ALU REPEAT FAMILY (ARF)- AND AMYLOID PRECURSOR PROTEIN (APP)- RNA EXPRESSION IN ALZHEIMER AND CONTROL FIBROBLASTS.
N.P. Dooley*, H.D. Durham, J. Nalbantoglu*, S. Gauthier and N.R. Cashman. MNI, McGill University, Montreal, Canada H3A 2B4.

We have investigated ARF- and APP-RNA in unstimulated, non-transformed fibroblasts grown from primary cultures. ARF, which is transcribed by RNA polymerase III predominantly in brain, has been implicated in the regulation of neuronal genes during postnatal neural development. We postulated that abnormalities in ARF may account for decreased mRNA in brains from patients with Alzheimer's disease(AD). Skin biopsies were obtained from AD patients and neurologically normal spouses. Total RNA was extracted using the guanidinium thiocyanate-phenol-chloroform method from cultures of the same low generation. Northern blots were probed for ARF RNA using the BC200 probe (Watson,J.B., Mol Cell Biol 7(9):3324-27, 1987) at high stringency. As a control for lane loading and signal intensity, these blots were stripped and rehybridized using a human actin probe (Gunning,P. Mol Cell Biol 3(5):787-95, 1983). ARF transcripts were detectable in all primary fibroblast cultures; preliminary results suggest decreased expression in 4 AD patients versus 6 controls ($0.20 > p > 0.10$).

APP deposition is believed to be a seminal event in AD pathogenesis and has been demonstrated in non-neuronal tissue. Northern blots were hybridized with a 3' APP cDNA probe followed by the actin probe. No significant difference between AD and control mRNA levels was observed.

322.2

BETA-AMYLOID PROTEIN INCREASES NEURONAL VULNERABILITY TO EXCITOTOXINS IN CORTICAL CULTURE.
J. Koh, L.L.Yang*, and C.W. Cotman, Dept. of Pyschobiology., Univ. of Cal., Irvine, CA92717.

Glutamate neurotoxicity has been proposed as a pathologic mechanism contributing to neuronal degeneration in a variety of diseases including Alzhemier's disease (AD). In addition to effects on extracellular glutamate levels, a particular disease may increase neuronal vulnerability to excitotoxins. In this study, we examined the possibility that β-amyloid protein found in plaques of AD acts in such a manner.

Mature mouse cortical cultures were exposed for 2 days to β-amyloid protein (β1-42, 100 μg/ml) in serum-free defined media and subsequently exposed for 5 min to 100 μM glutamate. Morphological observations and lactate dehydrogenase assay indicated that the pre-exposure to β1-42 significantly increased the glutamate neurotoxicity compared with control sister cultures. Similar potentiation by β1-42 was observed after 5 min exposure to 30 μM N-methyl-D-aspartate (NMDA) or 500 μM kainate. The potentiation of NMDA neurotoxicity was dependent on β1-42 concentration and exposure duration. Additionally, a shorter fragment of β-amyloid (β1-28) also potentiated excitotoxicity.

The present results demonstrate that prolonged exposure to β-amyloid protein enhances neuronal vulnerability to excitotoxic damage. We speculate that this toxicity-potentiating effect of β-amyloid protein could contribute to accelerated neuronal degeneration in AD.

322.3

Immunocytochemical Localization of A4 Amyloid Epitope in Plastic-Embedded Brain Tissue from Alzheimer's Disease Patients.
R.A. Brumback, C.A. Marotta, R.E. Majocha, D.L. Feeback,* R.W. Leech, J.L. Ketring,* L.K. Benningfield,* G.D. Miner. Univ. of Oklahoma and VA Medical Center, Oklahoma City, OK, Harvard Program in Neurosciences, Massachusetts General Hosp./McLean Hosp., Boston, MA, The Alzheimer's Foundation, Tulsa, OK.

Previously, we described improved histologic resolution of large brain specimens (without shrinkage and distortion artifacts of paraffin-embedding) using the technique of plastic embedding with methyl methacrylate (Ann Neurol 26:301, 1989). Cases of Alzheimer's disease studied with this technique show improved morphologic and spatial resolution of characteristic plaques and tangles in Bielschowsky-stained sections. However, since greater sensitivity in detection of the Alzheimer's disease-associated amyloid plaque protein is possible utilizing monoclonal antibodies developed to the A4 amyloid epitope (J Geriatr Psychiatry Neurol 1:65, 1988; PNAS 85:6182, 1988), we have applied these antibodies to plastic embedded tissue. Tissue sections (2 μm thick) mounted on glass slides, dried, and subsequently xylene immersed (to remove the plastic) were incubated with antibody to the A4 amyloid epitope. Positive immunostaining (standard avidin-biotin-HRP method) for the amyloid protein was readily identifiable in plaques and neurons and detailed localization was possible. [Supported in part by NIH AG02126 and Metropolitan Life Foundation (CAM); and Founders of Doctor's Hospital (GDM)]

322.4

EXPRESSION OF AMYLOID PRECURSOR PROTEIN mRNAs IN ENDOTHELIAL AND NEURONAL CELLS.
G.L.Forloni, S. Giorgi*, C. Bendotti , N. Angeretti* and S. Consolo, Istituto di Ricerche Farmacologiche "Mario Negri", 20157 Milano, Italy.

The origin of the β/A4 amyloid protein deposited in the neuritic plaque of Alzheimer disease (AD) is unknown. It has been demonstrated that the amyloid precursor protein (APP) gene encoding the precursor of the β/A4 amylod protein can be expressed as APP695 and/or APP751/770 depending on the presence of a Kunitz family protease inhibitor sequence. We investigated *in vitro* the expression of APP gene in endothelial and neuronal cells, by using two different probes recognizing the total APP mRNA or the APP751/770 mRNA. Total RNA was extracted from endothelial cells isolated from human umbelical cord grown to confluency and from rat cortical neurons dissociated at E17 and cultured *in vitro* for two weeks. The Northern blot analysis was performed using the APP ^{32}P-cDNA probe to detect the general APP sequence and with a ^{32}P-labelled oligonucleotide (40 mer) complementary to the sequence of the protease inhibitor. The APP mRNA transcripts were abundant in both cell types. APP751/770 mRNA was found in endothelial as well as in the neuronal cells, however in the neuronal cells the ratio APP751/770 mRNA/total APP mRNA was much lower than in the endothelial cells. We observed in the endothelial cells, similar increase of total APP mRNA or APP751/770 mRNA after interleukin-1 (100ng/ml) treatment. The present results demonstrate some differences in the APP expression between neuronal and endothelial cells and identify these primary cell cultures as a useful model to investigate the mechanisms related to the β/A4 amyloid protein deposition in AD.

322.5

A 35KD FRAGMENT OF AMYLOID β/A4-PROTEIN PRECURSOR IN ALZHEIMER'S DISEASE AND CULTURED CELLS.
L.A. Flanagan[1], G.M. Cole[1], E.R. Shelton*[2], H.W. Chan[2], and T. Saitoh[1].[1]University of California, San Diego, Dept. of Neurosciences, M-024, La Jolla, CA 92093, [2]Syntex Res. Inst. of Bio-Organic Chem., Palo Alto, CA 94304.

A 35Kd fragment of the amyloid β/A4-protein precursor (ABPP) has been recognized by antisera directed to the N-terminal portion of the precursor and found at higher concentrations in Alzheimer cortex than in control cortex (Cole et al., Neurosci. Lett., 100: 340-346, 1989). To identify this 35Kd fragment (ABPP-35K) and discover its significance we have undertaken a systematic analysis of this peptide. ABPP-35K is soluble at 60% saturated ammonium sulfate whereas the majority of other ABPP species precipitate. Immunostaining of Western blots suggests the presence of a doublet at 35K rather than a single band, and both bands appear to contain the Kunitz-type protease inhibitor region of the precursor protein. An antibody directed to amino acids 175–186 of ABPP has higher affinity for the lower band while other antibodies directed toward the N-terminal portion of ABPP detect both bands equally well.

We have previously suggested that degradation of ABPP occurs through lysosomal pathways (Cole et al., Neurochem. Res., 14: 933-939, 1989). We now have evidence that ABPP-35K may be generated by blocking lysosomal degradation of ABPP. Treatment of PC12 cells with leupeptin and ammonium chloride results in an increase in ABPP-35K. Further studies are underway to determine the C-terminal sequence of ABPP-35K and the pathways involved in its generation and degradation which may be altered in Alzheimers disease.

322.6

EFFECT OF NGF ON THE ALTERNATIVELY SPLICED βAPP mRNAS AND SECRETED βAPP DERIVATIVES IN PC12 CELLS.
S. Estus, T. Golde, G. Landreth, and S. Younkin., Case Western Reserve University, Cleveland, OH 44106

The factors regulating the extracellular accumulation of the soluble derivatives of βAPP_{695}, βAPP_{751}, and βAPP_{770} are unclear. These proteins could be secreted in direct proportion to the level of the corresponding mRNAs. Alternatively, regulation of the secreted forms could occur at the level of posttranslational processing. We have employed PC12 cells to examine this question because this cell line manifests a neural phenotype when stimulated by NGF and because our initial experiments showed that these cells express βAPP_{695} mRNA, a form found primarily in neurons that lacks the Kunitiz protease inhibitor (KPI) insert, as well as βAPP_{751} and βAPP_{770} mRNAs, forms that contain the KPI insert and are found in all types of cells. Analysis of the βAPP derivatives secreted from PC12 cells on immunoblots labeled with anti-APP_{45-62} revealed three large secreted forms: two proteins of ~ 134 and ~128 kDa that were also labeled with an antiserum specific for the KPI domain, and a protein at ~ 114 kDa that was not labeled with the KPI antiserum. To evaluate the changes induced by NGF, βAPP mRNAS were analyzed using a sensitive PCR based method and secreted βAPP derivatives were analyzed using ^{125}I-protein A to quantitate anti-APP_{45-62} immunoblots. As reported by others, NGF treatment produces a marked absolute and relative increase in the level of the secreted ~114 kDa KPI-free form. Moreover, our data indicate that this increase is due, at least in part, to an NGF-induced increase in the absolute and relative amount of APP_{695} mRNA. Additional studies are underway to characterize the posttranslational processing of the βAPP in NGF and control PC12 cells to determine whether changes in posttranslational processing also contribute to the NGF-induced change in secreted βAPP derivatives.

322.7

ß AMYLOID PROTEIN PRECURSOR DERIVATIVES IN AD . M. Palmert, T. Cheung*, M. Cohen*, V. Frazzini*, T. Kunishita*, J. Pasternack, M. Usiak*, and S. Younkin, Case Western Reserve University, Cleveland, OH 44106

Previously, we (i) identified ~125, ~105, and ~25 kDa soluble amino-terminal derivatives of the full-length, membrane-associated ß amyloid protein precursor (ßAPP) by purifying these proteins from human cerebrospinal fluid (CSF) and sequencing their amino termini, (ii) showed that the ~125 kDa form contains and the ~105 kDa form lacks the alternatively spliced Kunitz protease inhibitor domain, and (iii) demonstrated that the carboxyl-terminal region of both the ~125 and ~105 kDa forms contains at least part of the ß amyloid protein (ßAP). To determine if any of the ~125 or ~105 kDa derivatives in the AD or control brain are potentially amyloidogenic forms that contain full length ßAP, we are analyzing their carboxyl termini and have adapted our purification procedure to isolate the requisite large amounts of these proteins from human brain tissue. To identify quantitative changes in ßAPP derivatives that could reflect abnormal ßAPP processing that leads to amyloid deposition, we have analyzed CSF samples from 24 AD patients and 12 controls. Our results show (i) that in elderly (68 ± 3 year) as compared to young (45 ± 4 year) non-demented controls there is a significant increase in the relative amount of the ~25 kDa form, a corresponding significant decrease in the percentage of the ~105 kDa form, and significant increases in the absolute amounts of the ~25 kDa, ~105 kDa, and total ßAPP derivatives, (ii) that the age-dependent shift toward a higher percentage of the ~25 kDa form is significantly enhanced in AD cases as compared to age-matched controls, and (iii) that the changes in AD are correlated with mental status. To identify proteases that may be involved in normal or abnormal ßAPP processing, we are using substrate gel analysis (also called enzymography) to evaluate AD and control CSF. We have identified seven proteases between 45 and 155 kDa as well as several larger proteases in both AD and control CSF, and we are analyzing them to determine where they cleave the ßAPP.

322.8

EXPRESSION OF NORMAL AND VARIANT ALZHEIMER'S β-PROTEIN GENES IN VASCULAR AMYLOID OF HEREDITARY CEREBRAL HEMORRHAGE WITH AMYLOIDOSIS DUTCH TYPE. F. Prelli,* E. Levy,* S.G. van Duinen,* G.Th.A.M. Bots,* W. Luyendijk,* and B. Frangione NYU Med. Cent, NYC 10016; Univ. Med. Cent, Leiden, Holland

Amyloid fibrils deposited in cerebral vessel walls in Dutch patients with hereditary cerebral hemorrhage with amyloidosis (HCHWA-D) are formed by an amyloid protein similar to the amyloid β-protein of Alzheimer's disease (AD) and Down syndrome (DS). Sequence analysis of the genomic DNA isolated from 4 Dutch patients revealed a point mutation, cytosine for guanine, at position 1852 of the precursor β-protein gene. The HCHWA-Ds had 1 normal allele. The mutation was not detected in 5 AD, 2 DS patients and 11 normals. To ascertain whether the amyloid fibrils in HCHWA-D are composed of normal and variant β-protein, fibrils were isolated from brain tissue of 2 Dutch patients. HPLC fractionation of the tryptic digest of a fraction containing monomers yielded 4 peptides T1-T4; two peaks, T3a and T3b, had amino acid compositions identical to positions 17-28. Sequence analysis confirmed their identity, but T3a had GLN and T3b had GLU at position 22. Although GLN and ASN may undergo deamination, recovering GLN and ASN exclusively at positions 15 and 17, respectively, suggests that the GLU at position 22 expresses the normal β-protein allele rather than deamination of GLN. Thus both the normal and variant Alzheimer β-protein genes are expressed in the vascular amyloid of HCHWA-D.

322.9

THE β-AMYLOID PRECURSOR PROTEIN, A COLLAGEN BINDING PROTEIN, MEDIATES NEURAL CELL ADHESION.
Kieran C. Breen. Dept. of Pharmacology, University College, Belfield, Dublin 4, Ireland. (SPON: Brain Research Association).

The β-amyloid precursor protein (APP) is a glycoprotein which consists of at least four isoforms derived from a single gene by a process of alternative splicing. The 42 amino acid β-A4 region is located at the carboxy terminal of the protein, and is partially embedded in the membrane. Two isoforms, APP_{751} and APP_{770} contain an extra domain homologous with the kunitz-type protease inhibitor.

As a membrane-bound glycoprotein, APP has been suggested to have possible adhesive properties and to mediate neural cell adhesion. Purified APP from transfected cell lines has been demonstrated to be capable of mediating cell-substrate adhesion.

Fab' fragments of antibodies to different domains of the molecule were prepared These were demonstrated to inhibit neural cell adhesion to a collagen substrate. APP was further shown to bind specifically to type IV collagen-bound sepharose. Fab' fragments of the antibodies also inhibited neuron-neuron and neuron-glial cell adhesion, but did not influence glial-glial cell interaction.

These data suggest that APP plays a role in both cell-cell and cell-substrate adhesion in neural cells.

322.10

MOLECULAR MODELING OF THE PROTEASE INHIBITOR INSERT OF THE AMYLOID PRECURSOR PROTEIN. R.J. McClure, W.E. Klunk and J.W. Pettegrew. Laboratory of Neurophysics, Dept. of Psychiatry, Western Psych. Inst. & Clinic, Univ. of Pittsburgh, Pittsburgh, PA 15261.

Recent studies have demonstrated that the 751 amino acid form of amyloid precursor protein (APP-751) contains a 56 amino acid residue insert with sequence homology to the Kunitz family of serine protease inhibitors. Purified secreted APP-751 inhibits trypsin with high affinity (Oltersdorf, T. *et al.*, Nature, 341:144, 1989). The present study examines the 3-dimensional structure of this amyloid protease inhibitor (API) domain in order to help determine the role of a protease inhibitor in the increased deposition of β-amyloid in Alzheimer's disease (AD). The amino acids of API were modeled onto the molecular framework of bovine pancreatic trypsin inhibitor using the computer program FRODO and minimized using AMBER. This 3-dimensional model shows that API could adopt a conformation consistent with a high affinity inhibitor-trypsin complex. We postulate that AD may be a disease of protease dysregulation and that study of the tertiary structure of API may give insights into the molecular structure of this protease/protease inhibitor system which can be pharmacologically exploited.

322.11

CLIPSIN, A POSSIBLE β-AMYLOID GENERATING PROTEASE, IN HUMAN BRAIN. R. Siman, R.B. Nelson[1], M. Iqbal*, & J.C. Kauer, Cephalon, Inc., West Chester, PA. 19380, and [1]Dept. Neurobiology, Harvard Medical School, Boston, MA. 02115.

Proteolytic processing of the β-amyloid precursor proteins is required for deposition of the β-protein into neuritic plaques. We have designed chromogenic protease substrates with which to examine human brain fractions for activities capable of generating plaque β-amyloid. Here, we describe one such activity. Like the serine protease clipsin, previously described in rat brain, the human protease was detergent- and low ionic strength-insoluble, but could be extracted by high salt. The protease was found post-mortem in both Alzheimer's disease and normal brain. The human brain protease co-purified with authentic clipsin upon heparin-Sepharose and substrate affinity chromatographies. The protease, purified to homogeneity, had an apparent Mr~26 kDa and co-migrated with rat clipsin. The activity also co-migrated with authentic clipsin on substrate-containing enzymographic gels and, like clipsin, was inhibited by α1-antichymotrypsin. We therefore refer to the human brain protease as clipsin. Both human and rat brain clipsin readily hydrolyzed peptide substrates that two other chymotrypsin-like proteases, chymotrypsin and cathepsin G, did not degrade. These results indicate that not all members of the chymotrypsin family of serine proteases are capable of liberating the N-terminal of β-amyloid. Clipsin, on the other hand, is a candidate for such a role.

322.12

SELECTIVE ELEVATION IN ACTIVITY OF THE PROTEASE CLIPSIN IN ALZHEIMER'S DISEASE. M. J. Savage and R. Siman, Cephalon, Inc., West Chester, PA. 19380.

Abnormal activity of a chymotrypsin-like protease has been hypothesized to play a key role in the pathogenesis of neuritic plaques in Alzheimer's disease (AD). Recently, we identified a chymotrypsin-like protease present in both rat (J. Biol. Chem. 265:3638, 1990) and human brain (this volume), termed clipsin. To examine the hypothesis that aberrant clipsin activity might underlie ß-amyloid deposition into plaques in AD, we quantified the activities of clipsin and several other proteases using selective synthetic substrates. In parietal cortex grey matter, clipsin activity was significantly elevated by two-fold compared with age-matched controls (n=17). In preliminary analysis, this increase holds for other brain areas as well. Activity levels in white matter were about three-fold less than in grey matter, and were also significantly elevated in AD. The clipsin increase was selective, in that activity of the cytosolic enzyme prolyl endopeptidase was reduced by two-fold in AD, while that of the lysosomal enzyme cathepsin B was unchanged. Within the AD samples, clipsin activity did not correlate with the density of ß-amyloid deposits stained by Congo Red, but in control brains, clipsin activity was significantly lower along with the virtual absence of Congo Red staining. These results suggest that an aberrant increase in clipsin activity may contribute to the generation of ß-amyloid in AD.

322.13

PURIFICATION OF CLIPSIN, AN α_1-ANTICHYMOTRYPSIN-BINDING PROTEASE WHICH CLEAVES THE β-PROTEIN PRECURSOR. R. B. Nelson[1], R. Siman[2], and H. Potter[1]. [1]Department of Neurobiology, Harvard Medical School, Boston, MA 02115 and [2]Cephalon, Inc., 145 Brandywine Pkwy., West Chester, PA 19380.

Independent lines of evidence suggest that a chymotrypsin-like protease is involved in depositing β-protein into the amyloid plaque cores which form in Alzheimer's disease and Down's syndrome. First, a chymotrypsin-like protease is predicted to cleave at the site which generates the amino terminus of the β-protein from the β-protein precursor. Second, an α_1-antichymotrypsin-like protein (ACT) is an integral component of amyloid plaque cores. Third, the β-protein precursor includes a protease inhibitor domain having a high affinity for chymotrypsin. The discovery of clipsin, a chymotrypsin-like protease in rat brain which binds to ACT in an SDS-stable manner and preferentially degrades membrane-associated β-protein precursor, has recently been reported (*J. Biol. Chem.* **265**:3836). We report here the purification of clipsin to homogeneity. Detergent-extracted membranes from 6-day-old rat forebrains were treated with 1.0 M $MgCl_2$ to solubilize clipsin activity. Clipsin was then precipitated by dialysis against a low-ionic strength buffer, eluted at high salt from a heparin-agarose column, and finally purified to homogeneity using a custom-made substrate column (Ala-Pro-Phe-Sepharose 4B). Purified clipsin is a 25 kDa doublet which binds to ACT in an SDS-stable manner and demonstrates protease activity as measured *in vitro* and by enzymography. To obtain clipsin in a form suitable for micro-sequencing, the purified protease was exchanged into a volatile buffer on a Sephadex G-25 column. Internal protein sequence information from tryptic digests of clipsin is currently being obtained. Kinetic comparisons to other known chymotrypsin-like proteases already suggest that clipsin will be a novel enzyme. Partial sequences of clipsin will be used to raise domain-specific antibodies and to construct oligonucleotide probes for cloning clipsin from rat and human brain genomic and cDNA libraries.

322.14

ANALYSIS OF THE HUMAN APP PROMOTER: 96 BASE PAIRS UPSTREAM OF THE TRANSCRIPTIONAL START SITE ARE SUFFICIENT FOR FULL PROMOTER ACTIVITY. W.W. Quitschke and D.Y. Goldgaber, Department of Psychiatry, State University of New York at Stony Brook, Stony Brook, NY 11794.

The extracellular deposition of amyloid beta protein in cores of neuritic plaques and walls of blood vessels in brain and leptomeninges is a neuropathological manifestation of aging, Alzheimer's disease, Down syndrome, and hereditary cerebral hemorrhage with amyloidosis (Dutch type). The amyloid beta protein is derived from a larger amyloid beta protein precursor (APP). The gene encoding this protein is located on human chromosome 21. The APP gene encodes multiple transcripts for a family of secreted proteins that are generated by differential splicing. The APP gene is differentially expressed in all major tissues. In brain it is expressed primarily in neurons. The apparent overexpression of the APP gene in Down syndrome and in certain areas of the brain in Alzheimer's disease suggests that abnormalities in gene regulation might be a critical factor in the neuropathology associated with these conditions. We analyzed the promoter for the APP gene with the goal of identifying cis- and trans-acting factors that affect the expression of the APP gene. The promoter was analyzed by transfecting the mouse myogenic cell line C2C12 with APP promoter constructs fused to the chloramphenicol acetyl transferase gene (CAT). This cell line expresses the endogenous APP gene constitutively at moderate levels. The longest promoter fragment analyzed included 2.8 kb of sequence upstream of the transcriptional start site. Subsequent 5' deletions were introduced at positions -488, -308, -204, and -96. The results from transient transfection experiments suggest that full promoter activity is retained in a promoter fragment containing only 96 base pairs upstream of the transcriptional start site. Experiments are currently in progress to identify potential trans-acting factors that bind to this region. In addition, the promoter and its deletions are analyzed by stable transfection of additional cell lines of different origins that show varying levels of expression of the endogenous APP gene.

DISORDERS OF THE NERVOUS SYSTEM: DEVELOPMENTAL MODELS

323.1

TRYPTOPHAN (Trp), QUINOLINATE (QUIN) AND SEROTONIN (5-HT): I. ALTERATIONS IN CHILDREN WITH HYPERAMMONEMIA (HA). M. L. Batshaw, M. Heyes, S. Djali*, L. Rorke*, M.B. Robinson. Children's Seashore House, Depts. Pediatrics and Pharm, Univ. of PA, Phila., PA 19104 and NIH, Bethesda, MD.

Marked elevations in plasma ammonium levels in children can result in coma and subsequent mental retardation. Previous studies have demonstrated alterations in Trp and its metabolites in children and in animal models of HA. In this and the accompanying abstract (Robinson et al.), we report the effects of HA on Trp, 5-HT, 5-hydroxy-indoleacetic acid (5-HIAA), QUIN, and 5-HT receptors in children with congenital HA and 3 animal models of HA. Of the 13 children with urea cycle disorders and organic acidemias studied, three were neonates in coma, aged 3-7 d, with plasma ammonium levels >1000 μM (normal 20-40 μM). They had markedly elevated CSF levels of both QUIN and 5-HIAA: QUIN 252, 988, 672 nM (nl <35); 5-HIAA 1.55,1.17, and 9.19 μM (nl<.23). Ten were aged 2-22 yrs who had only modest elevations of plasma ammonium (30-71 μM). CSF QUIN levels were significantly elevated ranging from 24-71 nM (54±18, $p<0.01$) as were 5-HIAA levels, 110-680 nM (309±164, $p<0.0005$), compared to non-HA control children. Plasma ammonium levels were correlated with CSF Trp ($r=0.72$, $p<.05$) and 5-HIAA levels ($r=0.84$, $p<0.001$), but not with QUIN ($r=0.4$, $p>.05$). CSF Trp correlated with both 5-HIAA and QUIN ($r=0.48$, $p<0.05$; and $r=0.68$, $p<0.001$ respectively). Autopsy of the child with the highest levels of QUIN and ammonium revealed widely dispersed spongiform changes with neuronal necrosis and gliosis. These data suggest that alterations in 5-HT and QUIN may be factors in the neurologic alterations in children with HA.

323.2

TRYPTOPHAN (Trp), QUINOLINATE (QUIN) AND SEROTONIN (5-HT): II. ALTERATIONS IN ANIMAL MODELS OF HYPERAMMONEMIA (HA). M.B. Robinson, M. Heyes, E. Gorry*, N.J. Anegawa*, S. Djali*, I. Lucki, and M.L. Batshaw. Children's Seashore House, Depts. Pediatrics and Pharmacology, Univ. of PA, Philadelphia, PA. 19104 and NIH, Bethesda, MD.

The effect of HA on Trp and its metabolites was studied in 3 animal models: the ammonium acetate injected rat, urease infused rat, and the chronically hyperammonemic ornithine transcarbamylase deficient sparse fur (**spf/y**) mouse. In ammonium acetate injected rats, there was a 69% increase in cortical 5-HIAA levels compared to sodium acetate controls ($p<0.05$) with no alterations in CSF QUIN 1 h after injection. In these animals, cortical Trp significantly correlated with 5-HT ($p<0.02$). Cortical 5-HT correlated with both cortical 5-HIAA and CSF QUIN ($p<0.02$). To extend the duration of HA, rats were implanted with urease-containing mini-osmotic pumps. Urease infusion resulted in increased brain Trp and 5-HIAA ($p<0.05$) 12, 24 and 48 h after implant. Despite greater than two-fold elevations in brain Trp, no alterations in QUIN were observed in five brain regions, CSF, or plasma. In this model, plasma ammonium correlated with both brain Trp and 5-HIAA ($p<0.001$). Higher levels of 5-HIAA have also been observed in brain of the **spf/y** mouse compared to controls. Decreases in cortical ketanserin (-19%, $p<0.005$) but not 8-hydroxy-di-n-propylaminotetralin binding sites were found in the **spf/y** mouse. **Spf/y** mice also demonstrated a decreased 5-HT2 mediated, quipazine induced head twitch response ($p<0.005$). These data provide further support for altered serotonin metabolism in HA and also suggest that the pathways from Trp to QUIN and 5-HT may be differentially regulated in rats and children.

323.3

CUMULATIVE LEARNING DEFICIT IN NEONATALLY HYPERPHENYL-ALANINEMIC RATS: A RODENT MODEL OF MENTAL RETARDATION. B.J. Strupp, M. Bunsey, D.A. Levitsky* and K. Hamberger*. Div. of Nutritional Sci. and Dept. of Psychology, Cornell University, Ithaca, NY 14853.

Based on an analysis of the human mental retardation (MR) literature, we proposed an animal model that focuses on cumulative learning deficits (Strupp, B.J. and Levitsky, D.A., Adv. Infancy Res., 6:1990). The model proposes that the difference in problem-solving ability between "retarded" rats and controls will increase with successive problem presentations. The present study tested this hypothesis, using pre- and postnatal hyperphenylalaninemia (HP) (models of maternal and classic PKU, respectively) as exemplary mental retardation models. The results confirmed the predicted cumulative learning deficit in neonatally HP rats, suggesting that the relatively small deficits generally observed in animal models of MR syndromes may be due to the use of a single task rather than a series of related problems. Also discussed will be (1) the types of errors and response style that characterizes these groups, (2) the attentional dysfunction of the experimental animals, and (3) the points of correspondence between the pattern of intact and impaired cognitive processes in these animals and the pattern observed in human MR.

323.4

DISTURBED MYELINOGENESIS AND RECOVERY IN HYPERPHENYLALANINEMIA: BIOCHEMICAL AND IMMUNOHISTOCHEMICAL RESULTS. R. Burri*[a], R. Reynolds*[b], L. Bologa[a], N. Herschkowitz*[a]. [a]Univ. Berne, Dept. Pediatrics, Switzerland; and [b]Imperial Coll., Dept. Biochemistry, London Univ. UK.

To study myelinogenesis in hyperphenylalaninemia, rats were injected with phenylalanine (Phe)/alpha-methylPhe from days 3-17, and then allowed to recover until day 59. The developmental pattern of cerebroside sulfotransferase (CST), 2',3'-cyclic nucleotide 3'-phosphohydrolase (CNP), body weight, brain weight, brain protein and DNA were determined at 9 time points. CST and CNP activities (per mg brain) were reduced between days 10-17. CST recovered to normal values between days 17-24, CNP between days 35-59. However, brain weight was still significantly reduced at day 59. CNP and myelin basic protein immunostaining demonstrate a reduction in myelin in the corpus callosum (CC) by day 17 in test rats. Neurofilament protein (NF) positive axons were reduced in white and grey matter (GM). However, numbers of oligodendrocytes (CNP^+, $GalC^+$) and glial progenitors (G_{D3}^+, ganglioside$^+$) appeared normal throughout development. There was no obvious deficit in myelin in the CC at 59 days. The number of strongly NF^+ myelinated axons in the cortical GM was still reduced at 59 days. Our results suggest that axonal and not oligodendroglial maturation may be primarily affected by hyperphenylalaninemia.

323.5

PURSUIT EYE MOVEMENTS IN CEREBRAL PALSIED ADULTS. M.LeGare, J.Coltellaro* and J.Terdiman*. Dept. of Psychology and Biomedical Engineering Program, California State University, Sacramento 95819;Neuro-optometry Clinic, U.C. Berkeley 94720.

Pursuit movements of the left eye were measured in response to triangle wave stimuli (0.3 Hz, ±10 deg. 10 sec) presented to 5 moderate-severe cerebral palsied (CP) and 5 motorically normal (N) adult men using a monocular, noncontact videobased system (Micromeasurements S1200). Artifacts and quantization noise were removed from the raw signals by a rule-driven process followed by interpolation and 2-point averaging which provided movement signals comparable to those obtained simultaneously from a normal subject using an analog eye movement measurement device.

Plots of eye position superimposed on stimulus position showed more asymmetric hypo- and hypermetria, fixations and high velocity saccadic intrusions in CP as compared to N. Preliminary spectral analyses showed high spectral coherency between eye and stimulus positions (CP, N) with greater ranges of frequencies, power spectra and phase discrepancies for CP than for N in eye position. These data indicate good directionality with poor ramp pursuit control in CP at 0.3 Hz.

323.6

MYOTATIC REFLEX DEVELOPMENT IN NORMAL INFANTS AND CHILDREN WITH CEREBRAL PALSY. C.T. Leonard, T. Moritani, H. Hirschfeld and H. Forssberg. Depts. of Neurophysiology and Pediatrics, Karolinska Institute, Stockholm, Sweden and Physical Therapy Dept., Univ. of Montana, Missoula, MT 59812

Many neonatal neural projections are more diffuse than those in the adult animal. Neural exuberance and its retraction during post-natal life has been shown to exist in several species but not in humans. Damaging CNS structures during neonatal stages results in retention of some exuberant projections from functionally related but undamaged structures. The present study was undertaken to determine whether there was any evidence of neonatal neural exuberance and its retention following damage to the CNS in the human. Electromyographic (EMG) recordings were obtained from 6 lower extremity (LE) muscles following patellar and achilles tendon percussions in 20 normal children (ages 13 days to 16 years) and 14 children with spastic type cerebral palsy (CP) (ages 1 to 15 years). Previous studies have shown that normal adults and individuals with adult onset cerebral vascular accidents (CVA) exhibit electromyographic potentials only in the muscle being stimulated.

Results show that normal infants under the age of 2 often have reflex 'overflow' into adjacent muscles following a tendon tap. Children with CP, regardless of age, consistently exhibit reflex overflow into adjacent musculature. This reflex abnormality seen in children with CP, therefore, does not appear to be aberrant but rather a retention of an earlier phase of reflex development. The reflex overflow is most likely the result of either: a) group Ia or alpha motor neuron projection exuberance or b) the absence of a tonic inhibitory effects which, in the normal adult, may block reflex transmissions to other muscles. The fact that individuals with an adult-onset CVA do not exhibit reflex overflow provides support for the hypothesis that the overflow seen in normal infants and children with CP is the result of projection exuberance.

323.7

DALMATIAN CONGENITAL DEAFNESS: INCIDENCE AND PHENOTYPE CORRELATIONS. G.M. Strain, M.T. Kearney,* I.J. Gignac,* D.C. Levesque,* H.J. Nelson,* B.L. Tedford* and L.G. Remsen.* Vet. Physiol., Pharmacol., & Toxicol., Sch. Vet. Med., Louisiana State Univ., Baton Rouge, LA 70803.

Congenital deafness in the Dalmatian results from cochleo-saccular end organ degeneration. Deafness in most breeds is associated with the merle gene, which is absent in the Dalmatian. The apparent high incidence of unilateral and bilateral deafness in this breed suggests the potential for its use in studies of human hearing disorders, such as Waardenburg syndrome, while at the same time leading breeders to seek means for reducing its incidence. To assess deafness we performed brainstem auditory evoked potential (BAEP) measurements on 1,031 Dalmatians from three geographically separated testing locales (Louisiana/Texas, Arizona, northern California). Phenotypic markers were also assessed to determine possible markers correlated with deafness, including sex, hair coat color (black, liver, lemon, tricolor), completeness of eye rim and nose pigmentation, ear pigmentation, patch, spot size and marking, sire and dam BAEP status, and presence of iris and retina pigmentation. Combined data from all sites revealed 8.1% bilateral deafness (83/1031) and 21.6% unilateral deafness (223/1031), or an overall 29.7% incidence of hearing disorder. Significant ($P \leq 0.05$) correlations with deafness for the combined sites were seen for patch, sire and dam BAEP, iris pigment, and retina pigment. Dogs with patches were less likely to be deaf, while dogs without iris pigmentation (blue eyes, heterochromia iridis) or retinal pigmentation were more likely to be deaf. Unilateral sires or dams produced greater numbers of unilateral or deaf offspring. However, individual site significance levels differed for several phenotypic markers, showing no significance where grouped data were significant, suggesting the existence of multiple populations of deafness patterns. Insufficient data is currently available to determine if the genetic transmission of deafness follows the pattern of a dominant gene with incomplete penetrance seen with Waardenburg syndrome and blue-eyed deaf white cats.

323.8

INTERHEMISPHERIC MECHANISMS FOR VERTICAL MIDLINE STEREOPSIS: POSSIBLE SOURCES OF VISUAL CONFUSION IN DYSLEXIA. K.Brady. Dept. Psyc. Geo. Wash. U., Wash.,D.C. 20052.

Two mechanisms may accomplish stereopsis on the vertical midline:(a) convergence of monocular images via striatal callosal connections (b) a narrower nasotemporal overlap zone of intermingled ipsi- and contralaterally projecting fibers. These 2 pathways were compared in dyslexic and control adults using Poffenberger simple manual key-press RT to 50 ms light flashes at .5, 2, or 10^0 to right or left of fixation. The difference between crossed VF/hand RT, which requires callosal transfer of the stimulus, and uncrossed RT is considered a measure of interhemispheric transmission time. Abolition of the crossed-uncrossed difference (CUD) is interpreted as evidence that input is available to both hemispheres simultaneously. Controls demonstrated a CUD at all eccentricities consistent with previous literature. Dyslexics, however, showed no CUD at $.5^0$, consistent with the hypothesis of functional nasotemporal overlap. Retention of CUD at $.5^0$ in controls may reflect either absence or inhibition of overlap. At 2^0 dyslexics had both faster crossed and slower uncrossed RTs (after correction for overall slower RT). Net abolition of CUD at 2^0 may reflect group differences in the developmental processes of interhemispheric "pruning" and intrahemispheric proliferation of primary callosal fibers. CUD at 10^0 did not differ between groups.

323.9

QUANTITATIVE NEUROANATOMIC ANALYSES OF CEREBRAL CORTEX IN RHESUS MONKEYS FROM DIFFERENT REARING CONDITIONS. J.H. Morrison, P.R. Hof, W. Janssen*, J.L. Bassett[1], S.L. Foote[1], G.W. Kraemer[2]*, and W.T. McKinney[2]. Fishberg Res Ctr and Dept of Geriatrics, Mt Sinai Sch Med, New York, NY 10029, [1]Dept of Psychiatry, UCSD, La Jolla, CA 92093, [2]Dept of Psychiatry, Univ of Wisconsin Sch Med, Madison, WI 53792.

Behavioral studies have demonstrated that early maternal and peer deprivation causes long-lasting psychopathology in monkeys. The neuroanatomic substrates of this syndrome have not been elucidated. We have initiated a detailed anatomic analysis of several chemically-defined systems within the cortex of monkeys reared either in isolation (I) or a nuclear family (NF). Preliminary results with antisera to neurofilament protein (SMI32) and Ca^{2+}-binding proteins suggest that the density of SMI32-ir pyramidal cells and putatively GABAergic interneurons in several cortical areas is equivalent in the I and NF subjects. In contrast, initial counts of dopaminergic (DA) and serotonergic (5HT) varicosities by conventional microscopy suggested that dramatic differences existed between I and NF subjects in monoamine innervation density of cingulate, prefrontal and entorhinal cortex. However, more precise varicosity density measurements using a confocal laser scanning microscope demonstrated that the I subjects had a lower density of both DA and 5HT varicosities, but the differences were less dramatic than suggested by the conventional analysis. Further studies are in progress with the confocal microscope to determine the degree to which these differences are biologically and statistically significant. In addition, the analysis will be expanded to the amygdala and hypothalamus. Supported by the MacArthur Foundation.

323.10

A DEVELOPMENTAL MODEL OF CHOLINERGIC HYPOFUNCTION. T.J. Walsh, R.W. Stackman and G. Woertwein*. Rutgers University, Department of Psychology, New Brunswick, NJ 08903.

The cholinergic innervation of the hippocampus (HPC) and the cortex (CX) appears to be an essential neurobiological substrate of memory processes. This neurotransmitter system is susceptible to a wide variety of insults during the early post-natal period. For example anoxia, alterations in carbohydrate metabolism or hormonal status, vitamin deficiencies, and exposure to heavy metals, all produce impairments in the maturation or function of the cholinergic system as well as a persistent mental retardation. In previous studies we have used AF64A and colchicine to develop animal models of dementia and cholinergic hypofunction in adult rats (Chrobak et al., *Brain Res.*, 1988; Emerich and Walsh, *Brain Res.* in press). We are now evaluating whether these compounds will be useful tools in developmental studies of the cholinergic system and its behavioral properties. The present study examined the behavioral and neurochemical effects induced by intraventricular injection of either 175ng = 1.5nmoles/1µl of AF64A, 3.5µg/1µl of colchicine, or 1µl of artificial CSF into 21 day old weanling rats. Neither treatment altered body weight or the growth curve at any time point following surgery. Both treatments produced a 25-60% increase in locomotor activity that peaked 7 days following surgery and resolved by 45 days after surgery. Both groups were also impaired in the aquisition and retention of a spatial memory task in the Morris water maze. AF64A decreased ChAT activity in the HPC and frontal CX by approximately 40% while colchicine decreased ChAT activity only in the HPC by 16%. These initial studies suggest that AF64A and colchicine might be useful to produce developmental models of cholinergic hypofunction that will help to elucidate the biology of memory and memory disorders.

323.11

EARLY PRENATAL EXPOSURE TO SINGLE DOSE ETHANOL ALTERS OPEN FIELD ACTIVITY IN C57 MICE. R. Dumas*, A. Rabe, E. Sersen*, S. Delia* and A. Lidsky. NYS Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314

Our previous work has shown that early prenatal exposure to a single maternal oral dose of ethanol has resulted in craniofacial and brain malformations in the mouse fetus. These resemble those found in the fetal alcohol syndrome (FAS) child. Since functional impairment after early alcohol exposure has been reported in the absence of the typical FAS stigmata, our recent work has been concerned with extension of our mouse model to include the postnatal development of pups from alcohol treated dams that appear normal at birth. As an indicator of functioning, activity levels were measured in an automated open field apparatus at different ages in 3 groups of mice. At weaning (days 23 thru 25) we tested one male and one female from each of 60 litters: 17 untreated (C), 20 ethanol-exposed (E) and 23 isocaloric-dextrose-fed (D). A different pair of offspring from each of the litters was tested at a later age (day 37). Preliminary analysis of the data shows that the E mice spent significantly less time in ambulation and travelled a shorter distance than the C animals, but they reared more often. The D animals were between the C and E groups; they did not differ significantly from either. The lack of hyperactivity is in contrast to the reported hyperactivity in human FAS. This may reflect a species difference. (Supported in part by NIAAA grant 1R01-AA07060.)

323.12

MUSCARINIC CHOLINERGIC RECEPTOR CHANGES IN PRENATALLY INDUCED MICRENCEPHALIC RAT BRAIN. M.H. Lee, E.Alter*, A.Rabe, T. Lidsky and S.P. Banerjee. NY State Institute for Basic Research in Developmental Disabilities, Staten Island, NY 10314; Dept. of Pharmacology, CUNY Sch of Med, New York, NY 10031

The rat with methylazoxymethanol acetate-induced micrencephaly is a useful animal model of congenital brain defects. Born with severe forebrain hypoplasia, it consistently displays hyperactivity and impaired maze learning. Neurochemical bases for such behavioral changes have not been established. A recent study in our laboratory indicated hypersensitivity of the micrencephalic (M) rat to atropine: As compared to normal (C) rats, the magnitude of deficits in learning the Morris water maze increased in M rats as a function of dose (0, 10, 50 mg/kg), suggesting that the impaired learning and memory may be related to changes in the number and/or function of muscarinic cholinergic receptors (MCR). In order to further elucidate this potential relationship, we measured the density and apparent affinity of MCR in the cerebral cortex and basal ganglia of M and C rats, using [^{3}H]quinuclidinyl benzilate. In the M cortex, the densities of MCR binding sites decreased by 35%, while the affinity increased by about 50%. In contrast, there was a significant increase in the binding of QNB to MCR in the basal ganglia. Such region-specific changes in MCR may provide neurochemical explanation for the behavioral disorders of the M rats.

323.13

TRANSPLACENTALLY INDUCED MICRENCEPHALY HAS NO EFFECT ON TEMPORAL REGULATION OF BEHAVIOR. A.Rabe, M.Lee, and A. Heaney. NY State Institute for Basic Research in Developmental Disabilities, Staten Island NY 10314.

The micrencephalic rat has been used as a model for congenital brain defects as well as generalized cognitive impairment. Although it has been conclusively shown that it is hyperactive and poor at learning mazes, the nature of its defect(s) still remains to be characterized. We therefore studied its behavior on tasks that have rarely been used with this model. Severe cerebral hypoplasia was induced in all offspring (M group) by injecting the pregnant dam with 30mg/kg of methylazoxymethanol on GD 14. The controls (NC group) were from dams injected with saline. As adults, all offspring (N=62) were tested on 4 tasks with the following results: (1) Acquisition and retention of a step-down passive avoidance response; 3 baseline trials were followed by one footshock and retention tests 1 min, 1, 3, 7 d later. The M rats showed less avoidance at 1 min (36% vs 70%, $p<.025$) and at 1 d (26% vs 50%, $p<.05$). (2) Spontaneous alternation in a T-maze, one pair of trials daily for 3 d. The M rats alternated less (66% vs 81%, $p<.025$). (3) FI schedule with a 60 s delay; (4) DRL schedule with a 20 s delay. Training on both was for water, 30 min/d for 15 d each, followed by 3 d of extinction. On both timing schedules, the M rats had normal response rates, interresponse times and distribution of responses; the number of extinction responses were also the same for both groups. This pattern of results fits the hypothesis that the deficit in micrencephalic rats is cognitive and manifests itself primarily on tasks with spatial components.

323.14

VITAMIN A-INDUCED NEURAL TUBE DEFECT WITH CHIARI II MALFORMATION IN MICE. S. Nakahara*, W. Goossens*, R.G. Higbee, T. Inagaki* D.G. McLone and P.A. Knepper. Division of Neurosurgery, Children's Memorial Hospital and Northwestern University Medical School, Chicago, IL 60614.

Vitamin A induces phenotypic and biochemical alterations in vivo. In this study, we examined the effects of vitamin A on the process of neurulation and determined whether a neural tube defect (NTD) was associated with herniation of the contents of the posterior fossa, a Chiari II malformation. Pregnant C-57 BL/6J mice were given IP injections of 5000 IU of vitamin A at the start (gestation day 8.0 and 8.5 [group A]) and during the process of neurulation (day 8.5 and 9.0 [group B]). On gestation day 14 and 16, embryos were processed for SEM and for fluorescence microscopy using FITC-lectins.

The early administration of vitamin A (group A) resulted in a high percentage of NTD, a primary neurulation defect and intrauterine resorption. The later administration (group B) resulted in a low percentage of NTD (<5%) and a high percentage of tail defects (>80%), a secondary neurulation defect. In both groups, the presence of a caudal NTD was always associated a Chiari II. In embryos with only a tail defect, there was no Chiari II. These results indicate that vitamin A-induced NTDs in mice relate to the time of administration and a Chiari II occurred only if there is a primary neurulation defect. (Supported in part by the Kiwanis International)

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SYMPOSIUM. NINDS: FORTY YEARS OF PROGRESS. P. Goldman-Rakic, Yale Univ. Sch. of Med. (Chairperson); T.H. Bullock, UCSD; R.E. Burke, NIH; R.R. Llinas, New York Univ. Med. Ctr.; J. Wasmuth*, Univ. of California, Irvine; M.E. Raichle, Washington Univ.

The National Institute for Neurological Diseases and Stroke has, under its various former titles, been the major supporter of Neuroscience in the United States for four decades. From it have sprung the several additional NIH Institutes whose function is to promote research in specialized areas of Neuroscience. This Symposium celebrated the fortieth anniversary of NINDS by highlighting intra- and extramural achievements in the forty years of its existence. The Symposium focuses on selected areas of currently high topical interest including the contributions of basic research on simple nervous systems to the understanding of higher integrative functions, the nature of neuromuscular integration by spinal cord mechanisms, the fundamental membrane properties of central neurons, the molecular genetic basis of inherited neurological disease, and the application of modern imaging methods to CNS research. These are all areas in which NINDS has provided the incentive and critical support for major contributions to fundamental knowledge and to the understanding of neurological disease.

328.1

EXPRESSION OF THE c-HARVEY *ras* ONCOGENE ALTERS THE DIFFERENTIATED PHENOTYPE OF THE NEUROSECRETORY CELL LINE AtT20. N.C.Birnberg, L.M.Hemmick, R.E.Flamm, L.K.Kaczmarek, and P.J.Stork*†. Dept. of Pharmacology, Yale University Sch. of Med., New Haven, CT 06510 & †Vollum Institute, Oregon Health Sci. Univ., Portland, OR 97201.

Expression of the activated c-Harvey *ras* oncogene in most cell types studied results in increased cell proliferation and a tumorigenic phenotype. In neuroendocrine cell lines, however, the response to *ras* oncogene expression appears to be more closely related to a terminal differentiation event. In this study we have investigated the consequences of introducing the human EJ-*ras* oncogene into the mouse anterior pituitary cell line AtT20. We previously reported that the tetrodotoxin-resistant component of the voltage-dependent sodium current in normal AtT20 cells is extinguished following *ras* oncogene transformation (Flamm, R.E. et al., European Journal of Physiology, (1990) in press). We have found that expression of the *ras* oncogene induces a number of profound changes in cell morphology, hormone secretion and biosynthesis, and electrophysiology. We report here that secretion of the pituitary hormone ACTH and transcription of the gene encoding its precursor polypeptide, proopiomelanocortin, are markedly downregulated in EJ-*ras* transformed clones. Further, *ras* oncogene expression in AtT20 cells induces a change in the shape and duration of evoked action potentials and a significant increase in the density of voltage-dependent potassium channels. The cell line AtT20 has been a model system for the study of hormone biosynthesis and secretion, posttranslational processing and electrophysiology in a neuroendocrine cell line. The alteration of the differentiated phenotype of AtT20 cells following *ras* oncogene transformation makes this a valuable system for the study of the regulation of differentiation by *ras* oncogenes in a neurosecretory cell type.

328.2

A NOVEL ENZYME FROM NEUROINTERMEDIATE PITUITARY CATALYZES DEALKYLATION OF α-HYDROXYGLYCINE DERIVATIVES, THEREBY FUNCTIONING SEQUENTIALLY WITH PEPTIDYLGLYCINE α-AMIDATING MONOOXYGENASE IN PEPTIDE AMIDATION. Andreas G. Katopodis*, Dongsheng Ping* and Sheldon W. May. School of Chemistry and Biochemistry, Georgia Institute of Technology, Atlanta, GA 30332.

We report the isolation of a novel enzyme from bovine neurointermediate pituitary which catalyzes the conversion of α-hydroxybenzoylglycine to benzamide. This enzyme, termed HGAD (α-HydroxyGlycine Amidating Dealkylase), is a soluble protein with an apparent molecular mass of 47 kD, and no apparent cofactor requirement. Addition of HGAD to purified neurointermediate pituitary PAM (Peptidylglycine α-Amidating Monooxygenase EC 1.14.17.3) increases the rate of formation of amide products by an order of magnitude. Sequential additions of PAM and HGAD gave results consistent with PAM first catalyzing the formation of an intermediate which is subsequently, in a separate reaction, converted by HGAD to the final amide product. Purified neurointermediate PAM incubated with N-benzoylglycine produces a HPLC peak which co-elutes with authentic α-hydroxybenzoylglycine. Experiments with *trans*-benzoylacrylic acid and 4-phenyl-3-butenoic acid which are turnover-dependent inactivators of PAM, demonstrate that HGAD is not inactivation of PAM, and, correspondingly, that HGAD activity is not affected by inactivators of PAM. As expected, HGAD has no effect on the rate of PAM catalyzed sulfoxidation of (4-nitrobenzyl)thioacetic acid, where a reaction analogous to that occurring during amidation of glycine-extended substrates is not possible. On the basis of these results, we propose that peptide C-terminal amidation is a two step process, with PAM first catalyzing the conversion of a glycine extended peptide to the a-hydroxyglycine derivative, which is in turn converted to the final amide product by HGAD.

328.3

SOMATOMAMMOTROPIC TUMOR CELLS POSSESS A NEUROENDOCRINE-TYPE ACTIVE TRANSPORT SYSTEM FOR ASCORBIC ACID. E.I. Cullen, W. Huo *, and F. Mesaros *. Dept. of Pharmacology, New York Medical College, Valhalla, NY 10595.

Ascorbic acid (AH2) is a co-substrate in the amidation of peptides catalyzed by peptidyl-glycine α-amidating monooxygenase (PAM). Cells expressing PAM often possess a Na-dependent active transport system for AH2. PAM is expressed in rat somatomammotropic tumor cells (GH cells), and the cells can amidate peptides derived from transfected POMC cDNA (Cullen & Mains,'89 Endo 125: 1774). To determine if GH cells also possess an active transport system for AH2, they were incubated in 50 μM [14C]AH2. Cellular content of AH2 reached levels of 2.9 mM after 6 hr (using experimentally determined cell volumes). Substitution of choline chloride for NaCl diminished uptake by more than 90%. The initial rate of AH2 uptake was saturable and displayed a Km of 35 ± 3 μM and a Vmax of 1.4 ± 0.2 nmol/10(6) cell/hr. Efflux of AH2 was slow; the principle kinetic component was linear with time and represented a loss of 6%/hr of the initial cellular content. Growth for 7 days in 5 μM cortisol diminished cellular AH2 uptake and had the expected effects on GH and PRL (NIDDK RIA materials from Dr. Raiti, NHPP, U.Md.). These results reflect a previously unrecognized capability of GH cells, and support the hypothesis that the cells can amidate peptides. Supported by the Pharmaceutical Manuf. Assn. Found'n, Castle-Krob Res. Endowment, and NIH BRSG RR5398.

328.4

EVIDENCE FOR THE ENZYMATIC DEGRADATION OF CRF IN CORTEX, HYPOTHALAMUS AND PLASMA. J.C. Ritchie, C.B.Nemeroff, D. Knight, M.J. Owens. Dept. of Psychiatry and Pharmacology, Duke Univ. Durham, NC 27710.

Little is known concerning the ultimate fate of Corticotropin Releasing Factor (CRF) in the CNS, neuroendocrine complex, or the periphery. In order to investigate this area we have established and characterized a 2 step gradient HPLC scheme for the fractionation of rat/human CRF_{1-41}, its available synthetic fragments, analogues, and other neuropeptides. In this system ^{125}I-tyr^0-CRF elutes as a single peak. In an attempt to determine the degradation products of CRF_{1-41} in rat cortex (prefrontal/somatosensory), hypothalamus, and plasma both soluble and 10,000 x G particulate extracts were prepared. Extracts were incubated at 37°C for 120 mins. with iodinated tyr^0-CRF and excess noniodinated r/h CRF_{1-41}. Fractionation of the soluble enzyme digests showed a diminution of the CRF peak and 2 early eluting peaks. The particulate extracts gave a similar but diminished pattern of activity. The plasma preps exhibited a decrease in the CRF but no early eluting peaks.

These results indicate a similar pattern of CRF degradation in both the CNS and the neuroendocrine complex. Additionally, the enzyme/s responsible for CRF degradation appear to be of a soluble nature.

This work supported by NIMH grants Nos. MH42088 and MH-39393.

328.5

DISCOVERY AND COMPLETE CHARACTERIZATION OF A NOVEL PYROGLUTAMYL PEPTIDE DERIVED FROM CHROMOGRANIN B IN BOVINE ADRENOMEDULLARY CHROMAFFIN VESICLES. T. Flanagan, L. Taylor, Q.H. Viveros and E.J. Diliberto, Jr. Div. Medicinal Biochem. and Organic Chem., Wellcome Res. Lab., 3030 Cornwallis Road, Research Triangle Park, NC 27709.

We recently demonstrated the exclusive localization of a glutaminyl cyclase to the chromaffin vesicle of the bovine adrenal medulla. Thus, pyroglutamyl peptides are likely to be found within this subcellular compartment. While no pyroglutamyl peptide has yet been isolated and fully characterized from the bovine adrenal medulla, the presence of neurotensin immunoreactivity has been reported. Therefore, we developed an assay to isolate and characterize pyroglutamyl peptides in extracts of chromaffin vesicles. This assay consists of a spectrometric comparison of the mass of peptide molecular ions before and after the specific removal of pyroglutamyl residues with pyroglutamate aminopeptidase. We used this assay system to guide extraction, chromatographic isolation and the complete structural characterization of a novel pyroglutamyl secretory peptide from the bovine adrenal medulla (BAM-1745). Oligonucleotide probes deduced from backtranslation of BAM-1745 were used in a separate study to identify the peptide precursor as chromogranin B.

BAM-1745 is more abundant than enkephalins within the bovine adrenal medulla, and thus is a major secretory peptide. A Comparison of bovine, human, sheep, and rat chromogranin B sequence data, and the direct isolation and characterization of BAM-1745 homologs from bovine, human, sheep, goat, pony, and pig adrenal tissue indicates that this peptide sequence is both well conserved and also consistently processed into a pyroglutamyl adrenal secretory peptide among mammalian species. BAM-1745 is likely to serve a biological function, however, that function is currently unknown.

328.6

LIGHT AND ELECTRON MICROSCOPIC LOCALIZATION OF THE NEUROTENSIN DEGRADING ENZYME, NEUTRAL METALLOPEPTIDASE 24-16 IN RAT BRAIN. J. Woulfe, F. Checler* and A. Beaudet. Montreal Neurol. Inst., McGill Univ., Montreal, Que. H3A 2B4.

The neutral metallopeptidase 24-16 (NM 24-16) is an endopeptidase implicated in the biological inactivation of neurotensin (NT) through cleavage between pro and tyr in positions 10 and 11. In the present study, the regional and cellular distribution of NM 24-16 was examined by immunocytochemistry in rat CNS using a highly specific polyclonal antiserum generated against the purified enzyme. On light microscopic examination, immunoreactivity for NM 24-16 was ubiquitously distributed throughout all regions of the CNS. Areas of prominent labeling corresponded, in comparison with adjacent nissl-stained sections, to those of enriched neuronal density. On electron microscopic examination of ultrathin sections through the ventral tegmental area and substantia nigra, two areas which receive a substantial NT afferent input and exhibit high densities of NT binding sites, immunoreactivity for the peptidase was associated with both neurons and glia. In neurons, the labeling was restricted primarily to the plasma membrane and to multivesicular bodies. In glia, immunoreactivity for NM 24-16 was identified throughout the cytoplasm of astrocytic cell bodies and processes. The latter surrounded synapses established by axon terminals on dendritic shafts. These results provide anatomical support for NM 24-16's role in the endogenous metabolism and functional inactivation of NT.

328.7

EVIDENCE FOR REGIONAL SPECIFICITY OF NEUROTENSIN METABOLISM BY INTACT BRAIN SLICES. T.J. Gillespie* and T.P. Davis. Dept. of Pharmacology, Univ. of Arizona, Coll. Med Tucson, AZ 85724.

Our laboratory previously examined neurotensin (NT) metabolism by p SPM. This study examines regional differences in NT metabolism by rat brain micro slices, and describes specific peptidases involved. Male S.D. rats (280-360g) were sacrificed. Brains were sliced into 2mm coronal slabs, and caudate putamen (CPu) and nucleus accumbens (Acb) were micropunched (2mm). Hypothalamus (H) and hippocampus (Hi) were also punched. Slices (240μ) were incubated for 0 or 120 min. with 100μM NT in the absence or presence of specific peptidase inhibitors. After HPLC analysis NT fragments were collected and identified by amino acid analysis. NT loss after 120 min. incubation was Acb 50% ± 1.7, CPu 47% ± 2.0, H 45% ± 3.1, and Hi 38% ± 2.7, with significant ($p < .05$) differences between Hi and Acb or CPu. In the presence of 20μM phosphoramidon (PAM) and 100μM CPP-Ala-Ala-Phe-pAB, neurotensin did not significantly decrease in any region studied indicating that most of NT metabolism is due to neutral endopeptidase 3.4.24.11, and metalloendopeptidases including 3.4.24.15. NT loss was decreased 53% in CPu after 20μM PAM only ($p < .01$). In Hi, NT loss decreased 11% with PAM only. Therefore, 24.11 activity is much higher in CPu than Hi. Captopril (20μM) did not significantly change % loss of NT in CPu or Hi, however, a shift in NT fragment levels was evident. NT-(1-10) increased from 0.50 to 3.85 nmoles ($p < .01$) in CPu and NT-(1-8) decreased in both regions. This indicates that NT-(1-10) serves as a substrate for angiotensin converting enzyme (ACE), with ACE much higher in CPu than Hi. Since brain slices retain a greater degree of morphology and metabolic properties than homogenates, these data provide evidence for a regional specificity of NT metabolism dependent on the expression of specific endopeptidases. (Supp. NIH-MH 42600, DK 36289 and DA 06284).

328.8

CHOLECYSTOKININ ACTIVATION : EVIDENCE FOR AN ORDERED REACTION MECHANISM FOR THE TYROSYL SULFOTRANSFERASE RESPONSIBLE FOR THE PEPTIDE SULFATION. F. Vargas and O. Frerot. Unité de Neurobiologie et Pharmacologie, Centre Paul Broca de l'INSERM, 75014 Paris, France.

The kinetics of the forward tyrosylsulfotransferase reaction were examined using an assay based on the $^{35}SO_4$ transfer from 3'-phosphoadenosine 5'-phospho[^{35}S]sulfate ([^{35}S]PAPS) to tyrosyl residues of the non sulfated cholecystokinin derivate, BocCCK-8(ns). Tyrosyl sulfotransferase present in the microsomal membranes from rat cerebral cortex, was used for these studies. Initial velocity measurements performed over a wide range of PAPS and BocCCK-8(ns) concentrations, indicated that, the reaction follows an ordered mechanistic pathway. Thus, K_M value determined for BocCCK-8(ns) was 160 ± 18 μM, and that for [^{35}S]PAPS 0.15 ± 0.03 μM. 3'-Phosphoadenosine 5'-phosphate (3'-PAP) was found to be a strong product inhibitor with a Ki = 0.30 ± 0.02 μM. Adenosine 5'-phosphosulfate (APS) behaved as a dead-end inhibitor with a Ki = 3.0 ± 0.3 μM. ATP inhibited competitively the reaction when PAPS was the varied substrate with a Ki = 3.6 ± 0.5 μM. The results of product and substrate inhibition studies and the patterns of dead end inhibition obtained with APS are best fit by a steady state ordered Bi-Bi kinetic mechanism with PAPS and BocCCK-8(ns) as substrates. These results are of particular interest in view of the elucidation of sulfation reaction processes in the Golgi apparatus and Golgi membrane permeability.

328.9

Cloning of cDNAs encoding amphibian bombesin: evidence for the relationship between bombesin and gastrin-releasing peptide (GRP). Eliot R. Spindel, Bradford W. Gibson*, Joseph R. Reeve, Jr.* and Michele Kelly*. Div of Neuroscience, Oregon Regional Primate Res Ctr, Beaverton, OR 97006, Dept of Pharmaceutical Chemistry, UCSF, San Francisco, CA 94143 and Ctr for Ulcer Res and Education, VA Wadsworth and UCLA School of Med, CA 90073

Bombesin is a tetradecapeptide originally isolated from frog skin; its mammalian homologue is the 27-amino acid peptide, GRP. Bombesin and GRP have important functions as neurotransmitters, growth factors and as paracrine regulators of GI function. cDNAs encoding GRP have been cloned from diverse species, but no cDNAs encoding amphibian bombesin have previously been characterized. Mass spectrometry was performed to demonstrate the existence of authentic bombesin in the skin of *Bombina orientalis*. Next a cDNA library was prepared from *B. orientaiis* skin and mixed oligonucleotide probes were used to isolate cDNAs encoding bombesin. Sequence analysis revealed a 119 amino acid prohormone with two basic amino acids flanking the C-terminus of bombesin. The N-terminus of bombesin is not flanked by basic amino acids but is flanked by a chymotryptic-like cleavage site. RNA blot analysis demonstrated similarly sized bombesin mRNAs in frog skin, brain and stomach. Polymerase chain reaction and DNA sequence analysis demonstrated that the same bombesin prohormone is encoded in both skin and gut. Prohormone processing however differs between skin and gut. Chromatography of skin extracts showed only bombesin while chromatography of gut showed two peaks of bombesin immunoreactivity, one consistent in size with bombesin, and one closer in size to mammalian GRP. Thus the same bombesin prohormone is processed in skin to bombesin, but in stomach is processed to both a peptide similar in size to bombesin and to a peptide similar in size to mammalian GRP.

328.10

INFLUENCE OF NMDA RECEPTOR BLOCKADE ON THE LEVEL OF PREPROTACHYKININ AND PREPROENKEPHALIN mRNA IN THE RAT BASAL GANGLIA. D.L. Somers* and R.M. Beckstead. Dept. of Anatomy and Cell Biology, Medical University of South Carolina, Charleston, SC 29425

The influence of chronic (7 day) icv administration of APV, an NMDA receptor antagonist, on the level of striatal preprotachykinin (PPT) and preproenkephalin (PPE) mRNA was examined by in situ hybridization of [^{35}S]cDNA probes followed by autoradiography. Five rats received the drug via cannulas attached to osmotic minipumps loaded with 40mM APV in artificial CSF. The levels of message in the caudatoputamen on the side of infusion in the drug recipient rats were compared to those measured in 5 control rats that received CSF alone. Optical density measurements were taken from film autoradiograms of frontal tissue sections through the rostral, middle and caudal thirds of the caudatoputamen. The level of PPT mRNA was reduced by 34% in the rostral third, 22% in the middle third, and not at all in the caudal third of the striatum ($p < 0.025$). The level of PPE mRNA was reduced by 26% ($p < 0.01$) only in the rostral third of the striatum. Further analysis of the rostral levels revealed a gradient of PPT mRNA reduction ranging from 42% near the ventricle to 25% in the ventrolateral quadrant ($p < 0.025$). PPE mRNA showed a similar gradient of reduction with a maximum of 38% nearest the ventricle ($p < 0.025$). Experiments with the non-NMDA receptor antagonist, DNQX, revealed no such reduction in the level of either peptide message. The present findings are consistent with earlier studies of peptide message changes following the depletion of synaptosomal ASP and GLU levels, and provide further evidence for the role of excitatory amino acids as signals for the regulation of neuroactive peptide gene transcription. The data indicate further that the NMDA receptor subtype mediates this effect. Supported by NIH Grant NS24971.

328.11

EXPRESSION OF PROENKEPHALIN A AND PRODYNORPHIN GENES IN RAT BRAIN CELL CULTURES. R. Simantov and V. Höllt*. Dept. Mol. Gen. Weizmann Inst., Israel and NIDA Addiction Research Center, Baltimore, MD and Dept. Phy. U. of Munich, Germany.

The regulation of proenkephalin A (PEA) gene was studied extensively in adrenal chromaffin cells, but little is known about the control of this gene in nerve cells. Aggregating rat brain cell cultrues were used therefore to analyze the mechanism of PEA mRNA expression and translation to enkephalin. Depolarization of these cultures with potassium chloride or veratridine increase PEA mRNA levels in a time-dependent fashion, with a maximal effect of six folds. The same treatments, however, did not increase prodynorphin expression. PEA mRNA expression was also increased upon treatment with 8-Br-cAMP, phorbol 12-myristate-13-acetate (TPA) or the glucocorticoid hormone dexamethasone. Yet, neither of these agents increase the amount of enkephalin. The potent opioid agonist etorphine and the antagonist naltrexone did not alter PEA gene expression, suggestion that there is no feedback control of opioids on enkephalin biosynthesis. The aggregating brain cell cultures are useful therefore to investigate the mechanisms that regulate PEA and prodynorphin mRNA expression in neurons.

328.12

REGULATION OF CCK mRNA IN THE DOPAMINERGIC NIGRO-STRIATAL SYSTEM. X.Z. Ding*, E. Costa and I. Mocchetti^. Fidia-Georgetown Institute for the Neurosciences and ^Department of Anatomy and Cell Biology, Georgetown University, 3900 Reservoir Rd., N.W., Washington, D.C., 20007.

Although the coexistence of dopamine (DA) and cholecystokinin (CCK) in neurons of the substantia nigra (SN) is well documented, little is known about the interaction between these two putative neurotransmitters at the postsynaptic receptors. To shed some light on this interaction, we tested the hypothesis that DA could regulate CCK synthesis in the nigro-striatal system. Northern blot hybridization analysis was used to measure CCK mRNA, as an index of CCK synthesis. The induced release of DA evoked in rats by a single injection of cocaine (15 mg/kg s.c.), elicited, 8 hours later, a 50% increase in CCK mRNA content in striatum but not in SN. Seven daily injection of cocaine induced (3 fold) CCK mRNA in both SN and striatum but not in other brain regions. To gain insight of the dopaminergic nature of cocaine action we used haloperidol, a DA receptor blocker. Haloperidol, which decreased CCK mRNA by 50% in striatum, blocked the increase of striatal CCK mRNA elicited by cocaine. However, unlike cocaine, the effect of haloperidol was confined to the striatum suggesting that in striatum dopamine might regulate tonically CCK synthesis through a dopaminergic receptor mediated mechanism which is different from that operative in the SN. The mechanism of this interaction is currently under investigation in primary cultures of striatal and mesencephalic neurons.

329.1

G-PROTEIN REGULATION OF ω–CONOTOXIN BINDING SITES ON A NEURONAL CELL LINE. S. Bergamaschi*~, S. Govoni*, S. Del Monaco*^, F. Battaini~ and M. Parenti^, Dept. Pharmacobiol., Univ. Bari; ~Dept. Exp. Med. and Biochem. Sci., Univ. Rome; ^ Dept. Pharmacol., Univ. Milan, Italy.

G-proteins have been shown to modulate calcium currents (Hescheler , J., Nature, 325: 445, 1987) and the conformation of the binding site for dihydropyridines both in rat brain synaptic membranes and cultured cells (Bergamaschi S., Eur. Neurol. 30S2: 16, 1990). The present study investigates whether G proteins modulate the recognition site for ω-Conotoxin GVIA (CgTx), a peptide labelling high voltage-activated calcium channels. Both undifferentiated and differentiated (1 mM dibutyryl cAMP) neuroblastoma x glioma NG 108-15 cells were used. The specific binding of [^{125}I]-CgTx is higher in differentiated cells (undiff.: Bmax 2.3±1.2 fmol/mg prot., Kd 9±2.2 pM; diff.: Bmax 16.2±3.8 fmol/mg prot., Kd 16±2.8 pM). Addition of 10 μM GMP-PNP, a stable GTP analogue, to membranes prepared from differentiated cells decreases by 30-40% the binding of CgTx.This effect can also be observed in undifferentiated cells. Other nucleotides such as ATP and GDP-β-S were inactive. Pretreatment of the cells with Pertussis Toxin (PTx; 100 ng/ml medium for 20 hours) greatly reduces the effect of GMP-PNP (control: -32%; PTx: -14%). The results suggest that G protein activation decreases the labelling of calcium channels by CgTx, possibly through a conformational change in the binding site.

329.2

MUSCARINIC AND α-ADRENERGIC SUPPRESSIONS OF CALCIUM CURRENT (I_{Ca}) ARE BLOCKED ONLY PARTIALLY BY PERTUSSIS TOXIN IN RAT SYMPATHETIC NEURONS. L. Bernheim*, D.J. Beech* and B. Hille. Univ. of Washington Sch. of Med., Seattle WA 98195.

Neurons were acutely dissociated from adult rat superior cervical ganglia and current through voltage-gated Ca channels recorded under whole-cell voltage-clamp (CsCl pipette solution containing 0.1 mM BAPTA). In the bath was a 5 mM $CaCl_2$ Ringer solution with 10 mM TEA, 1 μM propranolol and 0.5 μM TTX. 100 μM oxotremorine-M (oxo-M) or 10 μM noradrenaline (NA) suppressed I_{Ca} by 83±3% (n=12) and 39±7% (n=10) respectively (mean±SEM). Increasing the pipette BAPTA to 20 mM reduced the suppression of I_{Ca} by oxo-M to 45±5% (n=7), but left unaffected the response to NA (37±10%, n=6). By measuring $[Ca]_i$ with fura-2 we showed that this effect of BAPTA was unrelated to Ca-chelating. Pertussis toxin (PTX) treatment divided both oxo-M and NA suppressions of I_{Ca} into two components. After neurons had been incubated with 50 ng/ml of PTX, either active (test) or boiled for 10 min (control), for 19-31 hrs at 37°C, oxo-M and NA suppressions of I_{Ca} fell from 89±3% to 47±5% and from 50±7% to 23±2% respectively. 9 PTX-treated and 9 control cells were tested for each agonist. Similar results were found using 150 ng/ml (n=8) or 500 ng/ml (n=19) PTX. Suppression of I_{Ca} by 0.5 μM somatostatin was assessed in the same cells. 50 ng/ml of PTX decreased the somatostatin response from 49±9% to 13±2%. (NIH NS08174, McKnight Research Award, Fond. Suisse Bourses Méd. Biol.)

329.3

ANALYSIS OF PRESYNAPTIC INHIBITION AND REDUCTION OF CALCIUM CURRENTS INDUCED BY BACLOFEN IN PYRAMIDAL AND NON-PYRAMIDAL NEURONS CULTURED FROM RAT HIPPOCAMPUS. K. P. Scholz, W. K. Scholz and R. J. Miller Dept. of Pharm. and Physiol. Univ. of Chicago, 60637.

The mechanisms underlying presynaptic inhibition produced by activation of GABAb receptors at excitatory and inhibitory synapses in the hippocampus were studied using whole-cell patch clamp techniques in cultured hippocampal neurons. We have characterized the differential developmental appearance of pyramidal neurons and GABA-ergic interneurons in the hippocampus and have taken advantage of these results to study the physiological properties of these different cell types. Baclofen inhibited two components of ICA in pyramidal neurons that showed different kinetics and differential sensitivity to nimodipine. The EC50 for this action was near 0.5 uM. This action was sensitive to block by phaclofen and 2-OH-saclofen. In addition, inhibition of ICA was enhanced and rendered irreversible by inclusion of GTP-γ-S in the pipette and abolished by pretreatment with PTX. In GABA-ergic interneurons, baclofen inhibited a single nimodipine insensitive ICA. Baclofen reduced both EPSCs and IPSCs by a presynaptic mechanism that was abolished by PTX pretreatment in both cases. Although 0.5 uM baclofen strongly inhibited ICA, effects on synaptic transmission required higher concentrations. Although both EPSCs and IPSCs were reduced by baclofen, EPSCs were sensitive to dihydropyridine agonists and antagonists whereas IPSCs were not. We are investigating the possibility that these synapses may use different calcium channels at the vesicle release site, and further examining the role these channels play in presynaptic inhibition induced by baclofen .

329.4

SINGLE POTASSIUM CHANNELS OF *HERMISSENDA* TYPE B PHOTORECEPTORS: SELECTIVE EFFECT OF PHORBOL-12-13-DIBUTYRATE (PDBU). R. Etcheberrigaray and D.L. Alkon. LMCN-NINDS, National Institutes of Health, Bethesda, MD 20892.

Conditioning-induced reduction of two macroscopic currents (I_A & I_C) of *Hermissenda* type B-photoreceptors can be mimicked by protein kinase C (PKC) activation (Alkon, D.L., Sci. Amer., 1989). At the level of single channels, we have recently described two different potassium channels, characterized by conductances of 65 pS (large) and 42 pS (medium), (R. Etcheberrigaray et al, submitted to Biophys. J.). In this study cell-attached (CA) and inside-out (IO) patch clamp modes were used to explore the effect of PDBU at the single channel level. Isolated eyes were incubated in PDBU 1μM for 30 to 90 min. before patch clamp experiments. Measurements of percentage open time (OP) in CA at resting potential showed a profound effect of PDBU on the large K-channel. OP decreased from 15.5 (control) to 5.0 (Mann-Whitney $p<0.005$). When a patch was ripped off a cell preincubated in PDBU, it remained almost completely silent. Occasional openings, nevertheless, allowed identification of the channel. When a patch was ripped off a cell preincubated in ASW channel activity remained very high (OP ≈ 80) . H-7 (50 μM) added before PDBU to the bath, prevented PDBU-mediated OP decrease of the large K-channel. CA and IO experiments revealed no differences in medium channel behavior after PDBU incubation (CA OP : control 16.0, SE 5.4, PDBU 12.6, SE 1.3; IO OP : control 48, SE 6.3, PDBU 57.8, SE 8.5) . H-7 plus PDBU also had no effect, and PDBU application to an IO patch was without effects. In summary, we have found selective effects of a PKC activator on a particular class of B-photoreceptor potassium channels. Preliminary evidence suggests that the same channels are regulated by classical conditioning.

329.5

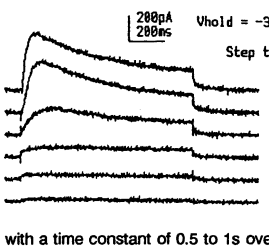
MODULATION OF CALCIUM AND POTASSIUM CHANNELS BY RAS ONCOGENE PROTEINS IN *HERMISSENDA* NEURONS. C. Collin, A.G. Papageorge' and D.L. Alkon. LMCN-NINDS and 'Laboratory of Cellular Oncology-NCI, NIH, Bethesda, MD 20892.

The early changes in excitability after iontophoretic injections of the proto-oncogenic (C) and oncogenic (V) forms of *ras* gene products, which are 20 KD G proteins, were studied on fully differentiated neurons (LP_1) of the snail *Hermissenda*, in two microelectrode voltage clamp experiments. C and V Harvey *ras* were expressed in E. Coli, purified and solubilized in a buffer containing (mM) EDTA, 1; $MgCl_2$, 5; Tris pH 7.8, 20. A third electrode containing 1 M KAc and 350 μg/ml of C and 150 μg/ml of V *ras* proteins was used for intracellular injections. Voltage clamp steps from V_H-60 to 20 mV under 0 Na^+ and 100 TEA ASW, elicited two voltage sensitive outward potassium currents (I_A and I_C) and a voltage sensitive inward current carried by Ca^{2+}. Ten minutes after V and 20 min. after C *ras* injections small increases in the inward currents were observed. After 40 to 60 min., progressive reductions of 15 to 30% in I_A and later I_C became clear. C *ras* but not V *ras* effects were reversible. Inward currents were isolated in other experiments using 3M $CsCl_2$ electrodes and (mM) 10 Ba^+ 5 4-AP and 100 TEA ASW. This sustained inward current, which activates at high thresholds and is blocked by 10 μM Nifedipine, was increased by 40% after *ras* injections. V *ras* effects were faster and more prolonged than C *ras*. Similar experiments on Type B photoreceptors showed no effects on Ca^{2+} currents, but significant reductions on both potassium currents. Heat inactivated *ras* injections had no effect. Finally, previous exposure to 10 μM Sphingosine prevented *ras* effects on potassium currents, but not Calcium currents in LP_1. This modulation of cell excitability by *ras*, and related G proteins (e.g. cp20, Nelson, T.J. et al., Science, 247:1470, 1990) may be a critical initial step for the cellular transformations of development, oncogenesis and memory storage.

329.6

FMRFamide REDUCES A DELAYED OUTWARD CURRENT IN LEECH HEART INTERNEURONS. T.W. Simon & R.L. Calabrese, Department of Biology, Emory University, Atlanta, GA 30322

FMRFamide can accelerate the leech heartbeat central pattern generator in concentrations as low as 10^{-9}M (Kuhlman, Li & Calabrese *J. Neurosci.* 5:2310-2317, 1985). In voltage clamp conditions, FMRFamide reversibly reduces a voltage dependent outward current, I_F, in leech heart interneurons. I_F activates at -20mV and appears fully activated at +10mV



The panel at the left shows I_F as a difference current in response to a range of depolarizing steps with and without 10^{-6}M FMRFamide in the bath. The saline contained in mM: 0 Na^+, 0 Ca^{++}, 1.8 Co^{++}. The activation time constant of I_F decreases from about 60 ms to 15 ms with depolarization. I_F inactivates with a time constant of 0.5 to 1s over this same voltage range. The steady state activation and inactivation levels of I_F are also voltage dependent. Pharmacologically, I_F is blocked by internal TEA and by 1 μM methohexital, a barbiturate, which blocks both I_A and the delayed I_{K+} in leech heart interneurons. We are currently engaged in experiments to specify further the kinetic and pharmacologic properties of I_F, and its ionic dependence.

329.7

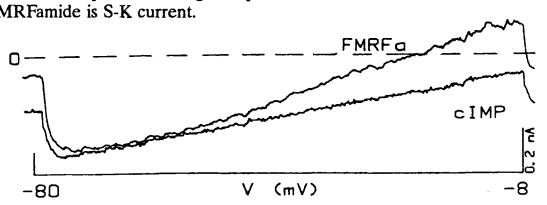
INTERPLAY OF 5-HT AND FMRFa CASCADES ON THE S-K+ CURRENT IN *APLYSIA* R.Shi and F.Belardetti. Dept. of Pharmacology, UT Southwestern, Dallas, TX 75235.

In *aplysia* mechanosensory cells (SNs) FMRFa has a dual action on the S-K+ channel: it increases its opening through the 12-lipoxygenase pathway of arachidonic acid (a.a.), and it "re-opens" channels closed by 5-HT or cAMP. We investigated the mechanism of the "re-opening" by voltage-clamping SNs at -35 mV and measuring the response to various agonists in the presence and absence of 5-HT (1-1000 nM). 1. Puff-applied a.a. (2-5 μM, 7 out of 9 experiments) or 12-HPETE (10-25 μM, 5/5) produced "re-opening", suggesting that a common pathway mediates both actions of FMRFa. 2. By constructing dose/response curves for each transmitter, we found that each transmitter *non-competitively* inhibits the other's action on the S-current. This rules out protein-phosphatase (PP) activation as a mechanism for "re-opening", unless PP is also inhibited by 5-HT. 3. High concentrations of 5-HT depressed also the response to a.a., indicating that this action is not primarily mediated by inhibition of the a.a. release. A 12-lipoxygenase metabolite might down-regulate a kinase, or alternatively act on the S-channel to make it a worse substrate for the kinase and/or a better substrate for PP. In the latter case, the channel might have a single receptor for a 12-lipoxygenase metabolite that mediates both effects of FMRFa, increased opening through a non-covalent change, and "re-opening" through de-phosphorylation.

329.8

FMRFamide and cGMP ACTIVATE INWARD CURRENT IN APLYSIA SENSORY NEURONS. N. Buttner & S.A. Siegelbaum. Ctr. for Neurobiol. & Behav., Columbia Univ., New York, NY 10032.

FMRFamide modulates several ionic currents in Aplysia sensory neurons, including Ca current, S-K current, and Ca-activated K current. We now report that FMRFamide also elicits a Na-dependent inward current. Normally, the increase in S-K current masks the inward current. Inward current is preferentially activated: 1. At low concentrations of FMRFamide (500 nM), 2. After whole-cell dialysis, and 3. In response to YGGFMRFamide. Application of 8-bromo-cGMP to sensory cells elicits the inward current (n=9), but occasionally also elicits outward current (n=2). 8-bromo-cIMP, a cGMP analog that activates cGMP-dependent phosphodiesterase, selectively activates the inward current (n=7). cIMP and cGMP occlude the inward current response to FMRFamide. The figure shows FMRFamide (500 nM) and 8-bromo-cIMP (1 mM) difference currents in response to voltage ramps. The additional outward current with FMRFamide is S-K current.



329.9

ROLE OF 5-LIPOXYGENASE METABOLITES IN MODULATING K⁺-CHANNELS IN BRAIN NEURONS. K.Koyano, J.J.Grigg, S.Nakajima and Y.Nakajima. Dept. of Anat. and Cell Biol. and Dept. of Pharmacol., Univ. of Illinois, College of Medicine at Chicago, Chicago, IL 60612.

The role of arachidonic acid metabolism on somatostatin-and substance P-induced responses in cultured locus coeruleus and nucleus basalis neurons of the rat was studied using the whole-cell clamp technique. The somatostatin-induced inwardly rectifying K⁺-conductance was reduced by phospholipase A_2 inhibitors (quinacrine, 50 μM; 4BPB, 5-20 μM), lipoxygenase inhibitors (NDGA, 3-5 μM; ETYA, 20 μM) and 5-lipoxygenase inhibitors (AA-861, Takeda, 50 μM; A63162, Abbott, 50 μM). In contrast, a cyclooxygenase inhibitor (indomethacin, 50 μM) and a 12-lipoxygenase inhibitor (baicalein, 50 μM) were ineffective. Furthermore, 5(S)HPETE (5 μM, a 5-lipoxygenase metabolite) enhanced the somatostatin-induced response as well as resting K⁺-conductance. The substance P-induced decrease in the K⁺-conductance was also suppressed by AA-861 but not by baicalein. These results suggest that 5-lipoxygenase metabolites are not directly involved in the signal transduction of the somatostatin or the substance P effects. Perhaps 5-lipoxygenase metabolites act to maintain or to enhance the functional state of the K⁺-channels. Supported by NIH grants, AG06093, NS24711.

329.10

ARACHIDONIC ACID-INDUCED DEPRESSION OF HIPPOCAMPAL POTASSIUM CURRENT IS MEDIATED VIA PROTEIN KINASE C AND OXYGEN FREE RADICALS. D.O. Keyser and B.E. Alger. Dept. of Physiology, Sch. of Med., Univ. of MD, Baltimore, MD 21201.

We have reported that arachidonic acid (AA) depresses whole-cell calcium current via protein kinase C (PKC) activation and oxygen free radical generation. We have now investigated outward currents including transient (I_A), steady-state (I_{SS}) and tail (I_{Tail}) currents and have tested the hypothesis that AA reduces both tail current and I_{SS}, by PKC activation and free radical generation.

Using acutely isolated adult guinea pig and primary cultured rat fetal hippocampal pyramidal cells we investigated outward currents under whole-cell voltage-clamp in control saline and 5 min following extracellular application of AA, 50 μM (n=37). I_{SS} and I_{Tail} (V_H=-50 mV; V_{STEP}=-10 mV), were reduced by over 50%. Robust effects were also observed when AA was administered intracellularly. Palmitate, an unsaturated fatty acid that does not activate PKC or generate free radicals, had no effect. Pretreatment with the peptide pseudosubstrate PKC inhibitor (19-36) or H-7 blocked the AA-induced current reduction by about 80%. Superoxide dismutase or a cocktail of free radical scavengers blocked the response by about 87%. I_A, (V_H=-110 mV; V_{STEP}=-10 mV), was not significantly depressed after 5 min in 50 μM AA. Our results show that AA preferentially depresses non-inactivating outward currents via activation of PKC and generation of oxygen free radicals.

329.11

DISTRIBUTION OF PROTEIN KINASE C (PKC) ISOZYMES PREDICTS THE ATTENUATION OF VOLTAGE-GATED K CURRENTS BY TWO CLASSES OF PKC ACTIVATOR IN CULTURED CEREBELLAR NEURONS. D.J. Linden, S.C. Sun, and J.A. Connor. Dept. Neuroscience, Roche Inst. of Mol. Biol., Nutley, NJ 07110.

A striking example of the differential distribution of PKC isozymes is found in the cerebellum, where Purkinje cells express PKCI, an isozyme which is strongly stimulated by both the classical activators (CA) such as diacylglycerols and phorbol esters, and the cis-unsaturated fatty acids (c-UFA), while granule cells express only PKC isozymes which are strongly stimulated by CA, but not by c-UFA (Shearman et al., 1989, FEBS Lett. 243:177). We hypothesized that voltage-gated K currents would be attenuated by both classes of PKC activator in Purkinje cells, but only by CA in granule cells. This hypothesis was confirmed in whole-cell perforated-patch recordings of cultured rat cerebellar neurons. Voltage-gated K currents were measured with depolarizing steps from a holding potential of -90 mV in TTX. The sustained component of the K current was measured 100 msec after the start of the depolarizing step. The following table shows the % attenuation (mean ± SEM) of this current at +30mV in the presence of PKC activators. Similar results were seen in measures of the inactivating component of the K current.

Treatment (N=5 for all groups)	Granule cells	Purkinje cells (% atten.)
PDAc, 20nM	45.2±3.8	37.1±2.6
PDAc, 20nM + staurosporin, 20nM	-6.9±2.5	4.3±2.4
oleate, 20uM	-3.2±1.3	47.4±4.5
oleate, 100uM	5.4±1.6	
methyloleate, 100 uM (inactive c-UFA)		5.7±2.9
oleate, 20 uM + staurosporin, 20nM		3.6±3.1

Currently, we are investigating the distribution of PKC isozymes among these cultured cerebellar neurons with immunohistochemical techniques.

329.12

MODULATION OF ELECTRICAL SYNAPSES BETWEEN CULTURED *APLYSIA* NEURONS: DEPENDENCE UPON PROTEIN SYNTHESIS. G.M. Carrow, M.P. Wilson*, and I.B. Levitan. Graduate Department of Biochemistry, Brandeis University, Waltham, MA 02254.

The efficacy of electrical synapses between weakly coupled pairs of cultured neurons from juvenile *Aplysia californica* is increased upon exposure to nanomolar concentrations of the lectin Concanavalin A (Con A). This change results from a two- to ten-fold increase in the junctional conductance between pairs of cells as determined directly by dual voltage clamp (Carrow and Levitan, J. Neurosci. 9:3657, 1989). The modulation of junctional conductance by Con A is dependent upon protein synthesis since it is reversibly blocked by 10 uM anisomycin; this concentration of anisomycin inhibits 95% of protein synthesis in *Aplysia* nervous tissue. It is unlikely that the anisomycin block of modulation is due simply to interference with gap junction formation or maintenance since (1) pairs of neurons are capable of de novo formation of electrical synapses when cultured in the presence of anisomycin and (2) long-term exposure (up to 1 day) of electrically coupled pairs of neurons to anisomycin does not reduce coupling. Instead, the block by anisomycin of the Con A-induced modulation of junctional conductance appears to be specific since incubation of nervous tissue from juvenile *Aplysia* with Con A does not lead to a general increase in protein synthesis but rather to changes in the synthesis of particular proteins as determined by SDS PAGE. Moreover, 10 uM anisomycin does not block the Con A-induced increase of a glutamate-sensitive inward current in these neurons. These data suggest that Con A may specifically regulate the synthesis of gap junction channels or proteins that, in turn, regulate the channels.
[Supported by NIH grant NS25366 to IBL]

330.1

MUSCIMOL DISRUPTS TEMPORAL DISCRIMINATION BY THE FM-FM AREA OF THE MUSTACHED BAT'S AUDITORY CORTEX. H. Riquimaroux, S. J. Gaioni* and N. Suga. Dept. of Biology, Washington Univ., St. Louis, MO 63130.

Neurons in the FM-FM area of the mustached bat's (*Pteronotus parnellii*) auditory cortex specifically respond to the combination of the FM sweep from the first harmonic of the emitted pulse (FM1) and a higher harmonic of the echo (FM2-4). They are tuned to particular echo delays. The best delay for these neurons systematically varies across the FM-FM area, suggesting that this area is important for processing echo delays, *i.e.*, target ranging (Suga, 1984). We tested this function of the FM-FM area using reversible ablation with muscimol, a GABA agonist (Riquimaroux *et al.*, 1989). Bats were trained on a discriminated shock avoidance task requiring a leg flexion. The stimuli were trains of artificial pulse FM1 - echo FM2 (P-E) pairs (*e.g.*, 31→21 kHz and 62→46 kHz). For S+, echo delays were always the same (*e.g.*, 4 ms), while for S- they were jittered between successive P-E pairs (*e.g.*, 4 ms and 6 ms). Following baseline training, we applied 0.1-0.2 µg of muscimol in saline bilaterally to the FM-FM area. The bats failed on previously successful discriminations (1-20 ms of jitter). Performance returned to baseline levels within 24 h. The FM-FM area is necessary for echo-delay discriminations, *i.e.*, target ranging. (Supported by AFOSR grant #87-0250.)

330.2

MULTI-COMBINATION-SENSITIVE NEURONS IN THE FM-FM AREA OF THE AUDITORY CORTEX OF THE MUSTACHED BAT N. Suga and H. Misawa* Dept. of Biology, Washington Univ. St. Louis MO 63130

In amphibians, avians, and mammals (bats), the auditory system creates combination-sensitive neurons for processing species-specific complex sounds. These neurons are tuned to combinations of two signal elements. In the mustached bat, a small population of neurons in the FM-FM area of the auditory cortex is tuned to combinations of more than two signal elements. An interesting question is how high the specialization of single neurons is for the processing of biologically important complex sounds. The aim of the present studies is to characterize the response properties of "multi-combination-sensitive (MCS)" neurons and their distributions within the FM-FM area. Out of 765 FM-FM neurons recorded, 156 were MCS neurons (80 FM1-FM2,4, 56 FM1-FM3,4, 6 FM1-FM2,3, and 14 FM1-FM2,3,4). They form two elongated 100 µm-wide bands along the boundary between the FM1-FM3 and FM1-FM4 subdivisions, and also along the boundary between the FM1-FM2 and FM1-FM4 subdivisions. A pulse FM1 is always one of the essential signal elements for the facilitative responses of MCS neurons. The best delays of MCS neurons are the same for different echo FM harmonics combined with the pulse FM1. When two echo components are combined with the pulse FM1 ("multi"-combination), the responses of an MCS neuron are either summation or facilitation of the responses to two "single" combinations. (Supported by PHS research grant NS 17333)

330.3

NMDA RECEPTORS ARE ESSENTIAL FOR DELAY-DEPENDENT FACILITATION IN FM-FM NEURONS IN THE MUSTACHED BAT.

J. A. Butman and N. Suga, Department of Biology, Washington University, St. Louis MO 63130

FM-FM neurons in the medial geniculate body of the mustached bat respond maximally to the combination of two signal elements, the biosonar pulse FM_1 and the echo FM_n, separated by a specific echo delay. The response at best delay consists of (1) a fast component due to a fixed latency initial spike followed by (2) a slow component due to a facilitative burst of spikes.

To investigate the synaptic mechanisms of this delay-dependent facilitation, the selective NMDA antagonist, APV, was iontophoresed onto thalamic FM-FM neurons while delay tuning curves were monitored. In 11/16 neurons, the burst (slow component) was eliminated, but the initial spike was little affected. This fast component still exhibited delay tuning, indicating that coincidence detection was unaffected. Kynurenic acid or non-specific doses of APV abolished this initial spike in some cases.

These data support the following model: Pulse and echo stimuli evoke fast EPSPs mediated by non-NMDA receptors. With NMDA receptors antagonized by APV, summation of these fast EPSPs depolarize the cell above spike threshold evoking only the initial spike. Normally, NMDA receptors are available, so that the summation of fast EPSPs at best delay depolarizes the cell sufficiently to unblock a slow NMDA mediated EPSP responsible for the facilitative burst. Delay tuning is thus the result of coincidence detection by fast EPSPs followed by a nonlinear multiplication of this response by the voltage-dependent NMDA receptor.

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330.4

THE SIZE AND NUMBER OF CORTICAL COLUMNS IN THE MUSTACHED BAT'S AUDITORY CORTEX. D. C. Fitzpatrick and N. Suga. Washington Univ., St. Louis, MO 63130.

The cerebral cortex is organized as arrays of modular elements (columns) with linear dimensions on the order of 0.5 mm. In most systems studied, the columns within a functional area are too numerous for detailed studies of identified columns to be possible. Here we report measurements that suggest that within the auditory cortex (AC) of the mustached bat, Pteronotus p. parnellii, each functional area contains sufficiently few columns for homologous columns in different animals to be mapped and studied. To determine the size of columns in the mustached bat's AC, injections of WGA-HRP or PHA-L were made in the AC of 4 bats. These injections labeled 21 cortico-cortical columns. The mean diameter of the columns was 368 ± 33.4 µm, after correcting for shrinkage. Sizes of individual functional areas were determined through multiunit mapping in 4 bats (39-51 penetrations/hemisphere). These areal sizes were then divided by the columnar size (described as closely packed hexagons, area = 0.102 sq. mm), yielding an estimate of the number of columns/functional area.

Area:	DSCF	FM-FM	CF/CF	Alp	Ala	DF	DM	VA	VF	VP	AC
Columns:	21	23	11	12	4	17	18	3	4	12	128

Since no injection (sizes 600-1200 µm) labeled more than 10 columns and 3 of the 4 injections labeled 5 columns or fewer, the connections between columns appear to be limited and specific. The small number of columns/area and their restricted connections suggest that it should be possible to map interactions between identified columns in this specialized cortex.

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330.5

FM NEURONS BECOME DELAY-SENSITIVE IN THE AUDITORY CORTEX OF THE *MYOTIS* BAT. M. Maekawa and D. Wong, Anatomy Dept., Indiana Univ. School of Medicine, Indianapolis, IN 46202.

Cortical neurons sensitive to single FM sounds are found throughout the auditory cortex of *Myotis lucifugus*, presumably to process the bat's emitted echolocation sounds. Delay-sensitive cortical neurons are also found in this FM bat, probably to provide target-distance information for echolocating bats in general. Our neurophysiological studies reveal that neurons sensitive to single FM sounds and to FM-FM sound pairs at specific time delays are not separate populations. Rather, cortical neurons exhibit brisk responses to single FMs at low stimulation rates, but become delay-sensitive to FM-FM pairs at higher stimulation rates. Responses to single FMs drop dramatically as facilitative responses emerge at specific delays. These stimulus conditions are analogous to the sound pattern that a bat hears when its sonar emission and echo return increase during echolocation. The change in the response properties suggests that individual neurons in *Myotis* cortex have the capacity for multi-dimensional analysis of target during auditory perception. (Supported by NIH grant DC00600).

330.6

ENCODING OF STIMULUS INTENSITY BY INFERIOR COLLICULAR NEURONS OF THE BIG BROWN BAT, EPTESICUS FUSCUS. M. Wu* and P.H.-S. Jen. Div. of Bio. Sci., University of Missouri, Columbia, MO 65211.

Using bats as a model system, we studied the isointensity and isofrequency discharge rate functions of inferior collicular (IC) neurons under free field stimulation conditions. For each IC neuron, the best frequency (BF), minimum threshold (MT), tuning curve and intensity rate function for the BF were first determined. Then at each of several chosen stimulus intensites, the number of impulses was measured for several frequencies which were incrementally chosen across the entire range of the neuron's tuning curve. A series of isofrequency discharge rate functions were also obtained by determining the intensity rate function for each of several frequencies chosen. Among 110 intensity rate functions measured at the BF of each IC neuron, 98 (89%) were non-monotonic and 12 (11%) were monotonic. However, within the same series of isofrequency intensity rate functions, a neuron might show both monotonic and non-monotonic functions when measured at different frequencies. Similarly, the profile of each intensity rate curve within a series of isointensity rate functions might also vary with stimulus intensity. Isointensity discharge rate functions of most IC neurons (62 neurons, 61%) were triangular shaped reaching a peak value when stimulated with BF. The remaining (39 neurons, 39%) IC neurons had isointensity functions in which different curves peaked at different stimulus frequencies. In some extreme cases, some curves had two peaks or fluctuated within a moderate range of discharge rate throughout the whole range of stimulus frequency. Such a variation is presumably due to the different dynamic ranges of a neuron when measured at different frequencies. Our studies suggest that encoding of a wide range of stimulus intensity involves more than the discharge rate of any one individual neuron. Different populations of neurons with different BFs have to work coordinately to overcome the limitation of the dynamic range of individual neurons and to solve the ambiguity created by the same discharge rate of each individual neuron to different combinations of frequency and intensity within its auditory response area.

330.7

PLASTICITY IN THE EFFERENT INNERVATION OF THE COCHLEA INDUCED BY NEONATAL DESTRUCTION OF THE OPPOSITE INNER EAR. A.F. Ryan and N.K. Woolf. Division of Otolaryngology, UCSD, La Jolla, CA 92093.

Unilateral cochlear ablations were performed on gerbils at 0 to 6 days after birth. In adulthood, retrograde transport of horseradish peroxidase or tritiated amino acids was employed to assess efferent innervation of the surviving cochlea. The numbers and distributions of lateral olivocochlear (OC) neurons were essentially normal. In contrast, only 45% of the normal numbers of medial OC neurons were observed. Most of these neurons were located ipsilateral to the surviving cochlea.

We found no evidence that OC neurons associated with the lesioned cochlea formed new projections to the surviving cochlea. Rather, many medial OC neurons degenerated or failed to develop projections, even those which normally project to the intact cochlea. This suggests that the OC neurons which degenerated received sustaining projections from the ablated cochlea. Lateral OC neurons of the surviving cochlea, unaffected by neonatal lesions, presumably do not receive sustaining projections from the contralateral cochlea. This implies that the lateral OC system is primarily an ipsilateral loop, with inputs deriving from the cochlea to which it projects, in contrast to the medial OC system which receives major input from the contralateral cochlea.

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330.8

SINGLE-UNIT RESPONSES IN THE COCHLEAR NUCLEUS OF YOUNG AND AGED RATS. E.M. Keithley, Division of Otolaryngology, UCSD, La Jolla CA 92093.

Aging in mammalian cochleas is associated with loss of spiral ganglion cells in the base and apex. Thus, responses to acoustic stimuli of cells in the ventral cochlear nucleus (VCN) may reflect this loss of afferent input. Neural responses were recorded from the VCN of 2 month old (MO) and 24-26 MO Sprague Dawley rats with glass microelectrodes. Two aged animals had spontaneously active units (1-10 spikes/s), but no stimulus evoked activity. Three other aged animals had units with thresholds at least 30 dB above the most sensitive units from the young animals. The characteristic frequencies of units from young animals extended from 0.4-55 kHz, while those from old animals ranged from only 0.9 -30 kHz. All classically described VCN unit-types were seen in both age groups. While the initial spike latency at 20 dB above threshold of most units from old animals was within the range of times for young animals, there were a few units with longer response times. Timing variability and paucity of units at low and high frequencies could contribute to a decrease in the accuracy of stimulus coding and thereby degrade the clarity of the acoustic message in the elderly.

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330.9

TOPOGRAPHIC ORGANIZATION OF THE PROJECTIONS OF THE COCHLEAR SPIRAL GANGLION TO THE VENTRAL COCHLEAR NUCLEUS IN CATS. P.A. Leake and R.L. Snyder* Epstein and Coleman Laboratories, Dept. of Otolaryngology, U494, Univ. of California San Francisco, CA 94143-0732

The morphological organization of inputs from restricted sectors of the cat cochlear spiral ganglion into the cochlear nucleus has been investigated previously by making focal extracellular injections of horseradish peroxidase (HRP) into the ganglion. Large injections intensely label sectors of the spiral ganglion which project into narrow "isofrequency laminae" extending across the entire lateral to medial dimension of the ventral cochlear nucleus (VCN). High frequency laminae are situated dorsally in the VCN and lower frequencies are found progressively more ventrally. Small injections label only part of an isofrequency lamina. Cells in the scala tympani portion of the spiral ganglion project to the lateral portion of VCN isofrequency laminae, and cells in the scala vestibuli portion of the ganglion project to the medial portion of the laminae.

In a corollary exeriment, restricted lesions of the anteroventral cochlear nucleus (AVCN) induced selective degeneration of spiral ganglion cells. Ablation of the lateral portion of AVCN isofrequency laminae results in degeneration of cells in the scala tympani portion of the ganglion, and medial lesions induce degeneration of the scala vestibuli portion of the ganglion for the frequencies effected by the lesion site. Since almost all cochlear nerve fibers bifurcate upon entering the brainstem, it is noteworthy that selective damage to the ascending branch is sufficient to induce degeneration of the ganglion. The distal axons display a similar selective pattern of degeneration.

The data indicate a topographic organization of the spiral ganglion and isofrequency laminae orthogonal to the frequency domain. That is, in addition to the spiral dimension of the ganglion (corresponding to the dorsal to ventral frequency map in the VCN), there is also an orderly, sequential topographic projection of inputs from the scala tympani to scala vestibuli dimension of the spiral ganglion across the lateral to medial dimension of the VCN. (Supported by NIH Grant DC00160.)

330.10

3-D ANALYSIS OF AUDITORY EVOKED POTENTIALS IN RAT NEOCORTEX. DANIEL S. BARTH and SHI DI *Departments of Neurology and Psychology, Univ.of California, Los Angeles, (U.S.A)*

A 8x8 channel microelectrode array was used to map epicortical field potentials, evoked by bilaterally presented click stimuli, from a 8x8 mm² area in the right parieto-temporal neocortex of 4 rats. In two rats, a 16 channel microelectrode array was also inserted into primary auditory cortex to record the laminar profile of auditory evoked potentials.

The epicortical responses began with a positive-negative fast wave followed by a positive-negative slow wave, similar to the previously reported P1, N1, P2, N2 complex. Topographical distributions of the potentials for each of these components were distinct, suggesting that they were produced by separate but overlapping populations of cells. Laminar recording revealed the asynchronous participation of supragranular and infragranular pyramidal cells in the generation of the evoked response complex. The surface recorded P1 was primarily produced by supragranular cells and the N1, by infragranular cells. The P2 and N2 were produced by temporally overlapping contributions from both cell groups.

We conclude that middle latency components of the auditory evoked potential complex reflect both sequential and parallel activation of subpopulations of pyramidal cells in primary auditory cortex.

330.11

THE GABAERGIC NEURONS IN CAT PRIMARY AUDITORY CORTEX (AI). J.J. Prieto, B.A. Peterson, and J.A. Winer. Department of Molecular and Cell Biolology, University of California, Berkeley, California 94720-2097.

The distribution, form, and number of GABA- or GAD-immunoreactive neurons in AI were studied in adult animals in 25 µm-thick sections. With one exception (see layers V and VI) all the cell types are non-pyramidal, and each was related to the neurons in Golgi material. The cells are: in layer I, horizontal, tufted, bitufted (vertical) and multipolar cells; in layer II, four kinds of multipolar neurons and bitufted cells; in layer III, two types of stellate cells, tufted and bitufted cells; in layer IV, three varieties of tufted cells, and large multipolar and double bouquet cells; in layer V, three kinds of multipolar cells, bipolar cells, and inverted pyramidal cells; and, in layer VI, two types of multipolar cells, two kinds of tufted neuron, bipolar cells, and inverted pyramidal cells. Thus, the common GABAergic cell types are: tufted, bitufted, multipolar, and bipolar cells; horizontal cells are found in layer I, and inverted pyramidal cells occur only in layers V and VI. Comparable cell types contain GABAergic members in other cortical sensory areas. GABA-immunopositive puncta were often found on the soma and dendrites of some GABAergic medium-sized stellate cells (layer III), medium-sized and large tufted cells (layer IV), and large multipolar cells (layers IV and V) (see Peterson et al. [1990]: this volume).

The number and proportion of GABAergic cells were determined in 1 µm-thick plastic sections, counterstained with toluidine blue. In 500 µm-wide samples at systematic intervals along the rostrocaudal and the dorsoventral extent of AI, GABAergic cells were 21.75±1.57% of the total, a value comparable to other cortical areas. The highest proportion of GABAergic cells (73%) is between 0-200 µm deep (layer I), falling to 20.3-23.7% between 200 to 1600 µm, and to 14.3-17.3% from 1600 µm to the white matter. The number and proportion of such cells within AI were not significantly different, suggesting that these values may be uniform across frequency or aural dominance regions.

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330.12

GABA-IMMUNOREACTIVE AXON TERMINALS IN CAT PRIMARY AUDITORY CORTEX (AI). B.A. Peterson, J.J. Prieto, and J.A. Winer. Department of Molecular and Cell Biology, University of California, Berkeley, California 94720-2097.

GABA is a major neocortical neurotransmitter but its distribution in primary auditory cortex (AI) has not been described. Since many cortical cells are GABAergic, knowledge of them (see Prieto et al. [1990]: this volume) would be useful in understanding how GABA influences the cortex. We studied the laminar distribution of axon terminals in plastic embedded, 1 µm-thick sections processed with GABA antiserum, and in 25 µm-thick frozen sections processed with GAD antiserum.

GABAergic axon terminals are found in all layers of AI but there are significant laminar differences in the form and locus of axon terminals. The main results are: (i) there is conspicuous immunoreactivity in the upper part of layer I and in layers III and IV that is related to both the density and size of axon terminals; (ii) layer I has the highest density (25-30 puncta/100 µm²) of GABAergic axon terminals; (iii) layers II, III, and IV have about half as many terminals; (iv) layers V and VI have still fewer terminals (5-8/100 µm²); (v) each layer has immunonegative cells with immunopositive axosomatic endings; and (vi) layers III and IV have many non-pyramidal immunopositive cells that receive immunopositive axosomatic endings, while layers I, II, V and VI have few.

The size and density of axon terminals in the thalamic recipient zones (layers IV and IIIb) is consistent with the idea that, in AI as well as primary visual cortex and somatosensory cortex, there are intrinsic inhibitory influences on or near geniculorecipient neurons. The soma and apical dendrite of pyramidal neurons are frequently covered with immunopositive axon terminals, suggesting significant influences on commissural and corticofugal pathways. Both immunopositive and immunonegative non-pyramidal cells, which may project in corticocortical pathways or intrinsic circuits, receive GABAergic axon terminals. It is likely that GABAergic cells and axon terminals have a different role in each layer, and participate in every cortical projection.

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331.1

APPEARANCE OF FUNCTIONAL VOLTAGE AND AMINO ACID GATED CHANNELS ON EMBRYONIC NEOCORTICAL NEURONS. J.J.LoTurco, M.G.Blanton and A.R.Kriegstein. Dept. of Neurology and Neurological Sciences, Stanford Univ. Sch. of Med., Stanford, CA, 94305.

The compliment of voltage and ligand gated channels present on immature neurons will constrain the possible activity-dependent mechanisms that can influence their differentiation. In order to determine which voltage and amino acid gated channels are present on neocortical neurons at different stages of development, we have made both whole-cell and outside-out patch-clamp recordings from different laminae of slices prepared from embryonic rat neocortex.

Cells in the ventricular zone had voltage activated sodium currents that were blocked by TTX. The predominant potassium current showed voltage-dependent activation and slow inactivation. Outside-out patch recordings indicated that these ventricular zone cells had GABAa receptor/channels on their membranes, however lacked NMDA receptor/channels. Cells in the cortical plate had sodium and potassium currents that were larger than those in ventricular zone cells. In addition, a faster inactivating potassium current that was blocked by 4-AP became apparent in cortical plate cells. Calcium currents, both low threshold and high threshold, were also first detectable in cortical plate cells. Another apparent difference between the ventricular zone cells and cortical plate cells was that outside-out patches from the latter contained both GABAa receptor/channels and NMDA receptor/channels.

These differences reveal the early assembly of neuronal properties, and indicate that embryonic cortical neurons at different stages of development will distinctively respond to extra- and intra- cellular signals.

331.2

ENDOGENOUS ACTIVATION OF NMDA RECEPTORS ON DIFFERENTIATING NEURONS IN EMBRYONIC NEOCORTEX. M.G. Blanton, J.J. LoTurco and A.R. Kriegstein. Dept. of Neurology and Neurological Sciences, Stanford Univ. Sch. of Med., Stanford, CA, 94305.

N-methyl-D-aspartate (NMDA) receptors may play an important role in the control of neuronal growth and differentiation. To determine when these receptors are expressed and activated in differentiating neocortex, we made whole-cell voltage-clamp recordings from neurons in slices of embryonic rat neocortex.

Focal application of glutamate (1 mM) activated a current that was sensitive to the NMDA receptor antagonist D-2-amino-5-phosphonovalerate (D-APV); fluctuation analysis of the evoked current revealed an underlying mean channel open time of 5.0 msec, typical of NMDA receptors. In the absence of exogenously applied agonists, similar spontaneous currents were recorded from neurons in the cortical plate beginning at embryonic day 15 (E15). The tonic currents were blocked by D-APV and the glycine site antagonist 7-chlorokynurenic acid. In addition the current was blocked in a voltage-dependent manner by magnesium. Analysis of single channels and fluctuation analysis of current noise in young cells revealed a conductance of 50 pS and a mean open time resembling that activated by exogenous glutamate. The currents were clearly distinct from spontaneous, discrete non-NMDA-mediated synaptic events that were detected later in embryonic life.

The activation in situ of NMDA channels prior to synaptic differentiation provides a physiological substrate for the control of early neuronal differentiation by NMDA receptors.

331.3

DISSOCIATED TECTAL NEURONS FROM *RANA PIPIENS* EXHIBIT A CALCIUM INFLUX IN RESPONSE TO EXCITATORY AMINO ACIDS. H.T. Cline and R.W. Tsien. Dept Physiol & Biophys. Univ Iowa. Iowa City, IA 52242 and Dept Mol. Cell. Physiol. Stanford Univ Medical School. Stanford, CA. 94305

NMDA receptor activation is required for normal development of the retinotectal projection in frogs. We have suggested that the NMDA receptor detects the correlated electrical activity of retinal ganglion cells with overlapping receptive fields and that the Ca influx through the NMDA channel initiates the stabilization of the coactive retinal inputs (Cline & Constantine-Paton '89 Neuron 3:413). Although retinotectal synaptic transmission has been shown to be glutamatergic, a Ca influx in the tectal neurons in response to glutamate has not yet been demonstrated.

We have used calcium imaging with Fura-2 to assay the responses of dissociated tectal neurons (Steen et al, '89 J. Comp. Neurol. 289: 467.) to depolarizing [K] and excitatory amino acids (EAAs). Tectal cells exhibit a resting [Ca] of 50-200 nM, which rises rapidly to 0.5-2 μM in response to 56 mM K or EAAs. The magnitude of the Ca rise and the % of responsive cells increases over the first 2 d in culture and remains constant over the next 3 d. In cultures where about 70% of the cells respond to high K, 70% of the excitable cells (50% of the total) respond to NMDA (10-100 μM) or glutamate (Glu; 1-10 μM). The NMDA-induced Ca transient is abolished in Ca-free Ringer; blocked by NMDA receptor/channel blockers, APV, Mg or MK801; and partially inhibited by Cd (0-50%) at concentrations that abolish the K response. The glu-induced Ca rise is completely blocked in Ca-free Ringer and partially blocked by APV. Quisqualate (up to 1 mM) did not cause a Ca rise, whereas kainate (10-100 μM) causes a small Ca rise of 100-300nM. Recovery from the Glu-induced Ca rise was slower (50% recovery at 7-10 min) than the recovery from the NMDA response (50% recovery at 1-2 min). These results indicate that dissociated tectal neurons respond to NMDA with a Ca influx through the NMDA channels, with a relatively small contribution from voltage-dependent calcium channels (VDCCs). Furthermore, Glu stimulation results in a Ca rise due to an influx through NMDA channels and VDCCs. Activation of the glutamate receptor does not appear to cause the release of Ca from intracellular stores.

331.4

NMDA RECEPTOR ACTIVATION DOMINATES SYNAPTICALLY-INDUCED CALCIUM INFLUX IN DEVELOPING RAT VISUAL CORTEX. R. Yuste and L.C. Katz. Laboratory of Neurobiology, The Rockefeller University, New York, NY 10021.

The entry of calcium through the NMDA receptor is considered a critical signal in models of developmental plasticity in the mammalian cortex. Using the calcium-sensitive indicator fura-2, we analyzed calcium influx mediated by excitatory amino acids and synaptic transmission in cortical neurons in rat visual cortex slices during the first postnatal week.

A single brief shock (100 μsec) applied to the cortical white matter induced a large increase in $[Ca^{2+}]_i$ of postsynaptic neurons in the upper cortical layers that lasted >10 sec. This synaptically mediated increase in $[Ca^{2+}]_i$ was due primarily to activation of NMDA receptors: AP5 (50 μM), a competitive NMDA antagonist, blocked 75±6% of the signal. The remainder of the signal was completely blocked by DNQX (5 μM), a selective quisqualate receptor antagonist. The peak of the DNQX-sensitive component occurred about 400 msec prior to the peak of the AP5-sensitive component. Similarly, approximately 95% of the increase in $[Ca^{2+}]_i$ induced by bath-applied glutamate (50 μM) was blocked by AP5.

NMDA and glutamate-induced increases in $[Ca^{2+}]_i$ could result either from Ca^{2+} entry directly through the NMDA channel, or indirectly via voltage-sensitive Ca^{2+} channels. To distinguish between these possibilities, we studied NMDA-induced responses in the presence of nimodipine, an antagonist of "L" type voltage-sensitive Ca^{2+} channels. Nimodipine (2.5-10 μM) attenuated the increase in $[Ca^{2+}]_i$ elicited by 5 μM quisqualate and 50 mM KCl by 40-60%, but had no effect on the response to 50 μM NMDA. Thus, in the developing cortex, most of the synaptically induced Ca^{2+} influx probably results from activation of the NMDA receptor and Ca^{2+} entry directly through the NMDA channel. Supported by the L.P. Markey Charitable Trust.

331.5

NIMODIPINE SELECTIVELY BLOCKS CA^{2+} INFLUX THROUGH VOLTAGE-GATED CA^{2+} CHANNELS IN CORTICAL NEURONS. J.M. Nerbonne and L.C. Katz, Pharmacology Dept., Washington Univ. Med. Sch. St. Louis, MO 63110, and Lab. Neurobiology, The Rockefeller Univ., New York, NY 10021.

To examine the contributions of different types of voltage-gated Ca^{2+} channels to depolarization-induced Ca^{2+} influx in isolated callosal-projecting (CP) and superior colliculus-projecting (SCP) visual cortical neurons, we combined in vivo fluorescent retrograde labelling to identify these distinct cortical cell types, with in vitro $Ca^{2+}{}_i$ imaging of dissociated cells loaded with fura-2. Resting $Ca^{2+}{}_i$ in isolated CP and SCP cells ranged between 25 and 50 nM. K^+-induced depolarizations (to ≈ -25 mV) resulted in rapid, reversible increases in $Ca^{2+}{}_i$ in both cell types. Although the magnitude of the responses varied, $Ca^{2+}{}_i$ increased in all cells by at least 3 fold, and returned to control levels within minutes of washing with normal medium. These K^+-induced increases in somal and dendritic $Ca^{2+}{}_i$ were reduced 50-80 % in the presence of 2 μM nimodipine. The putative "T" channel blocker, ethosuximide (10-50 μM), in contrast, did not measurably attenuate K^+-dependent increases in $Ca^{2+}{}_i$.

We conclude that depolarization-induced Ca^{2+} entry into both CP and SCP visual cortical neurons is predominantly through "L" type Ca^{2+} channels, and that these channels are effectively blocked by nimodipine. Because Ca^{2+} influx plays a critical role in excitotoxicity, these results suggest that nimodipine, which can cross the blood brain barrier, may be a useful therapeutic agent to prevent neuronal damage associated with cerebral ischemia. Supported by NSF #BNS-8809823, and the L. P. Markey Charitable Trust.

331.6

EFFECTS OF AP3 ON EAA-STIMULATED PI TURNOVER AND OCULAR DOMINANCE PLASTICITY IN THE KITTEN VISUAL CORTEX. S. M. Dudek, A. P. Bohner, and M. F. Bear. Center for Neural Science, Brown University, Providence, RI 02912.

It is now well known that certain excitatory amino acids (EAAs) can stimulate the hydrolysis of phosphoinositides (PI) in neurons. Potent agonists include glutamate, ibotenate, quisqualate, and ACPD. EAA-stimulated PI turnover is age dependent; in kitten visual cortex ibotenate-stimulated PI turnover correlates precisely with the critical period for ocular dominance (OD) plasticity (Dudek & Bear; *Science* 246: 673). Recently, it has been reported that the compound 2-amino 3-phosphonopropionate (AP3) selectively antagonizes EAA-stimulated PI turnover (Schoepp & Johnson; *J. Neurochem.* 53: 273). The aim of this study was to investigate the effects of AP3 on PI turnover *in vitro*, and on OD plasticity *in vivo*.

The ability of AP3 to inhibit ibotenate- and quisqualate-stimulated PI turnover was assayed in two different preparations: synaptoneurosomes and slices of kitten striate cortex. A striking difference was observed between the two systems; AP3 potently inhibited EAA-stimulated PI turnover in synaptoneurosomes, but had no effect in slices even at mM concentrations. Thus, the mechanism of action by AP3 is complex and appears to depend on factors differentially expressed by synaptoneurosomes and slices.

In the second part of this study, 50 mM AP3 was infused into striate cortex at 1 μl/ hr at the same time that the kitten was monocularly deprived by lid suture. AP3 infusion appears to interfere with the ocular dominance shift after 7 d. of MD, but the effect is age dependent. The shift contralateral to the deprived eye was nearly completely blocked by AP3 when MD was initiated between 50 and 60 days of age (n= 3); however, AP3 appears to be largely ineffective in blocking the OD shift in younger kittens. Taken together, these results suggest that AP3 may not be potent enough an inhibitor to block EAA-stimulated PI turnover, and consequently OD plasticity, at the height of the critical period. (Supported by NIH grant EY06929)

331.7

VISUAL ACTIVITY AND OCULAR DOMINANCE PLASTICITY IN CAT VISUAL CORTEX PERSIST FOLLOWING SPECIFIC BLOCKADE OF NON-NMDA GLUTAMATE RECEPTORS J. B. DeFreitas* and M. P. Stryker. Neuroscience Graduate Program and Dept. of Physiology, Univ. of Calif, San Francisco, CA 94143-0444.

Previous studies have shown that specific blockade of the NMDA subtype of glutamate receptor in cat visual cortex profoundly depresses neuronal activity and reduces ocular dominance plasticity following monocular deprivation (MD). We now investigate the effects of specifically blocking non-NMDA glutamate receptors on the activity and plasticity of cells in visual cortex. Cannulae attached to osmotic minipumps infused 0.5 µl/hr 4mM CNQX into area 17 of the right hemisphere of 4 week old cats. On the following day (in 2 animals) recordings of neuronal responses to iontophoresis of quisqualate (QA) and NMDA from multi-barrel pipettes were used to delineate the regions of cortex in which receptors were blocked, and visual responses were studied. In 4 additional animals, the left eyelid was closed 2 days after implanting the infusion cannula, and similar recordings were made 5-12 days later.

Close (<600µm) to the infusion cannula, both QA and NMDA iontophoretic thresholds were elevated, suggesting that CNQX was present in sufficient concentration to exert non-specific effects on NMDA receptors; and neural activity was profoundly depressed. Farther from the cannula, QA responses were selectively elevated, suggesting a specific blockade of non-NMDA receptors. In this region of cortex, visual responses were always evident and were commonly robust, while spontaneous activity was dramatically reduced in comparison to untreated cortex. The effects of MD were as pronounced in this region as in control cortex. While a reduction of ocular dominance plasticity was not evident under these conditions and is evident with infusion of NMDA-receptor blocker APV, these results do not provide support for a special role for NMDA receptors in ocular dominance plasticity, since visual responses were robust with CNQX but suppressed by APV.

(Supported by NSF BNS 8820406)

331.8

DARK-REARING KITTENS DELAYS THE DEVELOPMENTAL DECREASE IN CORTICAL NMDA RECEPTOR EFFICACY. K.FOX, N.DAW, D.CZEPITA Dept. Cell Biology, Wash. U. Med Sch, St. Louis, MO 63110

We have previously reported that APV becomes gradually less effective in antagonizing visual responses (VRs) in layers IV,V and VI of visual cortex as the animal develops from 3 to 4 weeks. This implies that NMDA-receptors contribute less to the VRs as the animal matures. We now report that the functional distribution of NMDA-receptors reaches an adult stage by 6 weeks for animals reared in the light but remains at the 3-week immature stage if reared in the dark. The NMDA component of the VR was estimated by iontophoresis of APV using a standard drug trial of 10nA for 3 minutes for each cell. Four groups of animals were studied. In animals dark-reared for 6 weeks VRs were substantially supressed by APV in all layers. In 6 week light-reared animals only VRs of layer II and III cells were antagonized by APV; adult animals were indistinguishable from them in this respect. VRs of layer IV cells showed the greatest divergence from age-matched light-reared animals. Animals dark-reared for 6 weeks then exposed to visual experience developed the adult insensitivity to APV in layers IV,V and VI by 10 days but only partially by 4 days. CNQX attenuated VR in layers IV,V and VI in cases where APV only affected spontaneous activity. The effectiveness of APV was independent of the magnitude of the control response. We conclude that visual experience leads to a decrease in the NMDA component of visual transmission in deep cortical layers during development.

331.9

INPUT-DEPENDENT TRANSIENT EXPRESSION OF RECEPTOR POPULATIONS IN KITTEN VISUAL CORTEX DEVELOPMENT. M.S. Cynader, V. Booth, Y.L. Liu, R. Dyck, and W.G. Jia, Dept. of Ophth., Univ. of British Columbia, Vancouver, B.C. Can.

We have previously found that over a dozen different classes of neurotransmitter receptors are transiently expressed in cells of certain layers of the kitten visual cortex during early postnatal development. These receptors alter their distribution during the critical period and may disappear or may be expressed by cells in different cortical layers, depending on the specific receptor under study. Here we show that receptor redistribution depends on thalamic input early in life.

Posterior thalamus, including lateral geniculate nucleus was removed in kittens 10-25 days of age. After 3 to 12 weeks, the kittens were perfused and examined with receptor autoradiography. We examined the following binding sites: NMDA, Kainate, GABA $_B$, 5-HT$_1$A, 5-HT$_1$C, Beta and Alpha Adrenergic, Muscarinic M1, M2, Adenosine A$_1$. Most receptors failed to undergo their normal age-dependent redistribution in the operated hemisphere. Instead receptors continued to be expressed in the same layers as they had been in young kittens. Adult lesions had no comparable effects. Despite the lesions, a few of the populations examined did undergo age-dependent redistribution. The implications for cortical plasticity of the differential responsivity of various receptor populalations will be considered.

331.10

POSTSYNAPTIC MEMBRANE POTENTIAL REGULATES SYNAPTIC POTENTIATION AND DEPRESSION IN VISUAL CORTICAL NEURONS. Y. Frégnac, D. Smith*, M.J. Friedlander, Neurobiology Res. Center, UAB, AL 35 294.

It has been suggested from in vivo recordings in cat visual cortex that changes in covariance between pre- and postsynaptic activity can potentiate or depress synaptic efficacy (Frégnac et al. Nature 333: 367, 1988). We have directly tested the role of membrane potential of the postsynaptic neuron for inducing such synaptic plasticity, by comparing, in vitro, the effects of paired afferent stimulation with concomitant depolarizing or hyperpolarizing current injection in the target cell.

450 µm thick frontal slices of primary visual cortex (VC) were taken from 4-5 week old kittens and adult guinea-pigs, and maintained in standard bicuculline free ACSF. 38 stable intracellular records were made in VC, mainly in granular and supragranular layers, using K+methyl-sulfate and biocytin filled micropipettes. Bipolar electrical microstimulation (STIM) was applied in white matter (WM) or at the layer VI /WM border. Evoked compound PSP's were studied in most cells (34/38) as a function of the stimulus intensity and/or of the postsynaptic membrane potential. Low frequency (0.1 to 1.0 Hz) pairing of STIM with a short depolarizing pulse (+4 to +7 nA, 50ms) resulted, in 2/4 cells, in a long lasting increase of the compound PSP, affecting mainly its peak amplitude and its decay rate. Pairing with a hyperpolarizing pulse (-2 to -4 nA, 50 ms, delayed by -10 ms) resulted in 8/11 cells in a transient depression of both early and late components of the PSP. Input resistance and resting potential were usually unchanged. No effect was observed for current values less than +/-1 nA. Temporal associativity was demonstrated by the lack of changes (0/6 cells) produced when imposed current pulses were applied out of phase (delayed from STIM by 140 ms) or uncorrelated with the PSP's occurence.

Similar changes were obtained in kitten and adult guinea-pig VC. These results demonstrate that the same compound PSP (involving mono- and polysynaptic excitatory and inhibitory pathways) can be potentiated or depressed by the temporal association of afferent stimulation with a local control of the polarization state of the postsynaptic cell, without pharmacological removal of intracortical inhibition.

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331.11

CORTICAL ORGANIZATION OF ORIENTATION SELECTIVITY EMERGES FROM INTERACTIONS BETWEEN ON- AND OFF-CENTER INPUTS. K.D. Miller, Dept. of Physiology, UCSF, San Francisco, CA 94143.

Last year I reported that Hebbian or similar synaptic plasticity mechanisms can yield orientation-selective cortical cells through interactions between ON- and OFF-center inputs. The resulting receptive fields consist of alternate bands of ON- and OFF-center inputs. This can occur if, during the time that orientation-selective cells are developing, ON- (OFF-) center inputs are better correlated in activity with other ON- (OFF-) center inputs at retinotopic separations smaller than a receptive field center diameter, but best correlated with OFF- (ON-) center inputs at larger retinotopic separations.

Here I report further studies of the cortical organization of orientation that emerges under this model. A locally continuous and periodic arrangement of orientation arises that resembles that seen experimentally. Dependence of this organization on biologically measurable quantities (the correlations among inputs, the geniculocortical connectivity, and the intracortical connectivity) is studied both through simulation and analysis. Analytically, simple Hebbian mechanisms produce an outcome in which cortical cell activity is correlated over distances at which cortical synaptic interactions are excitatory, and anticorrelated over distances at which those interactions are inhibitory. The correlation between two cortical cells depends on their preferred orientations, the retinotopic separation of their receptive field centers, and the spatial phase and frequency of their receptive field arrangements of ON- and OFF- regions. These considerations allow analysis of the spatial arrangements of receptive fields that should emerge under a Hebbian model. Previous models have not considered the role of spatial phase or frequency, which substantially alter predictions for the cortical organization of orientation. Experimentally, it will be important to study the simultaneous variation of all of these receptive field parameters in visual cortex.

Supported by an NEI Fellowship and a Human Frontiers Science Program Grant to M. P. Stryker (T. Tsumoto, Coordinator).

331.12

A MODEL OF VISUAL CORTICAL PLASTICITY WITH A SLIDING MODIFICATION THRESHOLD: COMPARISON OF THEORY WITH EXPERIMENT. E.E. Clothiaux*, M.F. Bear and L.N Cooper, Dept. of Physics and Center for Neural Science, Brown University, Providence, RI 02912

The response properties of neurons in striate cortex depend on the visual environment experienced during a critical period of postnatal development. For example, prolonged binocular deprivation broadens orientation selectivity, and binocular connections are modified after brief periods of monocular deprivation, strabismus, and reverse suture. The results of more recent experiments in which the cortical activity in one area of visual cortex is chronically recorded (Mioche and Singer, 1989) have provided detailed descriptions of the changes in binocular connections during periods of monocular deprivation and reverse suture. For example, Mioche and Singer found that during a reverse suture many neurons went through a "blind" period where they did not respond to stimulation of either eye. We have made a detailed investigation of the cortical plasticity model of Bienenstock, Cooper, and Munro (1982) with special attention to the fixing of parameters by comparison with experiment. Computer simulations capture the important features of the new monocular deprivation and reverse suture experimental results, while continuing to provide correct descriptions for normal rearing after monocular deprivation, binocular deprivation and strabismus. (Supported by ONR contract N00014-86-K0041)

332.1

INHIBITION OF EPINEPHRINE BIOSYNTHESIS. ENANTIOSELECTIVITY IN THE BINDING OF BENZYLAMINE INHIBITORS RELATED TO LY-134046 AND OF CONFORMATIONALLY-DEFINED PHENYLETHYLAMINE INHIBITORS TO THE ACTIVE SITE OF PHENYLETHANOLAMINE *N*-METHYLTRANSFERASE (PNMT). Gary L. Grunewald, Vidyadhar M. Paradkar*, Vilas Dahanukar*, Duane M. Stillions*, Feei Ching*, and Kevin R. Criscione*. Department of Medicinal Chemistry, University of Kansas, Lawrence, KS 66045.

In order to elucidate the role played by epinephrine in CNS function, a selective inhibitor of PNMT has been sought. Most currently available inhibitors of the enzyme also show high binding affinity toward other sites, most notably the α_2-adrenoceptor. To exploit as much as possible any subtle binding differences that may exist between ligands toward the active site of PNMT and to the α_2-adrenoceptor, we have prepared and evaluated binding affinities for enantiomers of inhibitors of the benzylamine class (typified by analogues of LY-134046) and of phenylethylamines (typified by conformationally-defined ligands in which the ethylamine side chain is held in an optimal fully extended conformation such as 8-amino-6,7,8,9-tetrahydro-5,8-methano-5*H*-benzocycloheptene and *anti*-10-amino-5,6,7,8-tetrahydro-5,8-methano-9*H*-benzocycloheptene). Where appropriate, X-ray crystal structures have been determined to establish absolute stereochemistry. A comparison of the binding modes of ligands of these two structural types to PNMT and to the α_2-adrenoceptor will be described and illustrated in terms of a computer graphics model of the active site of PNMT. Supported by HL 34193.

332.2

KINETIC MODELING OF 6-FLUORODOPA TIME COURSES IN PRIMATE BRAIN. J.E. Holden, B.D. Pate*, K.A. Hewitt*, W.R.W. Martin*, T.J. Ruth*, B.J. Snow*, and D.B. Calne*. UBC/TRIUMF PET Program, Vancouver, B.C. V6T 2A3, and Dept. of Medical Physics, U. of Wisconsin, Madison, WI 53705

Our previously reported graphical analysis of the kinetics of 6-fluoro-L-dopa (6FD) measured by dynamic PET (Martin et al., Ann. Neurol., 26:535-542, 1989) has been extended by fitting tissue time-course data to first-order kinetic models. A sequence of PET images was collected following bolus injection of 6FD into rhesus or cynomolgus monkeys, and time course data generated from both striatal and occipito-parietal cortical regions of interest. Plasma concentrations of 6FD and its chief circulating metabolite 3-O-methyl fluorodopa were determined at 25 time points during the 2 hour studies. The striatal and cortical time courses were fitted simultaneously, and the model parameters describing exchange of labeled compounds between tissue and plasma were assumed to be identical for both compounds and both anatomical regions. A third model parameter described the first-order irreversible trapping of striatal 6FD. Studies with cerebral decarboxylase inhibitors or elevated plasma amino acid demonstrated the ability of the model analysis to distinguish the transport and trapping processes. A model accounting for the slow loss of the trapped component was used to fit a preliminary series of 4 hour studies. The mean loss rate of 0.0034/min is consistent with literature values for striatal dopamine turnover rates.

332.3

LY274614, AN ANTAGONIST OF N-METHYL-D-ASPARTATE (NMDA) RECEPTORS, PROTECTS AGAINST AMPHETAMINE-INDUCED NEUROTOXICITY TOWARD STRIATAL DOPAMINE NEURONS IN RATS. R. W. Fuller, S. K. Hemrick-Luecke* and P. L. Ornstein. Lilly Research Laboratories, Eli Lilly and Company, Lilly Corporate Center, Indianapolis, IN 46285.

In rats treated with iprindole to block the usually rapid metabolism of amphetamine by para-hydroxylation, one dose of amphetamine depletes striatal dopamine and its metabolites for weeks (Fuller and Hemrick-Luecke, Science 209:305, 1980; Steranka, Brain Res. 234:123, 1982) and causes histologic evidence of neurotoxicity (Ricaurte et al, Brain Res. 291:378, 1984). In male Sprague-Dawley rats treated with iprindole (10 mg/kg i.p.), (±)-amphetamine sulfate (0.1 mmole/kg i.p.) caused about 40% depletion of striatal dopamine at one week. LY274614, (±)-decahydro-6-(phosphonomethyl)-3-isoquinolinecarboxylic acid (an NMDA receptor antagonist), dose-dependently antagonized this loss of striatal dopamine when injected i.p. at doses of 1-10 mg/kg 1 hr before amphetamine. LY274614 (10 mg/kg i.p.) protected against striatal dopamine depletion when it was injected during an 8 hr period preceding amphetamine or during a 4 hr period after amphetamine. LY274614 also antagonized methamphetamine-induced neurotoxicity in mice, consistent with findings of Sonsalla et al (Science 243:398, 1989) on other NMDA receptor antagonists. The NMDA receptor apparently plays a role in the nigrostriatal dopaminergic damage induced by amphetamines.

332.4

ROLE OF ANESTHESIA, ROUTE OF ADMINISTRATION AND CHRONIC TREATMENT WITH NICOTINE (NIC) ON THE NIC-INDUCED RELEASE OF DOPAMINE (DA) FROM RAT STRIATUM (St) AND NUCLEUS ACCUMBENS (NA) USING MICRODIALYSIS. Thomas C. Westfall and Lillian E. Vickery, Dept. of Pharmacology, St. Louis Univ. School of Medicine, St. Louis, MO 63104.

We have previously observed that the administration of NIC into microdialysis cannulae of freely moving conscious rats causes a release of endogenous DA and ^{3}H-DA newly synthesized from ^{3}H-tyrosine in the striatum (St). The present studies were designed to examine the role of anesthesia as well as the route of administration (i.v. or microdialysis cannula) on the NIC-induced release of DA, DA metabolites (DOPAC and HVA) and 5-hydroxyindole acetic acid (5HIAA) from two brain regions (St and NA) before and after chronic treatment of rats with NIC (1 mg/kg/day for 14 days). For studies on chloral hydrate anesthetized rats guide cannulae were implanted and microdialysis probes immediately introduced. For studies in conscious animals, rats were anesthetized with ketamine/acepromazine and a guide cannula stereotaxically implanted into the St or NA. The microdialysis probe was introduced into the guide cannula after 5-7 days. In both cases the microdialysis probe was perfused with Ringers solution (2 μl/min). Fractions were collected at 20 min intervals for analysis of DA and metabolites by HPLC-EC detection. NIC was administered i.v. (300 μg/kg) or into the microdialysis cannula (1 mM) 120 min after the start of perfusion. Basal release of DA from St was greater in anesthetized rats while there was an attenuation in the NIC induced release in these rats compared to conscious freely moving animals. Chronic treatment with NIC often resulted in an increase in basal levels of DA and a decrease in evoked DA release from both brain regions. Basal release of DA was lower in the NA compared to St while there was generally a greater NIC-induced release of DA from NA compared to St. (Supported by DA 02668).

332.5

SEROTONIN ACTS AT 5-HT$_{1A}$ RECEPTORS TO SELECTIVELY ATTENUATE GLUTAMATE-EVOKED RESPONSES OF LOCUS COERULEUS NEURONS. G. Aston-Jones[1], P. Charlety, H. Akaoka[1], R. Shiekhattar[1] & G. Chouvet, INSERM U 171/CNRS URA1195, CHLS, Pierre-Bénite, France; [1]Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann U., Philadelphia, PA, USA.

Extracellular recordings were obtained from individual LC neurons in halothane-anesthetized rats using an 8-barrel micropipette assembly (recording tip extending 12-18 µm). Iontophoretic Glu or acetylcholine (ACh) potently activated, and NE consistently inhibited, LC cells. In contrast, 5-HT had no consistent effect on LC spontaneous activity, but reliably suppressed responses of LC neurons to Glu (by 58 ± 3% overall) in 83% of 201 neurons. This effect was specific for Glu, as 5-HT did not reduce ACh responses in 10 of 11 neurons. Also, NE did not attenuate Glu-evoked responses of LC neurons.

Iontophoretic methysergide or methiothepin antagonized 5-HT's attenuation of Glu-evoked responses (11/15 & 4/5 neurons, respectively). Iontophoresis of the the 5-HT$_1$ agonist RU24969 mimicked the effects of 5-HT (10/13 cells), as did application of the 5-HT$_{1A}$ agonists 8-OHDPAT, PAPP and buspirone (18/25, 13/22 and 3/4 cases, respectively). Agonists at the 5-HT$_{1B}$ site, TFMPP and CGS 12066B, were less effective. The 5-HT$_2$ antagonist ketanserin did not attenuate (n=8), and the 5-HT$_2$ agonist DOI failed to mimic (n=6), 5-HT effects. Thus, a 5-HT$_{1A}$ receptor may be most involved.

Activation of LC neurons by sciatic nerve stimulation is mediated by excitatory amino acids (EAAs). After depletion of brain 5-HT with PCPA, LC neurons exhibited heightened sciatic responses (by 64%; n=32). However, microinfusion of 5-HT onto LC somata in intact rats did not reliably attenuate these responses. Thus, modulation of LC sensory responses by 5-HT may take place outside the LC, and 5-HT's attenuation of Glu responses may reflect an interaction with EAA receptor(s) that do not convey sensory responses of LC neurons. Support: CNRS, INSERM, the Philippe Foundation, the Simone del Duca Foundation, and PHS grants NS24698 & DA06214.

332.6

ENHANCEMENT OF SENSORY-EVOKED RESPONSES IN RAT LOCUS COERULEUS (LC) BY THE 5-HT$_2$ AGONIST 1-(2,5-DIMETHOXY-4-IODOPHENYL)-2-AMINOPROPANE (DOI). C. Chiang, R. Shiekhattar, and G. Aston-Jones. Div. Behav. Neurobiol., Dept. Mental Health Sci., Hahnemann Univ., Philadelphia, PA 19102

Hallucinogens, acting at 5-HT$_2$ receptors, decrease LC firing while increasing these cells' response to sensory stimuli (Aghajanian, 1980). Here, we systematically examined the effects of 5-HT$_2$ receptor activation on LC responses to noxious or non-noxious stimuli using systemic or local pressure application of the potent agonist, DOI.

In chloral hydrate-anesthetized rats, (±)DOI (50 µg/kg, i.v.) decreased LC spontaneous activity in all cells tested; it also markedly potentiated air puff-evoked responses in 7/10 cells (p=0.001; mean onset = 44 msec). Moreover, at this dose systemic DOI enhanced LC activation by subcutaneous electrical stimulation of the contralateral footpad (FS; 15/16 cells, mean increase = 53%, p<0.001). This enhancement was primarily the result of the induction of a second, late component of the FS response (onset approx. 64 msec, duration approx. 48 msec; mean increase of second component = 547% following DOI; p<0.001). After DOI pretreatment, local infusion of the non-NMDA antagonist, CNQX (50 µM, 60 nl), into LC reliably attenuated the first FS response component more than the second component (mean decrease = 71.5% and 11.5%, respectively). Similar infusion of the NMDA antagonist, AP5 (50 µM, 120-180 nl), attenuated the second response component but not the first in the one cell tested. Local pressure microinjection of DOI (0.5 µM or 20 µM, 30-240 nl) directly into LC had no effect on either spontaneous discharge or FS response of 13/13 LC cells. These results 1) show that, as with other 5-HT$_2$ agonists in the anesthetized rat, DOI can unmask non-noxious sensory responses in LC, 2) reveal that much of the enhancement of the LC FS response after systemic injection of DOI may be mediated by an input to LC acting at an NMDA receptor, and 3) support the hypothesis that DOI affects LC indirectly. Supported by PHS grants NS24698 and DA06214.

332.7

GENERALIZED OCCURENCE OF IMMUNOREACTIVITY TOWARDS A PEC-60 ANTISERUM IN CATECHOLAMINE NEURONS OF THE RAT. K. Fuxe, A. B. Tinner*, D.J. Morassutti, W. Staines, L.F. Agnati*, C.-G. Östensson, S. Efendic, B. Agerberth, M. Goldstein & V. Mutt. Dept of Histology and Neurobiology, Dept of Endocrin and Biochem, Karolinska Inst, Stockholm, Sweden; Dept of Human Physiol, Univ of Modena, Modena, Italy; Dept of Anatomy, Univ of Ottawa, Ottawa, Ontario, Canada, Neurochem Res Unit, NY Univ Med Center, NY, USA.

A peptide with a partial but distinct sequence homology to pancreatic secretory trypsin inhibitor (PSTI) has recently been isolated from the pig intestine and named PEC-60 (peptide with N terminal glutamic acid, C-terminal cysteine and a total of 60 residues). Using a standard formaldehyde picric acid fixation protocol it was possible to demonstrate immunoreactivity using a PEC-60 rabbit antiserum (2510) exclusively and within all central dopamine (DA), noradrenaline (NA) and adrenaline (A) neurons, (cell bodies, dendrites, axons, terminals) and within peripheral NAergic neurons and NA gland cells of the adrenal medulla using two colour immunofluorescence procedures. Absorption with PEC-60 but not PSTI (50 µg/ml) abolished the specific staining. Genetically modified TH-expressing cells failed to show IR against the PEC-60 antiserum and immunohistochemical controls run on Western blots showed that the high molecular weight PEC-60 immunoreactive protein was not a catecholamine synthetic enzyme. The IR, probably, is due to a peptide, or protein, with a fundamental role in CA transmission, since it is present in every single neuron of the CA nerve cell family.

332.8

STRESS INCREASES c-*FOS* EXPRESSION IN BOTH DOPAMINERGIC AND NON-DOPAMINERGIC NEURONS IN THE VENTRAL TEGMENTAL AREA. A. Y. Deutch[1], M. J. Iadarola[2], and M. Goldstein[3] [1]Departments of Psychiatry and Pharmacology, Yale University School of Medicine, New Haven, CT, 06510; [2]NIDR, Bethesda, MD, 20892; and [3]New York University Medical School, NY, NY 10016.

We have previously reported that mild stress increases dopamine (DA) metabolism in the ventral tegmental area (VTA) but not substantia nigra (SN). We now report that stress increases expression of the proto-oncogene c-*fos* in both DA and non-DA VTA neurons, but not neurons of the SN.

Rats were subjected to 30 min restraint stress or 30 min restraint in the cold (4°C). Animals subjected to restraint stress had an increased number of DA and non-DA neurons in the VTA (but not SN) which exhibited c-*fos*-like immunoreactivity (li). Cold restraint further increased c-*fos* expression in both VTA cell populations but not in the SN. The increased number of VTA c-*fos*-li DA neurons was most marked in the central linear nucleus and DA neurons in the dorsal raphe, and less marked in the nucleus parabrachialis pigmentosus. Stress also increased the number of c-*fos*-li cells in pontine nuclei innervating the VTA. These data indicate that stress increases c-*fos* in VTA DA and non-DA neurons. The stress-induced activation of VTA DA neurons my involve activation of excitatory pontine afferents to the VTA, and may also involve local circuit interactions with non-dopaminergic VTA neurons.

Supported by grant MH-45124 and by grants from the Scottish Rite Schizophrenia Research Program and the American Parkinson Disease Association.

332.9

COORDINATED REGULATION OF CATECHOLAMINE SYNTHESIZING ENZYMES IN THE CNS AND ADRENAL GLAND. T.H. Joh, D.M. Stone, K.T. Kim, D.H. Park, J.M. Carroll, C. Carver, H. Baker, K.S. Kim and T. Wessel. Cornell Univ. Med. College, The Burke Rehab. Center, White Plains, NY 10605.

Reserpine has been used extensively to study changes in catecholamine (CA) systems. The biosynthetic pathway consists of four enzymes: tyrosine hydroxylase (TH), aromatic L-amino acid decarboxylase (AADC), dopamine β-hydroxylase (DBH) and phenylethanolamine N-methyltransferase (PNMT). Previous biochemical studies demonstrated differences in the magnitude of changes in enzyme activity in response to reserpine in locus ceruleus (LC), adrenal gland (AG) and substantia nigra (SN). Recent studies have shown that the increase in TH activity can be attributed to increased gene transcription for this enzyme. In this investigation we combined immunohistochemistry, *in situ* hybridization and Northern blot analyses to investigate possible coordinate regulation of this pathway. Sprague-Dawley rats were treated with a single injection of reserpine (10 mg/kg SQ). Northern blots of AG mRNA 24h post injection showed clear increases in all four CA synthesizing enzymes: TH had the greatest up-regulation followed by PNMT and DBH while AADC showed a modest increase. *In situ* hybridization corroborated these results. Immunohistochemical staining at 96 h confirmed elevated protein. Similar results were obtained for the LC while the SN showed no changes. To investigate the role that the proto-oncogenes c-fos and c-jun may play in the regulation of the CA synthesizing enzymes, rats were reserpinized and perfused 1h post injection. *In situ* hybridization with DNA probes for c-fos and c-jun resulted in strong hybridization signal in the adrenal medulla and in LC while no signal could be detected in the SN or in controls. This interesting correlation suggests that AP-1 consensus sites in the 5'-upstream region of the TH gene and possibly the other CA synthesizing enzyme genes have functional significance in the living animal. Supported by MH 44043.

332.10

STRIATAL PATCH/MATRIX DISTRIBUTION OF FOS, SUBSTANCE P AND DYNORPHIN INDUCED BY APOMORPHINE IN INTACT AND 6-OHDA-LESIONED RATS. L.M. GRIMES, X. PING*, H.-K. JIANG*, AND J.-S. HONG. Bio Sci. Res. Cent., UNC-CH, Chapel Hill, NC 27599 and Lab. Mol. Int. Neurosci., NIEHS, RTP, NC 27709.

To determine the codistributions of immunoreactivity for fos, dynorphin (DYN), substance P (SP), 28 kD calcium binding protein (CaBP), and tyrosine hydroxylase (TH) in striatum, rats were injected intracisternally as neonates with 6-OHDA or vehicle and as adults were treated with apomorphine (AP) (5mg/kg, 7 days, bid). Twenty-four hours later, rats were given a final injection of APO or saline and perfused with 4% paraformaldehyde plus 0.2% glutaraldehyde after 1.5 hr. Immunocytochemical staining of 30 um Vibratome sections revealed that, in intact rats, fos, SP and DYN were restricted to striatal patches devoid of CaBP. In 6-OHDA-lesioned rats, areas depleted of TH showed expression of fos, SP and DYN in both patch and matrix compartments. These studies suggest that lesions of the striatal dopamine (DA) system cause alterations in matrix neurons to allow DA agonist-induced expression of fos, SP, and DYN gene products. Colocalization studies are in progress.

332.11

DOPAMINERGIC REGULATION OF TRANSCRIPTION FACTOR mRNAS IN STRIATAL NEURONS IN VIVO. A.J. Cole, P.F. Worley and J.M. Baraban. Depts. of Neurology, Neuroscience and Psychiatry, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Recent studies have shown that D_1-dopamine receptor agonists induce expression of the transcription regulatory factor (TF) protein c-Fos in deafferented rat striatal neurons (Robertson et al., Brain Res., (1989) 503:346-9). In light of previous findings that TF mRNAs are differentially regulated by neuronal stimulation in vivo, we have examined the effect of amphetamine on mRNA levels of several proven or putative TF genes using in situ hybridization. Amphetamine (2.5-10 mg/kg i.p.) causes a rapid but transient dose-dependent increase in zif/268 and jun-B mRNA levels in striatum and cortex. Striatal mRNA responses are blocked by pretreatment with the specific D_1 receptor antagonist SCH-23390 (0.2 mg/kg), but not by mianserin (1 mg/kg), a 5-HT receptor antagonist, suggesting that they are mediated by the dopamine releasing action of amphetamine. Paradoxically, several chemically distinct selective D_2 antagonists, eticlopride (0.1mg/kg), haloperidol or pimozide (1mg/kg) increase striatal mRNA levels for both of these TFs. Taken together, these results suggest that zif/268 and jun-B mRNA levels in the intact striatum are regulated by a complex interaction between D_1-and D_2-dopamine receptor subtypes.

332.12

DEVELOPMENTAL REGULATION OF THE ADRENERGIC PHENOTYPE. D.L. Wong, S.A. White and C.L. Bildstein*. Dept. of Psychiatry and Behavioral Sciences, Stanford University School of Medicine, Stanford, CA 94305.

Phenylethanolamine N-methyltransferase (PNMT), the final enzyme in epinephrine synthesis, is a marker of the adrenergic phenotype. In the rat adrenal gland, glucocorticoids regulate this enzyme and adrenergic expression in three ways. Initially, PNMT is steroid independent as it always appears on ~E17.5 irrespective of fetal steroid levels. Steroids subsequently become inductive, and can induce overexpression of PNMT. Finally, steroids become permissive, restoring PNMT to basal levels following depletion.

To determine the mechanisms for these various modes of steroidal regulation, we are examining a spectrum of PNMT indices, enzyme activity, protein and mRNA. From birth (P0) through P15, PNMT increases while adrenal weight changes insignificantly, consistent with adrenergic development. Up until ~P10, steroids are inductive. During this inductive phase, ACTH administration (1 I.U.) for 7 days, starting on P0 or P4, increases PNMT activity up to 35%. Similarly, corticosterone administration (10-100 mg) for 1 week elevates the enzyme up to 50%. Immunochemical data suggest that steroid-induced protein may be structurally different as the equivalence points and slopes of the immunotitration curves change. Between P10-12, glucocorticoids become permissive. PNMT activity cannot be hormonally elevated above basal levels. Hormones now regulate enzyme degradation. Hypophysectomy markedly depletes PNMT activity and protein. ACTH administration reverses these events. Simultaneously, PNMT mRNA changes insignificantly. Around P10, transsynaptic mechanisms become operant, which complement adult steroidal control of PNMT. Hormones, however, seem to be primary in adrenergic development. They appear critical to the expression and establishment of the adrenergic phenotype. Once the phenotype is established, steroids then subsume a permissive regulatory role working in conjunction with neural mechanisms to modulate adrenergic function.

333.1

THE DEVELOPMENT OF A RADIOIMMUNOASSAY AND AUTORADIOGRAPHIC DISTRIBUTION STUDIES WITH CI-977, A NOVEL ANALGESIC KAPPA OPIOID RECEPTOR AGONIST. C.M. Barksdale, W.P. McNally, G.D. Nordblom*, M.J. Coon*, P.D. DeHart*, T. Chang*, and D.S. Wright*. Pharmacokinetics/Drug Metabolism Department Parke-Davis Pharmaceutical Research Division, Warner-Lambert Company, Ann Arbor, Michigan 48105-2430.

CI-977, a novel kappa opioid receptor agonist currently undergoing clinical evaluation in humans, has high in vitro affinity for the κ opioid receptor in guinea-pig forebrain preparations: the Kd for the κ receptor is 0.11 ± 0.02 nM, while the Kd is 99.6 ± 4.3 nM for the μ receptor and 1036 ± 77 nM for the δ receptor. This results in a μ/κ selectivity ratio of 905 and a δ/κ selectivity ratio of 9418. We now report the development of a radioimmunoassay procedure for CI-977 and the autoradiographic distribution of [^{3}H]-CI-977 in rats.

Male rats given 40 μg/kg of [^{3}H]-CI-977 (31 μCi/μg) by IM injection were sacrificed at intervals from 10 minutes to 24 hours for whole body autoradiography. Autoradiograms showed activity widely distributed to tissues following IM dose, with highest levels seen in kidney, lung, spleen, salivaries, and liver at early time points. Activity was evenly distributed to areas of gray matter in brain and spinal cord, with higher concentrations in the choriod plexus, pituitary, cortex and hippocampal CA3 regions, previously reported to have significantly elevated kappa opioid receptor concentrations.

333.2

CHARACTERISTICS AND DISTRIBUTION OF CENTRAL *KAPPA* OPIOID RECEPTORS IN A SONGBIRD SPECIES (dark-eyed junco, *Junco hyemalis*). P. Deviche. Inst. Arctic Biology, Univ. Alaska Fairbanks, Fairbanks, AK 99775-0180.

The characteristics of the binding of ^{3}H-ethylketocyclazocine (^{3}H-EKC) to whole brain homogenates obtained from juncos were studied to identify and to characterize the central κ opioid receptors of this species. In the presence of DAGO and DPDPE (100 nM), ^{3}H-EKC binds to brain homogenates specifically, in a time-related fashion, and with a high affinity (Kd: 1.3 nM). Specific ^{3}H-EKC binding sites are present at a low concentration (Bmax: 35 fmoles/mg protein). Unlabelled ligands that have a high selective affinity either for μ or for δ opioid receptors have a low affinity for ^{3}H-EKC binding sites ($IC_{50} \geq 3\mu M$). Using *in vitro* autoradiography, we found that κ receptors are present at the highest density in the forebrain (olfactory bulb, ventral neostriatum, hyperstriatum), and at a lower density in mid- and hindbrain regions. They are virtually absent from the cerebellum. These results will be compared with those obtained for mammals and for domestic pigeons, the only other avian species in which the brain distribution of κ receptors has been determined so far.

333.3

PHARMACOLOGICAL CHARACTERIZATION OF NALORPHINE, A KAPPA$_3$ ANALGESIC. D. Paul, C.G. Pick, L.A. Tive and G.W. Pasternak. The Cotzias Lab. of Neuro-Oncology, Memorial Sloan-Kettering Cancer Center and Depts. of Neurology and Neuroscience and Pharmacology Cornell Univ. Medical College, New York NY 10021.

The observation that nalorphine antagonizes morphine analgesia, but itself produces analgesia led to the theory of Receptor Dualism and multiple opioid receptor types. Nalorphine binds with nanomolar affinity to the novel kappa$_3$ opioid receptor, as well as mu and kappa$_1$ receptors. We have now identified the receptor type that mediates nalorphine analgesia in the mouse tail-flick assay. Low nalorphine doses antagonized morphine analgesia, but at higher doses produced analgesia, replicating the original studies in man. Although it was blocked by naloxone, nalorphine analgesia was insensitive to the mu antagonist ß-funaltrexamine, the delta antagonist naltrindole, and the kappa$_1$ antagonist nor-binaltorphimine. Nalorphine was cross-tolerant with the kappa$_3$ agonist, naloxone benzoylhydrazone (NalBzoH), but not with the kappa$_1$ agonist, U50,488-H. In addition, nalorphine analgesia was 1000-fold more sensitive to antagonism by i.c.v. than i.t. administration of the opiate antagonist, WIN44,441, a pattern also seen with NalBzoH. These results support the conclusion that nalorphine produces its analgesic effect through supraspinal kappa$_3$ receptors.

333.4

QUANTITATIVE AUTORADIOGRAPHY OF OPIOID RECEPTORS IN BRAINS OF SUICIDE VICTIMS. A. Biegon and R. Gross-Isserof*. Dept. Neurobiol., Weizmann Inst. of Science, Rehovot, Israel.

Indirect behavioral and pharmacological data suggest the involvement of opioids in the pathophysiology of depression, but there are no direct studies of human brain opioid systems in this context. We have examined μ and δ opioid receptors in the brains of 12 suicide victims and 12 age and sex matched controls postmortem, using quantitative in vitro autoradiography. Drug- and neuropathology-free brains were frozen and sectioned in a whole body cryostat. μ and δ receptors were labeled with ^{3}H-DAGO and ^{3}H-DPDPE. Non specific binding was measured in the presence of naloxone. Computerized image analysis of the autoradiograms revealed large, statistically significant increases in μ receptor binding in several cortical regions of the suicide victims, especially temporal gyri. δ receptor binding was significantly reduced in the same brains, with the most pronounced differences in prefrontal cortical regions. These data suggest endogenous opioid systems play a role in depression and suicide via different receptor subtypes in specific brain regions.

333.5

AUTORADIOGRAPHIC DISTRIBUTION OF [^{3}H](+)-PENTAZOCINE BINDING SITES IN GUINEA PIG BRAIN J.M.Walker, W.D.Bowen, A.H. Roberts, B.deCosta, and K.C.Rice, Brown University, Providence, RI 02912 and National Institutes of Health, Bethesda, MD 20892

There has been a need for a selective (+)-opiate sigma ligand because evidence suggests differential binding of opiate and nonopiate-related sigma compounds. We now describe the autoradiographic distribution of specific binding of the selective sigma ligand [^{3}H](+)-pentazocine in guinea pig brain.

Alternate sections were incubated with either [^{3}H](+)-pentazocine or [^{3}H]-DTG. Blanks were determined with 10 μM haloperidol. After 8-11 week exposure, autoradiographs were quantified using a computer. Specific binding of 85% of the total occurred for both radioligands.

[^{3}H]-(+)-Pentazocine binding is concentrated sigma-rich areas including cingulate cortex, septum, ventromedial hypothalamus, periaqueductal gray, red nucleus, cerebellum and various cranial nerve nuclei. Binding was low in the caudate-putamen, ventral posterior nucleus of the thalamus, and most cortical areas. [^{3}H](+)-Pentazocine binding across brain areas correlated highly with the amount of [^{3}H](+)-DTG bound ($r=0.88$, $p<0.0001$), suggesting that it binds to sigma receptors. However, the ratios of the binding of the two ligands ranged from 0.8 to 3.4, suggesting that different populations of sites may be labelled by these two ligands.

333.6

***SIGMA* RECEPTORS IN IMMUNE AND ENDOCRINE TISSUES: DICHOTOMY BETWEEN BINDING AND FUNCTION.** S.A. Wolfe, Jr., L.G. Aguayo and E.B. De Souza. Neurobiology Laboratory, NIDA ARC, Baltimore, MD 21224, and Section of Electrophysiology, Laboratory of Physiologic and Pharmacologic Studies, NIAAA, Rockville, MD 20852.

PCP and the prototypic σ agonist N-allylnormetazocine (NANM) alter neuroendocrine and immune functions. We have previously described high densities of σ receptors (and the absence of PCP receptors) in human peripheral blood leukocytes and rat spleen, pituitary, adrenal, testis, ovary, and pineal. Since the pineal gland modulates CNS, endocrine and immune functions, we carried out electrophysiological studies to determine whether σ receptors are physiologically active in the rat pineal. In addition, we examined the functional nature of σ binding sites in the immune system. In whole-cell patch clamp recordings, PCP suppressed the delayed voltage-activated K^+ outward current in acutely dissociated rat pineal cells. The σ ligands butaclamol and 3-PPP were more potent than PCP in this regard. However, these electrophysiological studies failed to show the stereo-selectivity for enantiomers of butaclamol and 3-PPP which is characteristic of σ receptors in binding and autoradiographic systems. In the immune system, PCP and other σ ligands suppressed *in vitro* functional immune responses, but the pharmacological profile of immunosuppression was unlike that of σ receptors in binding assays. Recent data from several laboratories support the existence of multiple binding sites for σ ligands. Therefore the findings of the present study suggest that, although σ receptors appear to be present in high densities in pineal and immune cells, other receptor types in these cells may modulate the measured biological responses to σ ligands.

333.7

HETEROLOGOUS DOWN-REGULATION OF CHOLINERGIC RECEPTORS BY SIGMA RECEPTOR ACTIVATION. W.D. Bowen and P.J. Tolentino. Sect. Biochem., Div. Biol. and Med., Brown Univ., Providence, RI 02912.

We have previously shown that sigma ligands attenuate the ability of carbachol and oxotremorine-M (Oxo-M) to stimulate phosphoinositide (PPI) turnover in rat brain synaptoneurosomes (Bowen et al. *Eur. J. Pharmacol.* 149:399; Bowen et. al, *NIDA Research Monograph Series*, in press). The inhibitory potency correlated highly to sigma receptor binding affinity (r = 0.92; with oxotremorine-M as agonist). The major effect of sigma ligands is to reduce the maximal stimulation, without markedly affecting the ED50 of the cholinergic agonist.

We have investigated the effect of sigma ligands on cholinergic receptors. Pretreatment of membrane fragments with 50 uM (+)-pentazocine in Krebs-Henseleit/Hepes buffer had no effect on the binding of [3H]Oxo-M (assayed in same buffer). However, pretreatment of intact synaptoneurosomes with (+)-pentazocine resulted in a marked decrease in the number of [3H]Oxo-M binding sites, with no effect on the binding affinity. (+)-Pentazocine pretreatment had no effect on the binding parameters of the membrane permeant cholinergic ligand, [3H]QNB. These data suggest sigma-induced internalization of cholinergic receptors. In addition, when intact synaptoneurosomes were pretreated with (+)-pentazocine and then lysed in hypotonic buffer prior to Scatchard analysis of [3H]Oxo-M binding, an increase in Kd was observed, with no change in Bmax. The difference in results obtained when intracellular receptors are accessed with [3H]QNB (antagonist) and [3H]Oxo-M (agonist) may suggest that sigma activation also produces uncoupling of cholinergic receptors from G-proteins. Thus, sigma ligands appear to inhibit the cholinergic PPI response by heterologous desensitization. (Supported by PHS Grant NS-26746)

333.8

SIGMA (SR) AND OPIOID RECEPTORS (OR) IN HUMAN CANCERS. C.J. Coscia, W.T. Bem*, G.E. Thomas*, J.Y. Mamone*, B.K. Levy*, M. Szücs* and F.E. Johnson. St. Louis University School of Medicine, St. Louis MO 63104

SR and OR interact with cell signalling pathways which contain proto-oncogene products and regulate cell proliferation. OR modulate growth of developing brain and tumors. The putative presence of OR in various tumors has been based on binding assays using (±)^{3}H-ethylketocyclazocine (EKC). Since (+)EKC can bind to SR, we investigated SR and OR distribution in diverse tumors. Fresh specimens were obtained from 28 human neoplasms (19 brain tumors and 9 others) and 7 normal tissues. Binding parameters were determined by homologous displacement assays using ^{3}H-1,3-di-o-tolylguanidine for SR and either ^{3}H-U-69,593 or (-)^{3}H-EKC with unlabeled μ and δ suppressors for OR. Data were analyzed with the LIGAND program. SR were detected in 26/27 tumors. OR (κ) were found only in gliomas (4/4). OR were detected in desipramine-treated C6 rat glioma and 2 human glioma cell lines, but not in human neuroblastoma line SK-N-MC (which express SR). Bmax values for SR in renal, colorectal and meningeal tumors were greater than that of corresponding normal tissues. Intra-tumoral variability (ITV) in SR was compared with ITV in histology (assessed by computerized quantitative morphometry). ITV was modest for SR Bmax values but marked for histologic parameters. On the basis of these and previous studies, SR and OR differ in stereochemistry, subcellular distribution, and pattern of expression in tumors.

333.9

AUTORADIOGRAPHY OF ^{125}I-ß-ENDORPHIN BOUND TO OPIOID AND NON-OPIOID SITES IN TISSUE CULTURE. R.I. CONE, J. LAMEH* AND W. SADEE*. Dept. Pharmacy & Pharmaceutical Chemistry, UCSF, San Francisco, CA 994143-0446.

Detection of opioid binding sites on intact cells in culture is useful in monitoring the regulation of surface receptor affinity and density in human neuroblastoma cells, and in screening cDNA libraries for expression of genes encoding intact receptors following transfection in mammalian target cells. The presence of both μ and ∂ opioid receptors in the human neuroblastoma cell line, SK-N-SH, and its neuroblast subclone, SH-SY5Y have been demonstrated in this laboratory, and opioid receptor regulation in SH-SY5Y cells has been studied.

We have developed an autoradiographic method using ^{125}I-ß-endorphin (ß-E) for studying opioid receptor internalization in human neuroblastoma cell lines, and for screening a pCDM8 cDNA expression cloning library from SK-N-SH-SY5Y cells, complexity 2 x 10^7, after transfection into COS-7 cells for opioid expression.

Our results indicate that a large portion of ß-E binding in SK-N-SH, NG108-15 and COS-7 Cells is non-opioid. However, in the presence of blocking concentrations of ß-E (6-31), a fragment with intact c-terminus which binds only to non-opioid sites, ^{125}I-ß-E specifically labels μ and ∂ sites.

The K_d of ß-E binding to μ and ∂ opioid sites on intact neuroblastoma cells is between 0.2 to 1 μM, thereby allowing for sensitive detection of opioid receptors, either by filtration or by autoradiography. The ability of the tracer to cross the cell membrane or to internalize with the receptor will be investigated. Supported by NIDA grant DA 04166.

333.10

THE BIOSYNTHESIS OF MAMMALIAN MORPHINE: THE ROLE OF ENZYMATIC O- METHYLATION OF (R)- AND (S)-ENANTIOMERS OF NORCOCLAURINE, AND 3'-DEMETHYLNORRETICULINE IN THE FORMATION OF (S)-RETICULINE. C. R. CREVELING, M. E. BELL*, Y. SEKINE, D. TADIC, AND A. BROSSI. Labs. of Bioorganic and Analytical Chemistry, NIDDK, NIH, Bethesda, MD 20892.

Small amounts of the opioid alkaloids, morphine and codeine have been indentified in mammalian brain, adrenal glands, and skin, both as free alkaloids and as sulfate conjugates. The non-morphinan, reticuline, is converted in rat liver to the morphinan, salutaridine. The administration of salutaridine, thebaine, or codeine to rat results in increased levels of morphine in brain, liver, and intestine. These observations strongly suggest an endogenous biosynthetic rather than a dietary origin for mammalian morphine. Zenk et al. (1985) clearly demonstrated that in poppy plant, (S)-norcoclaurine, derived from an enzymatic and stereospecific condensation of dopamine with (4-hydroxyphenyl) acetaldehyde, is the first isoquinoline in the biosynthesis of the critical intermediate, reticuline. We have examined the O-methylation, catalyzed by mammalian catechol-O-methyltransferase, of the (S)- and (R)-enantiomers of norcoclaurine and demethylnorrecticuline. In both cases, the O-methylation was stereoselective, affording almost exclusively the 6-O-methylethers from the (S)-enantiomers, an obligatory pathway for the biosynthesis of (S)-reticuline. When the (R)-enantiomers were substrates the 7-O-methylethers were the major products. These results suggest that the putative pathway for the mammalian biosynthesis of (S)-reticuline may be identical to the pathway in poppy plants.

Zenk, M. H., Rueffer, M., Amann, M., Deus-Neumann, B., Nakagura, N. *J. Natural Products*, **48**, 725.

CELLULAR AND MOLECULAR STUDIES II

334.1

VITAL DYE ANALYSIS AND CONFOCAL IMAGING OF CRANIAL NEURAL CREST CELLS IN MICE. George N. Serbedzija, Scott E. Fraser and Marianne Bronner-Fraser. Developmental Biology Center, U.C. Irvine. Ca. 92717.

Analysis of cranial neural crest cell migration and differentiation in the mouse has been hampered by both the lack of a reliable cell marker and by the difficulty of manipulating the embryo *in vivo* at the stage of neural crest development. We recently have demonstrated that mouse truncal neural crest cells could be labelled successfully using the vital dye, DiI (Serbedzija, *et al.*, 1990 Develop. **108**:605-612). Here we use this approach in combination with an *exo utero* technique for manipulating mouse embryos (Muneoka *et al.*, 1986, *J. Exp. Zool.* **239**:289-293) to describe cranial neural crest cell migration pathways and sites of differentiation. On the eighth day of pregnancy, 1-4 somite stage embryos were exposed in the uterus and labeled with DiI. The mothers were then closed, and the embryos allowed to develop for an additional 24-48 hours. Embryos were analyzed both in whole mount using a confocal microscope and in serial sections using an epifluorescence microscope. After 24 hours, metameric streams of labeled cells were observed extending from the neural tube into the developing branchial arches, and single labeled cells could be seen at the levels of the forebrain and midbrain regions. After 48 hours, labeled cells also appeared in the cranio-facial ganglia, in the heart, and throughout the head (e.g. in the maxillary process, and around the eye and the olfactory pit). These results demonstrate the feasibility of using the *exo utero* technique to perform an *in situ* study of the timing and pattern of murine cranial neural crest migration and differentiation. Supported by USDHS HD 25138

334.2

DEVELOPMENT OF PERISYNAPTIC CELLS IN SKELETAL MUSCLES OF TRANSGENIC MICE. J. Weis, S. Fine*, and J. R. Sanes, Dept. Anat. & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

The adhesion molecules tenascin, N-CAM, and fibronectin accumulate near motor endplates in denervated skeletal muscle. Fibroblasts near synaptic sites selectively proliferate after denervation and are likely to be the cellular source of these molecules (Gatchalian et al., J. Cell Biol., 108, 1873-1890, 1989). This biosynthetic specialization could reflect distinct features of the perisynaptic environment that arise upon denervation and/or intrinsic differences between perisynaptic and extrasynaptic fibroblasts. We now report results that favor the latter possibility.

DNA constructs containing 2 kilobases of the MHC class I gene promoter, the coding portion of the E. coli ß-galactosidase gene (lacZ), and a 3' splicing and polyadenylation signal from SV40 were microinjected into the pronuclei of mouse embryos. Transgenic animals were identified by Southern blot analysis and PCR, and lacZ expression was detected histochemically. Surprisingly, the MHC promoter appeared inactive in this context and the transgene was apparently controlled by genomic regulatory elements near the integration site. Due to this control, the transgene was expressed in a distinct subset of cells in each line.

In one of nine mouse lines produced, cells close to neuromuscular junctions were lacZ-positive, whereas cells in extrasynaptic portions of muscle did not stain. Expression of lacZ was strong at birth and disappeared by 3 weeks postnatally. Electron microscopy revealed that some of the perisynaptic cells that expressed the transgene were fibroblasts. Other cells near synapses were also lacZ-positive; these may include Schwann, endothelial, and perineurial cells. However, no intramuscular cells (fibroblasts or others) located far from synaptic sites were lacZ-positive. These results support the notion that "perisynaptic" fibroblasts differ in their genetic program from "extrasynaptic" fibroblasts in muscle. (Support: NIH and Max Kade Foundation)

334.3

MIGRATION OF CLONALLY RELATED CELLS IN THE DEVELOPING CHICK SPINAL CORD. S.M. Leber and J.R. Sanes. Dept. Anatomy & Neurobiology and Pediatrics, Washington University School of Medicine, St. Louis, MO 63110.

To study the lineage and migration of chick spinal motoneurons, we infect progenitors with a ß-galactosidase-bearing retrovirus, then identify their progeny histochemically. We previously found that individual progenitors are pluripotent with respect to phenotype but that their progeny are restricted spatially along both the rostrocaudal (RC) and dorsoventral (DV) axes (Leber et al., J. Neurosci., in press). Here, we ask how this restricted distribution arises. First, we analyzed cell mixing in the ventricular zone (VZ). Some clones labeled at st. 8 spanned the entire DV extent of the VZ and up to 1½ segments along the RC axis. However, clones labeled at st. 12-14 were restricted to <½ segment length and to narrow DV sectors. These results imply that displacement of cells within the VZ becomes restricted between st. 8 and 12-14. Second, we studied the migration of cells out of the VZ by infecting embryos at st. 12-14 and varying the time of analysis. At st. 20-21, most clones consisted of radial arrays of cells, suggesting that the initial migration is predominantly radial. However, by st. 25, neurons in many clones turned orthogonally from their parent radial arrays and migrated along circumferentially oriented axons. By stage 34, clonally related cells were usually distributed in narrow transverse slabs in single quadrants of the spinal cord. Between st. 30 and 38, some white matter glia migrated up to several segments along longitudinal axons. We conclude that the distribution of clonally related cells results from: 1) limited mixing within the VZ that confines clonal relatives to the same RC position and, often, to either the alar or basal plate; 2) migration of cells from the VZ in spoke-like radial routes; 3) a secondary neuronal migration along circumferential axons; and 4) a late longitudinal migration of glia along white matter tracts.

334.4

VISUAL CALLOSAL DEVELOPMENT IN NEONATAL RATS: DO MIGRATING OR UNDIFFERENTIATED CELLS HAVE AN INTERHEMISPHERIC AXON? *C.S. Hernit, R.C. Van Sluyters, and K.M. Murphy*, Dept. of Molecular and Cell Biology and School of Optometry, Univ. of California, Berkeley, CA 94720, Dept. of Psychology, McGill University, Montreal, Canada H3A 1B1.

At birth (PND1) lamination in the visual cortex of the rat is incomplete. Neurons destined for layers II-III are undifferentiated and either still en route or newly arrived at the immature cortical plate[1]. In order to determine whether these cells have a callosal projection, the posterior neocortex of animals aged PND1 was injected with either Rhodamine Conjugated Latex Microspheres (RLM), Fast Blue (FB) or Fluorogold (FG). The radial positions of callosally labelled cells were examined on PND12, an age at which cortical lamination is complete.

In mature brains callosal cells are found in layers II-VI. Cells callosally labelled with RLM on PND1 were found only in lower layer IV and layers V-VI. A similar distribution of callosal cells was intensely labelled with FB and FG. Cells faintly labelled with FB were also found throughout cortical layers II-III and IV, as well as in the lateral geniculate nucleus *contralateral* to the injection. This faint labelling was likely due to leakage of FB from callosally labeled cortical cells. Some cells faintly labelled with FG were also visible in a region extending into lower layer II-III, and evidence indicates that they too were secondarily-labelled. The absence of supragranular cells intensely labelled with RLM, FB or FG suggests that at birth migrating or undifferentiated layer II-III cells do not possess a callosal axon.

1. Hicks, S.P. and C.J. D'Amato, *Anat. Rec.*, **160**:619-34, 1968.

Supported by NIH EY02193, NSF BNS84-18738 and a P.R. Harris Fellowship.

334.5

DEVELOPING ChAT-IMMUNOREACTIVE SPINAL CORD NEURONS MAY USE COMMISSURAL FIBERS AS MIGRATORY GUIDES. P. E. Phelps and J. E. Vaughn. Division of Neurosciences, Beckman Research Institute of the City of Hope, Duarte, CA 91010.

A "U"-shaped group of ChAT-immunoreactive (ChAT-IR) neurons was detected initially on embryonic day 16 (E16) directly adjacent to the ventral ventricular zone in rat cervical spinal cord. Since no cholinergic cells are observed dorsally at this or previous stages, we have hypothesized that the ChAT-IR neurons located in the dorsal and central spinal cord at later developmental times, and in adults, are derived from this E16 group of neurons (Phelps et al., *JCN*, 291:9, 1990). Closely spaced transitional stages suggest that some of these primitive cholinergic neurons that display short processes oriented dorsal-ventrally may follow the commissural pathway to reach their more dorsally-located, final positions. This pathway is formed by the early developing axons of commissural neurons that transiently express the cell surface glycoprotein TAG-1 (Yamamoto et al., *J Neurosci.*, 6:3576, 1986; Dodd et al., *Neuron*, 1:105, 1988). The coincidence of the distribution patterns of both the "U"-shaped group of ChAT-IR cells and the TAG-1-IR commissural fibers suggests the possibility that cholinergic cells could utilize such fibers as migratory guides. In addition, double-labeled immunocytochemical experiments provide preliminary evidence that some of these ChAT-IR neurons are associated with TAG-1-IR commissural fibers during the embryonic period (E16-17) when we propose that these neurons migrate from the ventral ventricular zone to their adult positions. *Supported by NIH grant NS 18858.*

334.6

LATERAL MIGRATION OF CELLS IN EMBRYONIC NEOCORTEX. S. A. Bayer, J. Altman, and R. Russo*. Dept. of Biology, Indiana-Purdue Univ. Indianapolis, IN 46205.

The embryonic (E) neocortex in rat brains from days E15 through E21 were three dimensionally reconstructed with a computer software package (SKANDHA, Univ. of Wash., Seattle). A prominent medial shift in the neocortical germinal matrix indicated that cells destined for the superficial layers (IV-II) in lateral cortical areas are generated in a medial position. Correlated [^{3}H]thymidine autoradiographic studies tracking the migratory pathways of young neurons indicated a "lateral migratory stream" in the intermediate zone. In addition, medial vs. lateral cortical cells have differential times of arrival in the cortical plate: dorsal cells are there in two days, lateral cells in three days, and ventrolateral cells in four days. (Support: NSF 8907219 and NIH NS23713.)

334.7

PRENATAL ASSEMBLY OF STRIATAL COMPARTMENTS IN THE MOUSE. M.A. Edwards and C.R. Gerfen. Developmental Neurobiology Dept., Shriver Center, Waltham, MA 02554, and Laboratory of Cell Biology, NIMH, Bethesda, MD 20892.

Previous studies have suggested that the patch-matrix organization of the rodent striatum emerges in the perinatal period. In the present study, the fetal development of striatal modules was studied using [3H]thymidine autoradiography and immunocytochemistry with monoclonal antibody RC2, which stains radial glia and immature astrocytes, and with an antibody to calbindin, known to stain predominantly neurons of the matrix zone. Results from variable survival periods after maternal [3H]thymidine exposure indicate that patch neurons arise largely between embryonic days E11 to E13 and initially become distributed uniformly to the striatal intermediate zone by E15. This distribution changes subsequently as later-born cells labelled on E14 and E15 migrate out to attain a matrix-like distribution between E17 and P0. Correspondingly, results with calbindin staining indicate that the neurons which express it become distributed in a matrix pattern in the period between E17 and E18. In the transition period between E14 and E17, RC2 staining reveals that radial glial fibers in the striatum changes from a uniform distribution into a pattern characterized by streams interposed by zones containing immature astroctyes. Sections double-labelled with RC2 immunostaining and autoradiography or with RC2 and anti-calbindin staining suggest that migratory columns of matrix neurons follow radial glial streams but earlier-born, patch neurons occupy the zones where immature astrocytes accumulate. These results suggest that radial glia organization may contribute to the migration of matrix neurons into zones which break up the initially uniform field of patch neurons. (Supported by HD21018).

334.8

TUMOR NECROSIS FACTOR (TNF) SELECTIVELY INHIBITS MITOSIS OF CULTURED BRAIN NEUROBLASTS. L-E. Chen, E. DiCicco-Bloom , and I. B. Black , Div. Dev. Neurol., Cornell Univ. Med. Coll., NY, NY 10021, and Dept. Neurosci. and Cell Biol. UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ.

During brain development, proliferation of neuronal precursors is highly reproducible spatially and temporally, suggesting precise regulation. Little is known, however, about mechanisms governing neuroblast mitosis. Previous studies in this lab have demonstrated that traditional growth factors as well as neurotransmitters stimulate neuroblast mitosis. We now report that TNF selectively and specifically inhibited the mitotic cycle of cultured brain neuroblasts.

A highly enriched population of granule neurons and dividing precursors derived from 7 day rat cerebella was cultured in fully defined medium, and assayed for mitotic activity, neurite outgrowth and cell survival. Exposure to recombinant murine TNF (rMuTNF) evoked a 40% decrease in ^{3}H-thymidine incorporation. In contrast, TNF did not affect survival, neurite outgrowth or protein synthesis, indicating that TNF selectively inhibited DNA synthesis. Moreover, inhibition reflected a decreased proportion of neuroblasts entering the mitotic cycle in culture and not simply diminished incorporation by a pre-existing mitotic population.

To define specificity, we examined a number of closely related cytokines. Although recombinant human TNF and lymphotoxin had similar effects on DNA synthesis, ED_{50}s were 10-fold higher than that of rMuTNF, consistent with previously defined species specificity. In contrast, interleukin-6 had no effect, suggesting that TNF is a highly specific inhibitor of neuroblast mitosis.

To characterize cellular specificity, the effect of TNF on cultured sympathetic neuroblasts was examined. TNF had no effect on DNA synthesis of sympathetic neuroblasts, suggesting that TNF may serve to regulate mitosis of specific populations of neuronal precursors.

In sum, TNF specifically inhibited neuroblast mitosis. Combined with previous evidence of mitogenic stimulation by growth factors and neurotransmitter, our observations suggest that both positive and negative epigenetic signals regulate neuroblast proliferation.

334.9

EGF AND FGF REGULATE MITOSIS OF CULTURED CEREBELLAR GRANULE CELL PRECURSORS. R.N. Cohen, E. DiCicco-Bloom, and I.B. Black. Div. of Developmental Neurology, Cornell Univ. Med. Coll. NY, NY 10021 and Dept. Neurosci. and Cell Biol., UMDNJ/ Robert Wood Johnson Medical School, Piscataway, NJ

Neurogenesis proceeds through a well-delineated sequence of events, yet underlying regulation remains undefined. Recent studies of the peripheral nervous system indicate that epigenetic signals regulate neuroblast mitosis. In contrast, numerous technical and theoretical constraints have impeded study of central neuroblasts. To overcome these limitations, we developed a fully-defined neuronal culture system in which central precursors undergo division. We found that physiologic concentrations of insulin promote cell survival, whereas insulin-like growth factor-I (IGF-I) additionally stimulates mitosis. We now report that both epidermal (EGF) and fibroblast (FGF) growth factors regulate DNA synthesis of central neuroblasts, but that multiple other factors are ineffective.

A highly enriched population of granule cells and precursors was obtained from postnatal day 7 rat cerebella (Hatten, J. Cell Biol., 1985); cells were cultured in fully-defined medium. To define potential growth factors for granule precursors, cells were cultured under varying conditions and were assayed for [3H]thymidine ([3H]dT) incorporation (Inc.) and survival. EGF increased Inc. 3-fold, with an EC_{50} of 1 ng/ml. To define the cellular basis for increased Inc., the proportion of cells that synthesized DNA (the "labeling index") was assessed by [3H]dT autoradiography. A suprasaturating dose of EGF (100 ng/ml) increased the labeling index 50%. This concentration, however, did not promote survival, suggesting that EGF specifically functioned as a mitogen. In contrast, bFGF increased both Inc. and survival (EC_{50} = 1 ng/ml), thereby exhibiting both trophic and mitogenic activity. The mitogenic effects appear to be highly specific. Both NGF and TGF-ß, which appear to regulate nervous system development, failed to stimulate Inc. Furthermore, depolarization, a mitogenic signal for peripheral neuroblasts, did not stimulate DNA synthesis of granule precursors. In sum, mechanisms regulating proliferation of central neuroblasts are apparently distinct from those operating in the periphery.

334.10

A METALLOPROTEASE SECRETED BY SCHWANN CELLS CLEAVES FIBRONECTIN INTO FRAGMENTS THAT INHIBIT PROLIFERATION. D. Muir, S. Varon and M. Manthorpe. Dept. of Biology, University of California at San Diego, La Jolla, CA 92093.

Rat sciatic nerve Schwann cell conditioned medium contains protein that inhibits the proliferation of mitogen-stimulated Schwann cell cultures. This antiproliferative protein can exist in 55 kD and >1000 kD forms and the 55 kD form can be dissociated from the >1000 kD form. During fractionation of the conditioned medium, a protease activity was found to copurify with the antiproliferative activity. In zymographic gels the purified 55 kD protease degraded gelatin and casein and was inhibited by EDTA and phenanthroline, indicating that it is a metalloprotease. When the 55 kD metalloprotease was tested for an ability to degrade rat laminin, fibronectin and native collagens only a limited proteolysis of fibronectin was observed. The resulting fibronectin fragments were separated by heparin-affinity chromatography and tested for the ability to affect Schwann cell proliferation. Results showed that a heparin-binding fraction (containing several fibronectin fragments with Mr=120-160 kD) possessed potent antiproliferative activity for mitogen-stimulated Schwann cells. Similar antiproliferative heparin-binding fibronectin fragments were found in serum-containing Schwann cell conditioned medium. These findings suggest that autocrine regulation of Schwann cell proliferation might involve an activation of fibronectin resulting from proteolysis by a secreted metalloprotease. Supported by NINCDS grants NS25011, 16349 and NSF grants 86-17034, 8808285.

334.11

AN INSULIN-LIKE GROWTH FACTOR BINDING PROTEIN GENE IS EXPRESSED IN THE EMBRYONIC EPIBLAST AND NEUROECTODERMAL DERIVATIVES IN EARLY AND MID-GESTATIONAL RAT EMBRYOS. T. L. Wood, K. H. Fliegner* and J. E. Pintar. Dept. Anatomy & Cell Biology, and Pathology, Columbia Univ., N.Y., N.Y. 10032.

The insulin-like growth factor binding proteins represent a family of proteins thought to mediate the effects of the insulin-like growth factors, IGF-I and IGF-II. Genes encoding three distinct IGF binding proteins have recently been cloned; two predict an RGD sequence that indicates possible additional functions for these proteins as matrix constituents. We here have used in situ hybridization to delineate the expression pattern for one of these IGF-binding protein genes, IGFBP-2, in early and mid-gestation rat embryos. IGFBP-2 gene expression represents a specific marker for cells of the early post-implantation epiblast, which ultimately gives rise to all three germ layers of the fetus. During gastrulation, ectoderm derived cells that will become both embryonic and extraembryonic mesoderm cease IGFBP-2 expression and ultimately begin to express IGF-II, whereas IGFBP-2 gene expression is maintained in surface ectoderm and in neuroectoderm throughout the rostral-caudal extent of the neural tube. By e11, IGFBP-2 expression in the neural tube (except for the floor plate) decreases to non-detectable levels in more caudal regions. In rostral brain regions (especially the telencephalon) IGFBP-2 expression is highest in cells of the ventricular layer and decreases as the cells move away from this proliferating zone and begin to differentiate. This expression pattern is complementary to that of the neurofilament related gene alpha-internexin which is expressed in the differentiating cells of the mantle zone in these regions. High levels of IGFBP-2 expression characterize other specific neuroectodermal derivatives in mid-gestation embryos including the epithelium of the choroid plexus, the floor plate of the spinal cord and the infundibular outpocketing of the diencephalon. Thus, in addition to a potential role in neuroblast cell cycle regulation, these expression patterns suggest that the IGFBP-2 gene product may have multiple roles during CNS development.

334.12

CELL KINETICS IN THE SUBEPENDYMAL LAYER OF THE MOUSE BRAIN. N. B. Manley, E. H. Lo, K. A. Frankel*, M. H. Phillips* and J. I. Fabrikant*. Research Medicine and Radiation Biophysics, Lawrence Berkeley Laboratory, Berkeley, CA 94720.

The subependymal layer of the mouse brain is an actively proliferating cell population with morphologically distinct subpopulations. Pulse labelling techniques with DNA precursors combined with high-resolution autoradiography demonstrate consistent regional differences in cellular kinetic parameters, such as labelling index (LI), growth fraction (GF), and mitotic index (MI), in different regions of the subependymal layer. These regional differences in brain cell kinetics may reflect locally specialized functions in the cytoarchitecture of the developing brain. Pulse-labelling methods provide useful techniques for studying the spatiotemporal patterns of cellular proliferation, differentiation, and migration in neural development. Focal charged particle radiation beams are used as non-invasive probes to perturb cell kinetics in the subependymal layer of the developing brain. Local changes in LI, GF, and MI are observed following irradiation with helium and neon ion beams. This research was supported by the Office of Health and Environmental Research, U.S. Department of Energy Contract DE-AC03-76SF00098.

$GABA_A$ RECEPTORS IV

335.1

DIFFERENCES IN RECEPTOR STRUCTURE REQUIREMENT FOR NEUROSTEROID AND BENZODIAZEPINE FACILITATION OF GABA-GATED Cl^- CURRENTS. G.Puia*, M.R.Santi, S.Vicini, P.H.Seeburg*§, and E.Costa. FGIN, Georgetown University, Washington, D.C. 20007 and §ZMBH, Heidelberg, FRG.

Using the whole cell voltage-clamp recording technique, we investigated the modulation of GABA-gated Cl^- channels by THDOC (3α,21dihydroxy-5a-pregnan-20-one), 3α-OH-DHP (3α-hydroxy-5α-pregnan-20-one) and PS (pregnenolone sulfate) on various $GABA_A$ receptor subtypes assembled in a mammalian cell line after transfection with cDNAs encoding for different $GABA_A$ receptor subunits. We also compared the activity of these modulators on primary neuronal cultures of newborn rats. Nanomolar concentrations of 3α-OH-DHP and THDOC potentiated GABA- activated Cl^- currents; micromolar concentrations of steroids elicited bicuculline- sensitive Cl^- currents. In these cultures micromolar concentration of PS negatively modulated GABA activated Cl^- currents. The interactions between 3α-OH-DHP and PS modulation will be discussed. Steroids facilitated GABA-gating equally well in artificially assembled homooligomeric and heterooligomeric channels, and in channels naturally expressed in primary neuronal cultures. Conversely, the γ and α subunits are required to optimize benzodiazepines (Bz) and imidazopyridines (Ip) positive modulation. However, variation in the molecular form of α and γ subunits may change efficacy and potency of Bz and Ip.

335.2

A NOVEL $GABA_A$ RECEPTOR SUBTYPE EXPRESSED IN CULTURED CEREBELLAR GRANULE CELLS. P.H. Seeburg[1], H. Monyer[1], K. Keinänen[1]*, T.A. Verdoorn[2], B. Sommer[1]* Center for Molecular Biology, University of Heidelberg and [2] Max-Planck-Institut für medizinische Forschung, 6900 Heidelberg, FRG.

A novel $GABA_A$ receptor subunit, α_6, was isolated from bovine and rat cerebellar cDNA libraries. Northern analysis and in situ hybridization demonstrate that the α_6 mRNA is expressed only in the cerebellum and, within this structure, predominantly by the granule cells. When this subunit is coexpressed with β_2 and γ_2 subunits in mammalian cells, a $GABA_A$ receptor is formed with a unique benzodiazepine recognition site. The only benzodiazepine ligands which bind to this site are Ro 15-1788 and Ro 15-4513. It is possible that this receptor mediates alcohol induced motor incoordination. To further investigate the functional properties of this receptor we analyzed the expression of α subunits in cultered cerebellar granule cells and studied electrophysiologically $GABA_A$ receptor properties in these cells. Amplification by PCR of cDNA constructed from cerebellar granule cell RNA revealed that α_6 is also expressed in vitro. Single channel currents in outside-out patches (-50mV) from these cells had a mean conductance of 25 +/-3.0 pS (n=3, 5μM GABA). Comparisons of these channel characteristics with channel currents from cells transfected with DNA encoding subunit combinations including α6 will be discussed.

335.3

PATCH-CLAMP STUDY OF GABA-EVOKED Cl$^-$ CURRENTS IN DOPAMINE (DA) VERSUS NON-DA NEURONS IN SLICE. G.Mereu and S.Vicini. FGIN, Georgetown University, Washington, D.C. 20007.

DA neurons were identified by their location and their electrophysiological characteristics in cell attached or current clamp (CC) mode. DA cells exhibited highly regular spontaneous firing (pacemaker like) of 0.5 - 5.0 Hz (more often observed at RP $\leq$ 56 mV) and spike duration $\geq$ 3.5 msec. In CC mode action potentials had high threshold (-38 mV), low frequency (< 8.0 Hz) repetitive firing, followed by huge ($\geq$ 10 mV) after hyperpolarization (AH) and spike duration $\geq$ 2.5 msec. On the other hand non DA, putative GABA, cells displayed modest AH, capability of high rate ($\geq$ 10 Hz) non rhythmic (rarely spontaneous) firing, and spike duration $\leq$ 2.5 msec. GABA-evoked Cl$^-$ currents in DA neurons were compared to that of non-DA neurons by monitoring these currents in rat thin slices by patch-clamp technique (Edwards et al.,1989, Pflugers Arch. 414:600). Interesting,in whole-cell voltage-clamp (VC) mode, the majority of cells (46/54) exhibited spontaneous synaptic currents both excitatory and inhibitory. By application of GABA in whole-cell VC mode, both DA and non-DA neurons generated Cl$^-$ currents which were modified by various benzodiazepine (BDZ) receptor ligands. The positive modulation elicited by diazepam, flunitrazepam, and zolpidem was about twofold more efficacious in non-DA than in DA neurons.

335.4

ZOLPIDEM MEDIATED POTENTIATION OF GABA$_A$ RECEPTOR RESPONSE IN RAT CEREBELLAR GRANULE CELLS. P. Vanderheyden*, M. Desarmenien*, P. Feltz and Ghandour, S., Lab de Neuroendocrinologie, Strasbourg,France.

The imidazopyridines zolpidem and alpidem were compared with the benzodiazepines diazepam and alprazolam for their action on GABA$_A$ receptor responses. For this, GABA-mediated currents were measured from rat cerebellar granule cells in primary culture (3-5 days) using the patch clamp technique in the whole-cell configuration. Both benzodiazepines, applied at 1 μM, increased the response to 5 μM GABA. Alprazolam was more potent than diazepam, causing a 96 % potentiation, versus 32 % for diazepam.

The BZD1 selective compound zolpidem caused a dose-dependent and reversible potentiation of GABA responses as well. The maximal effects were 63 and 34 % for 5 and 50 μM GABA applications respectively. This potentiation was reversibly blocked by the benzodiazepine antagonist Ro 15-1788 (0.5 μM), suggesting that zolpidem interacts at or near the benzodiazepine recognition sites. On the other hand, alpidem, a ligand that selectively binds to peripheral benzodiazepine sites, only slightly potentiated GABA responses in the cerebellar granule cells.

Our results confirm, on physiological grounds, the presence of a benzodiazepine1/GABA$_A$ receptor complex on cerebellar granule cells and the efficacy of zolpidem as a benzodiazepine receptor agonist.

335.5

CALCIUM MEDIATES THE HALOTHANE-INDUCED PROLONGATION OF SPONTANEOUS INHIBITORY POSTSYNAPTIC CURRENTS (sIPSCs) IN HIPPOCAMPAL NEURONS. I. Mody[1], M. B. MacIver[2] and D. L. Tanelian[2], Depts. of [1]Neurology & [2]Anesthesia, Stanford Univ. Sch. of Med., Stanford, CA.

The decay time constant (TAU_D) of sIPSCs is prolonged by anesthetic agents. Recently, volatile anesthetics have been shown to produce elevations in intramuscular and synaptosomal Ca^{2+} levels presumably through release of Ca^{2+} from intracellular storage sites. We have investigated the possibility that rises in intraneuronal Ca^{2+} may be responsible for the prolongation of sIPSCs by volatile anesthetics. Using whole-cell patch clamp recordings in hippocampal slices maintained at 34 ± 1°C we found halothane (1.2vol%) and pentobarbital (50 μM) to prolong the TAU_D of TTX-resistant GABA$_A$ receptor mediated sIPSCs by 275% and 230% respectively. When increases in intraneuronal Ca^{2+} were prevented by intracellular administration of the Ca^{2+} chelator BAPTA or the sarcoplasmic Ca^{2+} release inhibitor dantrolene, halothane's (but not pentobarbital's) effect was reduced by 70% ($p<0.005$, ANOVA). The contribution of GABAergic inhibition to the depression of field potentials by halothane was investigated in extracellular recordings. With inhibition intact or excitation enhanced by lowering extracellular Mg^{2+}, halothane depressed population spike amplitudes by 80-100%. When GABA$_A$-mediated inhibition was blocked by bicuculline (100 μM), halothane reduced the population spike amplitude by only 21%.

These findings demonstrate: 1) Transient elevations in intraneuronal Ca^{2+} enhance the function of GABA$_A$ receptor/channels; 2) Anesthetics which prolong sIPSCs do so via different mechanisms (unlike halothane, pentobarbital does not require an elevation of intraneuronal Ca^{2+}); 3) The foremost action of halothane is to enhance GABA$_A$ mediated inhibition rather than to depress neuronal excitability as previously thought.

335.6

ISOLATED CNS OF THE NEWBORN OPOSSUM (MONODELPHIS DOMESTICA) MAINTAINED IN VITRO: RESPONSES TO GABA, GLYCINE, GLUTAMATE AND NMDA. R.R. Stewart, D.J. Zou*, J.G. Nicholls and N.S. Saunders* Dept. Pharmacol. Biocenter, 4056 Basel, Switzerland and Dept. Physiol. & Pharmacol., Southampton Univ. Med. Sch., Southampton, England

The isolated CNS of the newborn opossum offers certain advantages for studying transmitter action. It survives for up to 4 days in medium as deficient as Krebs's fluid, and agents, such as TTX and Mg^{2+}, exert their effects rapidly and reversibly. In the present experiments we show that amino-acid transmitters such as GABA, glycine, glutamate, NMDA and their antagonists have rapid, reversible and reliable effects on spontaneous and evoked activity. For example, bicuculline, as expected, reversibly blocks GABA-A receptors but not the GABA-B receptors activated by baclofen; bicuculline can also cause synchronized bursts of activity in the CNS. In more suitable medium (BME), survival of the CNS has now been extended to over a week. Dose-response curves of the various transmitters at the end of a week remain unchanged. It is now possible to expose the CNS to TTX and other drugs for prolonged periods. For example, after 7 days in TTX, the CNS begins conducting compound action potentials (CAPs) within minutes after rinsing; in addition, the sensitivity of CAPs to GABA remains unchanged. By contrast, chronic exposure to low concentrations of certain amino acids markedly alters the sensitivity of evoked CAPs to GABA. These results suggest that the long-term regulation of receptor properties can be analyzed in detail in this CNS in vitro.

335.7

MODULATION OF [^{35}S]-t-BUTYLBICYCLOPHOSPHOROTHIONATE BINDING TO INTACT CEREBELLAR GRANULE CELLS BY THE TYPE II PYRETHROID DELTAMETHRIN. T.F. Murray and T.R. Jacobsen*. College of Pharmacy, Oregon State Univ., Corvallis, OR 97331.

An involvement of GABA$_A$ receptors in pyrethroid neurotoxicity has been posited. We have investigated the binding of [^{35}S]TBPS in intact cultured cerebellar granule cells to characterize the regulation of GABA$_A$ receptors in a defined neuronal population and to assess the interaction of deltamethrin with this recognition site. The binding of [^{35}S]TBPS at equilibrium was saturable with a Kd value of 60 nM and a Bmax value of approximately 650 fmol/mg protein. The specific binding of [^{35}S]TBPS was inhibited approximately 55-60% by the GABA receptor antagonist bicuculline methiodide (10 μM). The bicuculline methiodide-induced inhibition of [^{35}S]TBPS binding was reversed by muscimol in a concentration-dependent manner (0.01-1.0 μM). These results suggest that GABA$_A$ receptor activation effects a positive modulation of [^{35}S]TBPS binding presumably through an opening of the chloride channel. In contrast higher concentrations of muscimol in the range of 1-100 μM elicit an inhibition of [^{35}S]TBPS binding possibly reflecting an agonist-induced desensitization of the GABA$_A$ receptor complex. The pyrethroid insecticide deltamethrin modulated the equilibrium saturation binding of [^{35}S]TBPS in a complex manner. Concentrations of 1 and 10 μM deltamethrin reduced the [^{35}S]TBPS Bmax by 22 and 32%, respectively. In addition these concentrations of deltamethrin increased the affinity of [^{35}S]TBPS for the remaining sites. These results suggest that deltamethrin may act as both a noncompetitive antagonist and a positive allosteric modulator of [^{35}S]TBPS binding to the chloride channel domain of the GABA$_A$ receptor. (Supported by HHS Grant ES04891

335.8

GABA$_A$ RECEPTOR SUBTYPES AS A SITE OF ACTION FOR THE ANTICONFLICT AND ANTIPROCONFLICT ACTION OF BENZODIAZEPINE (BZ) LIGANDS. P.Giusti, A.Guidotti, and E.Costa. FGIN, Georgetown University, Washington, D.C. 20007.

The conditioned suppression of drinking in rats (Vogel test) - both in its conflict and proconflict paradigm - was used as a behavioral model to determine whether clinically effective BZ drugs with a preferential anti-panic action produce different behavioral effects by acting on the allosteric modulatory sites of a class of heteropolymeric GABA$_A$ receptor subtype. Pentylenetetrazole, a drug known to decrease GABA$_A$ receptor function and to produce panic-like syndrome in animals and man, was used to facilitate punished suppressed behavior in the proconflict paradigm. The anxiolytic drugs diazepam and chlordiazepoxide, which do not show a preferential potency in the treatment of panic disorders, presented similar anticonflict (AC) and antiproconflict (AP) potency in the Vogel test. The ED_{50} AC/AP ratio was close to 1. On the other hand, the 1-4 BZ clonazepam, the 1-4 triazolo BZ alprazolam and the 1-5 imidazo BZ bretazenil (Ro 16-6028), which have a preferential potency as anti-panic disorder agents, exhibited a higher AC/AP ratio as compared to diazepam. A further increase in the ratio was seen when animals that had received alprazolam were treated with CI-966 (1-(2-[bis(trifluoromethyl)-phenyl]-methoxylethyl)-1,2,5,6-tetrahydro-3-pyridine-carboxylic acid) (Park Davis), a GABA uptake inhibitor. On the contrary the AC/AP ratio for diazepam was only slightly affected in rats pretreated with CI-966. Flumazenil abolished either the AC or the AP activity of the above BZs. Interestingly, in rat striatum and spinal cord the density of the high-affinity binding sites of ^{3}H-clonazepam, ^{3}H-alprazolam, and ^{3}H-bretazenil was significantly lower than that for ^{3}H-diazepam. Thus, BZ ligands with clinical efficacy in the symptomatic treatment of panic disorders exhibited in rats an unexpected behavioral profile and a selective binding distribution as compared to the anxiolytic BZs, suggesting that they interact with a different population GABA$_A$ receptors.

335.9

NATURALLY OCCURRING ENDOGENOUS BENZODIAZEPINES: PURIFICATION, SYNTHESIS, AND PHYSIOLOGY. Rothstein JD, Garland W, Puia G, Guidotti A, Costa, E. Johns Hopkins Univ, Dept. Neurology, Baltimore, MD, 21205; FGIN-Georgetown Univ, Washington D.C., 20007; Hoffman-La Roche, Nutley NJ, 07110.

Endogenous benzodiazepine-like substances are naturally present in the CNS. These substances are present in tissue from man and animal, and in plants and fungi. Using selective extraction protocols and HPLC purification, receptor binding displacement studies, and selective anti-benzodiazepine antibodies, we have identified 6 benzodiazepine-like substances in rat and human brain. All material could displace [^{3}H]-flunitrazepam binding to central benzodiazepine receptors. One peak demonstrated greater activity at the "peripheral" benzodiazepine receptor. Electrophysiologically, some peaks potentiated GABA gated Cl^- channel opening, monitored in patch clamped cultured cortical neurons. Preliminary mass-spectroscopic analysis identified small amounts of diazepam (DZ) and desmethyldiazepam (DDZ) in purified peaks. However, the amount of DZ and DDZ in these peaks was a small fraction of the biological activity based on radioreceptor and electrophysiological analysis. Human CSF contained 3 pmole/ml of total BZ-like material, while human brain contained up to 150 pmole/gm. There was up to a 4 fold variation in regional human and rat brain content of BZ-like material. Cultured cerebellar granule cells, but not glial cells, could release the material in a calcium dependant, potassium stimulated fashion. The source of these substances may be endogenous synthesis or by dietary consumption. Furthermore, the ubiquitous fungus *Penicillium aurantiogriseum* synthesized BZ-like substances that appear to be similar to those purified from animals. Their synthesis is being studied. In conclusion, naturally occurring benzodiazepines exist in the CNS and may be active physiologically.

335.10

LOCALIZATION OF PERIPHERAL-TYPE BENZODIAZEPINE RECEPTOR- AND DIAZEPAM BINDING INHIBITOR-LIKE IMMUNOREACTIVITY IN HUMAN BRAIN AND CEREBRAL TUMORS. H. Alho[1], P. Helen[2], T. Harjuntausta[1*], A. Hervonen[1] and K. Krueger[3]. [1]Dept. of Biomed. Sci., [2]Dept. of Neurosurg., Univ. of Tampere BOX 607, 33101 Tampere, Finland; [3]Fidia- Georgetown Institute for the Neurosci., Washington DC 20007, USA.

The polypeptide diazepam binding inhibitor (DBI) inhibits benzodiazepine (BZ) ligand binding to both central benzodiazepine receptors (CBZR) and to so-called peripheral benzodiazepine receptors (PBZR). We studied the expression and distribution of DBI-like immunoreactivity (LI) and PKBS-LI (PK-11195 binding site, peripheral benzodiazepine receptor) in normal human brain and cerebral tumors by immunocytohistochemistry.

The immunohistochemistry was performed with routine PAP-method using human-DBI and rabbit-PKBS-antisera. In the normal cerebellum DBI and PKBS-LI was observed in astrocytes, no staining was identified in neurons. In astrocytomas the DBI- and PKBS-LI was identified in some of the astrocyte-like-cells in the tumors. In glioblastomas the numbers of DBI and PKBS immunoreactive cells were higher, large multinuclear anaplastic cells particular showed intense immunoreactivity. The role of PKBS and DBI in glia and in gliomas is unknown. DBI may modulate the function of CBZ- and PBZ-receptors, which are also found in gliomas, either by acting directly via these receptors or indirectly via neurosteroids regulated by the PKBS.

335.11

STATE-SELECTIVE MODULATION OF GABA CURRENTS BY γ-BUTYROLACTONES. K. D. Holland*, K.-W. Yoon, J. A. Ferrendelli, D. F. Covey*, and S. M. Rothman. Depts of Pharmacology, Neurology and Anatomy and Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

γ-Butyrolactones (GBLs) and γ-thiobutyrolactones are compounds which act at the picrotoxin receptor as either agonists or inverse agonists depending on the nature of the alkyl substituents. We examined the effects of two GBLs, α-ethyl-α-methyl-GBL (α-EMGBL) and α-isopropyl-α-methyl-GBL (α-IMGBL), on GABA currents and inhibitory postsynaptic currents (IPSCs) in cultured, voltage-clamped rat hippocampal neurons. α-EMGBL potentiated responses to low (0.5 μM) but not high (≥ 10 μM) GABA. α-EMGBL also decreased the rate of IPSC decay without altering IPSC peak amplitude. In the presence of higher GABA concentrations (30 μM) α-EMGBL blocked picrotoxin receptor agonists and inverse agonists. Thus, α-EMGBL acts as a mixed agonist/inverse agonist. In contrast to α-EMGBL, α-IMGBL had no effect on responses to either 0.5 μM or 30 μM GABA, or on IPSCs. α-IMGBL was able to block the effects of picrotoxin receptor agonists and inverse agonists. Thus, α-IMGBL acts as a pure antagonist at the picrotoxin receptor.

The main conductance state of the GABA-gated channel probably has two or more open states; an early, short monoliganded one and a diliganded open state. Using a computer model based on the single channel data of Weiss and Magleby (J. Neurosci., 1989) we were able to simulate our data by assuming that α-EMGBL altered only the opening and closing rate constants for the monoliganded open channel of the GABA receptor. These results suggest that some compounds may be "state" as well as site specific modulators of liganded channels.

Supported by the Seay Neuropharmacology Research Fellowship and NIH grants NS14834 and GM07805.

SPECIFICITY AND ENHANCEMENT OF REGENERATION

336.1

TOPOGRAPHIC SPECIFICITY OF PERIPHERAL AXON REGENERATION ACROSS ENCLOSED GAPS. T.M.E. Brushart. Departments of Orthopaedics and Neurology, Johns Hopkins Hospital, and The Raymond M. Curtis Hand Center, Baltimore, MD 21218.

These experiments evaluate the specificity of muscle reinnervation by sensory and motor neurons after entubated repair of a proximal nerve trunk. Both proximal sciatic nerves of nine female Sprague-Dawley rats were divided and the ends separated by 8 mms. within mesothelial tubes. Eighteen months were allowed for regeneration. The peroneal muscles were then injected with horseradish peroxidase to identify the sensory and motor neurons responsible for their reinnervation. Normal peroneal muscles were innervated by a mean of 386 motor neurons and 2897 sensory neurons. In experimental animals, these muscles were reinnervated by a mean of 210 motor neurons and 1982 sensory neurons. However, there was no return of motor function, with fixed ankle flexion and claw toe deformities. A mean of 53% of reinnervating motor neurons previously served the tibial muscles. The normal sensory innervation was from L2-4, centered on L3; neurons from L2-6 participated in reinnervation with random predominance of levels. Muscle was reinnervated by adequate numbers of neurons, but half of the motor neurons reinnervating the peroneal muscles had previously served the antagonistic tibial muscles and sensory levels were radically altered. Regenerating sensory and motor axons thus reinnervated muscle on a topographically random basis.

336.2

PERMANENT LABELING OF MOTONEURON POOLS: ACCURACY OF REGENERATION ANALYZED AT THE SINGLE NEURON LEVEL. R.D. Madison, S.J. Archibald, and S. Meadows. Departments of Surgery and Neurobiology, Duke University Med. Ctr., and Research Service, V.A. Hospital, Durham, N.C. 27710.

The motoneuron pool to the quadriceps muscle in the rat was labeled by exposing the terminal motor branch of the femoral nerve to the fluorescent dye DiI for 60 mins., and then repairing the motor branch by direct suture. Two weeks later, 5 of 14 animals had the parent nerve to the quadriceps muscle (femoral nerve) transected and repaired by direct suture, the other 9 animals served as non-transection controls. Four weeks later all 14 animals had the terminal motor branch re-exposed, a second tracer applied (Fluoro-gold) for 60 mins., and the nerve stump sealed into an empty polyethylene tube. Five days later animals were perfused and spinal cords were dissected and processed to display fluorescent cells. Corrected cell counts, mean+std. dev., were: (1) control animals, N=9, 396+37 double-labeled, 2+2 DiI alone; (2) femoral nerve transection, N=5, 351+62 double-labeled, 103+67 DiI alone. These results show that it is possible to permanently label a motoneuron pool with greater than 99% accuracy, and, in this model system the significant differences in DiI alone cells ($p<.001$) indicates many regenerating motor axons have been misrouted into the terminal sensory branch of the femoral nerve. Supported by NS 22404 and VA MR-Merit Review Program (RDM).

336.3

TRANSPLANTATION AND FUNCTIONAL INTEGRATION OF AN IDENTIFIED INTERNEURON THAT CONTROLS RESPIRATORY BEHAVIOR IN LYMNAEA. N.I. Syed, R.L. Ridgway, A.G.M. Bulloch and K. Lukowiak, Department of Medical Physiology, HSC, University of Calgary, Calgary, Alberta, Canada, T2N 4N1.

An identified interneuron, Visceral Dorsal 4 (V.D4), of the pond snail Lymnaea stagnalis is involved in the control of respiratory behavior. Semi-intact preparations in which V.D4 was ablated have suggested that this neuron in necessary for respiratory behavior to occur. In the present study we made critical tests of the importance of this neuron through transplantation experiments. We selectively removed or destroyed interneuron V.D4 from the visceral ganglion of an animal. Using in vitro culture techniques, neuron V.D4 from another animal was then transplanted into this ganglion, in the same position where the original V.D4 had been located. Within 3 - 5 days, the transplanted V.D4 extended neurites and restored the functional circuitry underlying the respiratory behavior. Interestingly, when a V.D4 was implanted into a ganglion in which the host V.D4 had not been ablated earlier, the second (implanted) V.D4 displayed an aberrant morphology and failed to make appropriate synaptic connections; it did, however, make inappropriate connections. This result suggests that transplanted interneurons have the capacity to extend neurites and to form synaptic connections. Additionally, these neurons can re-establish connections, with appropriate targets, but these do not occur in the presence of the host interneuron.

Supported by MRC (Canada).

336.4

ADULT RAT RETINAL GANGLION CELL AXONS ESTABLISH CONNECTIONS WITH COCULTURED VISUAL MIDBRAIN TARGETS. M. Bähr and G.W. Eschweiler* Dept.Neurology, University Tübingen and Max-Planck-Institut für Entwicklungsbiologie, D-7400 Tübingen, F.R.G.

The ability of adult rat retinal ganglion cells (RGC) to regrow axons into appropriate target regions was investigated in vitro. Retinal explants were obtained from adult rats 5 days after optic nerve crush and cultured with organotypic midbrain cultures including the primary visual target area (colliculus superior) from 15 day old (E15) rat embryos. Explant cultures were placed at a distance of 200-500 µm and cocultured for 10-14 days. The origin and cellular morphology of different RGC types projecting to the midbrain cultures could be determined by placing small crystals of Di-I (Molecular Probes) into the explants. RGC axons were anterogradely labeled inside the cocultured explant, but no midbrain neurons were labeled retrogradely, suggesting that midbrain axons avoided to grow into adult retinal explants. The projections which were established with midbrain targets by adult RGC showed some similarity to those seen in vivo. In electrophysiological experiments neuronal activity could be recorded in the adult retina and in the midbrain explants suggesting that a functional reconnection of adult rat RGC with target neurons might be observed in our tissue culture system.

G.W.E. was supported by the Boehringer Ingelheim Fonds.

336.5

LOW ENERGY LASER IRRADIATION AT DIFFERENT WAVELENGTHS ALTERS PHOTOSTIMULATION EFFECTS ON THE RATE OF RAT FACIAL NERVE REGENERATION. J.J. Anders, S. Woolery*, R. Borke and W. Vandemerwe*. Dept. of Anatomy and Laser Biophysics Center, USUHS, Bethesda, MD 20814.

Low energy laser irradiation (8.5mW, 90 min, 632nm) increases the rate of rat facial nerve regeneration after crush injury (Anders, J.J., Neurosci., 15:316, 1989). The purpose of this study was to establish the wavelength with the greatest photostimulatory effect on the rate of facial nerve regeneration. The facial nerve was crushed unilaterally in anesthetized rats for 90 secs. The wound was sutured and transcutaneously irradiated daily with a tunable dye or ND-YAG laser at 8.5 mW for 90 min for 7, 8 and 9 days at various wavelengths (1060, 720, 632, 514, 457 and 361nm). HRP was injected subcutaneously, on the side of the face supplied by the injured nerve, before aldehyde perfusion. The number of HRP-labeled neurons in the facial motor nucleus was used as an assay of the degree of regeneration. Laser irradiation at all wavelengths examined caused an increase in the rate of regeneration as compared to the non-irradiated controls. 632nm had the greatest effect on the rate of regeneration and increasing or decreasing the wavelength from 632nm decreased the stimulatory effect.

336.6

NERVE GROWTH FACTOR (NGF) PROMOTES CHOLINERGIC AXONAL REGENERATION INTO THE DENERVATED ADULT RAT HIPPOCAMPAL FORMATION. T. Hagg, H.L. Vahlsing*, C. Portera-Cailliau*, M. Manthorpe and S. Varon, Dept. Biol., M-001, UCSD, La Jolla, CA 92093

Cholinergic axons of the adult rat regenerate vigorously into peripheral nerve grafts placed between the disconnected septum and hippocampal formation (HF) but do not regenerate well beyond the end of the nerve bridge into the hippocampal tissue. Since cholinergic septal neurons are known to be supported by NGF we tested whether NGF administration would promote their regeneration into the HF. Adult female Sprague-Dawley rats received bilateral aspirative fimbria-fornix transections, fresh septo-hippocampal nerve autografts and a concurrent one month unilateral infusion with 7S NGF or vehicle i) into the lateral ventricle proximal to the graft or ii) into the hippocampal tissue 2 mm (dorsal HF) or 7 mm (ventral HF) distal to the graft. With intraventricular NGF the number of ChAT-positive septal neurons which axotomy reduced to 35% was increased to 85% of normal, but the number of AChE-positive axons in the nerve graft and HF was significantly decreased. Intraventricular NGF also induced the appearance of a cholinergic plexus in the septal region facing the ventricle and away from the fimbria-fornix region. This suggests that NGF promotes and directs axonal regeneration toward the site of infusion. A one month intrahippocampal infusion of NGF into the dorsal and to a lesser degree into the ventral regions dramatically enhanced and accelerated the entry and penetration of cholinergic axons into the dorsal HF. The improved regeneration occurred without a concomitant increase in the number of ChAT-positive neurons in the septum or of AChE-positive axons in the nerve graft, suggesting that hippocampal NGF infusion promoted regeneration by improving tissue penetration and not by recruiting additional neurons or axons. Support: NINCDS grants NS-16349, NS-25011 and NSF grants BNS-88-08285, BNS-86-17034.

336.7

WALLERIAN DEGENERATION OF NERVE ALLOGRAFTS AND IMMUNOSUPPRESSION. T.E. Trumble, Orthopaedic Research Lab., Univ. of Washington Sch. of Med., Seattle, WA 98195

The transplantation of Wallerian degenerated grafts were investigated as a means of decreasing the immunogenicity of the grafts and therefore the requirement for systemic immunosuppression. Short-term immunosuppression has been shown to decrease the host rejection in previous studies. This study evaluated the functional recovery in the animal with Wallerian predegenerated grafts, both alone and in combination with short- term immunosuppression with cyclosporin-A (CyA). Eight groups of animals were used and included allografted groups (grafts transplanted from Brown-Norway to Lewis rats), control groups with isografts with and without predegeneration, and a separate group that was not grafted. CyA concentrations were evaluated by radioimmunoassay one month following transplantation. Recovery of neurologic function was evaluated using sensory testing, gait analysis (de Medinaceli sciatic function index, or SFI), hind limb flexion contractures, tibialis anterior (TA) muscle weight, and hydroxyproline concentration, as well as axonal counts. Three months after surgery the CyA was terminated and the animals were observed for another month to allow for signs of rejection to occur.

Results: All the animals receiving CyA had therapeutic serum levels. The animals receiving allografts alone had the greatest flexion contractures (44°±13°) which was significantly higher than the animals with the predegenerated allografts without CyA (36°±7°, $p<.01$). Both of these groups were higher than allografted animals that receive CyA or the isografted animals ($p<.01$). The muscle weight ratios revealed that all grafted groups received some reinnervation, with the allograft-only group having the smallest return of muscle weight. The hydroxyproline concentrations revealed a significant difference between the allograft-only group and the allograft group with predegenerated grafts but without CyA ($p<.01$). The allografted groups without CyA did better than those without CyA whether or not they had predegenerated grafts ($p<.01$). Axonal counts did not reveal a significant difference between the grafted groups. In sum, predegenerated allografts allowed a better functional result when cyclosporin was not used.

336.8

MORPHOLOGY OF REGENERATED DORSAL ROOTS IN SEMIPERMEABLE TUBES IS ALTERED BY TUBE INNER SURFACE COMPOSITION. J.H. Lustgarten*, M. Proctor*, A.M. Avellino* and M. Kliot. Dept. of Neurosurgery, Columbia Univ., New York, NY 10032.

Centrally-directed fibers of lesioned dorsal roots in the adult rat regenerate up to the PNS/CNS interface but not across it. Providing a permissive local environment, e.g. an implant coated with embryonic astrocytes, enhances their outgrowth [Kliot et al., 1990]. Semipermeable acrylic polymer tubes in combination with biological substrates provide a defined microenvironment in which to clarify conditions influencing dorsal root regeneration. Centrally-directed processes of transected dorsal roots were placed in blind-ended tubes. The extent and pattern of axon outgrowth was studied using 1 µm plastic sections. Tubes having a smooth inner surface and impregnated with laminin support outgrowth of a discrete central cable of myelinated axons across a 2.7 mm gap. At 4 weeks regenerated axons represent <5% of the total root axons placed in the tube; this increased three-fold by 8 weeks. Tubes having a rough inner surface support more modest outgrowth of myelinated axons splayed within a diffuse cellular infiltrate. Rough tubes impregnated with dexamethasone suppressed this cellular infiltrate and axonal regeneration. We conclude that limited regeneration of dorsal roots can occur in semipermeable conduits even in the absence of a distal stump; and that morphology of the regenerated root is highly dependent on the tube inner surface composition.

336.9

THE EFFICACY OF MODIFIED ACELLULAR NERVE ALLOGRAFTS IN THE REPAIR OF PERIPHERAL NERVES. A.K. Gulati. Department of Anatomy, Medical College of Georgia, Augusta, GA 30912.

Genetically different nerve allografts are unable to support host axonal regeneration as they are immunologically rejected. The present study describes the ability of experimentally prepared nerve allografts in the repair of rat peripheral nerves. Nerve allografts were prepared by first freezing and thawing a six week in situ degenerated rat peripheral nerve. This procedure removed the degenerated myelin and killed the cellular elements within the nerve. The Schwann cell basal lamina was however preserved. Prior to transplanting these nerves to repair nerve gaps, as was done in a recent study (Gulati and Cole, J. Neurosurg. 72:114-122, 1990), these nerves were passaged through an intermediate host or cocultured with macrophages to rid the grafts of dead cell debris. After each of these procedures the nerves were harvested and used to repair surgically created nerve gap in the host rat. Appropriate control cellular allografts (i.e. not frozen and thawed) and genetically identical isografts were performed for comparison. The graft length was 2cm in all cases. Grafts were evaluated morphologically at 1, 2, 4 and 12 weeks. The control cellular isografts supported the axonal regeneration best. The cellular allografts were rejected and were unable to support axonal regeneration. The acellular allografts after passage through an intermediate host or after culture were successful in supporting axonal regeneration through its entire length, as were acellular isografts. It is concluded that experimentally modified acellular allografts are capable of supporting axonal regeneration and exhibit reduced immunogenicity.

336.10

FIBRONECTIN-LAMININ COMBINATION ENHANCES PERIPHERAL NERVE REGENERATION ACROSS LONG GAPS. A.L. Woolley*, J.P. Hollowell*, and K.M. Rich. Dept. of Otolaryngology, Dept. of Neurosurgery, and Dept. of Neurobiology, Washington Univ. School of Medicine, St. Louis, MO 63110.

An exogenous fibronectin-laminin (FN-LAM) solution was added into a silicone chamber to determine the effects on peripheral nerve regeneration across 18 mm long gaps. The sciatic nerves of adult rats were sutured into silicone tubes 20 mm in length, creating a 18 mm gap between the proximal and distal nerve stumps. The tubes were filled with either a mixture of fibronectin and laminin (500 μg/ml each) or a solution of cytochrome C (1 mg/ml) as the control. After six weeks, the animals were sacrificed and the chambers were examined for regeneration. Seventy percent of the animals from the FN-LAM group demonstrated regeneration across the 18 mm gaps, compared to only thirty percent in the control group. The combination of FN-LAM significantly increased the number of axons that grew into the distal end of the chamber (FN-LAM, 1325 $\pm$ 442; cytochrome C, 153 $\pm$ 51; $P < 0.05$). Examination of the distal tributary nerves revealed axons only in the FN-LAM group; none were found in the control group. Quantitative analysis of horseradish peroxidase labeling of the distal sciatic nerve through the regenerated segment revealed a greater number of sensory and motor neurons in the FN-LAM group had extended axons across the 18 mm gap compared to the control group. Morphometric studies revealed that the mean area of the regenerated segment in the FN-LAM group was 37% larger than the controls, and ultrastructural analysis demonstrated a more mature nerve. This is the first *in vivo* demonstration that this combination of fibronectin and laminin significantly enhances the regeneration of myelinated axons across a long nerve gap in the rat sciatic nerve. Supported by NIH grant 5T32NS07057-11.

336.11

EFFECT OF SERINE PROTEASE AND SERINE PROTEASE INHIBITOR ON NERVE REGENERATION IN VIVO. P. Lee[1], R.H. Soriano, J.G. Spector[1] and D.G. Roufa, [1]Dept. of Otolaryngology, Washington University and CNS Diseases Research, Searle R&D, St. Louis, MO 63198.

Serine proteases (e.g., thrombin) and serine protease inhibitors (e.g., Protease Nexin-1) have been implicated in neurite outgrowth activity. We have examined the activities of thrombin and protease nexin-1(PN-1) in a rat sciatic nerve regeneration model.

A 4-5mm sciatic nerve segment was removed and the nerve ends were implanted in a 10mm silastic tube to create an 8mm gap. Each tube was prefilled with either 1.5IU thrombin, 50ug/ml or 1mg/ml of PN-1, or saline. Thrombin, at day 1 and 4 after implantation, reduced the length of the blood clot propagating from both the proximal and distal nerve stumps. PN-1 at 50ug/ml did not interfere with clot progression at day 1 and day 4. At day 17, the number of nerve regenerates containing myelinated axons in the middle of the tube was 1/6 for thrombin, 6/9 for PN-1 (50ug/ml), and 6/10 for saline. Nerve regenerates had no statistically significant increase in the number of myelinated axons at day 21, with a higher dose of PN-1 (1mg/ml).

In conclusion, we demonstrated that thrombin interfered with clot progression and reduced the number of myelinated axons. PN-1 did not affect clot progression nor did it affect the number of myelinated axons.

DEGENERATIVE DISEASE—PARKINSON'S

337.1

ABSOLUTE NUMBER OF NEURONS IN THE SUSTANTIA NIGRA IN PARKINSON'S DISEASE. A. Møller, B. Pakkenberg, H.J.G. Gundersen, A.M. Dam* & H. Pakkenberg*. Neurological Research Laboratory, Hvidovre University Hospital, Copenhagen and Stereological Research Laboratory, Aarhus University, Denmark.

Using an unbiased stereological technique, the total number of pigmented and non-pigmented neurons was estimated in the substantia nigra of seven patients and seven controls. In the control group the average total number of pigmented neurons was 550.000 and non-pigmented was 260.000 with a variation of about 20%. There was a large reduction in the neuron number for both the pigmented neurons, 66%, and the non-pigmented neurons, 24%, in the Parkinson's diseased patients with no overlap in the figures between the two groups. A significant correlation ($r = 0.81$, $2p = 0.028$) existed between the total number of pigmented and non-pigmented neurons in the controls, whereas a similar correlation in the patients fell just short of significance ($r = 0.27$, $2p = 0.069$). The unbiased stereological method is a few orders of magnitudes more efficient than the previously used conventional and biased methods. The amount of time used to count each substantia nigra was approximately one hour.

337.2

IRON AND ALUMINUM INCREASE IN THE SUBSTANTIA NIGRA OF PATIENTS WITH PARKINSON'S DISEASE: AN X-RAY MICROANALYSIS
Hirsch E.C., Brandel J.P., Galle P., Javoy-Agid F. and Agid Y.
INSERM U289, Hôpital de la Salpêtrière, 47 Bd de l'hôpital, 75013 Paris and INSERM SC27, 94010 Créteil, France.

Parkinson's disease is characterized by the degeneration of melanized dopaminergic neurons in the substantia nigra and the presence of Lewy bodies. In order to analyze the putative role of trace metal in nigral cell death we analyzed the level of different elements by X-ray microanalysis in the substantia nigra and the central grey substance of patients with Parkinson's disease, progressive supranuclear palsy and matched controls. In control brains, only iron, potassium, silicium, sodium, sulfur and zinc were within the detection limit of the technique. The elements were present at similar concentrations and distributed uniformly in the two regions except sulfur levels which were higher on neuromelanin aggregates in the substantia nigra than in nigral regions lacking neuromelanin or in the central grey substance. The concentrations of the other elements were similar in both structures. In Parkinson's disease, but not in progressive supranuclear palsy, nigral iron levels increased in regions devoid of neuromelanin and decreased on neuromelanin aggregates and were unchanged in the central grey substance, when compared to control values. Concentrations of the other elements in the central grey substance and substantia nigra were not different from controls in brains from patients with Parkinson's disease and progressive supranuclear palsy. Analysis of Lewy bodies in the parkinsonian substantia nigra, revealed high levels of iron and the presence of aluminum. Trace metal abundance was not affected in progressive supranuclear palsy, in spite of the nigral cell death. This suggests that the increased iron levels and the detection of aluminum observed in the substantia nigra of patients with Parkinson's disease are not solely the consequence of the neuronal degeneration.

337.3

SPECIFIC DOPAMINERGIC CYTOTOXICITY IN SERA OF IDIOPATHIC PARKINSONIAN PATIENTS. R. Dal Toso, G. De Fazio*, D. Benvegnù*, Minozzi M.C.*, A. Cananzi[1]* and A. Leon. Fidia Research Laboratories, 35031 Abano Terme, Italy and [1]Dept. of Neurology, Vicenza Hospital, 36100 Vicenza, Italy

We here provide evidence, that sera from patients affected by Parkinson's Disease (PD) contain complement-dependent cytotoxic activity specific for dopaminergic (DA) neurons in vitro, and mainly present in sera of idiopathic PD patients. In particular, heat inactivated serum from PD patients or neurological controls were added to serum-free mesencephalic-striatal co-cultures on day 4 "in vitro". After 24 hrs, reconstituted rabbit complement was added for 30 min and DA and GABA uptake thereafter assessed. We found that 14 out of 16 sera of idiopathic PD patients produced a complement-dependent reduction of DA uptake without affecting GABA uptake. This reduction was correlated with an equivalent loss of tyrosine-hydroxilase immunoreactive neurons, thereby indicating specific cytotoxicity for DA neurons. No inhibitory activity was observed in 12 out of 14 non-idiopathic PD and all neurological control sera. The correlation between this cytotoxic activity in serum and that reported in CSF (McRae et al., Neurochem. Res., 13: 679, 1988) as well as its relevance in pathophysiology of PD remain to be elucidated.

337.4

MICROSTIMULATION OF VENTRAL INTERMEDIUS NUCLEUS OF THALAMUS IN HUMAN PATIENTS WITH PARKINSONIAN TREMOR. A.L. Benabid, D.M. Gao*, D. Hoffman* and P. Pollak*, INSERM U. 318, Department of Neuroscience, Grenoble University, 38043 Grenoble - France.

Chronic stimulation of the thalamus has been demonstrated to suppress the parkinsonian tremor in human patients. The effects of the stimulation of various sites and the stimulation parameters were investigated in 41 patients with parkinsonian tremor or other sorts of tremors during stereotaxic surgery and were used for precise location of the chronic stimulating electrode. The optimal stimulation point was identified in the presumed nucleus ventralis intermedius (Vim) of the thalamus by multi-unit recording and microstimulation and compared with the determination of Vim from ventriculographic landmarks. The threshold intensity in Vim required to suppression of the tremor was minimal (0.39 ± 0,095 mA at 130 Hz), where the relatively higher neural noises were recorded, and stimulation intensities higher than the threshold intensity could induce paresthesias in the contralateral side of the body, which was considered to be caused by the diffusion the stimulation current to the somatosensory nuclei of the thalamus (VPL or VPM). At sites which were 1 or 2 mm posterior to the optimal stimulation, multi-unit responses to the superficial stimulation of the contralateral body were often recorded. This tremor suppression effect was frequency-dependent and stimulations with a frequency from **60 to 1000 Hz** were effective, but the optimal stimulation frequency was about **130 to 150 Hz.** The function and the possible mechanism of action of the presumed Vim nucleus are discussed in the scope of a feed-back loop model.

337.5

A STRIATAL-DERIVED NEUROTROPHIC FACTOR (NTF): RELATIONSHIP TO PARKINSON'S DISEASE (PD). E.S. Lo, L.R. Ptak*, S.T. Nath*, E.J. Mufson (1), H.L. Klawans*, and P.M. Carvey. Rush Medical College, Chicago, IL 60612. 1-Inst. for Biogerontology Research, Sun City, AZ

The dopaminergic degeneration of PD is associated with dopamine (DA) receptor proliferation and increased DA synthesis as compensatory mechanisms. Extracts of the caudate (Cau), putamen (Put), and cerebellum (Cb) of 4 Lewy body positive PD patients were evaluated for their growth effect in an E13 rat mesencephalic culture system to determine if enhanced NTF production also represents a DA cell loss compensatory mechanism. Number of cells in a low density culture system or DA uptake and number of tyrosine hydroxylase (TH) positive neurons in a high density system were used as indices of growth. Put and Cau extracts from PD patients rescued more cells than extracts from age-matched Lewy body negative controls in the low density system. Put extracts from PD patients also tended to enhance DA uptake as well as the number of TH positive neurons relative to PD Cau and control extracts, although this effect was not seen in all PD patients. Cb extracts from all patients had low level effects. These data suggest that physical denervation secondary to PD leads to an enhanced production of a target-derived NTF which is similar to that seen following pharmacologic denervation (Carvey, this meeting). Enhanced NTF production may represent a compensatory mechanism for loss of striatal DA tone. Since chronic DA agonism reduces this NTF, levodopa therapy in these PD patients may account for the inconsistent increases observed.

337.6

A UNIFORM RATING SCALE FOR PARKINSONIAN FEATURES IN NON-HUMAN PRIMATES.

D.M. Gash[1], M.H. Kim*[1] and R. Kurlan*[2]. Departments of Neurobiology and Anatomy[1] and Neurology[2], University of Rochester, Rochester, NY 14642.

Many laboratories are now using primates with MPTP-induced nigrostriatal lesions as a model of human Parkinson's disease (PD). While parkinsonian monkeys are proving valuable for evaluating new therapeutic approaches for the treatment of PD, the behavioral tests employed for assessing the motor impairments and recovery of function following treatment vary widely. This has made the comparison of results between laboratories difficult and to some extent impeded progress in this field. It is important that the research community using primates agree upon and utilize standardized tests to overcome this problem. As one step in this direction, we have developed a 24 item rating scale based on the human clinical PD rating scales for assessing parkinsonian features (e.g. bradykinesia, tremor), general behavioral features (e.g. social interaction, grooming behavior, defense reactions) and treatment related side effects (e.g. dyskinesias, vomitting) in Rhesus monkeys. This rating scale has proven to be quite sensitive in detecting L-dopa induced improvements in our unilateral and bilateral parkinsonian monkeys. In order to improve interrater reliability and consistency, specific definitions for scoring have been developed and are illustrated on videotape.

Supported by NIH NS 25778 and the PEW Foundation.

337.7

HEMIPARKINSONISM IN MONKEYS AFTER UNILATERAL STRIATUM INFUSION OF MPTP: CAUDATE NUCLEUS VS PUTAMEN. H.IMAI, T.NAKAMURA*, K.ENDO*, and H.NARABAYASHI*, Dept.of Neurology, Juntendo Univ. Sch. of Med., Tokyo 113, Japan.

In an attempt to clarify the role of the dopamien nerve terminal and cell body in MPTP toxicity and construct a model for pure hemiparkinsonism, we first administered 4 mg of MPTP HCl directly into the unilateral caudate nucleus of crab-eating monkeys via an Alzet 200 µl osmotic minipump for 14 days. The monkeys exhibited a persistent flexed posture and hypokinesia of the contralateral limbs, and spontaneous circling toward the MPTP-treated side. After treatment with apomorphine the circling motion was reversed. Almost total nigral dopamine cell loss was produced in the MPTP-treated side. After infusion of 4 mg of MPTP into the unilateral putamen the monkey exhibited a clear hemiparkinsonism behaviorally and histologically, virtually the same as seen in those animals given 4 mg of MPTP into the caudate nucleus. Infusion of 0.4 mg of MPTP into the caudate nucleus exhibited no clear hemiparkinsonism except apomorphine-induced circling away from the MPTP-treated side, and produced a partial nigral cell loss mainly rostrally and clusters of spared cells were seen. Infusion of 0.4 mg of MPTP into the putamen exhibited a mild hemiparkinsonism but no apomorphine-induced circling, and produced a partial nigral cell loss mainly caudally and ventrolaterally.

337.8

DELAYED AND ACUTE STEREOTACTIC CO-GRAFTING IN THE TREATMENT OF HEMIPARKINSON MONKEYS. R.A.E. Bakay, C. Herring, R. Watts, and L. Byrd. VA Medical Center, Yerkes Primate Center of Emory Univ., Atlanta, GA 30322.

A delayed stereotactic technique has been developed that has the potential for improving graft survivability by increasing the availability of neurotrophic factors at the time of grafting, and by the creation of a cavity for implantation of solid tissue. The delayed technique consists of x-ray confirmed stereotactic implantation of catheters into targets in the caudate and putamen one week prior to grafting. At the time of grafting, the catheters are removed and the tissue placed in the tract developed by the catheters.

Nine male M mulatta (2-4 years old) were rendered hemiparkinsonian by an intracarotid injection of MPTP. After stabilization of the lesion over a 2-4 month period, the subjects were randomized to either an acute stereotactic grafting of adrenal medullary tissue and sural nerve or a delayed technique with or without co-graft tissue. Apomorphine-induced rotation was decreased at three months by 45% in the acutely grafted monkeys and by 81% in the monkeys in which a delayed technique was used. Follow-up demonstrated that this decrease in drug-induced behavior was maintained over a six-month period.

Support: APDA, VAR&D, Yerkes Regional Primate Research Center (RR-00165) and NIH (RO1-NS24340)

337.9

RELATIONSHIP BETWEEN EXCESS S-ADENOSYLMETHIONINE (SAM) DEPENDENT METHYLATION AND PARKINSON'S DISEASE (PD). C. G. Charlton, B. Crowell, Jr. and R. Benson*. Department of Physiology, Meharry Medical College, Nashville, TN 37208.

The primary symptoms of PD are tremor, hypokinesia and rigidity due to degeneration in the nigrostriatal pathway. Tyrosine hydroxylase (TH), dopamine (DA) and melanin are depleted, and the relative concentration of methylated metabolites of DA are increased. During therapy, l-dopa is methylated, accompanied by a depletion of SAM. The cause of PD is still unknown. Any proposed cause should embrace both the etiology and the therapy of PD. An excess of SAM may embrace both, since an increase in SAM-dependent methylation can cause similar changes to those occurring in PD, and SAM seems to be involved in the action of l-dopa. We tested the proposal by injecting SAM into the brains of rats and by administering l-dopa and quantifying its effects on SAM. Acute icv injection of SAM caused tremors, hypokinesia, abnormal posture, etc. and DA depletion in the caudate nucleus (49.1% ipsilateral and 9.5% contralateral). Chronic treatment caused neurodegeneration - localized damage to the CN, hippocampus and septum; and remotely, in the substantia nigra (SN), a reduction in size, cell loss and gliosis occurred accompanied by a reduction in TH and disruption in its cellular compartments. Chronic l-dopa increased the activity of methionine adenosyl transferase (MAT), the synthesis enzyme for SAM, by 32%. Most of the motor deficits cause by SAM are similar to those occurring in PD. The depletion of DA and TH and the remote degeneration that occurred in the SN also parallel the neurological findings in PD. It is known that l-dopa depletes SAM, therefore, our finding that chronic l-dopa can increase MAT is an indication that a rebound increase in SAM may contribute to the reduced efficacy of l-dopa during long term l-dopa therapy. (Supported by NIH RR03032, NSF RII-H70421 and NSF 8714805)

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS VI

338.1

UNC-116, A GENE INVOLVED IN PROVIDING POSITIONAL INFORMATION FOR AXONAL GUIDANCE DURING C. ELEGANS NEURAL DEVELOPMENT. N.Patel and J.R. Mancillas. UCLA, Los Angeles, CA, 90024.

Unc-116 is a genetic locus identified by mutation during a screen for genes involved in axonal guidance. Unc-116 (e2312) mutants display abnormal locomotion, primarily during backward movement. Anatomical abnormalities involve the misplacement of axons normally occupying a lateral position to more dorsal positions and abnormal hypodermal nuclear migrations along the circumferential axis (Thierry-Mieg and Mancillas, in preparation), suggesting that unc-116 plays a role in a system signaling position along the circumferential axis. Genetic analysis placing unc-116 chromosomes over deficiencies spanning the locus reveals that e2312 is a hypomorph. This appears to be due to transcription of the transposon (TC5) whose insertion caused the mutation in e2312. We have isolated cDNAs of various sizes from 2 cDNA libraries and identified genomic fragments covering the entire coding region. Its temporal pattern of expression is currently being analyzed by probing blots of C. elegans poly A+ mRNA isolated from distinct developmental stages.

338.2

PIONEER NEURON GUIDANCE CUES IN GRASSHOPPER LIMB BUDS. J. S. Duerr and M. L. Condic. Dept. of Mol. and Cell Biology, Univ. of Calif., Berkeley, CA 94720.

In the grasshopper limb bud, the Ti1 pioneer neurons extend their axons from their origin in the periphery along a stereotyped pathway to the central nervous system. In a semi-intact preparation, the behavior of DiI-labeled growth cones of these identified pioneer neurons can be observed at the level of resolution of single filopodia with computer-enhanced video microscopy. Along their normal pathway, the cells encounter several possible sources of navigational information, including immature neurons and different regions of limb epithelium. Recent experiments have indicated that a single filopodium in contact with an immature neuron can reorient the extension of the growth cone. In the experiments reported here, sensory neurons of an identified type (femoral chordotonal organ) are taken from donor limbs and placed on the epithelium near the normal pathway of DiI-labeled pioneer neuron growth cones. The behavior of the pioneer neurons' growth cones as they approach and pass or touch these cells is observed with fluorescent video microscopy. In addition, the behavior of the transplanted sensory neurons in their new environment is monitored to see if they respond to general cues present in the limb epithelium.

338.3

NEURITIC OUTGROWTH PATTERNS FROM SEVERED GIANT AXONS IN *LUMBRICUS TERRESTRIS*. A.W. Lyckman, S.M. Thomas* and G.D. Bittner. Dept. of Zoology, Univ. of Texas, Austin, Texas 78712.

We have previously shown that severed giant axons in the earthworm *Lumbricus terrestris* regenerate functional connections that restore electrical coupling and impulse propagation across the lesion site. This functional restoration is specific in that medial giant axons (MGAs) reconnect only with MGAs, and lateral giant axons (LGAs) reconnect only with LGAs (Balter *et al., J. Exp. Neurol.* 211: 395, 1980; Birse & Bittner, *J. Neurophysiol.* 45: 724, 1981). We have now examined the pattern of neuritic outgrowth of MGAs and LGAs severed by transection of the ventral nerve cord (VNC). After various postoperative intervals, severed giant axons were injected with Lucifer yellow and prepared for epifluorescence microscopy.

The probability of producing at least one neurite from a severed MGA or LGA averaged 57% for the 1st through 7th postoperative weeks (POW). Severed MGAs and LGAs produced 1.2 ± 0.2 S.E.M. (n = 74) neurites which emerged from the cut end of the axon. The number of neurites showed little dependence on POW, except that outgrowth was minimal in the 1st POW. For severed MGAs or LGAs, proximal stumps produced significantly ($p<0.01$) more neurites than distal stumps (which may have cytoplasmic contact with nucleated axons). Severed earthworm giant axons grew very straight for most of their length. The initial and final angles of growth were typically less than 30° from the longitudinal axis. On average, neurites encountered appropriate giant axons for 37±7% of their total length, inappropriate giant axons for 7±3%, while spending 18±5% in the lesion site and 40±7% in other regions of the VNC. 86% of the severed axons had some contact with an appropriate giant axon, while 46% had some inappropriate contact. Supported by grants TAT #194 and NSF ECS 8915178 to GDB.

338.4

CELL-SPECIFIC CONTACT SELECTS THE RESPONSE TO 5-HT DURING REINNERVATION OF AN IDENTIFIED LEECH NEURON. D. Merz* and P. Drapeau. McGill Univ. Centre for Research in the Neurosciences, Montreal, Canada.

When serotonergic Retzius neurons of the leech contact pressure sensitive (P) neurons in culture, they selectively reduce a cationic response to 5-HT by uncoupling protein kinase C activation and reform the inhibitory, Cl-dependent synapse seen in vivo.

Retzius cells fixed in a mild aldehyde solution prior to pairing still reduce the cation response of the P cell (Drapeau et al, J Neurosci 9(7):2502-2508, 1989). Treatment of the Retzius cell with trypsin prior to fixation and pairing prevents the loss of the cationic response of the P cell.

To examine the cellular specificity of this interaction, P cells were paired in culture with other identified leech neurons. Contact with other sensory neurons (T or N cells), or with a motor neuron (AE) had no effect on 5-HT responses of the P cell.

We conclude that the selection of responses may be mediated by surface proteins with a restricted cellular distribution. The early clearing of the non-synaptic (excitatory) response to transmitter appears to be a prelude to (inhibitory) synapse formation.

Supported by the MRC and FRSQ of Canada.

338.5

EXPRESSION DURING DEVELOPMENT OF A NEURAL ANTIGEN SHARED BY GRASSHOPPER AND *DROSOPHILA*.
R. von Bernhardi and M. J. Bastiani. Dept. of Biology, University of Utah, Salt Lake City UT 84112.

During development most growing neurons extend their axons along the surface of established axon bundles. Molecules expressed in the axolemma may be specific cues for pathway choice or may be related to the extension of growth cones.

We have characterized the expression of an antigen (Ag) recognized by the monoclonal antibody (MAb) 5C1using immunofluorescence and immunoperoxidase labeling techniques, both with light and electron microscopy. In grasshoppers 5C1 begins to recognize the nervous system at approximately 30% of development. Initially the label is on cell processes in the CNS axon pathways and the major nerves. After 40% of development, labeling becomes prominent around neuronal somata. This pattern persists through embryonic development and into adulthood, always with a conspicuous granular appearance.

Live labeling indicates that the MAb recognizes a surface Ag and electron microscopy shows expression with a granular pattern on axons and filopodia. Finally, this MAb also recognizes an epitope expressed in the CNS of *Drosophila* embryos making it possible in the future to address the question of its physiological function using genetic techniques.

The spatial pattern of expression and its relative abundance on filopodia suggest that this molecule could participate in the recognition, affinity, or extension of a developing axon. We are currently assaying the effect of the Ab on axon growth and pathfinding.

Supported by NIH grant NS25378 and the McKnight Foundation.

338.6

STEREOTYPY AND VARIABILITY IN THE PATTERN OF DYE COUPLING BETWEEN NEURONS IN THE GRASSHOPPER EMBRYO.
Paul Z. Myers and Michael J. Bastiani. Dept. of Biology, University of Utah, Salt Lake City, UT 84112.

We have examined quantitatively the strength and frequency of dye-coupling between a set of early neurons in the embryonic grasshopper. The Q1 neuron, which pioneers a fascicle in the posterior commissure, initially extends its growth cone anteriorly along the longitudinal pathway pioneered by the MP1 neuron. After growing past the anterior and posterior corner cells, the Q1 growth cone turns medially to grow over the MP1 soma to the midline, where it contacts the growth cone of its contralateral homolog. Very shortly after axogenesis begins and for 10% or more of development, Q1 and the corner cells are robustly and consistently dye-coupled to one another. Q1 and MP1, however, are only observed to be dye-coupled approximately 50% of the time overall, although there is a likelihood of roughly 85% of observing strong dye-coupling when or shortly after their growth cones contact each other's somata.

Our observations suggest that there is a transient period of strong dye-coupling between MP1 and Q1 that occurs when growth cones contact cell bodies, and that this dye-coupling persists only weakly or for a variable length of time. In contrast, dye-coupling between Q1 and the corner cells is maintained at a uniformly vigorous level over a substantial portion of embryonic development. We are currently investigating the significance of these different temporal patterns of intercellular communication by ablating neurons before they become dye-coupled.

Supported by 5T32 CA09602, NIH NS08656 and NS25378, and the McKnight Foundation.

338.7

A NOVEL SUBSET OF AXON PATHWAYS IN GRASSHOPPER EMBRYOS IS LABELED BY THE 10E6 MAB
M. J. Bastiani and E. M. Carpenter. Dept. of Biology, University of Utah, Salt Lake City, UT 84112

The 10E6 MAb was generated by immunosuppression of mice (Hockfield, Science 237, 1987) with body wall tissue from 50% grasshopper embryos followed by immunization with adult nerve cord. Labeling using this antibody is first seen at 33% of development on a pair of cells that pioneers a pathway in the anterior commissure and on the midline cells that pioneer the median fiber tract. As axonogenesis begins in these neurons their cell bodies, growth cones, and filopodia all label with the MAb. However as their axons extend, the labeling fades from the cell bodies but remains on the axons, growth cones, and filopodia. At 40% of embryonic development the 10E6 MAb labels all sensory neurons and their associated peripheral nerves, a single ventral pathway in the CNS connectives, and 3 CNS commissural pathways. At this stage approximately 10 CNS neurons are labeled. The ventral connective pathway is quite interesting in that it is pioneered by a pair of CNS neurons and then followed by the processes of sensory neurons which had been waiting in the ventral sensory neuropil region. By 70% of development, labeling of the commissural pathways appears to fade and is not seen at later stages of development or in cryostat sections of the adult, while the ventral sensory pathway is very prominent and remains prominent in the adult. All the peripheral sensory neurons seem to label while less than 1% of the CNS neuronal somata and axonal processes label in the adult. Incubation of live embryos with the MAb results in the same pattern of labeling suggesting that the MAb binds to a surface epitope. Immuno EM examination confirms the surface membrane localization of the antigen. We are currently attempting to characterize the antigen recognized by the 10E6 and determine what effects the MAb may have on normal neuronal development.

Supported by NIH grants NS25378, NS08404, and the McKnight Foundation.

338.8

A CHROMOSOMAL DEFICIENCY IN *DROSOPHILA* ALTERS NEURITIC PROJECTIONS IN AN IDENTIFIED MOTONEURON.
G.P. Swain*, R.J. Wyman† and M.D. Egger. Dept. of Neuroscience & Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ 08854; † Dept of Biology, Yale University, New Haven, CT 06511.

Baird (1988) noted that in *Df(1)16-3-22/FM6 Drosophila*, which are heterozygous for a deficiency at the base of the X-chromosome, a branch of the medial neurite of the motoneuron innervating the tergotrochanteral (TTM) muscle invariably crossed the midline, whereas in wildtype strains this midline crossing occurred rarely. Earlier, however, Koto (1983) had reported that in *Df(1)16-3-22*/C-S flies, the medial neurites of the TTM motoneuron did **not** cross the midline. We reinvestigated the influence of *Df(1)16-3-22* on TTM motoneuron morphology. HRP was applied to the TTM muscles of wildtype (C-S, n=32; O-R, n=17) strains, as well as to *Df(1)16-3-22*/C-S (n=31) or *Df(1)16-3-22*/O-R (n=40). Reaction product could be seen in at least one TTM cell body in 80-100% of the flies in each of the four groups. Of those flies in which at least one cell body was labelled, one or both medial neurites were **well filled** in 65% of the C-S flies, 50% of the O-R, 39% of Df(1)16-3-22/C-S, and 55% of Df(1)16-3-22/O-R. Rates of midline crossing were then assessed in these flies. **No** midline crossings were observed in the C-S flies. However, in contrast to Koto's observations, a neuritic branch crossed the midline in 58% (7/12) of *Df(1)16-3-22*/C-S flies, some extending 25-30 μm from the parent neurite. In 2/8 O-R flies, neurites crossed the midline, but in only one of these was this a major projection, contrasting strikingly with the *Df(1)16-3-22*/O-R flies, in which a branching process of the medial TTM neurite extended beyond the midline in 82% (18/22) of our sample. In more than half of these flies, the midline-crossing neurite extended 30-45 μm from the parent neurite. In summary, the deficiency, *16-3-22*, promotes midline crossings of branches of the medial neurite of the TTM motoneuron. These midline crossings occurred with greater frequency and magnitude than in control wildtype strains.

PROCESS OUTGROWTH, GROWTH CONES AND GUIDANCE MECHANISMS VII

339.1

EFFECTS OF MANIPULATION OF GAP-43 EXPRESSION ON MORPHOLOGY IN PC12 CELLS AND CULTURED HIPPOCAMPAL NEURONS.
S.A. Fidel, L.R. Dawes, K.A. Neve, R.L. Neve. Dept. of Psychobiology, Univ. of California, Irvine, CA 92717 and V.A. Medical Center, Portland, OR 97207.

To analyze the role of the neuronal protein GAP-43 in neurite outgrowth and regeneration, we designed oligonucleotides and recombinant constructions of GAP-43 that allow us to alter the levels of GAP-43 within neuronal cells. Recombinant sense and antisense GAP-43 clones were transfected into PC12 cells and recombinant GAP-43 RNA levels in each were analyzed by PCR amplification of cDNA synthesized using vector-specific primers. Immunoblot analysis of stable transfectants revealed that cells expressing high levels of RNA from the sense construct contained higher levels of GAP-43 protein than controls; these cells displayed more and longer processes than control undifferentiated cells. The cells expressing antisense GAP-43 mRNA contained lower levels of GAP-43 protein than controls, and displayed a morphology similar to that of PC12 cells treated with glucocorticoid. HPLC analysis of dopamine levels in medium conditioned by these cells is underway.

Antisense oligonucleotides complementary to GAP-43 mRNA were added to primary hippocampal neuronal cultures. Antisense, but not sense, oligonucleotides inhibited process outgrowth from these neurons. The extent of inhibition of outgrowth was dependent on the concentration of antisense oligonucleotide that was added to the culture.

339.2

EXPRESSION OF GAP-43 mRNA AND PROTEIN IN THE CHICK EMBRYO
L. Baizer, G. Ciment, and K. Stocker* Neurological Sciences Institute, Good Samaritan Hospital, and Department of Cell Biology and Anatomy, Oregon Health Sciences University Portland, Oregon 97209

Growth-associated protein-(GAP)-43 is a neuron-specific phosphoprotein expressed at elevated levels during periods of axonal growth and regeneration. To begin to analyze the expression of GAP-43 in the early stages of neuronal development we recently isolated a cDNA for chicken GAP-43 (Mol. Brain Res. 7:61-68 (1990). We have used this cDNA as a probe in Northern blot and in situ hybridization analysis to investigate the time-course and location of GAP-43 mRNA expression in the chick embryo. We have also performed immunocytochemical analysis of GAP-43 protein expression.

Results indicate that GAP-43 mRNA and protein expression are first detectable on embryonic day 2 (e2) in the chick brain, neural tube, and neurectodermal placodes. Northern blot analysis demonstrates that GAP-43 mRNA levels in brain reach peak levels at e13 and diminish rapidly thereafter. On e7 and e10, GAP-43 mRNA is detectable by in situ hybridization analysis in the brain and cranial ganglia, spinal cord, and sympathetic, dorsal root, and nodose ganglia. Thus, GAP-43 is expressed by tissues derived from neurectoderm, neural crest, and ectodermal placodes, and is among the first neuron-specific gene products to be expressed in the developing nervous system.

In situ hybridization analysis reveals that GAP-43 mRNA is also expressed in the distal regions of the limbs on e7. This result is substantiated by Northern blot analysis, which indicates that GAP-43 mRNA is expressed in the limbs transiently, from e5 to e9. Our results thus suggest a possible role for GAP-43 in limb development.

339.3

THE GROWTH ASSOCIATED PROTEIN, GAP-43, IS PRESENT IN DENDRITIC PROCESSES AND GROWTH CONES. M.I.Johnson and S.M.Schuh. Univ. of Ariz. Col. of Med., Tucson, AZ 85724 and Wash. Univ. Sch. of Med., St. Louis, MO 63110.

The membrane bound phosphoprotein, GAP-43, is elevated in axons and axonal growth cones during neuronal development and regeneration. In this study an in vitro model is utilized to determine whether GAP-43 is present in developing dendrites. Dissociated embryonic rat superior cervical ganglion neurons, maintained in serum free medium in the absence of nonneuronal cells elaborate primarily axonal processes (Bruckenstein and Higgins, 1988). After 3-7 days dendrite development was induced by addition of 25-50 μg/ml of Matrigel to the medium. Cultures were double stained immunocytochemically to simultaneously detect GAP-43 and dephosphorylated neurofilament proteins. Whenever a process was identified as dendritic by the presence of dephosphorylated neurofilament proteins, GAP-43 was also present. The fluorescence in dendrites was decreased in intensity compared to axons. As dendrites developed, neurofilament bundles were observed to fill the broad proximal segments of the dendrites, taper, and end at the base of the growth cone. GAP-43 was found in the entire dendrite including the growth cone. The difference in these results from those reported in hippocampal neurons (Goslin et. al., 1988) may be secondary to the different neuronal system, antibody, or antibody concentrations used.

339.4

NEURITE INITIATION AND THE ESTABLISHMENT OF POLARITY BY NEURONS *IN VITRO*. C.L. Smith and E.M. Munro*, Laboratory of Neural Control, NINDS, NIH, Bethesda, MD 20892.

Neurite formation signals the initiation of a neuron's differentiation and the establishment of its functional polarity. We are studying neurite formation by embryonic chick neurons grown *in vitro* in order to understand how the shapes and polarities of neurons are determined. Cultures of dissociated neurons are examined with video microscopy on an inverted microscope enclosed in an incubator. Our initial studies focused on regenerating dorsal root ganglion neurons. Although these neurons often retain a remnant of their original axon during dissociation, their axons usually disappear within 1 hour after plating. The neurons extend a flat, veil-like lamellipodium arround their entire circumference. Filopodia protrude from the lamellipodium. The first filopodium that contacts another cell often develops into a neurite. The filopodium develops a thickening at its base which moves centrifugally toward the tip. The thickened area often sprouts new filopodia as it moves. After the thickening reaches the tip, the process begins to elongate. Immunocytochemical staining of filopodia undergoing this transformation showed that the thickened area contained actin, microtubules and GAP-43, a protein known to be enriched in growth cones. Most sensory neurons developed only one or two neurites, but some became multipolar. Although sensory neurons appeared polarized prior to neurite formation, with eccentric nuclei and centrally-located centrosomes, the positions at which they formed neurites did not bear a consistent relationship to their initial polarities. However, if a neurite formed on the same side of the cell as the nucleus, then the cell body rotated so as to move the nucleus to the opposite side. Thus, the polarities of regenerating sensory neurons *in vitro* can be influenced by extrinsic factors. Preliminary observations on the initial outgrowth of processes from sympathetic ganglion neurons generated *in vitro* from dividing precursor cells indicate that the polarities of newly-generated neurons also can be influenced by their contacts with other cells.

339.5

ANTISENSE B-50 (GAP-43) PREVENTS NEURITE OUTGROWTH IN PC12 CELLS. P. Schotman*, E.R.A. Jap Tjoen San, M. Schmidt-Michels* and W.H. Gispen* (SPON: European Neuroscience Association) Div.of Molec. Neurobiol. IMB, Dept. of Physiol. Chem., Rudolf Magnus Institute, University of Utrecht, Padualaan 8, 3584 CH Utrecht, The Netherlands.

It has become clear, that B-50 expression and its (trans)location into growth cone membranes correlate well with neuronal development, but whether this is a prerequisite for neurite outgrowth, is still unknown.

NGF-induced neuritogenesis was studied in PC12 cells. After 24 hrs of culture B-50 immune reactivity (B-50 IR) was determined with an B-50 ELISA and average neurite length of the same cells was determined by video image analysis.

Using a small, antisense oligomer to B-50, we were able to specifically block the NGF-induced enhancement of B-50 IR. This treatment also prevented the NGF-induced neuritogenesis of the PC12 cells completely.

It has been reported that dexamethasone interferes with B-50 expression at the transcriptional level. Pretreatment of PC12 cells with dexamethasone interfered with the NGF-induced increase of B-50 IR and neurite outgrowth in a similar way as the antisense oligomer to B-50 did. Thus, interfering with the NGF-induced B-50 expression in PC12 cells, either at the transcriptional level by dexamethasone or at the translational level by an antisense oligomer prohibited the NGF-induced neurite outgrowth of these cells.

We conclude that enhanced B-50 expression is a prerequisite for NGF-induced neurite outgrowth in PC12 cells.

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339.6

DIFFERENTIAL EFFECTS OF ANTIBODIES TO PKC SUBSTRATE B-50 (GAP-43) AND KINASE INHIBITORS ON CALCIUM- AND PHORBOL ESTER-INDUCED NA RELEASE FROM PERMEATED SYNAPTOSOMES. P.N.E. DeGraan, J.J.H. Hens*, L.V. Dekker*, M. De Wit, A.B. Oestreicher* and W.H. Gispen*. Div. Mol. Neurobiol., Rudolf Magnus Inst. and Inst. Mol. Biol. and Med. Biotechnol., Univ. of Utrecht, Padualaan 8, 3584 CH Utrecht, NL.

Antibodies raised against the neuron-specific growth-associated protein B-50 (GAP-43) inhibit protein kinase C (PKC)-mediated phosphorylation of B-50 and Ca^{2+}-induced ^{3}H-noradrenalin (NA) release from Streptolysin-O permeated synaptosomes (Dekker et al., *Nature*, 342: 74, 1989). The present study was designed to characterize Ca^{2+}- and phorbol ester-induced NA release from highly purified permeated synaptosomes and to study possible differences between the two secretagogues using anti-B-50 antibodies and kinase inhibitors. Both secretagogues induce a concentration-dependent release of NA; their stimulatory effects are not additive. Anti-B-50 IgG completely inhibits Ca^{2+}-induced release, but has only minor effects on phorbol ester-induced release. The kinase inhibitors H-7 and polymyxin B, which inhibit B-50 phosphorylation, inhibit phorbol ester-induced release, but only polymyxin B attenuates Ca^{2+}-induced release. Our data indicate that Ca^{2+} and phorbol esters stimulate NA release through different mechanisms. Alternatively, besides phosphorylation, other properties of B-50, such as calmodulin binding, may also be important for the regulation of NA release from permeated synaptosomes. To investigate this latter possibility we studied B-50/calmodulin binding. With homobifunctional crosslinkers we identified a 70 kDa B-50/calmodulin complex *in vitro* and in synaptosomal plasmamembranes. The complex is only formed in the absence of Ca^{2+} and is not found after prephosphorylation with PKC. Our data are in line with the hypothesis that both B-50 phosphorylation and calmodulin-binding are regulatory factors in NA release.

339.7

PC12 CELLS DEVOID OF GAP-43 PROTEIN SYNTHESIZE A TRUNCATED FORM OF GAP-43 mRNA E.E Baetge[1], C.M.Sampson[1] J.P.Hammang[2] [1]CNS Molecular Biology, Bristol-Myers Squibb Pharm. Res. Inst. Wallingford,Ct 06492.[2]Univ. Wisconsin-Mad., Pathobio. Sci. Madison, WI. 53706.

We have characterized a PC12 cell line, (PC12B), that is devoid of GAP-43 protein and mRNA as determined by immunocytochemical, western and northern blot analysis.

GAP-43 probed northern blots containing 10ug of Poly A+-mRNA isolated from PC12(B) cells revealed a diffuse mRNA hybridization signal that was more than 3 orders of magnitude lower than an equivalent amount of PC12(A)(GAP-43 containing) Poly A+-mRNA. In addition, the size was shorter by 100-200 nt. To further characterize these findings, RNA from PC12(A) and PC12(B) cells was reverse transcribed using random primers and PCR-amplified using oligonucleotides flanking the entire coding region of rat GAP-43. The predicted 708 bp GAP-43 coding sequence was amplified from both cells and both were identical to the published rat cDNA as determined by complete DNA sequence analysis. In contrast, when RNA was oligo-dt primed and amplified as above, the 708 bp coding fragment could only be amplified from PC12(A) RNA. Further analysis of PC12(B) RNA using a 3' PCR-oligo complementary to the last 24 nt of the 3'UT region of GAP-43 mRNA, did not produce the expected 1207 bp DNA fragment suggesting that these cells express a truncated mRNA missing a portion of the 3'UT region and lacking a poly A tail. The missing 3' end may predispose the truncated GAP-43 mRNA to rapid nuclear/cytoplasmic degradation thus preventing functional translation and expression of the GAP-43 protein.

339.8

THE C6 GLIOMA CELL LINE EXPRESSES THE GROWTH-ASSOCIATED PROTEIN GAP-43 IN A DEVELOPMENTALLY REGULATED FASHION.

[1]J.P. Hammang, [1]A. Messing and [2]E.E. Baetge.

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The growth-associated protein, GAP-43, is expressed in developing neurons during axonal outgrowth and in peripheral neurons during regeneration. This membrane-associated phosphoprotein is particularly concentrated in the axonal growth cones and is involved in various aspects of synaptogenesis and synaptic function. Until recently, this protein was considered neuron-specific. However, new evidence suggests that the GAP-43 protein is expressed in primary cultures of neonatal rat cortical astrocytes (Vitković et al., PNAS 85 1988). As these astrocytes differentiate in culture, GAP-43 levels decrease.

The C6 glioma cell line has been studied extensively as a model system for glial differentiation. This differentiation can be induced with the addition of dBcAMP to the culture medium. We report that monolayer cultures of C6 glioma cells (35-45th passages) express GAP-43 mRNA and protein and low levels of GFAP. When the cells are treated with 1 mM dBcAMP, GAP-43 protein levels fall as GFAP levels rise. The C6 line should prove to be a valuable model system for investigating the role of the GAP-43 protein in glial development.

339.9

Electrophysiological Properties of GAP-43-Containing and GAP-43-Negative PC12 Cells Exposed to NGF. Valentin K. Gribkoff[1], E. Edward Baetge[2], and Joseph P. Hammang[3]. CNS Pharmacology[1] and Molecular Biology[2], Bristol-Myers Squibb Pharm. Res. Institute, 5 Research Parkway, Wallingford, CT 06492, and Pathobiol. Sciences[3], Sch. Veterinary Med., Univ. of Wisconsin-Madison, Madison, WI 53706.

PC12 cells differentiate into neurons when exposed to NGF. During the process of differentiation, neurites are formed and the cells become capable of forming functional synaptic contacts. PC12A cells contain GAP-43, and it has been shown that levels of this protein are elevated by NGF, leading to the conclusion that GAP-43 plays an important role in the response to NGF. Recently, a mutant cell line (PC12B) was characterized that does not contain GAP-43 or functional GAP-43 mRNA, yet which responds to NGF with neurite extension and by expressing other neuronal characteristics (see abstract by Baetge et al.). Using intracellular recording techniques, we have begun to characterize these cell lines electrophysiologically. PC12A cells (n=26) had Vm ($\bar{x}\pm$sem)= -51.4±2.8 mV; PC12B cells (n=25) had Vm ($\bar{x}\pm$sem)=-51.5±2.2 mV. The input resistance of all cells exceeded 100 MΩ. No cells exhibited spontaneous action potentials, and clearly-defined postsynaptic potentials were not observed. In response to depolarizing intracellular current injection most PC12A cells (23/26) responded with a well developed action potential; fewer PC12B cells (10/25) displayed current-evoked action potentials under these conditions.

339.10

LOCALIZATION OF GAP-43 mRNA IN RAT CEREBELLUM AND IN BRAINSTEM NUCLEI PROJECTING TO THE CEREBELLAR CORTEX: A DEVELOPMENTAL STUDY. Linda Console-Bram,[1] James G. McElligott,[1] and Sandra G. McElligott.[2] [1]Temple University, Dept. of Pharmacology Phila PA. 19140, [2]E.I. Du Pont, Medical Products Dept., Glasgow DE. 19702

Distribution of GAP-43 within the cerebellum has been shown to coincide with early postnatal development and synaptogenesis. Within the first two weeks after birth, rat cerebellar cortex undergoes extensive remodeling leading to the formation of three distinct layers. Our study was designed to determine the location of GAP-43 mRNA, employing an ^{35}S-labeled oligonucleotide probe, in the cerebellum and brainstem nuclei projecting to the cerebellum in both immature and adult rats. At postnatal day (PD) 6-7, the GAP probe was found to hybridize strongly in the molecular layer (ML) and moderately in the internal granule cell layer (IGL). Expression of GAP-43 mRNA in the ML during this time period corresponds to the descent of granule cells from the premigratory zone to the IGL. The olivary nuclear complex in the brainstem, the source of cerebellar climbing fibers (CF), also exhibited intense hybridization. Both the external germinal cell layer and Purkinje cells were devoid of GAP message. By PD 21, GAP-43 message was confined to the granule cell layer of the cerebellum. The olive remained positive for GAP-43 mRNA but with a reduction in hybridization in comparison to that of the first postnatal week. In the adult rat, hybridization patterns were similar to those found at PD 21. Previous studies demonstrated the presence of GAP-43 protein in the ML of both immature and adult rats and GAP-43 mRNA in granule cells of adult rats. Since this study is the first to demonstrate GAP-43 mRNA in both granule and olivary cells of immature and adult animals, GAP-43 protein in the ML is most likely derived from two sources: parallel fibers (PF) and CF. This study supports developmental regulation of GAP-43 expression in the central nervous system as the most intense hybridization was observed during periods of axonal outgrowth and synaptic organization within the cerebellum. Because GAP-43 is also thought to be associated with synaptic plasticity, the presence of GAP-43 mRNA in the adult rat suggests that the PF and CF synapses may continue to be plastic in adults.

339.11

EVIDENCE THAT NEUROMODULIN (GAP-43, B-50, pp46, F1) IS NOT ESSENTIAL FOR EARLY NEURITOGENESIS. J.T. Megerian, W.L.Klein Northwestern Univ. Inst for Neurosci., Evanston IL. 60208

Neuromodulin is a calmodulin binding protein found in developing neurites and the presynaptic endings of certain adult brain regions. Its role is unclear, having been implicated in axon outgrowth, neurotransmitter release, and plasticity of adult synapses. We report here evidence against a requirement for neuromodulin in neurite outgrowth. Mouse and human neuroblastoma cells were cultured in conditions promoting and inhibiting neurite growth and examined for the presence of neuromodulin using a monoclonal antibody (courtesy of J.H.P. Skene) capable of recognizing four known isoforms. Immunoblots and cell fluorescence showed neuromodulin was present equally in neurite promoting and inhibiting cultures. In cultures lacking neurites, neuromodulin appeared in a halo around the periphery of the cell, suggesting association with the plasma membrane. A second study looked at the production of neuromodulin in 1 day old cultures of E15 rat basal forebrain cells. These cultures contained cells undergoing a neuroblast- neuron transition and sprouting neurites de novo. Immunofluorescence showed that 80% of the cells that had neurites lacked neuromodulin. Double labeling showed that neurofilament was present in many of the neurite-positive, neuromodulin-negative cells. The two studies are consistent with the hypothesis that neuromodulin is neither sufficient nor necessary for early neurite outgrowth.

339.12

MONOCLONAL ANTIBODIES DEMONSTRATE THE REGULATION OF GAP–43 AMOUNT AND PHOSPHORYLATION BY KINASE C DURING AXONOGENESIS *IN VIVO*. K.F. Meiri & J.E. Schwob. Depts. Pharmacology & Anatomy & Cell Biology, SUNY Health Science Center, Syracuse NY 13210.

The neuron-specific protein GAP-43 is highly enriched in the membrane skeletons of growth cones and is a substrate for protein kinase C. Initial studies *in vivo* determined that GAP-43 synthesis is induced during axonogenesis but did not provide a precise temporal correlation with the onset of axon out- growth, nor did they investigate the role of kinase C phosphorylation in this process. We have generated a panel of monoclonal antibodies monospecific for GAP-43 that are able to discriminate between GAP-43 that has been phos- phorylated by kinase C (exemplified by 2G12) and GAP-43 that has not (exemplified by 10E8). We have used these antibodies to study early axon outgrowth *in vivo* and have obtained two major results.

First, with regard to kinase C phosphorylation of GAP-43, the phosphorylated form is **spatially** restricted to the distal axon and growing tip; it is never seen in either cell bodies or dendrites, even those that contain unphosphorylated GAP-43. Kinase C phosphorylation of GAP-43 is also **temporally** restricted and occurs subsequent to the onset of axonogenesis. Furthermore, the lag between the onset of axon outgrowth and the appearance of phosphorylated GAP-43 in different types of neurons is variable.

Second, with regard to the amount of GAP-43 present during axonogenesis, 10E8 immunoreactivity is present at the onset of axonal growth in those types of neurons that we have examined. Taken together these results indicate that the regulation of GAP-43 and hence its role in axon outgrowth is more complex than previously appreciated, and emphasize that the process of axon outgrowth is likely to have multiple components.

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339.13

RELATIVE DISTRIBUTIONS OF KINASE C-PHOSPHORYLATED AND NON-PHOSPHORYLATED FORMS OF GAP-43 IN THE ADULT NERVOUS SYSTEM. J.E.Schwob & K.F.Meiri. Depts. Anatomy & Cell Biology and Pharmacology, SUNY Health Science Center, Syracuse NY 13210.

The neuron-specific protein GAP-43, which is highly enriched in the membrane skeletons of growth cones, is also found in the pre-synaptic terminal in parts of the adult nervous system where it is a substrate for protein kinase C. While information about the distribution of GAP-43 is available from studies utilizing polyclonal antisera, nothing is known of the relative distribution of phosphorylated vs. non-phosphorylated forms. To address this issue we have used the monoclonal antibodies 2G12, which is specific for the kinase C phosphorylated form of GAP-43, and 10E8, which recognizes both phosphorylated and nonphosphorylated forms (Meiri and Schwob, presented at this meeting). While the distribution of 10E8 immunoreactivity resembles that described previously using antisera, the relative amount and distribution of the kinase C phosphorylated form varies between neuronal types. For example, the spatial distributions of kinase C phosphorylated and unphosphorlyated GAP-43 are similar in the neuropil of the adult cerebral cortex, (in contrast with the differences seen in the developing cerebral cortex, Meiri and Schwob, this meeting), although the intensity of the 2G12 immunoreactivity is consistently less. Conversely, in both sympathetic and parasympathetic postganglionic axons of the adult autonomic nervous system, little if any 2G12 immunoreactivity is detectable under normal conditions, despite the presence of 2G12 immunoreactivity during the initial innervation of the target organs in the embryo. These results demonstrate that in the adult nervous system, as during axonogenesis, the regulation of GAP-43 by kinase C phosphorylation is under complex control.

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339.14

DEPENDANCE OF AXONAL BRANCHING AND GROWTH CONE STRUCTURE ON THE TARGET CELL. S.A. Berman and S.Bursztajn. Baylor College of Medicine and M.D. Anderson Cancer Center. Houston, Tx 77030

The growth cone is a crucial structure which guides neuronal growth in response to environmental cues and appropriate targets. It has been shown that GAP43, a neuron specific growth-associated phosphoprotein, is selectively distributed in axons of growing neurons and their growth cones, but is absent from dendrites. We have utilized an antibody to GAP43 and immunofluorescent microscopy to determine the changes in growth pattern and polarity the growing neurons undergo when they contact a "proper" target. We have used ciliary neurons, cocultured with myotubes or plated alone, since these neurons form functional synapses with myotubes. Our results show that ciliary neurons plated alone have bipolar or tripolar axons, but when cocultured most cells have 4 or 5 axons showing GAP43 immunoreactivity. The mean number of axons per cell soma was 1.9+ 0.1(SEM) when ciliary neurons were plated alone, and 3.4+ 0.1 when ciliary neurons were cocultured with myotubes. Differences in growth cone behavior was readily apparent in these two types of cultures. The size occupied by the neuronal growth cone lamelopodia showed significant differences. The majority of growth cone lamelopodia in coculture were 20-30μm^2 with an average area of 25.0+ 2.3μm^2 (SEM), whereas those neurons plated alone occupied an average area of 56.3μm^2. The number of filopodia of the growth cones depended on culture conditions. In neuronal cocultures, the average number of filopodia per growth cone was 3.6+ 0.2(SEM), whereas in ciliary cultures the number of filopodia was 6.8+ 0.4(SEM). These results suggest that muscle cells or the factors they release can regulate the growth and topography of axons and their growth cones.

339.15

ELEVATED LEVELS OF EXPRESSION OF GAP-43 AND CALCYCLIN mRNAS DURING TPA-INDUCED DIFFERENTIATION OF A HUMAN NEUROBLASTOMA CELL LINE. G. Allan,* J.M. Moerschbaecher, III and N.G. Bazan. LSU Eye Center and Neuroscience Center, New Orleans, LA 70112.

The cell line SH-SY5Y undergoes morphological differentiation in response to 13-*O*-tetradecanoyl-12-phorbol acetate (TPA) as characterized by neurite extension. Maximal neurite extension is achieved after 7-8 d exposure. In the present study, however, we show that the mRNA coding for GAP-43, a protein associated with growth cones and nerve endings undergoing remodeling, dramatically increases in SH-SY5Y cells exposed to 10ng/ml TPA for 24 h and does not rise above these levels over an interval of 8 d. We also note that there is a low but significant level of GAP-43 mRNA in the untreated cells. While this expression can be explained by the low level of spontaneous neurite extension observed, we also observe a similar level of GAP-43 mRNA in the retinoblastoma cell line Y79, which exhibits no overt signs of neurite extension. TPA treatment of the neuroblastoma line also elevates levels of the mRNA coding for calcyclin, a putative calcium-binding protein specific for the G_1 phase of the cell cycle. This suggests that the increase in GAP-43 mRNA in these cells is accompanied by significant alterations in cell-cycle kinetics. By contrast, levels of cyclophilin mRNA, which encodes a constitutive "house-keeping" protein, do not change over this time course. We have previously shown that the lipid mediator platelet-activating factor (PAF) elevates calcyclin mRNA expression in the same cell line, and that TPA and PAF activate c-*fos* expression in an additive manner. Thus it may be that there are convergent pathways acting in response to differentiating signals and signals of cell damage, which trigger neuronal regeneration (some neuroblastoma cell lines respond to PAF exposure with a differentiation response). We are currently studying the effects of factors on the differentiation/regeneration response of this system. Supported by NIH grant NS23002.

339.16

POSTTRANSCRIPTIONAL REGULATION OF GAP-43 mRNA LEVELS DURING PROCESS OUTGROWTH. N. I. Perrone-Bizzozero, N. Irwin*, S. E. Lewis, I. Fischer*, R. L. Neve and L. I. Benowitz McLean Hosp. (Belmont, MA, 02178), Harvard Med. Sch., E. K. Shriver Center and U.C. Irvine

The expression of GAP-43 is a well-characterized correlate of the growth and reorganization of neural connections. Although the expression of the protein is known to depend on the mRNA levels, the mechanisms that control the latter are poorly understood. Using Northern blots and nuclear run-on assays, we analyzed the synthesis and accumulation of GAP-43 mRNA in (a) developing rat cortical neurons, (b) the regenerating goldfish optic nerve, and (c) NGF-induced PC12 cells. In all of these instances, significant levels of the nascent transcript were detected even when cells were not growing, and this changed little during differentiation. These data suggested that GAP-43 mRNA levels are regulated via a transcription-independent pathway. To begin to study the nature of the mechanism(s) involved, we examined the half-life of the mRNA in naive and NGF-treated PC12 cells. Experiments using pulse-chase labeling or actinomycin D treatment both showed that NGF induced a 3-fold increase in the stability of the mRNA. Thus, changes in the rate of degradation of the mRNA seem to be the key control mechanism that regulates GAP-43 levels during neurite outgrowth. The presence of putative instability-conferring sequences in the 3' untranslated region of GAP-43 mRNA lends further support to this possibility. *Supported by NIH EY 05690 and the Scottish Rite Schizophrenia Res. Foundation.*

SPROUTING AND SPROUTING MECHANISMS

340.1

PEPTIDE-IMMUNOREACTIVE FIBERS IN HIPPOCAMPUS FOLLOWING SEPTAL LESIONS. R. Loy, D. Heyer* and M. Johnson. Dept. Neurology, Univ. Rochester Sch. Med., Rochester, NY 14642 and Dept. Neurobiology, Harvard Med. Sch., Boston, MA 02115.

Septal deafferentation of the rat hippocampus leads to ingrowth of noradrenaline (NA)-containing collaterals of vascular sympathetic fibers. In normal cerebral vascular afferents, neuropeptide Y (NPY) is co-localized with NA. We have investigated whether or not NPY immunoreactivity (NPYir) is also present within putative sympathetic fibers in the hippocampus following septal lesions. In addition, we have evaluated adjacent sections for substance P (SPir), as this peptide is present in sensory fibers innervating the cerebral vasculature. Rats received aspiration lesions of the medial septum 4 days after birth and were allowed to survive 3-10 weeks. Sections from normal and lesioned brains were stained free-floating in NPY (Incstar, 1:1000), SP (Incstar, 1:500), or in nerve growth factor receptor (NGFR, 192-IgG, 1 µg/ml, courtesy of P.S. DiStefano). In lesioned brains, both NPYir and SPir fibers are associated with penetrating and fine collateral blood vessels adjacent to the hippocampal fissure and within the parenchyma. These are not as numerous, however, as fibers immunoreactive for NGFR, which are present in a characteristic pattern along the granule and pyramidal cell layers, as well as adjacent to blood vessels. While it remains to be determined if the SP- and NPY-containing fibers are in fact peripheral in origin, or if all sympathohippocampal fibers contain both NA and NPY, it appears likely that reorganization of pathways containing these peptides occurs following septal deafferentation.

Supported by NS25169 (RL) and NS02253 (D.D. Potter).

340.2

ALTERED PHOSPHORYLATION OF A GROWTH-ASSOCIATED PROTEIN (GAP-43) IS ASSOCIATED WITH LESION-INDUCED TERMINAL SPROUTING IN THE CNS. L. H. Lin*, B. Costello*, and J. J. Norden. Dept. of Cell Biology, Vanderbilt University, Nashville, TN 37232.

Reactive synaptogenesis, a widely used model of synaptic plasticity known to involve axon growth, was induced in the hippocampus by lesioning the ipsilateral entorhinal cortex in rats. Decreased *in vitro* phosphorylation of GAP-43 was observed in ipsilateral dorsal hippocampal tissue from animals sacrificed at 4 days(when terminal proliferation begins), 6 days (maximal terminal proliferation) and 30 days (synaptogenesis ends) following entorhinal cortex lesions, as compared to that of the contralateral dorsal hippocampi of the same animals. The amount of GAP-43 in the hippocampus also was determined since a change in the observed incorporation of ^{32}P may represent a change in the amount of substrate protein available for phosphorylation. A significant decrease in GAP-43 levels in the ipsilateral hippocampus was seen 2-3 days following lesion. Levels of GAP-43 in ipsilateral hippocampi were lower but not significantly different from that of contralateral sides at 4, 6, 15, 30, 60, and 120 days following lesion. When the phosphorylation of GAP-43 was expressed as incorporation of ^{32}P per unit of GAP-43, a significant decrease was observed in the ipsilateral hippocampi of animals sacrificed 6 days following lesion, but not at 4, 15, 30, 60, and 120 days post lesion. This decrease in *in vitro* phosphorylation coincides with the period of maximal proliferation of presynaptic terminals. These data suggest that GAP-43 phosphorylation state is associated with lesion-induced axonal growth in the CNS of adult mammals. (Supported by NIH Grant NS25150 to JJN)

340.3

ASTROCYTES MAY BE INVOLVED IN THE HOMOTYPIC SPROUTING OF 5-HT FIBERS IN THE HIPPOCAMPUS, F.C. Zhou, and S. Bledsoe. Department of Anatomy, Indiana University School of Medicine, Indianapolis, IN 46202

We have previously reported that partially removing 5-HT fibers in the hippocampus induced a homotypic sprouting (Zhou & Azmitia, Brain Res, 1984, 1986). Extract from partially denervated hippocampus increased the survival, fiber growth and transmitter level of fetal 5-HT neurons when grafted in adult brain for bioassay (Zhou et al., J. Neurosci. Res. 1987; Zhou & Azmitia, Brain Res., 1990). A sprouting factor, SNTF, is being proposed in this experimental model. We currently report that the sprouting of 5-HT fibers can be induced directly by removing neurons in the hippocampus. The role of astrocytes on release of SNTF and sprouting of 5-HT fibers are hypothesized.

Ibotenic acid (IB) injected into hippocampus of Sprague-Dawley rats removes the intrinsic neurons. Three days after IB-lesion, the intrinsic neurons in the hippocampus had degenerated in the injection site as shown by Nissl staining. Immunocytochemistry showed that two patterns of laminin immunostaining were increased, small punctiform in the granular layer, and the sheath form on the vessel; neo-microvessels were observed; reactive astrocytes elevated glial fibrillary acidic protein (GFAP) level; double immunostaining of laminin and GFAP showed that the small punctiform laminin was produced by astrocytes; meanwhile, the 5-HT fibers retained similar fiber density,. At 8-day stage, both patterns of laminin continued at elevated levels, Astrocytes expressed high activity of GFAP, and 5-HT fiber density was highly increased in the hippocampus with a similar innervation pattern. The hyperinnervation reduce to a less degree but sustained above normal scale at the stage 24 days after lesion. The 5-HT inneravaton pattern maintained in the same manner of normal without the local target neruons. A number of important result were observed in this study (a) astrocytes became reactive and produced large quantity of GFAP and laminin, (b) micro-vessels are actively formed, (c) 5-HT innervation fiber to the hippocampus was maintained without contacting hippocampal target neurons, (d) 5-HT fiber sprouting occurred at 8-day stage; SNTF would be induced in this stage, (e) astrocytes may be involved in the sprouting of 5-HT fiber in the injured hippocampus. Supported by NIH grant NS23027 to FCZ.

340.4

Axonal Sprouting Induced by Striatal Implants of Genetically Modified Fibroblasts that Produce Nerve Growth Factor *in vitro*. M.D. Kawaja[1], M.B. Rosenberg[2], K. Yoshida[1], T. Friedmann[2]*, and F.H. Gage[1]. Depts. of Neurosciences[1] and Pediatrics[2], University of California, San Diego, La Jolla, CA 92093

Cultured skin fibroblasts of Fischer 344 rats were infected with a retroviral vector containing the cDNA for mouse nerve growth factro (NGF). These cells synthesized and released NGF *in vitro*. The purpose of this study was to assess the biological effects NGF-producing fibroblasts have in the adult rat nervous system. Female Fischer rats received implants of genetically modified (NGF-producing) primary fibroblasts into the right striatum; uninfected primary fibroblasts or those infected with a retroviral vector containing the cDNA for *E. coli* ß-galactosidase were implanted into the left striatum. At 1, 2, 3, and 8 weeks after grafting, the rats were perfused, and sections of their brains were processed for immunocytochemistry and electron microscopy. At 1, 2, and 3 weeks, a modest to robust aberrant plexus of NGF receptor-positive axons was found adjacent to the right striatal grafts only. At 3 and 8 weeks, immunoreactive axonic profiles were also found within the right striatal grafts. NGF receptor-positive axons were not observed adjacent to or within left striatal fibroblast grafts. Also, aberrant monoaminergic striatal inputs were not seen near the right or left striatal grafts. Ultrastructural data revealed that all grafts were composed of fibroblasts and collagen, and possessed numerous capillaries. Small, unmyelinated axons were evident near the grafts in the right striatum only. Axonic profiles within these grafts were surrounded by astrocytic processes and/or basal lamina. These results reveal aberrant sprouting of NGF receptor-positive axons in response to NGF, putatively released from genetically modified fibroblasts grafted into the striatum. These axonic profiles later penetrate the grafts within host-derived astrocytic sheaths. Our data provide evidence for NGF-induced tropism *in vivo* and new insight into the substrates that promote axonal sprouting in the adult rodent central nervous system. (Supported by the N.I.A.)

340.5

PRUNING AND REGENERATIVE CAPACITY IN THE CNS IS ALTERED IN PERINATALLY MORPHINE-EXPOSED RATS. A.Gorio, A.M.Di Giulio, B.Tenconi*, M.L.Malosio*, M.L.Donadoni*, S.De Biasi* and F.Cattabeni. Dept. of Med.Pharmacol.;Inst. of Pharmacol. Sci.; Dept. of General Phisiol. and Biochem.; Univ. of Milano, Italy.

The major aim of this laboratory in the past several years has been centered upon neuronal plasticity and regeneration both in normal and pathological conditions. More recently we have focused our attention on brain plasticity in rats perinatally exposed to morphine. The most striking effects of the treatment were confined to the development of the striatal innervation, with reduced dopaminergic innervation and hyperinnervation by met-enkephalinergic neurons. No traces of morphine were ever found in the brain of treated animals. Following neonatal lesions by the neurotoxin 5,7-HT the serotoninergic neurons of morphine-exposed rats did not show any pruning capacity, while the rate of regeneration was slightly reduced. In addition, these animals showed a higher susceptibility to neurotoxic lesions compared to control animals. Our hypothesis is that some permissive factor is lacking or present in insufficient quantities.

340.6

A STRIATAL-DERIVED NEURONOTROPHIC FACTOR: ENHANCEMENT BY CHRONIC HALOPERIDOL TREATMENT. P.M. Carvey, L.R. Ptak, J.M. Kerns, D.K. Sierens*, H.L. Klawans*. Rush Medical College, Chicago, IL 60612

Crude extracts of rat striatal tissue, contain a neuronotrophic factor (NTF) which induces dopamine (DA) neuron growth, as well as functional activity (DA uptake), in a dose-dependent fashion when added to rat E-13 mesencephalic cultures. Boiling the extract removes this activity. Extracts from animals with unilateral kainic acid lesions of the striatum produce significantly less growth in culture than extracts from the contralateral striatum. We therefore examined the effects of chronic haloperidol (HAL) or amphetamine (AMPH) treatment on the growth effect extracts have in culture. Striatal, but not cerebellar extracts from animals rendered behaviorally hypersensitive to apomorphine by 1 month chronic HAL (1.25 mg/kg) treatment, enhanced growth and DA uptake in culture relative to saline treated controls. Extracts from chronic AMPH (2.5 mg/kg) treated animals had the opposite effect. The growth effect therefore appears to be inversely related to chronic DA tone. Electron microscopic examination of the striatum (dorsolateral) in 4 of these animals revealed that HAL treatment led to an 18% increase in the overall number of synapses and a 121% increase in the number of perforated synapses suggesting alterations in neuronal cytoarchitecture. This data suggests that HAL induced increases of a striatal-derived DA NTF and its subsequent effect on synaptic plasticity may, in part, be responsible for the development of DA hypersensitivity behaviors.

340.7

CHANGES IN DORSAL AND MEDIAN RAPHE NEURONS DURING LESION-INDUCED REORGANIZATION OF THE AREA DENTATA SEROTONERGIC PLEXUS. J.H. Haring, J. Young*, A. Bartoli* & J. Bergeron*. Dept. Anat. & Neurobiol., St. Louis Univ., St. Louis, MO 63104.

Six weeks after partial lesions of the median raphe (MRN) a proliferation and reorganization of serotonergic (5HT) axons is seen in the area dentata (AD) using immunocytochemistry (Haring, Soc. Neurosci. Abstr. 1595, '88). This response mediates the recovery of 5HT content to low normal levels (Marshall & Haring, Soc. Neurosci. Abstr. 15:90, 89). The purpose of this study was to determine what, if any, changes were occurring in the dorsal raphe (DRN) and MRN during the 6 weeks following partial MRN lesion.

Adult, male, Sprague-Dawley rats were anesthetized with nembutal and injected with 5,7 DHT (3ug in 250nl artificial CSF) in the caudal MRN. Control rats were injected with vehicle. The status of MRN and DRN neurons with projections to the dorsal AD was studied by fluorescent retrograde tracing at 2 and 6 weeks post-lesion. The cross-sectional area of MRN and DRN 5HT-immunopositive neurons was studied at 2, 4 and 6 weeks. No significant change has been detected in either numbers or distribution of MRN and DRN neurons with projections to dorsal AD 2 and 6 weeks post-lesion except for the MRN cell loss. A few cases appeared to have a rostral shift in the distribution of DRN neurons, but tracer spread into overlying cortex accounts for this result. A significant increase in the area of 5HT neurons of both MRN and DRN was seen at 2 weeks. AT 4 and 6 weeks MRN and DRN cell areas were in the normal range. These data suggest that recovery of the AD 5HT plexus is mediated only by AD-projecting cells that survive the lesion. Changes in MRN and DRN cells correspond to the time of increased cell activity following the lesion, but the cells are normal at the time when sprouting is most evident in the AD. Support: NS25752.

340.8

METAMORPHIC REORGANIZATION OF TRIGEMINAL MOTONEURONS IN RANA PIPIENS. F.F. Omerza* and K.E. Alley. Ohio State University, Columbus, OH 43210.

During metamorphosis trigeminal motoneurons of R. pipiens larvae are redeployed from degenerating larval myofibers to newly formed adult myotubes. The objectives of this study were to describe: 1) the ontogeny of neuromuscular junctions (NMJs) and 2) the transfer of axonal processes from larva to adult. Ag/AChE histochemistry was used to identify NMJs. The morphometric analysis of NMJs consisted of an assessment of the following parameters: synaptic length, synaptic density and the number of polyinnervated NMJs. Examination of 647 synapses on 101 myofibers from 24 animals demonstrated that both synaptic length and density expanded during early and mid-larval stages reaching a plateau in late larval and metamorphic stages. In contrast, NMJs containing multiple axons peaked during the late larval period and then declined. A striking feature, evident on observation of the staining patterns of larval and adult muscles, was the redistribution of the synapses. In the larva NMJs were diffusely distributed, whereas in early juveniles the banded adult pattern was already evident. In order to examine axonal transition we followed the cellular features of the trigeminal motor terminals during myofiber turnover. In an analysis of 725 adult NMJs, a vast majority of innervation (91%) occurred by means of nerve sprouts that arose from the neighboring larval junctions while only a small contingent (09%) arose as collateral branches off the preterminal axon. These observations demonstrate that new adult NMJs received innervation from axons previously distributed to larval myofibers and suggest that the premetamorphic elevation of synaptic length, density and polyinnervation may ready the motor axons for the rapid redeployment to new adult fibers.

340.9

INCREASE OF THE PRIMARY AFFERENT INPUT TO AUTONOMIC AREAS OF THE RAT SACRAL CORD AFTER NEONATAL SPINAL TRANSECTION. K.Chung. School of Allied Health Sciences & Marine Biomedical Institute, Univ. of Texas Medical Branch, Galveston, TX 77550.

The present study examines the plasticity of the primary afferent input to the lumbosacral spinal cord of the rat after neonatal spinal transection. The antibody against calcitonin gene-related peptide (CGRP) is used as a primary afferent marker. In neonatal rats, spinal transection is performed under hypothermic anesthesia. Controls are litter mates subjected to a sham operation. All operated rats are returned to the mother and then raised to adulthood. Three-four months after operation, the animals are perfused with 3% paraformaldehyde, 3% glutaraldehyde in 0.1M phosphate buffer. The L6 and S1 segments are removed, vibratome sectioned, and immunostained for CGRP by peroxidase-anti-peroxidase method. CGRP immunoreactivity in the sacral autonomic areas of the cord in the neonatal spinal transected rats is greatly increased compared to that of the control litter mates. Data suggest that many more primary afferent terminals led in the autonomic areas of the spinal cord. (supported by NS11255)

340.10

MK-801 INDUCES AN INCREASE IN CENTRAL AND PERIPHERAL CGRP IMMUNOSTAINING WITHOUT ALTERING PRIMARY AFFERENT NEURONAL NUMBERS. D.L. McNeill, K.A. Sharkey, R.E. Papka, J.M. Galbraith*, and R.L. Shew. Dept. of Anat. Sci., Univ. of Oklahoma, Oklahoma City, OK 73190 and Dept. of Med. Physiol., Univ. of Calgary, Calgary, AB, Canada

MK-801 induces an increase in the density and distribution of CGRP immunoreactive (IR) fibers in the rat dorsal spinal cord. In the present study, the increase in CGRP-IR following MK-801 treatment was quantified in the dorsal spinal cord and dorsal root ganglia (DRG). In addition, the stomach and distal ileum were examined to determine if a similar increase in CGRP-IR occurred in peripheral processes of DRG neurons. Five Sprague-Dawley rats received 1.0 mg/kg/day of MK-801 for 30 days. Four additional untreated rats served as controls. On day 30, rats were perfused and the L5 DRG, L5 spinal cord, stomach and ileum were removed and immunostained for CGRP. Image analysis revealed a significant increase in CGRP-IR in the dorsal grey commissure ($p < 0.05$) and deep dorsal horn laminae ($p < 0.01$) in MK-801 treated rats. However, the number of CGRP-IR neurons in the DRG was not significantly different between the two groups. Furthermore, there was an apparent increase in the number of CGRP-IR nerves in the stomach of MK-801 treated animals, but no difference in CGRP-IR in the ileum. These data indicate that MK-801 induces an increase in CGRP-IR processes of DRG neurons, both central and peripheral, without a concomitant change in the number of CGRP-IR perikarya. (Supported by the Oklahoma Center for the Advancement of Science and Technology and the KWNPF.)

340.11

SYNAPTIC PLASTICITY IN LAMINA II OF DEAFFERENTED SPINAL CORD. B.Zhang , M.E.Goldberger, L.-F.Wu*, and M.Murray. Dept. Anatomy, Medical College of Pennsylvania, Phila., PA 19129 and Dept. Histology, West China University of Medical Sciences,Chengdu, Sichuan,PRC.

Projections to the dorsal horn change in adult mammals after complete or partial deafferentation. We are using quantitative EM to study the related changes in specific classes of terminals in the dorsal horn. We first examined complex terminals (CT) which originate from dorsal roots. The CT and the post-synaptic densities (PSD) associated with CT were measured in lamina II of L5 and L6 in cats subjected to unilateral spared root (L6) dorsal rhizotomies and compared to CT in the control side. After acute partial deafferentation, the number of CT decreased but the length of their PSD increased. Later the number of CT was restored and both the number and length of PSD were increased. The changes in CT suggest reactive reinnervation (sprouting) from the spared dorsal root fibers. Spared root deafferentation thus elicits compensatory changes in presynaptic terminals of the spared root and also in post-synaptic neurons. After complete lumbosacral deafferentation, the number of synaptic terminals remains constant, indicating replacement of lost dorsal root terminals by newly formed terminals from spared intrinsic systems. Quantitative LM immunocytochemistry has shown marked changes in densities of SP and 5HT projections which may permit identification of the replacement terminals with EM immunocytochemistry.

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340.12

SINGLE SUBLETHAL DOSE OF IRREVERSIBLE CHOLINESTERASE INHIBITOR PRODUCES ULTRATERMINAL AND NODAL NERVE SPROUTING IN MAMMALIAN MUSCLE. W.M.Cintra, M.Kawabuchi*, S.S.Deshpande* and E.X.Albuquerque. Dept. Pharmacol. Exp. Ther., Univ. Maryland Sch. of Med., Baltimore, MD 21210.

A single sublethal dose of the irreversible anticholinesterase agent sarin produced severe damage of the post-junctional membrane of rat muscle (Kawabuchi et al., Synapse 2:139-147,1988). To study the effects on the presynaptic terminal, young female Wistar rats were injected s.c. with a sublethal dose of sarin and their soleus (SOL) muscle was examined with a combined cholinesterase (ChE)-silver staining for light microscopy on 1, 3 and 6 days after injection. By day 1 neural degeneration affecting the motor nerve with selective retraction of distal motor nerve was seen in 22% of endplates. Recovery from sarin injury was evidenced by restoration of the size and density of ChE sites, decreased extent of neural degeneration and enhanced sprouting. At 6 days 18% of the endplates showed nodal sprouting and 3% showed ultraterminal sprouting. Membrane depolarization was observed, being more pronounced at the endplate region of the SOL muscle (-73±3 mV (n=251) for control and -53±10 mV (n=10) at 1.5-2.0 days after sarin injection; -57±7 mV (n=24) at 5 days after sarin injection) than on the extra-junctional regions (-59±11 mV (n=19) and -67±7 mV (n=30) for 1.5-2.0 days and 5 days after drug injection, respectively). The depolarizing and morphological changes were more pronounced on the SOL than on the extensor digitorum longus (EDL) muscles. 3-6 days after one dose of sarin injection, miniature endplate potentials (mepps) were recorded on the SOL muscle fibers 800-1500 μm from the endplate region. These mepps, some quite abnormal in shape, had smaller amplitudes and lower frequency than those recorded from the endplate region in the same fibers. Thus, after initial nerve retraction and reduction of terminal branches due to sarin, nerve sprouting was observed on rat SOL muscles, and the presence of mepps suggests that these newly formed ectopic nerve-muscle contacts were functional. Support: U.S.Army Med. Res. & Devel. Comm. Contr. DAMD17-88-C-8119.

340.13

DIRECTIONAL SPROUTING AT ENDPLATE SITES ON INTACT MUSCLE FIBERS IN RESPONSE TO DAMAGE OF NEARBY MUSCLE FIBERS IN LIVING MICE. P. van Mier and J.W. Lichtman, Dept. Anat. & Neurobiol., Washington Univ. Sch. Med., St. Louis, MO 63110.

In muscles in which some fibers had unintentionally been injured, we were surprised to observe sprouts originating from nerve terminals on healthy muscle fibers. To determine more precisely what the sprouting stimulus was, we used a laser ablation technique to destroy single muscle fibers in living mice. In each sternomastoid muscle (N=26) one vitally stained muscle fiber was damaged by exposing it to 1-15 laser pulses (3 nsec, 503 nm), focussed through the microscope (spot diameter < 1 μm). The sites of laser damage were at least 300 μm from the endplate band to avoid axonal or nerve terminal injury. Seconds after laser irradiation evidence of striations began to disappear in the treated fiber, and by 2 d. such muscle fibers had completely degenerated. Typically, the nerve terminal on the damaged muscle fiber retracted within 2 d. after ablation (see Rich & Lichtman 1989, Neuron 3:677-688). Within 3-4 d. a new muscle fiber was generated within the original basal lamina ghost. In 15 muscles (57%), at the time the new muscle fiber was being generated, nerve terminals on the muscle fibers immediately adjacent to the ablated muscle fiber began to extend sprouts, whereas in normal vitally stained muscle observed over time sprouts rarely occurred (< 1 per 100 endplates). In 14 of the laser damaged muscles (93%), the sprouts were either directed towards or already growing on the newly forming muscle fiber. In 2 of these muscles, nerve terminals on nearby but not adjacent muscle fibers also extended sprouts towards the young muscle fiber. The results of our experiments show that nerve terminals nearby damaged and regenerating muscle fibers are induced to sprout, and that newly formed muscle fibers seem to present a favorable substrate for these sprouts. We are presently testing, by repeated laser ablation, whether sprouting of nearby nerve terminals is caused by muscle fiber degeneration or regeneration.

340.14

PLASTICITY OF DORSAL ROOTS IS ASSOCIATED WITH A TRANSIENT INCREASE IN GAP-43 FOLLOWING SPINAL CORD HEMISECTION IN THE ADULT CAT. M.E. Helgren[1] and M.E. Goldberger[2]. [1]Regeneron Inc. Tarrytown, NY. and [2]Dept. of Anatomy, Medical College of Pennsylvania, Philadelphia, PA.

High basal levels of GAP-43 immunoreactivity are observed in the superficial laminae of the adult cat spinal cord. The protein is localized in the terminals of primary afferent fibers presynaptic to dorsal horn neurons (Skene,J.H.P, 1989). The area occupied by GAP-43 staining in the dorsal horn is significantly increased unilaterally following low thoracic hemisection sparing the dorsal columns. A moderate increase is seen 8 days post-operatively (dpo), peaks by 14 dpo and then gradually returns to basal levels. The region of GAP-43 staining in the spinal cord overlaps with the projection areas of dorsal root fibers mapped with MAb RAT-102 and the descending serotoninergic projections. Following hemisection there is a 4-5 fold increase in the density of Rat-102 immunoreactivity on the lesioned side along with a permanent decrease in the density of 5-HT staining. The increase in GAP-43 immunoreactivity may indicate a growth phenomenon of dorsal root projections in response to hemisection. These results suggest that a process of reactive reinnervation by primary afferent systems occurs in response to a loss of descending projections. This response may be one mechanism mediating recovery of motor control. It is consistent with behavioral studies using the hemisected spinal cord model which shows increases in reflex activity.

Supported by NIH grants NS24707 and NS16629.

340.15

SPROUTING OF ATYPICAL, NEUROPEPTIDE Y-IMMUNOREACTIVE SYMPATHETIC NERVES INTO NEONATALLY DENERVATED CONTRALATERAL TARGET. P.G. Smith, T.L. Hoffman* and H. Reddy*. Dept. of Physiology, University of Kansas Medical Center, Kansas City, KS 66103

Sympathetic innervation to lateral cranial targets normally is distributed strictly ipsilaterally. However, unilateral superior cervical ganglionectomy of neonatal but not older rats results in formation of projections from the contralateral ganglion to denervated targets. This study was conducted to determine if differences in neuropeptide phenotype exist in sympathetic neurons providing ipsilateral and contralateral innervation.

Superior tarsal smooth muscle (STM) of the ipsilateral or contralateral orbit was injected with Fluoro-Gold (1 μl, 2%) and Neuropepide Y-immunoreactivity (NPY-ir) of labelled ganglion neurons quantified. 21±3% of somata labelled by ipsilateral injections displayed NPY-ir; in contrast, 85±3% of the contralaterally projecting cells were NPY-ir ($p<0.001$). Examination of ipsilaterally innervated STM revealed infrequent NPY-ir nerves; after contralateral reinnervation, NPY-ir nerves were observed throughout the muscle. It is concluded that sympathetic outgrowth following neonatal unilateral superior cervical ganglionectomy results in establishment of an atypical NPY-ir pathway to non-vascular orbital smooth muscle. The presence of this non-native neuropeptide in the contralateral pathway may contribute to the slower time course for STM contraction and relaxation which occurs with electrical stimulation of sympathetic nerves.

Supported by NIH NS23502.

340.16

MAJOR CYTOSKELETAL CHANGES PRECEDE DENDRITIC SPROUTING FOLLOWING CLOSE AXOTOMY OF LAMPREY GIANT CENTRAL NEURONS. G.F. Hall+, V.M.-Y. Lee+ and K.S. Kosik+. +Harvard Med. Sch. Brigham and Women's Hosp., Boston, MA 02115 and +Dept. of Pathology, Univ. Penn., Sch. Med., Philadelphia, PA 19104.

Close axotomy of giant central neurons (ABCs) in the lamprey within 500 μm of their somata results in axonal regeneration from the dendritic tips (dendritic sprouting), while distant axotomy results in sprouting from the axon stump. We show that mAbs recognizing highly phosphorylated (RM034) and dephosphorylated (RMd015) epitopes on lamprey neurofilaments and total neurofilament protein (RM0270) reveal a large increase in the number and degree of phosphorylation of neurofilaments in the somata and dendrites of ABCs by 4-6 days following close axotomy. This is paralleled by a large increase in staining for tyrosinated tubulin. These changes occur well before dendritic sprouting begins at 10-15 days post axotomy. After distant axotomy, such changes do not appear until after the onset of sprouting.

Dendritic sprouts have higher levels of RM034 and RM0270 staining than do dendrites following close axotomy, and unlike dendrites, have generally low levels of tubulin. Dendrites which give rise to sprouts resemble sprouts rather than dendrites in these characteristics.

These early changes may thus be an important preliminary to dendritic sprouting in ABCs, and may therefore have a role in the maintenance of normal polarity in injured neurons.

340.17

SPROUTING OF IDENTIFIED MOLLUSCAN NEURONS IN VITRO: PROMOTION BY MURINE NERVE GROWTH FACTOR (β-NGF). R.L. Ridgway, N.I. Syed, Y. Fujito, K. Lukowiak, and A.G.M. Bulloch. Dept. of Med. Physiol., Univ. of Calgary, Alberta, Canada T2N 4N1.

Sprouting by adult neurons of the pond snails Lymnaea stagnalis and Helisoma trivolvis normally requires the presence of "conditioning factor(s)" produced and released by brains. Since the actions of these as yet unidentified endogenous factors resemble those of known vertebrate neurotrophic factors, we examined whether murine β-NGF could elicit sprouting in molluscan neurons. Identified Lymnaea and Helisoma neurons cultured in vitro in defined medium plus β-NGF extended neurites within 18-24 h of plating on poly-L-lysine substrate. The sprouting was dose-dependent, but the response varied with cell type. For most cell types the threshold concentration of β-NGF was 50-100 ng/mL; the optimum was 300-400 ng/mL. Motor neurons (e.g., Ce.A and Pe.A Cluster Cells) and certain interneurons (e.g., R.Pe.D1 and V.D4) exhibited the most extensive outgrowth, whereas known neurosecretory cells (e.g., Ce.LGCs, Ce.CDCs, V.YCs, and Pa.YGCs) were unresponsive to β-NGF. In contrast, virtually all identified Lymnaea and Helisoma neurons sprouted when cultured in brain-conditioned medium. Adsorption of defined medium containing β-NGF with an affinity purified polyclonal antiserum (to murine β-NGF) blocked sprouting by ordinarily responsive neurons. Interestingly, sprouting by these neurons was also inhibited when brain-conditioned medium was absorbed with the same antiserum; non-immune serum had no effect. This suggests that an endogenous NGF-like neurotrophic substance may be present in molluscs.

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340.18

GLUTAMATE MODULATES SPROUTING OF ADULT HELISOMA NEURONS BY ENHANCING CONDITIONED MEDIUM. A.G.M. Bulloch, R. L. Ridgway and G. Hauser. Department of Medical Physiology, University of Calgary, 3330 Hospital Dr. N.W., Calgary, Alberta, T2N 4N1 Canada

A primary goal of our laboratory is to identify molluscan neurotrophic factors and mechanisms by which they are regulated. A number of our recent studies have indicated that the amino acid, L-glutamate, can enhance sprouting by both intact and isolated Helisoma neurons. Specifically, glutamate enhances the conditioning of medium by ganglionic rings of Helisoma. Such medium is effective in evoking sprouts from undamaged neurons in a semi-intact preparation, as well as from cultures of isolated neurons. Previous studies have indicated that glutamate does not exert a direct effect on neurons, rather its effect appears to be mediated via macromolecular trophic factors.

We are attempting to isolate and characterize the neurotrophic factors present in conditioned medium, particularly those that are enhanced by glutamate. To this end, we have analyzed conditioned medium by SDS-PAGE and have shown the presence of a number of polypeptides. Two of these molecules, which have nominal molecular weights (under reducing conditions) of approximately 16 and 25 kD, are enhanced by glutamate. It therefore appears that the action of glutamate on sprouting is by enhancement of specific polypeptides, presumably acting either to increase their synthesis and/or release. The precise mechanism by which glutamate acts, and also the nature of the trophic factors, are currently under investigation. In this context, preliminary Western Blots indicate that the 25kD molecule is immunoreactive for NGF.

Supported by MRC (Canada).

TROPHIC INTERACTIONS I

341.1

PROENKEPHALIN mRNA IS EXPRESSED BY DIFFERENT SUBPOPULATIONS OF DEVELOPING MURINE ASTROCYTES IN CULTURE: COMBINED *IN SITU* HYBRIDIZATION AND GFAP IMMUNOCYTOCHEMISTRY. A. Stiene-Martin, J.G. Osborne and K.F. Hauser. Dept. of Anat. and Neurobiol., Univ. of Kentucky College of Med., Lexington, KY 40536-0084.

Different studies have shown that confluent, astrocyte-enriched cultures produce proenkephalin mRNA; and, that Met-enkephalin suppresses astrocyte growth in primary, mixed-glial cultures isolated from 1 day old mouse cerebral hemispheres after 5 or 6 days *in vitro*. We were therefore interested in ascertaining whether cultured astrocytes produce proenkephalin mRNA after 5 days *in vitro* when approximately 50-60% of the cells are astrocytes. A method was developed to double label cells by colocalizing proenkephalin mRNA by *in situ* hybridization using a cRNA probe and the immunocytochemical identification of glial fibrillary acidic protein (GFAP). GFAP-positive cells could be separated into 3 morphologic categories: flat (type I), bipolar, and multipolar process-bearing (type II) astrocytes. Astrocytes were classified according to morphology and evaluated based on the presence or absence of mRNA for proenkephalin. Between 60% and 70% of type I astrocytes contained mRNA for proenkephalin, whereas virtually all bipolar astrocytes and essentially none of the multipolar astrocytes contained mRNA for proenkephalin. The heterogeneous expression of proenkephalin mRNA by astrocyte subpopulations suggests that there are age- and/or region-dependent differences in opioid expression by these cells. Moreover, a subset of astrocytes may act as critical intermediaries in opioid-dependent regulation of neural maturation through the local synthesis of proenkephalin peptides. Supported by NIDA grant DA 06204 and NIH grant RR-05374.

341.2

LOCALIZATION OF ENKEPHALIN IMMUNOREACTIVITY AND PROENKEPHALIN mRNA BY *IN SITU* HYBRIDIZATION IN PURKINJE CELLS OF THE DEVELOPING RAT CEREBELLUM. J.G. Osborne[1], M.S. Kindy[2]* and K.F. Hauser[1]. Depts. of [1]Anat. and Neurobiol., and [2]Biochemistry, Univ. of Kentucky College of Med., Lexington, KY 40536-0084.

Enkephalins have been reported to transiently appear in neuronal progenitor cells of the cerebellar external granule layer (EGL) both *in vivo* and *in vitro*, and have been implicated in the regulation of EGL cell growth. Moreover, by manipulating opioid systems (opioids and their receptors) it is possible to alter the rate and pattern of dendritic growth, and spine number in Purkinje cells of developing rat cerebella. To identify potential sources of endogenous opioid production in the developing cerebellum, immunocytochemistry and *in situ* hybridization was performed on coronal and sagittal sections of cerebella from pre-weaning male Sprague-Dawley rats. Briefly, the pups were anesthetized and perfused with 4% paraformaldehyde in phosphate buffer (pH 7.25). Enkephalin immunoreactivity was examined on frozen sections (10 μm) using previously characterized antisera specific for met- and, to a lesser extent, leu-enkephalin (donated by Dr. B.E. Maley). *In situ* hybridization was performed using a [^{35}S]-labeled cRNA probe specific for proenkephalin mRNA. We have previously described proenkephalin gene products in Golgi cells and some glia. We now additionally report the localization of these products as being confined to a subpopulation of Purkinje cells which varies with age as well as region of cerebellum sampled. The local production of proenkephalin by developing Purkinje cells might be a mechanism by which opioids modulate cerebellar growth including the rate of EGL cell production. Supported by NIDA grant DA 06204 and NIH grant RR-05374.

341.3

SOLUBLE AXOLEMMA STIMULATES PROLIFERATION OF CELLS OF THE OLIGODENDROGLIAL LINEAGE. J.B. Grinspan, B.Q. Kreider, J.L. Stern, M.L. Williams and D. Pleasure. Neurology Research, Children's Hospital of Philadelphia, Philadelphia, PA 19104

The importance of contact with neurons in regulating glial cell proliferation has been demonstrated in both PNS and CNS. Chen and DeVries (J Neurochem., 52:325, 1989) showed that particulate axolemma has a mitogenic effect on GC$^+$ oligodendroglia, and that this effect could be inactivated by heat or trypsin. We have extended their observations using soluble axolemma derived from the brains of three day old rats by a modification of the method of Ratner et al (PNAS USA, 85:6992, 1988). Soluble axolemma was added to cerebral white matter cultures from 6 day old rats. Using indirect immunofluorescence, we found that the number of O2A oligodendroglial-precursor cells and of GC$^+$ oligodendroglia increased more than three fold after three days of treatment and more than 6 fold after a week of treatment. The mitotic index of both the O2A cells and oligodendroglia was more than doubled with either 24 or 48 hours of treatment. Axolemma had no effect on the proliferation of astrocytes in these cultures. Platelet-derived growth factor (PDGF) added in combination with axolemma further augmented recruitment of both O2A cells and oligodendroglia. Incubation of soluble axolemma-treated cells with anti-PDGF antibodies causes a 50% inhibition of the axolemma-induced cell increase, suggesting either that soluble axolemma contains PDGF activity or that endogenously secreted PDGF is a necessary cofactor for the axolemmal mitogen.

341.4

TARGET HIPPOCAMPUS REGULATES LOCUS COERULEUS NEURON SURVIVAL IN DISSOCIATED CELL CULTURE. L.J. Robinson*, I.B. Black and C.F. Dreyfus. Div. Devel. Neurol., Cornell Univ. Med. Coll., NY, NY 10021 and Dept. Neurosci. and Cell Biol., UMDNJ Robert Wood Johnson Medical School, Piscataway, NJ 08854

Increasing evidence suggests that environmental signals regulate development and function of neurons in the brain. For example, recent studies of the noradrenergic (NA) locus coeruleus (lc) indicate that depolarizing influences increase tyrosine hydroxylase (TH), the rate-limiting enzyme in NA biosynthesis. We have extended this work to determine whether the target hippocampus (hi) also regulates development of this diffusely projecting brain nucleus. Dissociated lc was grown alone, or co-cultured with hi cells in a serum-free, fully-defined medium that limited non-neuronal growth. Lc grown with target hi exhibited a 2-fold increase in TH activity after 6 days. Moreover, TH+ cells increased 2-fold, suggesting that hi enhanced lc survival. Increased cell number was accompanied by a striking increase in neurite length and branching. The target effects were apparently selective, since total neuron number, estimated with neuron specific enolase or total protein, was unaffected by hi. To approach the issue of survival more directly, hi cells were added to lc dissociates at zero time, or after 2 days. TH+ cell number was increased only by hi cells added initially, suggesting that the target does, indeed, foster neuron survival. Our observations suggest that hi regulates survival and neurite elaboration of afferent lc neurons. (Support:NIH grants HD23315, NS10259, DA05132)

341.5

IN VITRO DEVELOPMENT OF CHICKEN EMBRYONIC MOTOR NEURONS IN MEDIA CONDITIONED BY VARIOUS CELL TYPES. S.J. Jeong, T.H. Oh, G.J. Markelonis and M.B. Clark. Dept Anatomy, Univ of Maryland School of Medicine, Baltimore, MD, 21201

In vivo studies suggest that cell-cell interactions may influence naturally occurring spinal motor neuron (MN) death observed early in embryonic development. To mimic these interactions, media conditioned by various cells (conditioned media; CM) were collected and tested for their trophic activities on MN. Purified chicken embryonic MNs were cultured for 6 days in media conditioned by skeletal muscle (SKM-CM), heart muscle (HM-CM), dorsal root ganglia (DRG-CM), Schwann cells (SC-CM) and astrocytes (AS-CM). All CMs supported the survival and outgrowth of neurites from MNs. Survival of MNs grown in the presence of SKM-CM, SC-CM or AS-CM was higher than that of MNs grown in HM-CM or DRG-CM. Choline acetyltransferase activity of MNs was enhanced by SKM-CM, AS-CM and DRG-CM but not by SC-CM or HM-CM. Interestingly, certain CMs induced distinct patterns of neuritic arborizations produced by cultured MNs. MNs grown in SKM-CM, DRG-CM or SC-CM had extremely complex branches at the end of a relatively long process containing a prominent growth cone. MNs grown in AS-CM or HM-CM had large numbers of simple, rather straight primary or secondary processes. This study suggests that various supporting cells have qualitatively different effects on motor neuron survival and neuritic outgrowth and appear to influence neuronal morphology and cholinergic differentiation. (Supported by NIH grant NS 15013 and by the Bressler Research Fund).

341.6

DISSOCIATION OF FOS ONCOGENE EXPRESSION FROM CELL PROLIFERATION AND DELTA-OPIOID RECEPTOR EXPRESSION IN N4TG1 NEUROBLASTAMA CELLS. W.X. Lu* and K.-J. Chang. Division of Cell Biology, Burroughs Wellcome Co., Research Triangle Park, NC 27709

Effects of fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF-1) and others on fos oncogene expression, cell proliferation and delta-opioid receptor activity were studied in N4TG1 neuroblastama cells. Using Northern Blot, FGF was found to stimulate dose-dependently and transciently fos oncogene expression. Both subtypes of FGF, acidic and basic FGF, possessed a similar effect. Cycloheximide, a protein synthesis inhibitor, potentiated and prolonged the effect of FGF on fos expression, but IGF-1, other growth factors, neuropeptides and neurotransmitters did not significantly change fos expression. When ^{3}H-thymidine incorporation was used to measure proliferation, IGF-1 and insulin dose-dependently increased thymidine incorporation at 24 h after administration, but FGF, other growth factors, neuropeptides and neurotransmitters did not. N4TG1 cell has a high density of delta-opioid receptor. IGF-1, insulin and dibutyryl cAMP (Bt$_2$cAMP), but not FGF and other growth factors, decreased opioid receptor binding activity after at least 1 day treatment. After 2 days treatment of Bt$_2$cAMP, the withdrawal of Bt$_2$cAMP on the third day induced partial recovery of opioid receptor synthesis. Results suggest that fos expression is independent from or insufficient for cell proliferation and opioid receptor expression in N4TG1 cells.

341.7

GLIAL-MEDIATED EFFECTS OF 5-HT AND NIALAMIDE ON IN VITRO DEVELOPMENT OF RAPHE AND SUBSTANTIA NIGRA NEURONS. J.Liu*, M.B.Wilkie* and J.M.Lauder, Dept.of Cell Biol.& Anat. Univ. N.C.Sch. Med., Chapel Hill, NC 27599 USA

The importance of neurotransmitters in embryonic neuronal-glial interactions was studied in cell cultures from the embryonic day (E)14 rat brain containing rostral raphe (RR) 5-HT neurons, substantia nigra (SN) tyrosine hydroxylase (TH) neurons, RR or SN radial glia/astrocytes (RG/A). 1) 5-HT and/or nialamide added to the medium promoted survival and growth of 5-HT neurons, with minimal effects on TH neurons. 2) When co-cultured with RR or SN RG/A and treated in the same way, or 3) exposed to conditioned medium from treated glial cultures (GCM), survival and growth of 5-HT neurons was enhanced, but some inhibitory effects of 5-HT were seen, especially in co-cultures where the numbers of RG/A were greatly increased. In both types of glial experiments, TH neurons were much less affected suggesting that they respond differently to the same glial signals. Regional differences in treatment effects on 5-HT and TH neurons indicate that populations of embryonic glia may produce different trophic factors in response to the same neuroactive substances.

341.8

OUTGROWTH OF STATOACOUSTIC GANGLIA EXPLANTS IN RESPONSE TO COCHLEAR-DERIVED CONDITIONING FACTORS. L.M. Bianchi, R.J. Salvi, and C.S. Cohan, Departments of Communicative Disorders and Anatomical Sciences, SUNY at Buffalo, Buffalo, N.Y. 14214

Previous studies have suggested the presence of cochlear-derived conditioning factors which are necessary for guiding the growth of acoustic nerve fibers. The present experiments evaluated the existence of such factors in the embryonic chick auditory system and the period in which these factors were active.

Otocysts and statoacoustic ganglia (SAG) were removed from chick embryos at various stages of development. Three to four otocysts were co-cultured with two to three ganglia in a serum-containing medium on plastic tissue culture dishes. Control conditions consisted of SAG explants in the absence of otocysts. Neurite outgrowth occurred at stages 22-24 (preinnervation stages) and 27-29 (early innervation stages) in the co-culture conditions. Neurites extended within 24-72 hours and continued to grow for up to ten days. Degeneration of neurites became visible within 14-20 days. The majority of control explants did not extend neurites. In a few control explants a brief period of neurite outgrowth was followed by neurite retraction within 24-48 hours. Thus, a cochlear-derived conditioning factor promotes neurite outgrowth at early stages of SAG development.
(Supported by grants from the Deafness Research Foundation and NIH #NS25789)

341.9

GRAFTS OF FETAL CNS TISSUE RESCUE AXOTOMIZED CLARKE'S NUCLEUS NEURONS IN ADULT AND NEONATAL OPERATES. B.T. Himes[1], M.E. Goldberger[1], and A.Tessler[1,2]. [1]Dept. of Anatomy, Medical College of PA and [2]VA Medical Center, Philadelphia, PA.

Why CNS neurons die following injury is still incompletely understood. Many elements are thought to be involved, including maturity of the cell at the time of injury, ability to re-establish or maintain target contact and dependence upon trophic factors produced by targets. We have therefore studied axotomized Clarke's nucleus (CN) neurons after thoracic hemisection in neonatal and adult rats and compared cell loss following hemisection to that following the same lesion with insertion of a fetal CNS transplant.

In the rat, hemisection axotomizes many CN neurons and causes extensive cell death. Neonatal T8-T9 hemisection causes a 40-50% loss of neurons in CN at L1 ipsilateral to the lesion. Similar surgery in the adult causes a 20-30% cell loss. In both groups it is primarily the larger neurons that either die or atrophy. Cell death is complete within one month of axotomy in neonates and 2-3 months in adults. A transplant of embryonic (E14) cerebellum or spinal cord prevents the death of most axotomized neurons. In both neonates and adults only 10% of CN neurons at L1 die when a transplant is placed in a hemisection cavity at T8-T9. Transplants rescue axotomized CN neurons by providing trophic support. Supported by grants NIH grant NS 24707, USAMRDC-51930002, and The VA Medical Research Service.

341.10

GASTRIC TRANSPLANTS RESCUE NEURONS IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS AFTER AXOTOMY. L. Rinaman and P. Levitt, Dept. of Anatomy, Med. Coll. of Pennsylvania, Phila. PA 19129.

Death of axotomized neurons is more rapid and complete in the dorsal motor nucleus of the vagus (DMV) than in any other motoneuronal population. Axotomized DMV motoneurons may degenerate because they are deprived of factors normally supplied by their gastrointestinal enteric targets. To test this, we compared DMV degeneration following cervical vagotomy in rat neonates to that in animals which first received an implant of gastric tissue at the site of vagal lesion. A 1 mm^2 piece of the stomach wall was dissected from E17 donors and implanted next to the carotid artery at the level of the omohyoid muscle in P0 hosts. Control animals received no transplant or received a transplant of uterus, bladder, or heart tissue. Unilateral vagotomy was performed below the nodose ganglion of all animals 24 hr later. Animals were sacrificed 1-25 days subsequent to the lesion and the DMV examined in Nissl-stained sections. In some animals, CT-HRP was injected into the transplant (or into the area of nerve lesion in non-transplant controls) 48 hrs prior to sacrifice. DMV motoneuronal loss was first evident 3-5 days post-vagotomy, and appeared complete by 8-10 days in control animals, including those that contained healthy, non-gastric transplants. CT-HRP labeled DMV motoneurons were rarely observed on the lesioned side in control animals. In contrast, animals that received gastric implants had DMV motoneurons on the lesioned side as long as 25 days post-vagotomy, although their number was reduced as compared to non-lesion controls. Injections of CT-HRP into gastric transplants routinely labeled surviving neurons in the lesioned DMV. Examination of gastric transplants revealed a well-developed mucosa, enteric neurons, and intramural nerve fibers. These results suggest that the survival of axotomized DMV motoneurons during early postnatal development depends on their access to gastric tissue, and that other peripheral tissues are ineffective. Whether this involves neuronal or non-neuronal gastric components remains to be determined. Supported by NIMH Grant MH45507.

341.11

MITOTIC SYMPATHETIC NEUROBLASTS REQUIRE TROPHIC SUPPORT. Emanuel DiCicco-Bloom, David W. Pincus, and Ira B. Black. Div. of Devel. Neurol., Cornell Univ. Med. College, NY, NY 10021. Dept. Neurosci. and Cell Biol., UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854.

While targets govern neuronal survival after innervation, the role of trophic support earlier in development is largely unexplored. Specifically, when do developing neurons acquire dependence on exogenous molecules for survival?

To examine trophic chronology, we employed a previously defined sympathetic neuroblast culture system containing a virtually pure population of neurons and dividing precursors. Superior cervical ganglia from gestational day 15.5 rat embryos were dissociated and cultured at low density in serum-free medium. In the presence of mitogenic concentrations of insulin (10μg/ml), approximately one-third of plated cells divide *in vitro*. The fate of dividing cells was documented by serial time-lapse photomicrography.

Observation of newly-born cells revealed that 20% died within 24 h of plating, whereas 75% were lost by 48 h. In contrast, addition of two sympathetic trophic molecules, VIP and NGF, rescued virtually all mitotic cells. Remarkably, treatment with either factor alone failed to reproduce these effects, suggesting that multiple factors interact to generate stable neuronal populations. Indeed, combined factor treatment allowed multiple rounds of division by single neuroblasts *in vitro*.

In aggregate, our observations suggest that trophic molecules play a major role in survival of mitotic neurons. Consequently, trophic mechanisms may govern neuronal ontogeny at times earlier than previously considered, during neuroblast division or immediately thereafter. Since the majority of neurons at this developmental stage have not established target connections, trophic molecules may be elaborated locally, from ganglionic sources. (Supported by the NINDS, NICHD, and the Dysautonomia Foundation, Inc.)

341.12

MULTIPLE FACTORS REGULATE CULTURED BASAL FOREBRAIN (BF) NEURONS THROUGH DIFFERENT MECHANISMS. M. Yokoyama, R.S. Morrison, I.B.Black, & C.F. Dreyfus. Cornell U. Med. Coll., NY, UMDNJ/Robert Wood Johnson Med. Sch., NJ, and R.S. Dow Neurol. Sci. Inst., OR

Numerous studies suggest that Nerve Growth Factor (NGF) plays a critical role in regulation of BF function. We sought to determine whether BF neurons are responsive to NGF exclusively, or whether other known factors also govern function directly or indirectly. Initially, we defined the effects of NGF, basic Fibroblast Growth Factor (bFGF; Synergen), Epidermal Growth Factor (EGF) and Transforming Growth Factor-α (TGF-α; Oncogen Inc.) on BF cholinergic cells in culture. All factors elevated activity of the acetylcholine synthetic enzyme, choline acetyltransferase (CAT). To determine whether non-neuronal cells mediated the observed effects, a mitotic inhibitor, 5-fluorodeoxyuridine (FDUR), was added to the cultures to eliminate dividing support cells. The actions of EGF and TGF-α were completely blocked by the addition of FDUR. However, NGF and bFGF continued to elevate CAT activity in the presence of FDUR. Consequently, NGF and bFGF may regulate forebrain cholinergic function directly, whereas EGF and TGF-α may affect cholinergic cells indirectly through support cells. NGF influences both cholinergic and GABA populations in the BF. To detemine whether FGF regulates the same neuronal populations, the activity of glutamic acid decarboxylase (GAD), a GABA synthetic enzyme, was monitored. FGF elicited a significant increase in GAD activity: the effect was completly abolished by addition of FDUR. Thus, FGF may act directly on cholinergic neurons and indirectly on GABA cells. Our study suggests that multiple factors potentially regulate cell function through different mechanisms. The multiplicity of factors and mechanisms, and apparent redundancy, may play a critical role in normal ontogeny. (Support: NINDS, NICHD, March of Dimes, Mc Knight fellowship & Bristol Meyers)

341.13

COMBINATORIAL EFFECTS OF INSULIN-LIKE GROWTH FACTOR I AND NERVE GROWTH FACTOR ON CULTURED SEPTAL CHOLINERGIC NEURONS. N.T. Neff and C.A.H. Friedman*. Cephalon, Inc., West Chester, PA 19380.

Nerve growth factor (NGF) and insulin-like growth factor-1 (IGF-1) each enhance choline acetyltransferase (ChAT) activity in cultured embryonic rat neurons (J. Neurosci 8: 2967, 1988; J. Neurosci., 10:558, 1990). To determine potential differential actions of these factors on cholinergic neurons, their effects on ChAT activity and cholinergic cell survival were investigated in embryonic age (E17) rat septal cultures. Saturating concentrations of NGF (20 pM) or IGF-1 (50 nM) enhance ChAT activity by 40-60% when added separately to cultures at plating. When added together, their effect on ChAT activity is additive and results in a 2-fold increase in ChAT activity over uninduced 5 day cultures. A greater than additive effect on ChAT activity is observed in the continual presence of IGF-1 when NGF is added 3 days after seeding. To discriminate between the effects of these factors on enzyme regulation or cholinergic neuron survival, acetylcholinesterase (AChE) cytochemistry, which is a reliable marker for ChAT positive neurons in rat septal cultures, was performed. IGF-1 increases the number of AChE positive neurons by 3 to 4-fold, whereas NGF results in no increase. The number of AChE positive cells increased by the joint presence of the factors is the same as that elicited by IGF-1 alone. These results suggest that under the culture conditions employed, IGF-1 has a greater effect on cholinergic cell survival, while NGF regulates (increases) ChAT activity in existing cholinergic neurons.

341.14

Effects of Estrogen and Fimbria/Fornix Transection on NGF Receptor Immunoreactivity in the Rat Basal Forebrain. R.B. Gibbs and D.W. Pfaff. Lab of Neurobiology & Behavior, Rockefeller University, New York, N.Y. 10021.

Effects of estrogen on expression of the nerve growth factor (NGF) receptor in the medial septum (MS) and horizontal limb of the diagonal band (HDB) of normal and injured animals were examined using immunocyto-chemical (ICC) techniques. Thirty adult, ovariectomized, Sprague Dawley rats were used. Silastic capsules (5 mm) containing 17-β-estradiol crystals (E) were implanted subcutaneously into 16 animals. Two days later, 6 animals with implants and 6 animals without implants received a bilateral transection of the fimbria/fornix. Ten animals (5 with implants and 5 without implants) received sham surgery. Two weeks later, these 22 animals were sacrificed. Their brains were removed, sections through the MS and HDB were cut and processed for ICC detection of the NGF receptor protein using the mouse monoclonal antibody 192 IgG (Dr. E. Johnson). Remaining animals had their implants removed and were allowed to survive an additional 2 weeks prior to sacrifice.

As expected, transection of the fimbria/fornix resulted in a significant decrease (45.8%) in the number of NGF receptor-IR cells observed in the MS. Interestingly, E-treatment alone also resulted in a significant decrease (22.8%, $p<0.01$) in the number of NGF receptor-IR cells observed in the MS. Animals in which E-treatment and fimbria/fornix transection were combined showed the same degree of cell loss (47.6%) as the non-E-treated animals, suggesting that E-treatment influences NGF receptor expression within the same cells that are affected by fimbria/fornix transection. The effects of E were reversible - the number of NGF receptor-IR cells in animals from which E-capsules had been removed were not significantly different from non-E-treated controls. No effects of estrogen and/or fimbria transection on the number of NGF receptor IR cells in the HDB were observed. These data demonstrate that (1) NGF receptor expression in the rat basal forebrain can be influenced by estrogen, and (2) the effects of estrogen are region specific.

Supported by NIH grant #HD05751.

341.15

NGF REGULATES THE DEVELOPMENT OF RAT CEREBELLAR PURKINJE CELLS IN CULTURE. S. Cohen-Cory, C.F. Dreyfus and I.B. Black. Lab. of Neurobiol., Rockefeller Univ., Div. of Dev. Neurol., Cornell Univ. Med. Coll., New York, N.Y. 10021, and Dept. Neurosci. and Cell Biol. UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, N.J.

In the cerebellum, Nerve Growth Factor (NGF) and NGF receptors are highly expressed during early development in several species. The localization of biologically active, high-affinity receptors to developing Purkinje cells suggests that NGF may play an important role in the ontogeny of this cell type.

To begin defining the mechanisms by which NGF may regulate development, we have studied the effects of several epigenetic signals on the survival and maturation of Purkinje cells. Responses to NGF and excitatory neurotransmitters were analyzed in dissociated cell culture. Purkinje cells were identified immunocytochemically with anti-Calbindin D28K antibodies (a gift of Dr. S. Christakos). NGF markedly increased the survival and morphological maturation of Purkinje cells grown in the presence of general depolarizing agents, such as potassium or veratridine. Furthermore, NGF together with the excitatory neurotransmitters, aspartate or glutamate, promoted a 2-fold increase in survival of Purkinje cells. The combination of agents also elicited a marked increase in neurite branching. Effects on survival or neurite elaboration were not evoked by exposure either to trophic factor or transmitters alone. Our results suggest that the trophic factor, NGF, and excitatory amino acid transmitters act in concert to regulate survival and differentiation of cerebellar Purkinje cells. (Supported by NINDS, NICHD, March of Dimes and McKnight fellowships)

341.16

VIP AND NGF SUPPORT SYMPATHETIC NEURONAL SURVIVAL AT DIFFERENT DEVELOPMENTAL STAGES. David W. Pincus, Emanuel DiCicco-Bloom, and Ira B. Black. Cornell Univ. Med. College, NY, NY 10021, UMDNJ-Robert Wood Johnson Med. Sch., Piscataway, NJ 08854.

Current models of trophic factor action suggest that developing neurons are initially independent of survival factors and develop requirements for target-derived trophic support with maturity. We recently found that vasoactive intestinal peptide (VIP) regulated multiple ontogenetic processes of cultured rat sympathetic neuroblasts, including survival. This putative transmitter was localized to the developing superior cervical ganglion (SCG), reflecting presynaptic and/or perikaryal sources. Thus, transmitter molecules of local origin may provide trophic support to developing neurons prior to target innervation.

The present study defines the trophic actions of VIP on SCG neurons at different ages. We compared VIP to nerve growth factor (NGF), since sympathetics develop absolute dependence on the factor with maturation. In fact, at gestational age 15.5 (E15.5), either VIP or NGF rescued ~90% of cells initially plated. In the absence of either factor, 50% of cells survived. At older ages, VIP rescued fewer cells, while NGF dependence increased. By birth, all SCG neurons required NGF for survival. Further, the time course of VIP effects on neuronal survival correlated with expression of peptide in the SCG. While total VIP levels/ganglion increased with maturity, VIP concentration/ug protein decreased with age from a peak at E15.5. This developmental profile was unique when compared to VIP expression in other neuronal populations.

In sum, our findings indicate that sympathetic neurons may derive trophic support from VIP or NGF as a function of embryonic age. Since the temporal profile of VIP responsivity parallels the ontogenetic expression of VIP in the SCG, these results may reflect *in vivo* mechanisms. Consequently, young neuronal populations that have not yet reached targets may derive trophic support from presynaptic or local factors.

341.17

EFFECTS OF BASIC FIBROBLAST GROWTH FACTOR AND HEPARIN ON TRANSPLANTED FETAL DOPAMINERGIC NEURONS AND ASTROCYTES IN THE DENERVATED RAT CAUDATE-PUTAMEN. H.W.M.STEINBUSCH, M.J.DOLLEMAN- VAN DER WEEL, A.NIJSSEN and F.FULLER*. Dept. Pharmacology, Vrije Universiteit, Amsterdam, The Netherlands and *California Biotechnology, Mountain View, CA, U.S.A.

Neurotrophic factors appear to be essential for the growth, maintenance, and repair of neurons. Basic fibroblast growth factor (bFGF) has been recognized as a trophic factor for fetal CNS cells in vitro. It has been shown to exert a positive effect on the survival of fetal astrocytes and in vivo a preserving effect on damaged adult septal cells. The aim of this study was to investigate the morphological and functional effects of heparin with or without bFGF on grafted dopaminergic neurons. The experimental design consisted of rats with an unilateral 6-OHDA lesion, grafting of a fetal (E 15) dopaminergic cellsuspension in the caudate-putamen and various behavioral tests using rotation turning behavior with amphetamine and apomorphine. The cellsuspension was mixed either with vehicle, heparin or a combination of bFGF with heparin. All rats were processed for DA- and GFAP-immunocytochemistry. All groups showed surviving dopaminergic transplants. A difference was observed with regard to the fiber outgrowth, the heparin group alone showed the highest fiber outgrowth. No differences were noted in diameter of the transplants or the number of surviving dopaminergic cells. The behavioral data revealed that the heparin group resulted in a marked decrease in apomorphine-induced rotation, while the bFGF/heparin group was not different from the control group. The amphetamine-induced ipsilateral rotation did not differ between groups. The comparison of both the amphetamine conditions with the pretransplant or lesion condition revealed no differences in recovery. Heparin alone had a positive effect on morphology and behavior, while the combination bFGF/heparin showed a positive tendency towards changes in morphology and enhanced recovery towards behavior.

341.18

MANNOSE-6-PHOSPHATE POTENTIATES IGF-II STIMULATED NEURONAL CELL DIVISION BUT NOT NEURITE OUTGROWTH. E.L. Feldman and A.E. Randolph. Department of Neurology, University of Michigan Medical Center, Ann Arbor, MI 48109.

Mannose-6-phosphate (M6P) and insulin-like growth factor II (IGF-II) bind to separate sites on the same cell surface receptor. M6P is known to increase the affinity of the shared receptor for IGF-II, potentiate the effects of IGF-II on phosphotidylinositol turnover in kidney cells and increase IGF-II endocytosis in IM9 cells. We postulated that M6P might modulate the known effects of IGF-II on neuroblastoma cells. We report that M6P alone increases neuronal cell doubling when compared to serum-free media, and in the presence of IGF-II, M6P potentiates IGF-II induced neuronal cell doubling, but has no effect on IGF-II enhanced neurite outgrowth.

SH-SY5Y neuroblastoma cells were grown in 6 well plates in either serum free DMEM (SF DMEM), or with the addition of 5 mM M6P, 10 nM IGF-II, or a combination of M6P and IGF-II. Cell number was determined on days 2, 3, and 7 with corresponding DNA assays. Compared to SF DMEM cell counts on days 2, 3, and 7, M6P increased cell number by 7, 17 and 43%, IGF-II by 73, 116 and 110%, and IGF-II/M6P by 90, 135 and 235%, respectively. This same pattern was observed with quantitative DNA assays. In a separate set of experiments, under the same conditions, neurite outgrowth was assayed by the method of Sonnefeld and Ishii (1982). On days 1 through 7, there was no difference in neurite outgrowth between SF DMEM or SF DMEM with M6P. IGF-II alone stimulated outgrowth by 70% over SF DMEM by day 3; the addition of M6P to IGF-II did not alter this stimulation. We speculate that M6P can potentiate selective effects of IGF-II on neuronal cells.

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341.19

MUSCARINIC ACETYLCHOLINE RECEPTOR BINDING IN PARASYMPATHETICALLY ANEURAL CHICK ATRIA. T. L. Creazzo. Dept. of Anatomy, Medical College of Georgia, Augusta, GA 30912-2000.

Chick hearts were made cholinergically aneural by bilateral ablation of the nodose placodes and the neural crest which seeds pharyngeal arches 1-3, prior to cell migration (Kirby, 1988, J Neurosci, 8:1089-1095). Hearts with this lesion do not have postganglionic parasympathetic intracardiac ganglion cells. The animals were sacrificed on day 11, the atria separated from the ventricles and stored in liquid nitrogen until used. The atria were pooled (approx. 15 per determination) and assayed for muscarinic receptors (MAChR) with [^{3}H]-QNB as previously described (Creazzo TL, Hartzell HC, 1985, J Neurochem, 45:710-718). Scatchard analysis showed no significant difference in the number of MAChRs in aneural atria compared to sham-operated controls (sham: 221±34 fmol/mg±S.D., n=3; aneural: 241±47, n=3). In competition with carbachol, approx. 30% & 70% of MAChRs were in low- and high-affinity states, respectively, and in 50 μM GPP(NH)P, essentially all of the MAChRs were in the low-affinity state for both sham-operated and aneural (sham: K_I=7.1±3.7μM, n=2; aneural: K_I=4.4±1.1μM, n=2). The results show that, unlike nicotinic ACh receptors in skeletal muscle, MAChR development in chick atria does not depend on cholinergic innervation.

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341.20

Ca^{+2}-INDUCED VESICULATION IN DIALYZED SQUID GIANT AXON. Harvey M. Fishman and Kirti P. Tewari* Department of Physiology and Biophysics, University of Texas Medical Branch, Galveston, TX 77550-2779

Formation of vesicles within cut ends of squid giant axons following transection in divalent cation (DC)-containing artificial sea water (ASW) was recently reported (BBA, Biomemb. 1023:421, 1990). Internal dialysis of axons was implemented to determine whether vesiculation is caused by entry of Ca^{+2} or by the injury. A technique for dialysis of axons was modified so that continuous DIC video microscopy was possible during dialysis. Control solution consisted of (mM) 440 K glutamate + 2.5 EGTA + 5 TrisCl adjusted to 989 mOsm by adding glycine. Control solution in the bath and as dialysate produced minimal vesiculation after 1 hr of dialysis. Replacing glutamate by Cl^- in the dialysate produced a slight increase in vesiculation compared to that of control. Dialysis with DC-free ASW (430 Na^+) produced a further increase in vesiculation. Vesicle production was greatest when Ca^{+2} was added to the DC-free ASW. Ca^{+2}-induced vesicles were evident at dialysate [Ca^{+2}] of 100 μM and axosomes (50 μM dia., fused vesicles) formed at dialysate [Ca^{+2}] above 1 mM. No vesiculation was apparent beyond the porous region of the dialysis tubing within axons. These data show that a rise in axoplasmic [Ca^{+2}] to 100 μM or more without axonal injury is sufficient to induce axonal vesiculation and that Na^+ and Cl^- enhance the induced vesiculation effect of Ca^{+2}.

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341.21

TWO-DIMENSIONAL ELECTROPHORETIC ANALYSIS OF GLIAL PROTEINS RELEASED BY VIP. D.E. Brenneman. Laboratory of Dev. Neurobiology, NICHD, NIH, Bethesda, Maryland 20892

Previous studies indicated that nonneuronal cells mediate the neurotrophic action of VIP (J. Cell Biol. 104:1603, 1987). To investigate the glial proteins which may participate in this action, the release of S^{35} methionine-labeled proteins was measured in astroglial cultures treated with VIP. Conditioned medium was collected from rat cortical astrocyte cultures during one hour incubations. A 2-fold increase in labeled proteins was detected in the medium after treatment with 0.1 nM VIP. A similar stimulation of secreted proteins was observed after treatment with an active phorbol ester; however, the addition of 8-Br cAMP or forskalin increased protein efflux by < 40% as compared to controls. Two-dimensional polyacrylamide gel electrophoresis combined with quantitative image analysis revealed an increase (> 50% of control) in 15 out of the 333 medium protein spots after VIP treatment. These proteins had molecular weights estimated to be from 17 to 117 kDaltons and all had acidic pI's. By optical density of the autoradiograms, the VIP-stimulated proteins comprised 18-22% of the total protein released from the glial cultures. The three proteins showing greatest (9-25 fold) increase from controls were between 45 and 52 kDaltons. PHI-27, a closely homologous peptide to VIP, had no detectible effect on any of the VIP-stimulated proteins. These data indicate that low concentrations of VIP release a complex mixture of acidic proteins of high molecular weight from glial cultures and suggest that VIP-mediated secretion of glial proteins may not exclusively involve cAMP-linked actions.

342.1

NGF RECEPTOR EXPRESSION IN RAT CIRCUMVENTRICULAR ORGANS (CVO) HB Clark, Q Yan, EM Johnson, Jr. and WF Hickey*. Pathology, So. Ill. Univ., Springfield, IL 62781 and Pharmacology & Pathology, Washington Univ., St. Louis, MO 63110

Previously we have shown that in the neurohypophysis (NH) NGF receptors (NGFR) are localized on cells with the ultrastructural appearance of "perivascular microglia" (PVM). Presently we have examined NGFR expression in other CVO using immunostaining with monoclonal antibody 192-IgG by light microscopy (LM) and electron microscopy (EM). NGFR immunoreactivity (NGFRI) and immunostaining for other cellular markers, CD45 for bone marrow-derived monocytes and GFAP for fibrillary glia, were compared by LM and EM. In median eminence the LM staining pattern for NGFR and GFAP was similar, and by EM the NGFRI was primarily on tanycytes. In choroid plexus the LM staining for NGFR and CD45 corresponded and by EM was localized to PVM. In NH and area postrema (AP) the staining for NGFR, CD45 and GFAP did not correlate well by LM. EM showed NGFR and CD45 on PVM in both NH and AP. In AP occasional cells with neuronal morphology had NGFRI and in NH some cells resembling pituicytes had NGFRI. Cells and processes reactive for CD45 were seen in the neuropil of the AP and NH. In summary, CVO have NGFR localized to PVM but also to other cells that differ among the CVO and include neurons and glia. Some "microglial" cells with NGFR may be present in the neuropil of AP and NH. The function of NGFR on these various types of cells in CVO is unclear.

342.2

NERVE GROWTH FACTOR (NGF) RECEPTOR EXPRESSION IN THE BRAIN AND RETINA OF CHICK EMBRYOS. **C.S. von Bartheld, J.G. Heuer*, M. Bothwell. Depts. of Otolaryngology (RL-30) and Physiology & Biophysics (SJ-40), University of Washington, Seattle, WA 98195.**

Expression of NGF receptors was investigated in chicks from 5 days of incubation to 14 days posthatch with *in situ* hybridization. NGF receptor mRNA is expressed *transiently* in hippocampus, neostriatum, habenula, optic tectum (layers 3, 10), all nuclei of the isthmic complex, all motor nuclei, cerebellum (Purkinje cells, Golgi cells), inferior olive, and retina (inner plexiform layer, ganglion cell layer). High levels of NGF receptor mRNA persist in nucleus subrotundus, mesencephalic nucleus of the trigeminal nerve, locus coeruleus /subcoeruleus and reticular nuclei of the medulla.

NGF receptors are expressed predominantly, but not exclusively in cholinergic neurons and always *precede* the onset of cholineacetyltransferase immunoreactivity. Levels of NGF receptor mRNA are high during periods of target dependence and *cell death* (e.g., isthmo-optic nucleus, retina, optic tectum, cerebellum, motor nuclei), suggesting a role of NGF receptors in cell survival. High levels of NGF receptor transcripts in locus coeruleus are remarkable because rat locus coeruleus normally lacks NGF receptors. Surprisingly, NGF receptors are *not* expressed in cholinergic neurons of the basal forebrain, instead, these neurons express high levels of Fibroblast growth factor (FGF) receptors (Heuer et al., submitted). The type of growth factor that is required for avian cholinergic basal forebrain neurons (FGF, but not NGF) thus differs from that in mammals, possibly reflecting differences in the embryonic origin of the targets. **Supported by NIH grants DC0019, GM07270, NS23343.**

342.3

DISTRIBUTION AND QUANTIFICATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR mRNA IN THE CENTRAL NERVOUS SYSTEM

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Brain-derived neurotrophic factor (BDNF) is a protein that is both functionally and structurally related to nerve growth factor (NGF). The BDNF gene is strongly expressed in the adult central nervous system, from which BDNF was originally isolated. The distribution of BDNF mRNA in the adult mouse brain was examined by quantitative Northern blot analysis. All brain regions studied were found to contain two transcripts, 1.5 and 4.2 Kb in length. The highest levels of both transcripts were found in the hippocampus followed by the cerebral cortex. Quantitative determinations indicated that in the hippocampus, which is also the site of highest NGF gene expression in the central nervous system, there is about 50-fold more BDNF mRNA than NGF mRNA. In other brain regions, such as the cerebellum, the difference between the levels of BDNF and NGF mRNAs are even more pronounced. These data suggest that BDNF may play an important role in the CNS for a wide variety of adult neurons.

342.4

LOCALIZATION OF BDNF mRNA IN AGED RAT BRAINS

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Brain Derived Neurotrophic Factor (BDNF) is a neurotrophic factor, related to NGF, for which the sequence has recently been determined. We wanted to determine if there were any changes in BDNF concentrations in the aging brain. For initial studies of regional distribution young (21 days) Fisher 344 rats were decapitated and the brains dissected into regions. Tissue pieces were frozen on dry ice and the RNA was isolated using acid guanidinium. Northern blots were hybridized to an ^{32}P-labeled antisense 460 bp partial rat BDNF riboprobe overnight and exposed to X-ray film. Exposures were quantitated by densitometry. Two species of mRNA hybridized with the probe, one at 4.5 and one at 1.7 kilobases. BDNF mRNA was found to be widespread in rat brain. Concentrations in dorsal and ventral mesencephalon were intermediate. These findings suggest a role for BDNF in the function of a number of neuronal populations. The highest concentration was found in the hippocampus and the lowest concentration in the striatum. Results will be presented comparing the concentration of BDNF mRNA in various regions of young adult (6 months) and old (26 months) rat brains.
SAJ was supported by NIA AG07909.

342.5

CILIARY NEURONOTROPHIC FACTOR (CNTF) IMMUNOREACTIVITY IN RAT CNS. S. Varon, T. Hagg, O. Prospero-Garcia, J.M. Conner and M. Manthorpe Dept. Biol., M-001, UCSD, La Jolla, CA 92093

A peptide corresponding to the carboxy terminal residue Nos. 181-200 of rat nerve CNTF (Stöckli et al., *Nature* 342:920, 1989) was synthesized and coupled to keyhole limpet hemocyanin and the complex used to immunize rabbits. The resulting anti-peptide antibodies were affinity-purified with sepharose-immobilized peptide. Anti-peptide antibodies reacted with purified rat sciatic nerve CNTF protein by ELISA and by immunosequestration of CNTF neurotrophic activity toward 8 day chick ciliary ganglionic neurons. Adult and neonatal rats were transcardially perfused with 4% paraformaldehyde and 30 µm sections cut from the brain and spinal cord on a freezing microtome. Immunostaining for CNTF-peptide was carried out on selected sections using a sensitive ABC-peroxidase method. In normal neonatal and adult rat, anti-peptide immunoreactivity appeared in most neuronal and glial cell bodies of the brain and spinal cord as a perinuclear vesicular staining. This staining pattern appeared to be associated with the perinuclear Golgi system. Electron microscopy is being undertaken to determine the exact intracellular location of brain CNTF. The widespread staining pattern detected in the CNS is in contrast to the selective staining of Schwann cells in the PNS (Rende et al., *Soc. Neurosci. Abstr.*, 16, 1990). The above results suggest that central nervous system CNTF is more widely distributed than might be expected for a specific target-derived neuronotrophic factor. Support: NINCDS NS-16349, NS-25011 and NSF BNS-88-08285, BNS-86-17034.

342.6

Survival effect of ciliary neurotrophic factor (CNTF) and other factors on embryonic motoneurons in culture.

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CNTF has a potent survival effect on various embryonic neurons in culture, such as ciliary, dorsal root sensory and sympathetic neurons. Recent investigations showed that CNTF prevents the degeneration of motoneurons in new-born rats after lesion (M. Sendtner et al., *Nature*, 1990, *in press*). The question arose as to whether CNTF also has a survival effect on embryonic chick motoneurons at the developmental stage where physiological cell death occurs. To study this, we improved the culture method for chick embryonic motoneurons using metrizamide gradient centrifugation, which provided a high purity (more than 80% demonstrated by retrograde labelling) and a high survival rate of motoneurons (virtually 100% after 3 days in culture in the presence of muscle extract). Under these conditions, CNTF (1.5ng/ml) supported 64% of initially plated motoneurons (EC_{50} 0.023ng/ml). NGF, brain derived neurotrophic factor (BDNF) and neurotrophin-3 (NT-3) had no survival effect. Basic FGF supported maximally 51% of motoneurons (EC_{50} 0.28ng/ml) and acidic FGF supported 35% of motoneurons in the presence of 1 µg/ml heparin. Small effects at high concentrations were observed by insulin (16%) and insulin-like growth factors I and II (both at 15%) (basal survival rate was 5%). Many other mitogens and cytokines had no survival activity. The effect of CNTF and basic FGF was additive, resulting in a 100% survival even after 6 days. Thus, CNTF also supports embryonic motoneurons and, together with basic FGF, results in a 100% survival even after 6 days.

342.7

LOCALIZATION OF BRAIN-DERIVED NEUROTROPHIC FACTOR mRNA AND PROTEIN. C. Wetmore, P. Ernforst, H. Perssont, L. Olson, Dept. of Histology & Neurobiology, and Department of Medical Chemistryt, Karolinska Institute, 104 01 Stockholm, Sweden.

For many years, nerve growth factor (NGF) was the only trophic factor known to exert specific effects upon the growth and differentiation of neurons. In the past year, two new neurotrophic factors with striking homology to NGF have been purified and cloned: brain-derived neurotrophic factor (BDNF) and neurotrophin 3 (NT3). While all three factors show extensive similarities in amino acid sequence, molecular weight, conservation of six cysteine residues and of the intraprotein disulphide bonds, these factors effect neurotrophic responses from somewhat overlapping yet distinct populations of neurons. BDNF, unlike NGF, appears not to promote the growth of sympathetic neurons in culture, yet does support fiber outgrowth of placode-derived nodose ganglion neurons.

In the present study, we have used oligonucleotide probes to study the distribution of BDNF in the adult pig and rat brain. The oligonucleotide, derived from a stretch of porcine BDNF sequence, was labeled at the 3' end with [^{35}S]-dATP, applied to slide-mounted, 14μm sectioned brain tissue, and allowed to hybridize for 6 weeks at -20°C. Measurements of grain densities were carried out at high magnification using bright field illumination and clustering over specifically labeled cells was well over (20-40 fold) background levels as evaluated by computer-assisted image analysis.

BDNF mRNA shows a distribution in the brains of both species similar in several respects to that known for NGF mRNA, with strong labeling in granule cells of the dentate gyrus, as well as pyramidal and hilar cells in the hippocampus. Additionally, BDNF mRNA was detected in small cells around the subependymal area of the pig, and in scattered neurons of cortex cerebri, and claustrum of both pig and rat. These results indicate that in the brain, BDNF, like NGF, has neuronal rather than glial sites of synthesis. Immuno-histochemical localization of the BDNF protein with antibodies raised against BDNF peptides in rabbits shows a distribution similar to that of BDNF mRNA as detected with *in situ* hybridization.

342.8

PROTEIN KINASE INHIBITORS K-252A AND K-252B INHIBIT THE TROPHIC ACTION OF NGF, BUT NOT bFGF AND INSULIN ON CNS NEURONS IN CULTURE. F. Hefti and B. Knüsel. Andrus Gerontology Center, Univ. of Southern California, Los Angeles, CA 90089.

The protein kinase inhibitor K-252a has been shown to inhibit NGF induced neurite growth in PC12 cells and in primary cultures of dorsal root ganglion cells (Matsuda, Y. and Fukuda, J., *Neurosci. Letts.* 87:11, 1988; Hashimoto, S., *J. Cell Biol.*, 107:1531, 1988). We studied actions of K-252a and the structurally closely related K-252b in primary cultures of embryonic rat CNS cells in presence and absence of growth factors. Treatment for 5 days with 10 to 200 nM K-252a depressed ChAT activity and dopamine uptake in cultures of medial septum and ventral mesencephalon, respectively, to 10 - 40 % of control. 300 to 1000 nM K-252a restored both parameters to control values or higher. K-252b, up to 2500 nM, slightly depressed ChAT activity and dopamine uptake in a dose-dependent manner. No stimulatory effect was observed with this compound. Protein content was only mildly decreased with either compound, but the cultures showed signs of toxicity of K-252a (>30nM) in phase-contrast observation. Tested with growth factors, both, K-252a and K-252b completely abolished the action of NGF on septal cholinergic neurons. High concentrations of the two compounds did not diminish the stimulatory actions of bFGF and insulin on septal cholinergic and mesencephalic dopaminergic neurons. Insulin, however, exacerbated the deleterious effect of low concentrations of K-252a on ChAT activity in septal cultures.

We conclude from our findings, that the actions of K-252a and possibly also K-252b in the CNS are twofold: (i) selective inhibition of the action of NGF on basal forebrain; (ii) non-selective inhibitory and stimulatory actions on different transmitter-specific populations of neurons. Since the two compounds inhibit various protein kinases with different selectivity (Kase, H., et al., *Biochem. Biophys. Res. Comm.*, 142:436, 1987), our results are suggestive of multiple roles of protein kinases in biochemical differentiation of CNS neurons.

342.9

A DOPAMINERGIC NEUROTROPHIC FACTOR (DNTF) IS SECRETED BY SCHWANN CELLS GROWN IN CULTURE. J.E. Springer, L. Iacovitti, B.A. Maguire,* and T.J. Collier. Center for Neurological Research and the Hahnemann Institute for Neuroscience, Hahnemann University, Philadelphia, PA 19102, and Dept. of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, New York, 14642 (TJC).

Specific polypeptide molecules termed neurotrophic factors may be required for the development, survival, and normal function of certain neuronal populations in the central nervous system (CNS). We have previously shown that a soluble factor(s), designated as dopaminergic neurotrophic factor (DNTF), is secreted from sciatic nerve and exhibits trophic actions on embryonic midbrain dopamine neurons. In the present study we have determined that Schwann cells grown in culture may be a potential source of DNTF. Ventral midbrain tissue containing the substantia nigra was isolated from rats at 15 days gestation, dissociated and plated at a density of 300,000 cells per culture well. These cultures were grown in culture medium containing 10% fetal calf serum (FCS) for 48 hours. The cultures were then incubated in conditioned medium from an immortalized Schwann cell line (kindly provided by G. Owens, Washington University) diluted with culture medium containing 1% FCS. For comparison, additional cultures were grown in the presence of known growth factors including basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), insulin-like growth factor I, (IGF-I), and nerve growth factor (NGF). The cultures were maintained in the low serum/growth factor conditions for 3-5 days and processed for the immunocytochemical localization of tyrosine hydroxylase (TH). Cultures grown in the presence of Schwann cell conditioned medium (SCCM) exhibited a concentration-dependent increase (150-200%) in the number and neurite outgrowth of TH-positive neurons. Cultures treated with bFGF showed a similar, but less robust effect compared to SCCM treated cultures (38%). None of the other growth factors tested showed any effect with the exception of IGF-I, which decreased the number of TH-positive neurons by 20%. These results demonstrate that one of the factors secreted by Schwann cells exhibits trophic activity on dopaminergic neurons in vitro (see also Collier and Springer, this volume). Supported by NIH grants AG-08969 (JES) and AG-08133 (TJC).

342.10

A PERIPHERAL NERVE GROWTH FACTOR AUGMENTS THE BEHAVIORAL AND MORPHOLOGICAL EFFECTS OF CO-GRAFTED DOPAMINE NEURONS. T.J. Collier and J.E. Springer, Dept of Neurobiology and Anatomy, Univ. of Rochester Sch. of Med., Rochester, NY 14642; and Dept of Neurology, Hahnemann Univ. Sch. of Med., Philadelphia, PA 19102.

We have demonstrated that explanted segments of adult rat sciatic nerve placed in co-culture with embryonic rat mesencephalic dopamine (DA) neurons increase the number of tyrosine hydroxylase (TH) positive neurons and enhance their neurite outgrowth (*Soc. Neurosci. Abst.* 15:1354, 1989). The current study examined whether intraventricular implants of sciatic nerve could serve as a source of potential DA neuron growth-promoting factor, and would augment the behavioral and morphological recovery produced by embryonic rat ventral mesencephalic tissue grafted into the denervated striatum of unilateral 6-hydroxydopamine (6OHDA) lesioned rats. One week following lesion, rats were tested for baseline rotational behavior in response to intraperitoneal injection of 5mg/kg amphetamine. Subjects then received simultaneous implantation of a single block graft of E15 rat ventral mesencephalon into the DA-depleted striatum, combined with a hollow fiber implant into the ipsilateral lateral ventricle. Controls (n=6) received an empty fiber , co-grafted subjects (n=10) received a fiber containing a 5mm segment of adult rat sciatic nerve. Amphetamine rotation was retested 1,3,5,7,10 and 12 weeks after grafting. Co-grafted rats exhibited enhanced recovery of rotational behavior: 8 of 10 co-grafts showed complete recovery of rotation over the 12 week interval, while only 1 of 6 controls recovered. Co-grafted rats also exhibited an average 86% increase in the number of surviving grafted TH neurons in the striatum, combined with a prominent increase in neurite outgrowth, such that a larger proportion of the striatum was reinnervated in co-graft subjects and behavioral recovery was achieved even by small grafts that were behaviorally ineffective in control rats. These results support the view that peripheral nerve produces a factor with growth-promoting properties for central DA neurons, and that supplying a source of this factor in the environment of grafted embryonic DA neurons provides significant advantages. Supported by R29-AG08133 (TC) and R29-AG08969 (JS).

342.11

VASOPRESSIN PROMOTES HIPPOCAMPAL NERVE CELL GROWTH IN CULTURE. R.E. Brinton, Sch. of Pharmacy and Depart of Biology, Univ. of Southern California, 1985 Zonal Ave, Los Angeles, CA 90033.

The present study seeks to address the hypothesis that if memory formation is subserved by structural changes in nerve cell morphology, then factors which influence memory formation should influence neuronal growth. Vasopressin (AVP) has been shown to enhance memory formation (DeWied, 1971; 1984) through a NE dependent mechanism (Kovacs et al., 1979). AVP also acts as a neuromodulator to potentiate NE-induced cAMP formation in the hippocampus (Brinton and McEwen, 1989). In an initial test of the above stated hypothesis we found AVP to act as a neurotrophic factor in cultured embryonic Xenopus nerve cells (Brinton and Gruener, 1987). Because the site of behavioral and neuromodulatory effects of AVP involve the hippocampus, the influence of AVP upon hippocampal nerve cell growth in culture was determined. Hippocampal nerve cells from E18 rat pups were cultured in serum containing media and allowed to attach to polylysine coated coverslips whereupon the media was changed to serum free. Thirty min exposure to 10 uM AVP resulted in a significant increase in the length of filopodial extensions when compared to the change in filopodial length during the control period of observation (control = -0.89 $\pm$ 0.51 um, n=102; AVP treated = 1.76 $\pm$ 0.37 um, n=102). The significant increase in filopodial length persisted for 20 minutes and returned to control values at 30 min. Exposure for 18 hr to 100 nM AVP in serum free medium resulted in a significant increase in the # of neurites (c=0.13 $\pm$ 0.06; AVP=0.64 $\pm$ 0.13, n=66 cells, p <.0001) and # of filopodia (c=1.0 $\pm$ 0.27; AVP=6.23 $\pm$ 1.1, n=66 cells, p <.0001). Exposure for 48 hr, to 100 nM AVP in serum free medium resulted in loss of neuritic extensions suggesting that prolonged exposure to AVP is toxic to nerve cells. Experiments to determine calcium dependency of AVP effects are in progress. This work supported by NIH grant MH-46036 to R.E.B.

342.12

BRAIN-DERIVED NEUROTROPHIC FACTOR IS EXPRESSED IN CENTRAL OLFACTORY REGIONS AND BY TARGET AREAS OF BASAL FOREBRAIN CHOLINERGIC NEURONS. H.S. Phillips, J.M. Hains*, G.R. Laramee*, K. Nikolics, and J.W. Winslow, Dept Dev. Biology, Genentech, 460 Point San Bruno Blvd., South San Francisco, CA 94080.

Brain-derived neurotrophic factor (BDNF) is a homolog of NGF which exhibits a different pattern of target specificity in the peripheral nervous system and which appears to be expressed in brain at higher levels than NGF (1). We report here the distribution in rat forebrain of messenger RNA (mRNA) for BDNF as determined by *in situ* hybridization. Sense and anti-sense 35-S labelled RNA probes were employed for hybridizations which were analyzed with a combination of sheet film and emulsion autoradiography. Cells hybridizing intensely for BDNF message were located within the major targets of the basal forebrain cholinergic system, i.e., hippocampus, neocortex and amygdala. High concentrations of strongly hybridizing cells were also observed in several structures associated with the olfactory system, including the anterior olfactory neucleus, piriform cortex, tenia tecta, and endopiriform nucleus. These findings suggest that trophic activity of BDNF may not be restricted to primary sensory neurons, and point to basal forebrain cholinergic cells and olfactory pathways as potential central targets for BDNF.

(1) Y.A. Barde, et al., *EMBO J.*, **1**, 549 (1982); A.M. Davies, *TINS*, **11**, 243 (1988); J. Leibrock et al., *Nature* **341**, 149 (1989); Y.A. Barde, *Neuron* **2**, 1525 (1989).

342.13

HUMAN BASIC FIBROBLAST GROWTH FACTOR AND TRANSPLANTATION OF HUMAN AND RAT FETAL MESENCEPHALIC DOPAMINE CELLS. C.R. Freed, Y.B. Zhang,* D. Bhaskaran, and C.A. Kruse, Depts. of Med., Pharm., and Surg., Univ. of Colo. Health Sci. Ctr., Denver, CO 80262.

To improve survival of fetal mesencephalic dopamine cells during transplantation to adult brain, we have studied the effect of human basic fibroblast growth factor (bFGF, Amgen) on fetal human and rat cells in culture and after transplant. Human fetal dopamine cells were acquired from therapeutic abortion as previously described (Arch. Neurol. 47:505-512, 1990). Rat mesencephalon was obtained from embryonic day 15 to 16 Sprague-Dawley fetal rat. Tissue was preserved in high potassium, calcium and magnesium free media at 10 degrees. On the day following acquisition, fetal cells were placed in culture. Rat and human tissue was cultured in N5 media with NSF as previously described (Brain Res. 384:84-93, 1986). Human bFGF 10 ng/ml and heparin 1 ug/ml were added. Tissue was harvested after one week in culture and assayed for dopamine. Dopamine concentration from bFGF/heparin treated cultures was compared to untreated cell cultures. In addition, some cultured tissue was directly transplanted into rats with unilateral striatal dopamine depletion. Rats receiving human fetal tissue were immunosuppressed with daily injections of cyclosporine (10 mg/kg i.p.).

342.14

EXPRESSION OF C-SIS ONCOGENE-RELATED PROTEINS IN DEVELOPING AND ADULT RAT CENTRAL NERVOUS SYSTEM.

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The expression and distribution of the c-sis/PDGF-B gene product within the mammalian CNS is still controversial (Oberdick et al., Neuron, 1988; Johnston and van der Kooy, PNAS, 1989; Pringle et al., EMBO J, 1989). In order to address this issue we have used three monoclonal antibodies (mabs) for morphological and biochemical analysis of rat CNS: one mab was directed to residues 1-18 (Microbiol. Assoc.) and two mabs (C-1975 and C-3893) were developed against residues 22-38 (from C. Hart, Zymogenetics Inc.) of the PDGF-B chain. Detergent solubilized polypeptide fractions of human platelets, developing and adult rat cerebellum and forebrain were reduced, alkylated and processed for 1-D Western Blotting. On immunoblots of platelets all mabs recognize a 28 Kd band, the molecular weight (m.w.) of the c-sis gene product. A band of the same m. w. is seen for cerebellar fractions when using the two mabs to residues 22-38; the mab to 1-18 residues recognizes a 18 Kd band (corresponding to the PDGF-B chain m.w.) and a 33-35 Kd doublet on both the developing cerebellum and forebrain. Immunostaining of neural antigens on tissue sections is obtained by the latter mab. During prenatal development, regions corresponding to the main fibre pathways of the neural tube and forebrain are labelled. The developing fibre tracts of the postnatal cerebellum are also immunoreactive. However, in adult CNS fibre pathways are not immunolabelled, while dendrites of pyramidal neurons and Purkinje cells are well stained. We are carrying out analyses of cultured glial cells, prepared from prenatal rat cortex and preliminary data show cytoplasmic staining of the astrocytes with the C-3893 mab. The present study confirms that PDGF is expressed in the rat CNS and suggests that different post-translational products of the c-sis oncogene can be detected in the developing CNS in locations that are different from adult.

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342.15

FATE OF INTRACEREBRALLY INJECTED ^{125}I-FIBROBLAST GROWTH FACTOR. L.S. Carman, A.M. Gonzalez*#, F.H. Gage, M. Buscaglia*#, C.W. Shults and A. Baird*#. Dept. of Neuroscience, U.C. San Diego and #The Whittier Institute for Diabetes and Endocrinology, La Jolla, CA 92037.

Basic fibroblast growth factor (bFGF) is a member of the FGF family that shows multifunctional biological activity. It is localized and synthesized in several different tissues and particularly in the brain. It is a potent neurite-promoting agent for a variety of CNS cell types *in vitro*. In addition, infusion of bFGF *in vivo* has been shown to enhance survival of septal cholinergic neurons following axotomy. In an effort to understand the fate of bFGF following intracerebral injection, we injected ^{125}I-bFGF into striatum or ventricle of normal or lesioned rats and measured activity in dissected tissue or processed tissue for autoradiography. Thus, we hope to determine (1) how much bFGF remains in brain, (2) whether it is stable, and (3) where it binds.

By 4 hours after injection into striatal parenchyma, 62% of injected radioactivity remained at the injection site. By 2 days and 7 days, this decreased to 36% and 6%, respectively. There were no differences in diffusion or binding between normal and previously-lesioned rats. Western blot analysis showed that after 2 days, the bFGF present in the tissue corresponded to the 18-kD molecular form. Tissue autoradiography showed that injected ^{125}I-bFGF remained mainly bound to cells and matrix at the site of injection. Following injection into the ventricle, most ^{125}I-bFGF bound to ependymal cells, lining the ventricle and very little of it diffused into adjacent parenchyma.

These observations suggest that, as in other tissues, injected bFGF binds locally in the brain. The biological significance of this binding and potential exploitation of this characteristic is in progress. DK-18811.

342.16

TYROSINE PHOSPHORYLATION OF A 70 kDa PROTEIN BY INSULIN AND INSULIN-LIKE GROWTH FACTORS IN CULTURED FETAL CHICK NEURONS. K.A. Kenner* and K.A. Heidenreich. Dept. of Medicine, UCSD, La Jolla, CA 92093

Insulin and insulin-like growth factors have been implicated in neuronal growth and differentiation. Previous studies in our laboratory showed that cultured neurons derived from embryonic day 7 chick forebrain have both insulin and IGF-I receptors (5.7×10^3 and 4.5×10^4 high affinity sites/cell, respectively), which undergo autophosphorylation in response to ligand binding. Tyrosine phosphorylation of other proteins by the activated receptor kinases was not detected in neurons cultured for 5 days. In this study we examined neurons cultured for less time, using immunoblot analysis with antiphosphotyrosine antibodies. Neurons cultured 3-5 hrs contained a predominant protein M_r 70,000 (pp70) that was phosphorylated on tyrosine in response to insulin, IGF-I, or IGF-II. Ligand-stimulated phosphorylation of pp70 was not detected in neurons cultured longer than 24 hrs. Phosphorylation of pp70 was detected within 30 sec of ligand exposure and was maximal by 5 min. All 3 growth factors stimulated phosphorylation of pp70 to a similar extent at concentrations ranging from 1-50 nM. The time course and dose-response of pp70 phosphorylation paralleled that of insulin and IGF-I receptor autophosphorylation, suggesting that this protein represents an endogenous substrate for these receptor kinases in neurons.

342.17

NEUROTROPHIC ACTIVITY CONTAINED IN RAT HIPPOCAMPUS IS REDUCED BY CHRONIC EXPOSURE TO ETHANOL. D.W. Walker, M.B. Heaton, M.A. King, B.E. Hunter, Veterans Administration Medical Center and University of Florida College of Medicine, Gainesville, Florida, U.S.A. 32610.

The objective of this research is to determine if chronic ethanol treatment (CET) reduces the NGF-like neurotrophic activity contained in the rat hippocampus (HPC). A bioassay performed in cultures of dissociated dorsal root ganglion cells (DRG cells) from E7-8 chick embryos was used to determine the relative NGF-like neurite-promoting activity contained in the HPC of rats exposed to CET or control diets for 20-24 weeks. We compared the effect of the following four culture media conditions on the survival and neurite outgrowth of DRG cells after 24 and 48 hrs in culture: 1) control medium, 2) control medium + NGF (20 ng/ml) as positive control, 3) medium containing HPC extract (5% w/v) from a CET rat and 4) medium containing HPC extract (5% w/v) from a pair-fed control rat. The most dramatic difference in neurotrophic activity observed was a 40-50% decrease in both the total extent and average length of neuronal processes extended in the cultures maintained in medium containing HPC extract from CET rats as compared to HPC control extract or NGF control conditions. These data support the hypothesis that CET reduces the neurotrophic activity contained in the HPC which may compromise the structural and functional integrity of the septohippocampal system due to chronic deprivation of normal neurotrophic influence. We are currently conducting experiments to determine what proportion of the neurotrophic activity contained in the HPC extract is NGF-like, and what proportion may be due to other neurotrophic factors.

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342.18

TGF-BETA ISOFORMS IN THE CENTRAL AND PERIPHERAL NERVOUS SYSTEM. K. Unsicker, K.C. Flanders*, D.S. Cissel*, S. Marascalco*, R. Lafyatis*, A.B. Roberts* and M.B. Sporn*. Dept. Anatomy and Cell Biology, University of Marburg, F.R.G., and Lab. Chemoprevention, NIH, NCI, Bethesda, MD 20892.

TGF-ßs are multifunctional growth factors with a wide tissue distribution. We have studied the localization of the TGF-ß1, 2 and 3 proteins and TGF-ß3mRNA in the adult rat and embryonic chick nervous systems using immunohistochemistry, Northern blot analysis and in situ hybridization. TGF-ß2 and 3 immunoreactivities are strictly co-localized and occur in neurons, radial glial cells, white matter astrocytes and Schwann cells. TGF-ß1 is confined to meninges and choroid plexus. In the rat brain, highest densities of TGF-ß immunoreactive neuronal cell bodies are found in brainstem motor nuclei, cerebral cortical laminae 2, 3 and 5, hippocampus, hypothalamus and amygdala. In other areas, like spinal cord dorsal horn, periaqueductal gray and thalamic nuclei, neuronal perikarya containing TGF-ßs are very scarce. Northern blots reveal approximately equal levels of TGF-ß3mRNA in cerebral cortex, hippocampus, striatum and cerebellum. In situ hybridizations suggest synthesis of TGF-ß3 in neurons and glial cells.

343.1

SUBSEIZURE AFFERENT STIMULATION INCREASES NGF mRNA IN RAT DENTATE GYRUS GRANULE CELLS.

M.W. Oliver, P.J. Isackson and C.M. Gall. Department of Anatomy and Neurobiology, University of California, Irvine CA 92717.

Previous work in our laboratories demonstrated that recurrent seizures increases NGF mRNA expression in the dentate gyrus granule cells. In the present study in situ hybridization techniques, using ^{35}S-cRNA probes complementary to the full rat preproNGF and c-Fos mRNA sequences, were used to evaluate whether subseizure stimulation of the perforant path is sufficient to alter NGF and c-Fos mRNA expression in GC.

In anesthetized Sprague-Dawley rats, either high frequency (HFS; 100-400Hz, 5 pulses ,repeated 10x) or low-frequency (LFS; 10Hz, 15-20 pulses, repeated 2-4x) stimulation was delivered to the angular bundle; continuous recordings from the dentate gyrus verified orthodromic activation and the absence of epileptiform activity. In only 2 of 18 cases, HFS dramatically increased (>7-fold) hybridization of the NGF cRNA probe within GC. LFS was more effective: in 3 of 6 cases hybridization of the NGF cRNA probe was increased 3-fold or more. In all cases, hybridization was only increased ipsilateral to stimulation. In control rats, with electrodes lowered but no stimulation, NGF cRNA hybridization was only slightly increased (<50%) within ipsilateral GC and olfactory cortex. Increases in hybridization to c-Fos mRNA correlated with NGF cRNA labeling in GC of stimulated rats. These data demonstrate that subseizure activity regulates the expression of neural trophic factors and immediate-early genes by adult forebrain neurons. (Supported by PEW Foundation & NS26748 to C.G. and NS24747 to P.I.)

343.2

SEIZURES INDUCE INCREASES IN NGF mRNA THROUGHOUT CEREBRAL CORTEX.

J.C. Lauterborn, P.J. Isackson, and C.M. Gall. Department of Anatomy & Neurobiology, University of California, Irvine CA 92717.

Nerve growth factor (NGF) application has been demonstrated to affect the levels of both choline-O-acetyltranferase and NGF receptor expression within the basal forebrain. These observations are intriguing in that they suggest that changes in the amount of endogenous NGF may be of consequence to specific neuronal circuitries. Recently, our laboratories have reported that hilus lesion (HL) induced limbic seizures lead to a rapid and dramatic increase in the expression of NGF mRNA in neurons within hippocampus followed by increases in NGF mRNA throughout broad fields of neocortex (Science, 1989). In the present study, the full distribution of seizure-induced increases in NGF mRNA within rat forebrain was examined by in situ hybridization using a ^{35}S-labeled riboprobe complementary to the full rat preproNGF sequence and autoradiographic techniques. By 24 hrs following HL, hybridization to NGF mRNA was observed throughout the entire rostral to caudal extent of the neocortex as well as the entorhinal and piriform cortices, anterior olfactory nucleus, amygdaloid complex, and endopiriform nucleus. Increases in hybridization to NGF mRNA within neocortex were most prominent within layers II, III, and VI. In one case, densiometric analysis of the increased cRNA hybridization within lateral neocortex revealed a 30-fold increase in the HL animal as compared to control. These data demonstrate that physiological activity can stimulate the expression of NGF mRNA in a variety of cortical neurons. (Supported by NS26748 to C.G. & NS24747 to P.I.).

343.3

BRAIN DERIVED NEUROTROPHIC FACTOR mRNA EXPRESSION IS INCREASED IN ADULT RAT HIPPOCAMPAL NEURONS AFTER SEIZURE INDUCTION. P.J. Isackson, M.M. Huntsman*, K.D. Murray*, and C.M. Gall. Department of Anatomy and Neurobiology and Department of Biological Chemistry, University of California, Irvine, CA 92717.

Brain derived neurotrophic factor (BDNF) and nerve growth factor (NGF) are homologous target-derived neurotrophic factors that affect different neuronal populations. We have previously shown that the induction of limbic seizures results in a rapid, transient increase in NGF mRNA expression in hippocampal dentate granule cells. We have used in situ hybridization and S1 nuclease protection assays with an RNA probe prepared from a PCR-generated cDNA clone for rat BDNF to demonstrate that BDNF is regulated by a similar mechanism in the adult rat hippocampus. The level of BDNF mRNA is dramatically increased in the dentate gyrus granule cells, bilaterally, following recurrent limbic seizures induced by unilateral electolytic lesion of the dentate hilar region as well as following one hippocampal discharge (30-45 sec duration) induced by 10 Hz stimulation of the perforant path. Hybridization is increased greater than 10-fold above normal levels in the hilus lesion rats. In contrast to NGF, BDNF mRNA is increased in pyramidal cells of the CA1 and CA3 regions of the hippocampus in addition to the dentate gyrus granule cell layer. These results suggest that homologous members of the NGF neurotrophic family are regulated similarly by physiological stimulation and may play an important role in activity-dependent plasticity in the adult CNS. (Supported by NS24747 to P.I. and NS26748 to C.G.).

343.4

IN SITU LOCALIZATION OF bFGF RECEPTOR mRNA IN THE ADULT RAT CNS. A. Wanaka, E.M. Johnson, Jr., and J.D. Milbrandt. Departments of Pharmacology and Pathology, Washington University School of Medicine, St. Louis, MO 63110.

Basic FGF receptor (bFGF-R) mRNA expression in the adult rat CNS was examined with Northern blot analysis and in situ hybridization histochemistry with the use of cRNA probes for rat and human bFGF-R. Northern analysis showed a distinct 4.3 Kb transcript in the various regions of the CNS. In situ hybridization revealed widely distributed, but specific, populations of the cells that express bFGF-R mRNA. The most intense hybridization signals were observed in the hippocampus and in the pedunculopontine and laterodorsal tegmental nuclei. The amygdala central nucleus, supraoptic nucleus, median eminence, substantia nigra pars compacta, locus coelureus, motor nuclei and cochlear nucleus showed robust labeling. Cerebellar granule cells were labeled intensely. Various types of neurons in the spinal cord were positive for bFGF-R mRNA. We did not find any signals in the basal forebrain cholinergic neurons. These results are generally consistent with retrograde transport studies using ^{125}I-bFGF (Ferguson et al.). The distribution of bFGF-R mRNA significantly differed from that of NGF-R mRNA with a little overlap. The present study strongly suggests that FGF may act as neurotrophic or modulating factors on specific neuronal populations in the mature CNS. bFGF-R mRNA expression in the injured CNS is now under investigation.

343.5

bFGF UNDERGOES RECEPTOR-MEDIATED RETROGRADE TRANSPORT IN CNS NEURONS. Ian A. Ferguson, Akio Wanaka, and E.M. Johnson, Jr. Dept. of Pharmacology, Washington University School of Medicine, St. Louis, MO 63110

Neurons in the adult rat CNS which respond to bFGF by internalizing and retrogradely accumulating the growth factor were identified by autoradiography following injections of ^{125}I-bFGF. Neuronal populations which showed specific (saturable) retrograde accumulation of ^{125}I-bFGF included the pyramidal cell neurons of the hippocampus, neurons in the subiculum, paracentral thalamic and central medial thalamic nuclei, parafascicular nucleus, the lateral hypothalamus, supramammilliary nucleus, substantia nigra compacta, pedonuclopontine and laterodorsal tegmental nuclei. Basal forebrain cholinergic neurons, which retrogradely accumulated ^{125}I-NGF, did not accumulate ^{125}I-bFGF. Neurons which retrogradely accumulated ^{125}I-bFGF also expressed mRNA for the bFGF receptor as shown by in situ hybridization studies. The retrograde accumulation of ^{125}I-bFGF in neurons was specific as co-injection of excess unlabeled bFGF or co-injection of WGA (which non-specifically blocks the binding of bFGF to its receptor) abolished the retrograde labeling. The time course of labeling was consistent with labeling being a retrograde transport-mediated phenomenon. The analogy with NGF is striking and strengthens the possibility that bFGF may be a neurotrophic factor for some CNS neuronal populations.

343.6

DIFFERENTIAL INHIBITION OF EARLY NGF-INDUCED mRNAs BY PURINE ANALOGUES. A. Batistatou, C. Volonté, L.A. Greene*. Department of Pathology and Center for Neurobiology and Behavior, College of Physicians and Surgeons, Columbia University, New York, N.Y. 10032.

Purine analogues [exemplified by 2-aminopurine (2-AP) and 6-thioguanine (6-TG)] inhibit some of the actions of NGF when applied to PC12 cells, but not others. We have extended our studies to the effects of purine analogues on the NGF induction of several early mRNAs in PC12 cells. Our data suggest that NGF induces early genomic effects via at least three distinct pathways. One pathway is sensitive to 2-AP but not to 6-TG and includes the NGF-induced increases of c-*myc*, c-*fos*, c-*jun* and TIS11 mRNAs. A second pathway is sensitive to both 2-AP and 6-TG; 2-AP delays the NGF-induced increase of TIS1 (also designated as NGFI-B or nur77) mRNA, while 6-TG inhibits this increase. A third pathway is insensitive to both 2-AP and 6-TG; neither of these compounds decrease the NGF-induced increase of TIS8 (also designated as NGFI-A or egr-1) mRNA. Prior studies indicate that these compounds inhibit protein kinases. Both 2-AP and 6-TG block PKN, an NGF-regulated serine protein kinase (Volonté, C. et al., J. Cell Biol., 109:2395, 1989) at concentrations similar to those used in these experiments. Thus far 6-TG appears to be specific for PKN. 2-AP affects other kinases as well and this may explain its wider action. These data support the hypothesis that multiple distinct kinase pathways are involved in the NGF-mediated increase of early gene expression and argue for the requirement of PKN in one of these pathways.

343.7

MOLECULAR CHARACTERISTICS OF AN NGF-ACTIVATED PROTEIN KINASE (PKN). C. Volonté and L.A. Greene*, *Department of Pathology, Columbia University, New York - N.Y., 10032.*
Past work established a cell-free assay for the detection of an NGF-activated protein kinase activity designated protein kinase N (PKN). PKN is a serine kinase which is activated by NGF and other factors in PC12 cells and other cell lines. Through the use of purine analogs which inhibit PKN, we have been able to suggest a role for PKN in certain, but not other, of the multiple pathways of the NGF mechanism of action. The aim of this work is to further characterize the biochemical properties of PKN. We now have found a new purine analog, 6-methylmercaptopurine riboside (6-MMPR), which is very potent (IC_{50} ~ 5nM in vitro) and apparently specific for PKN inhibition . The structure of 6-MMPR is important for PKN inhibition : similar compounds which lack the methyl or riboside groups are not as active in inhibiting the kinase. 6-MMPR also prevents some of the biological actions of NGF in PC12 cells, including neurite regeneration and ODC induction, again suggesting a role for PKN in certain NGF pathways. Preparations highly enriched for PKN have been prepared with good recovery of activity by ion exchange chromatography on Mono S and Q resins and by elution from Mono S with ATP. By density gradient centrifugation the kinase exhibits a Svedberg coefficient of 4.6. On a Superose12 FPLC column PKN elutes as major peak at 40-50kD and minor peak at 100-130kD. PKN can be affinity labelled with 8-azido-(^{32}P)ATP and shows apparent mol wt of about 47kD by SDS-PAGE. The affinity labelling of PKN is inhibited by purine analogs and shows competition with ATP and GTP, both phospate donors for PKN.

343.8

REGULATION OF NERVE GROWTH FACTOR mRNA BY INTERLEUKIN-1 IN THE RAT CENTRAL NERVOUS SYSTEM R. P. Hellendall, D. Casper, C. Lackner*, F. Berkenbosch, and M. Blum. Fishberg Ctr. for Neurobiology, Mt. Sinai Sch. of Med., New York, NY 10029
Interleukin-1 (IL-1), a cytokine released by activated macrophages and microglia, has been shown to induce the synthesis of nerve growth factor (NGF) mRNA in explant cultures of rat sciatic nerve (Lindholm, D., et. al., Nature 330:658, 1987). We are studying the regulation of NGF mRNA by IL-1 in the CNS to examine if a similar regulation occurs. Astrocytes, purified from PN2 Sprague-Dawley rat hippocampus, showed significantly increased levels of NGF mRNA in non-confluent cultures following exposure to IL-1β (10U, 3 hours; Genzyme Corp.). To determine if there are regional differences in the astroglial regulation of NGF mRNA by IL-1β, we compared the response to IL-1β in astroglia cultured from the hippocampus, hypothalamus and cortex. Astroglia from all of these areas produced significantly incresed levels of NGF mRNA in response to IL-1β. In contrast, preliminary experiments with cultured neurons isolated from E18 rat hippocampi gave no NGF mRNA induction subsequent to IL-1 application. Since it has been demonstrated that the degree of NGF release can be growth-phase dependent, we hypothesize that the ability of NGF gene expression to be induced by IL-1 may also be correlated to the proliferative state of the cell. Consistant with this idea, injection of 10U of IL-1β (ICV, 3 hr. survival) into adult Fisher 344 rats, where NGF mRNA has been localized to neuronal perikarya, gave no NGF mRNA induction. Thus astroglia may be susceptible to IL-1 induced NGF gene expression during periods of growth and cell division; these periods may include development of the CNS and proliferative responses following injury. Current experiments are examining the effect of the stage of astrocyte proliferation on this inducibility. We are also further clarifying the differences between the glial and neuronal cultures.

343.9

INCREASED NERVE GROWTH FACTOR RECEPTOR mRNA IN CONTUSED RAT SPINAL CORD. M. Reynolds, N. Brunello, J.R. Wrathall and I. Mocchetti. Department of Anatomy and Cell Biology, Georgetown University, Washington, D.C. 20007.
Sciatic nerve transection has been shown to increase the expression of nerve growth factor receptor (NGFR) mRNA in spinal cord motoneurons (Enfors *et al., Neuron* 2:1605, 1989). To test whether a similar increase occurs after injury to the spinal cord, NGFR mRNA content was determined in spinal cords of adult rats after a standardized mild contusive injury (Wrathall *et al., Exp. Neurol.* 88:108, 1985). A cRNA probe for NGFR mRNA was synthesized from the p5B plasmid (Buck *et al., Develop. Brain Res.* 44:259, 1988) and used in Northern blot analysis of total RNA. By 4 days after contusion NGFR mRNA was significantly increased in thoracic segments of the cord that contained the injury site. The induction was maximal at 7 days, about 5-7 times the level of uninjured controls, and remained about 4-fold control level at 14 and 28 days after contusion. The cellular localization of this increased NGFR mRNA is currently unknown but *in situ* hybridization studies should allow us to identify the sites. The increase in NGFR mRNA after contusive injury suggests that axotomy in the CNS may trigger the molecular mechanism(s) leading to up-regulation of NGFR gene expression. One possibility is that the receptor mRNA may increase in response to the higher levels of NGF protein that we find in the spinal cord after contusive injury. (Supported by NIH-BRSG RR05360 and Genentech, Inc.).

343.10

INCREASE IN NERVE GROWTH FACTOR (NGF) AND DECREASE IN CHOLINE ACETYLTRANSFERASE (ChAT) ACTIVITY FOLLOWING MILD CONTUSIVE INJURY. J.R. Wrathall, M. Armanini, G.L. Bennett*, W-L. T. Wong* and C. Bakhit. Dept. of Anatomy and Cell Biology, Georgetown Univ., Washington, DC, 20007 and Genentech, Inc., South San Francisco, CA, 94080.
A standardized mild contusive spinal cord injury can be produced in rats using a weight-drop technique (Wrathall, *et al., Exp. Neurol.* 88:108, 1985). Initially there is a severe functional deficit that is attenuated over time to reveal by three weeks the mild chronic deficits characteristic of this degree of trauma. As cholinergic neurons are widely distributed and functionally important in the spinal cord, we measured ChAT activity in different segments of the spinal cord before and after injury. We also determined the levels of NGF, a trophic factor for some CNS cholinergic neurons, using a specific two-site ELISA. In normal spinal cord we found a significant correlation (r = .74) between ChAT activity and NGF levels at different segmental levels of the cord. Mild contusive injury resulted in a significant decrease in ChAT activity which reached a nadir at 1 week then partially rebounded at 2 and 4 weeks after injury. The changes in ChAT activity were mirrored by changes in NGF levels that increased dramatically to reach a maximum of about ten-fold greater than normal at 1 week, then declined at 2 and 4 weeks as ChAT activity rebounded. These results suggest the possibility of a role for NGF in promoting the recovery of cholinergic function after spinal cord injury. (Supported by Genentech, Inc.)

343.11

QUANTITATIVE ANALYSIS OF NGF-RECEPTOR GENE EXPRESSION IN THE DEVELOPING RAT CNS. B. Zhao, S. Koh, and G.A. Higgins. Univ. Rochester. Med Ctr., Rochester, NY 14642, and Gerontology Res. Ctr., NIA/NIH, Baltimore, MD 21224.
During the first 3 weeks of postnatal development, NGF-responsive basal forebrain neurons undergo a program of differentiation, including profuse dendritic growth and cellular hypertrophy. *In situ* hybridization shows that levels of NGF-receptor mRNA appear to peak within basal forebrain neurons at postnatal day (PD) 15 in the rat. In order to better understand the developmental profile of NGF-R gene expression, we have quantified NGF-receptor mRNA levels at PD 1, 5, 10, 15, 22, 30 and adult rat brain, using a combination of densitometric measures of *in situ* hybridization and the competitive reverse transcription - polymerase chain reaction (RT-PCR). For RT-PCR, the same oligonucleotide primers are being used to amplify endogenous NGF-receptor mRNA, as well as known amounts of exogenously added control NGF-receptor RNA. The control RNA was synthesized from a plasmid which was engineered to produce a different-sized product after RT-PCR. These studies should provide a more detailed quantitative measure of changes in NGF-receptor mRNA levels during rat brain development.

343.12

RECOMBINANT HUMAN NGF LABELS NOVEL HIGH AFFINITY BINDING SITES IN RAT BRAIN. M. Dugich-Djordjevic, L. E. Burton++, G. L. Bennett*, and C. A. Altar. Developmental Biology, ++Recovery Process R & D, and *Immunology Res. and Assay Tech. Genentech, Inc., South San Francisco, CA 94080.
Iodinated recombinant human nerve growth factor ([^{125}I]rhNGF) was used to characterize displaceable, high affinity NGF binding sites throughout rat brain. [^{125}I]rhNGF stimulated neurite formation in PC12 cell systems with an EC50 of 49 pg/ml in two studies, compared with 39-52 pg/ml for rhNGF. Using quantitative ligand autoradiography, the *in vitro* equilibrium binding of [^{125}I]rhNGF was saturable, reversible, and displaced by up to 74% with rhNGF or muNGF, but not by 100 nM bFGF, EDF, rhGH, cytochrome c or IGF-1. [^{125}I]rhNGF bound to these sites with high affinity (K_d = 52-85 pM) and low capacity (B_{max} values ≤ 13.2 fmol/mg protein). Displaceable [^{125}I]rhNGF binding varied by 10-fold throughout the brain and labelled areas not identified previously with [^{125}I]muNGF or with antibodies to the murine NGF receptor. These novel areas include the nucleus accumbens, olfactory tubercle, subiculum, pineal gland, medial geniculate nucleus, dorsal hippocampus, dentate gyrus, amygdala, paraventricular thalamus, and frontal, parietal, occipital, and cingulate cortices. Most brain binding sites for [^{125}I]rhNGF are in the caudate-putamen, which also contained a 2 to 3-fold medial-lateral gradient of increasing NGF binding density in rat and rabbit. The nucleus accumbens contained a two-fold lateral-medial gradient. [^{125}I]rhNGF binding sites were also found in most areas labeled by [^{125}I]muNGF. [^{125}I]rhNGF binding sites were absent in the pedunculopontine and parabrachial cholinergic nuclei and their thalamic, hypothalamic, and tectal projections, and in areas replete with low affinity NGF binding sites, including the olfactory bulb, circumventricular organs, myelinated fiber bundles, and the choroid plexus.

343.13

SURFACE PROTEIN PHOSPHORYLATION IN PC12 CELLS: INTERACTION WITH NGF. Z. Pawlowska*, M.V. Hogan* and Y.H.Ehrlich. CSI/IBR Center for Developmental Neuroscience, City University of New York, Staten Island, NY 10301.

The regulation of neuronal function by extracellular ATP may involve the activity of ecto-protein kinases that phosphorylate proteins located at the cell surface. To investigate the role of ecto-protein kinase (ePK) in neuronal growth and differentiation, we have identified and characterized the surface phosphoproteins in PC12 cells, a cloned cell line used extensively in studies on the action of nerve growth factor (NGF). Cells were assayed in 48-well plates, attached to collagen, in Krebs-Ringer Hepes buffer. 0.1μM of γ-^{32}P-ATP were used in ePK reactions and intracellular ATP pools were labeled by incubating cells with ^{32}Pi. Ecto-protein kinase activity in PC12 cells was very rapid; phosphorylation of proteins with apparent MW of approx. 20K, 40K and 105K was detected within 10 seconds of the addition of AT^{32}P to the extracellular medium. Surface phosphorylation of proteins with MW of approx. 97K, 116K and 125K was observed after longer incubations. In contrast, the early substrate for intracellular phosphorylation in cells labeled with ^{32}Pi was a protein with MW of 56K. Addition of NGF (50 ng/ml) to the ePK reaction medium 5 min before the AT^{32}P stimulated selectively the phosphorylation a 20K protein. Furtheremore, in PC12 cells treated for 3 days with NGF there was a significant increase in the phosphorylation of the 20K protein by ePK. The surface phosphorylation of this specific protein by extracellular ATP may play an important role in the functional consequences of the interaction of NGF with neurons. Supported by AFOSR.

343.14

217c, A TUMOR-ASSOCIATED ANTIGEN MONOCLONAL ANTIBODY RECOGNIZES THE RAT NGF RECEPTOR. M.G. Fiori, G. Ferrari, M. Fabris*, S.D. Skaper, P. Polato* and Q. Yan (1). Fidia Research Labs., Abano Terme (PD), Italy and (1) Genentech Inc., South San Francisco, CA.

The monoclonal antibody 217c, has been shown to be a marker for Schwann cells in culture. We now report that the antigen recognized by 217c is the rat NGF receptor. The distribution of 217c immunoreactivity closely paralleled that observed for NGF receptors using 192-IgG, with both cultured Schwann cells and NGF-primed PC12 cells as well as a number of central nervous system neurons in vivo. 217c immunoprecipitated an ^{125}I-NGF/receptor complex having a major band at 90 kDa, as reported for 192 IgG. However, 217c recognized an epitope on the rat NGF receptor different from 192-IgG because: a) the two monoclonal antibodies differed in their capacity to increase NGF binding to PC12 cells at 4C°; b) a combination of 192-IgG and 217c stained more strongly NGF receptor-positive neurons than did either antibody alone; and, most importantly, c) 217c did not compete with ^{125}I-192-IgG binding to PC12 cells. Thus, the 217c antibody can now be considered an important tool in the study of NGF receptors.

343.15

THE USE OF XENOPUS OOCYTES FOR DETECTION AND CLONING OF NEUROTROPHIC FACTORS. A.Lam*, F.Fuller, J.Kloss, B.Cordell, S.Varon^{+} and M.Manthorpe^{+}. California Biotechnology Inc. 2450 Bayshore Pkwy, Mountain View, CA 94043; $^{+}$Dept. Biology, UCSD, La Jolla, CA 92093.

The rat glioma cell line, C6, produces several neurotrophic factors (Westermann, R., et al. J. Neurochem., 50:1747, 1988). We have developed a highly sensitive assay capable of detecting responses of E8 chick ciliary neurons to polypeptide hormones. At least two factors present in extracts of C6 cells elicited a response in this assay - basic FGF, and a distinct ciliary neurotrophic factor (CNTF). Injection of Xenopus oocytes with C6 mRNA elicited only the non-FGF CNTF activity. The physical behavior of this expressed CNTF was identical to that of rat sciatic nerve CNTF. A cDNA library was prepared from C6 cell mRNA using a transcription vector. Injection of oocytes with synthetic mRNA prepared from this library revealed the presence of a clone encoding a functional, rat CNTF molecule. Further characterization indicated that a C6 cell contains only about 1-5 copies of CNTF mRNA. Thus, oocyte injection represents an extremely sensitive method for the detection and cloning of neurotrophic factors.

343.16

COMPARISON OF EXPRESSION AND FOLDING OF MOUSE RECOMBINANT ß-NERVE GROWTH FACTOR (NGF) IN BACTERIAL SYSTEMS. G.-L. Hu*, Y. Luo*, D. E. Timm*, and K.E. Neet. Department of Biochemistry, Case Western Reserve University, Cleveland OH.

We have examined three plasmid vectors for high level expression of recombinant mouse ß-NGF in *E. coli*. Biologically active NGF has been produced in each system with varying yields and varying biological specific activity. Our original pAS1 system produced mature NGF in a soluble form at a low level of about 50 μg/L culture of which 0.1% was biologically active; these results were attributed to problems of both folding and proteolysis [Gene 70, 57 (1988)]. The pRIT2T system produced a Protein A-NGF fusion protein that accumulated in insoluble inclusion bodies with a yield after purification of about 5 mg/L culture; after cleavage about 600 μg of pure NGF could be obtained that contained about 10-20% biologically active NGF before further refolding. The pT7-7 plasmid system yielded about 4 mg/L culture of mature NGF in inclusion bodies that had low biological activity but could be readily purified. From these data we conclude that this small protein can be protected against bacterial degradation in inclusion bodies with or without fusion to another protein. A protocol has been developed for refolding recombinant or authentic mouse NGF from guanidine hydrochloride under controlled redox conditions that yields NGF with high specific activity. The disulfide formation and secondary structure of these proteins are being characterized. (Supported by an NIH grant, NS24380)

343.17

NGF INDUCES A DIFFERENTIAL INCREASE IN THE MULTIPLE mRNA's FOR CALMODULIN IN PC12 CELLS G. Bai* and B. Weiss, Department of Pharmacology, Medical College of Pennsylvania, Philadelphia, PA 19129

We previously reported (Soc. Neurosci. 15:501, 1989) that nerve growth factor (NGF) increases the activity of calmodulin (CM) and the amount of CM mRNA in PC12 cells and that these changes are coincident with cellular differentiation. Recently, it has been shown that in rat tissue CM is encoded by 3 distinct genes which transcribe five different mRNA's (0.9, 1.4, 1.7, 2.3 and 4.1 kb). In this study, we examined whether the multiple mRNA's for CM also exist in PC12 cells and what effect NGF has on the expression as well as the turnover of these mRNA's. RNA was isolated from PC12 cells incubated with NGF for 30 min to 16 days. The CM mRNA's were analyzed by Northern Blot using a cRNA probe, which is able to reveal all the CM mRNA's when used at conditions of moderate stringency, and oligonucleotide probes designed to detect the calmodulin mRNA's for each of the calmodulin genes. Densitometric measurements of the developed autoradiograms were calibrated using cyclophilin mRNA as a standard. The results showed that PC12 cells have all the mRNA's for calmodulin; the relative abundance of the calmodulin mRNA's was 1.7 > 1.4 > 4.1 > 2.3 > 0.9 kb. Treatment of the PC12 cells with NGF caused a differential increase in the mRNA's; the 1.4 kb transcript was increased earlier and to a greater extent than any of the other mRNA's. No significant changes in the CM mRNA's were seen in the cells not treated with NGF. The half-lives (t1/2) of the mRNA's were estimated by adding actinomycin D (1 ug/ml) after treating the cells for two days with NGF, then measuring their rate of decline. These studies showed that there were no significant effects of NGF on the t1/2 of any of the CM mRNA's. However, the t1/2 of the CM mRNA's were different from each other; the t1/2 for the 1.4, 1.7, 2.3 and 4.1 kb transcripts were 12, 17, 20 and 26 hr, respectively. We conclude that NGF-induced cellular differentiation may involve the selective induction of certain types of CM mRNA's.

343.18

EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR (NGFR) mRNA IN THE RAT RETINA IS DEVELOPMENTALLY REGULATED AND INCREASED AFTER OPTIC NERVE SECTION. M.C. Comelli*, L. Bonfanti*[1], A. Merighi*[1], G. Carmignoto and L. Maffei*[2]. Fidia Research Laboratories, Abano Terme (PD), [1] Dept. of Morphophysiology, University of Turin, Turin and [2] Institute of Neurophysiology, CNR, Pisa, Italy.

Using RNA blot analysis, NGFR mRNA in the rat retina was found to be expressed at its highest levels on postnatal day 2 sustained at a somewhat lower level throughout development and in adulthood. Optic nerve section in the adult rat resulted in a 2 to 7 fold increase in NGFR mRNA levels in the lesioned retina compared with the unlesioned control, consistent with an increased need for trophic support to damaged retinal ganglion cells. We previously showed that NGFR in the retina is localized on ganglion cells and Müller cells. Immunocytochemical studies using the monoclonal antibody ^{192}IgG for NGFR are in progress to investigate whether an increase in NGFR mRNA is paralleled by an increase in NGFR protein on Müller cells and/or ganglion cells.

343.19

EXPRESSION OF NERVE GROWTH FACTOR RECEPTOR (NGFR) ON ADULT RAT RETINAL GANGLION CELL (RGC) TERMINALS. G. Carmignoto, P. Candeo*, C. Comelli*, G. Calderini and L. Maffei*[1]. Fidia Research Labs, Abano Terme (PD) and [1]Institute of Neurophysiology, CNR, 56100 Pisa, Italy.

A recent immunohistochemical study using the monoclonal antibody 192-IgG for rat NGFR demonstrated the presence of NGFR immunopositive fibers at the level of the Superior Colliculi (SC) and Lateral Geniculate Nuclei (LGN), the main targets of RGC projections (Yan and Johnson, J. Comp. Neurol., 290:585, 1989). After optic nerve section we found that immunopositive fibers contralateral to the lesion site practically disappeared in the LGN and were reduced in the SC, demonstrating that the NGFR is clearly associated with RGC axonal terminals. Preliminary results indicate that following ligation of the optic nerve, ^{125}I-NGF injected into the target regions of RGC projections accumulates at the site of ligation. Axonal terminals of RGCs appear to bind, internalize and retrogradely transport NGF to the cell bodies, supporting the hypothesized role of NGF as a target derived factor for rat RGCs.

SENSORY SYSTEMS—DEVELOPMENT AND PLASTICITY II

344.1

PRESYNAPTIC SITES PERSIST FOLLOWING DEGENERATION OF THEIR POSTSYNAPTIC PARTNERS AT PHOTORECEPTOR SYNAPSES OF THE FLY'S LAMINA. I.A. Meinertzhagen, I.H. Brandstätter and H.S.Seyan. Life Sciences Centre, Dalhousie University, Halifax, N. S. CANADA B3H 4J1. Terminals of photoreceptor cells in the first optic neuropile, or lamina , beneath the fly's *(Musca domestica)*, compound eye lose afferent synaptic sites as part of normal adult development. At each site a presynaptic ribbon abuts a postsynaptic tetrad invariably comprising processes of two monopolar interneurons, L1 and L2, unique occupants of each lamina module, or cartridge. To study the effects of degenerating these postsynaptic elements at the photoreceptor synapses, we severed the axons of L1, 2 in the chiasma, the output tract of the lamina, and examined the structure and frequency of synapses by single-section EM. The head of 7d-adult flies was opened and a lesion made with a fine tungsten needle, then closed and the animals left to recover in a moist chamber from 0.5 to 48hr. Control animals received only a stab wound. After the lesion, L1, 2 degenerated extremely rapidly, shrinking, becoming dark and exhibiting other signs of electron-dense degeneration, even by 0.5hr. post-lesion. Despite the rapidity of this degeneration we found no difference after 0.5hr in the tetrad synaptic frequencies between receptor terminals with L1, 2 degenerated and controls, only a moderate (ca.16%) decrease after 1 - 4hr post-lesion, and around a 30% decrease after 18hr, following which the frequency then increased slightly up to 48hr. Thus, many presynaptic ribbons outlive their postsynaptic elements, and although L1, 2 were heavily degenerated their spines preserved contact with the presynaptic site as long as a ribbon was present. We found no detached presynaptic ribbons, as occur in L2 following the degeneration of the receptor terminals at feedback synapses, suggesting that the tetrads' presynaptic ribbon dismantles directly at the presynaptic site. Other effects following postsynaptic degeneration included: in some cases extensive lamina glial hypertrophy, changes in membrane recycling activity in the receptor terminals, a decrease in the number of receptor mitochondrial profiles, and receptor terminals completely deprived of synaptic vesicles after 48hr post-lesion. Supported by NCE (Ottawa), and NIH (EY-03592) grants to I.A.M..

344.2

SENSORY EXPERIENCE AND THE CONSTRUCTION AND LATER MAINTENANCE OF THE SUPERIOR COLLICULAR MAP OF AUDITORY SPACE IN THE GUINEA PIG. M.J. Keating, K.E. Binns and D.J. Withington-Wray*, National Institute for Medical Research, Mill Hill, London NW7 1AA, UK.

Topographic order in the superior collicular map of auditory space in the guinea pig appears about 32 days after birth (DAB). The 4 day period 26-30 DAB constitutes an experience-dependent crucial period for the normal emergence of the map. Visual or auditory deprivation limited to this period prevents the normal emergence of the map. Bimodality auditory and visual experience limited to this period is sufficient for map emergence. The experience-dependence of the auditory space map is not, however, limited to this single developmental crucial period. Visual or auditory deprivation from 32 DAB disrupts an already established map. Conversely, animals deprived of a normal map by sensory deprivation until 34 DAB generate an ordered map if permitted subsequent visual and auditory experience. The map's responsivity to normal or abnormal sensory experience appears to have an upper time limit. Animals visually-deprived until 100 DAB are unable to generate a map even if permitted subsequent normal experience.

344.3

AUDITORY INPUT TO THE SUPERIOR COLLICULUS (SC) OF ALBINO GUINEA-PIGS: INDICATION OF AN ANOMALY OF THE 'ACOUSTIC CHIASM'. S. Grant* & K. E. Binns* (Spon: BRA). National Institute for Medical Research, London NW7 1AA, UK.

In pigmented guinea-pigs auditory neurons of the SC respond to sounds from restricted spatial locations. At threshold sound intensities for these neurons this location-sensitivity is monaural, based upon excitatory drive from the contralateral ear, but at 15-20dB above threshold requires inhibitory input from the ipsilateral ear (Palmer & King, 1985). We have compared the contributions of the two ears to the spatial sensitivity of auditory SC neurons in pigmented versus albino guinea-pigs, using (i) plugs to (reversibly) occlude an ear and (ii) unilateral cochlea ablation. We found that the ipsilateral ear exerts less influence upon neurons of the albino SC than in pigmented animals: they can maintain appropriate location-sensitivity at suprathreshold sound intensities when this ear is plugged or its cochlea destroyed. Albinism has long been associated with anomaly of the optic chiasm, whereby the ipsilateral eye gains little access to centers of vision. Our results are consistent with an analogous anomaly of the 'acoustic chiasm' in this mutation.

344.4

TRAJECTORY PATTERNS AND MULTIPLE ENDINGS: IMPLICATIONS FOR DEVELOPMENT OF CALYCIFEROUS AXONS IN AUDITORY BRAINSTEM J.M.Zook and N.Kuwabara Dept. of Zool. & Biomed. Sci. & Basic Sci., COM, Ohio Univ., Athens, OH 45701

We have classified calyciferous axons in the auditory brainstem by intraaxonal dye-labeling in a tissue slice preparation. Slices were taken from gerbils, *Meriones unguiculatus*, and mice, *Mus musculus*, of different ages as well as from adult bats, *Eptesicus fuscus*. Each calyciferous axon originates from a globular-bushy cell of the ventral cochlear nucleus, crosses the brainstem in the trapezoid body, and gives rise to a single, large, perisomatic ending (a calyx of Held) within the contralateral medial nucleus of the trapezoid body (MNTB). Each of these axons is characterized by an abrupt change in trajectory just prior to calyx formation. We will discuss this trajectory change in terms of the development of topographic maps in the MNTB.

In tissue slices from mature animals (greater than four weeks old), labeled calyciferous axons usually terminated with a single calyx. In slices taken from young animals (less than two weeks old), around 5 % of the labeled calyciferous axons formed multiple calyces. These axons commonly branched two or even three times, with each branch terminating as a calyx of Held upon a different target cell. Calyx-forming branches usually diverged from the main axons at widely different points, but almost always formed calyces on either two neighboring cells or two distant, but vertically aligned cells. These patterns will be discussed in terms of possible age-related pruning and map refinement in the auditory brainstem. (Supported by NIH Grants NS 26304, NS 01394 and the OUCOM)

344.5

VISION INSTRUCTS THE AUDITORY SPATIAL TUNING OF NEURONS IN THE OPTIC TECTUM OF DEVELOPING BARN OWLS. E.I. Knudsen and M.S. Brainard. Dept.Neurobiology, Stanford University, Stanford, CA 94305.

The optic tectum (superior colliculus) contains auditory and visual maps of space that are mutually aligned: neurons in the tectum respond to auditory and visual stimuli originating from the same restricted region of space and are organized according to the locations of their auditory and visual receptive fields to form a multimodal map of space. The alignment of auditory with visual spatial tuning indicates that tectal neurons are tuned for the values of sound localization cues arising from sound sources at the location of their visual receptive fields.

We have found that the tuning of tectal units for sound localization cues is guided by vision. Barn owls were raised from the day of eyelid opening (14d) viewing the world through displacing prisms that shifted the visual field 23° to the left or right. Once the owls were older than 60 days, they were prepared for extracellular tectal recording. Auditory receptive fields were measured with a movable free field speaker in a sound isolation chamber; visual receptive fields were plotted on a hemispheric screen. Tectal units with visual receptive fields that were located within the optically displaced field of the prisms had auditory receptive fields that tended to be shifted in the direction and, in most cases, by an amount equal to the optical displacement of their visual receptive fields. In contrast, units with visual receptive fields located in areas that were blocked by the spectacle frames exhibited little if any systematic shift in auditory spatial tuning.

The data demonstrate that different portions of the auditory space map are calibrated independently by vision and suggest that the underlying mechanism depends on coincident auditory and visual stimulation from restricted portions of space. Supported by NIH (R01 DC00155-11)

344.6

THE INFERIOR COLLICULUS IS A SITE OF PLASTICITY IN THE VISUAL CALIBRATION OF AUDITORY SPATIAL TUNING IN DEVELOPING BARN OWLS. M.S. Brainard and E.I. Knudsen. Dept. Neurobiology, Stanford University, Stanford, CA 94305.

The optic tectum (superior colliculus) contains aligned auditory and visual maps of space. Barn owls raised with laterally displacing prisms which optically shift the visual map, develop an auditory map of space in which the representation of azimuth is shifted by an approximately equivalent amount (Knudsen and Brainard, Soc. Neurosci. Abstr., vol. 18, 1990). Because the azimuthal tuning of tectal neurons is largely due to their tuning for interaural time difference (ITD), this shift in auditory receptive fields corresponds to a shift in neuronal tuning for ITD. We have found that these vision dependent changes in tuning for ITD reflect changes occurring before the level of the optic tectum, in the external nucleus of the inferior colliculus.

Owls were raised with displacing prisms that shifted the visual field by 23° to the left. Extracellular unit recordings were used to compare the representations of ITD in the central and external nuclei of the inferior colliculus (ICc and ICx) and in the tecta of normal and prism reared birds; ITD tuning was measured using dichotic stimuli delivered via earphones placed in the ear canals.

In normal owls, units at the rostral end of the tectum, ICx and ICc were tuned to 0 μsec ITD (corresponding to a sound source located on the midsagittal plane). In contrast, in prism reared owls, neurons at the rostral end of the tectum and ICx were tuned to 50 μsec right ear leading, indicating that the representation of ITD in both of these nuclei had been altered by prism rearing. However, neurons at the rostral end of the ICc, which provides input to the ICx, were still tuned to approximately 0 μsec ITD. The data suggest that displaced vision alters the pattern of neural connectivity between the ICc and ICx, at the site where the auditory map of space is synthesized.

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344.7

AUDITORY EXPERIENCE MODIFIES THE TUNING FOR SOUND LOCALIZATION CUES OF NEURONS IN THE BARN OWL'S OPTIC TECTUM AND INFERIOR COLLICULUS. J. Moqdans and E.I. Knudsen. Dept. Neurobiology, Stanford University, Stanford, CA 94305.

Neurons in the barn owl's optic tectum (OT) and external nucleus of the inferior colliculus (ICx) have restricted spatial fields as a consequence of their tuning for both interaural intensity differences (IID) and interaural time differences (ITD). Experiments in which barn owls were raised with monaural earplugs have shown that experience with abnormal interaural difference cues during early development leads to an adaptive change in the spatial tuning of tectal neurons.

In the present study we used dichotic stimulation to measure the tuning for IID and ITD of neurons in the OT and ICx in normal birds and in birds raised with monaural earplugs. We found that in earplugged birds the tuning of neurons in both the tectum and the ICx was altered so as to compensate for the monaural impairment: IID tuning was shifted by up to 10 dB and ITD tuning was shifted by up to 50 μs towards the unplugged ear compared with the tuning of neurons at comparable sites in normal animals. These adaptive changes to abnormal auditory cues that occur during early development must therefore be implemented prior to the formation of the auditory space map in the ICx, possibly at the first stages of binaural interaction in the brainstem. We are currently attempting to identify the site in the auditory pathway where these experience-dependent changes take place.

Supported by a DAAD-fellowship and NIH grant R01 DC00155-11.

344.8

NEURONAL ACTIVITY REGULATES GROWTH AND RETRACTION OF ASTROCYTIC PROCESSES

K. S. Canady and E. W Rubel, Hearing Development Laboratories, Univ. of Washington, Seattle, WA 98195

Previously we have shown that blockade of 8th nerve action potentials results in rapid growth of astrocytic processes in the ipsilateral cochlear nucleus. The increase in processes immunoreactive for glial fibrillary acidic protein (GFAP) is greater than two-fold within 3 hours of blocking 8th nerve activity. To assess the reversibility of this astrocytic reaction, we first blocked 8th nerve activity for a minimum of 3 hours with a single intralabyrinthine injection of tetrodotoxin (TTX) and then allowed recovery of normal 8th nerve activity. We then measured the density of astrocytic processes immunoreactive for GFAP in nucleus magnocellularis (NM, the avian cochlear nucleus).

Subdermal electrodes were used to record the click-evoked auditory brainstem response (ABR) in 7 deeply anesthetized 8-10 day old chicks. Peak-to-peak amplitude of the ABR was measured before and after injection of 1 μl 0.3 mM TTX. Animals were re-anesthetized and re-tested 3 hours later to confirm that the duration of activity blockade was sufficient to produce an astrocytic reaction, and again on subsequent days to confirm full recovery of inner ear function. One week after TTX injection, the animals were sacrificed and tissue sections from the brain stem were prepared for GFAP immunocytochemistry.

Chicks sacrificed during the blockade of 8th nerve action potentials showed a mean increase in GFAP immunolabeling of 120% in the ipsilateral NM. This confirms that astrocytic processes proliferate during periods of prolonged neuronal inactivity. In contrast, chicks allowed to recover normal 8th nerve activity after TTX injection showed no difference in GFAP immunolabeling between the control and experimental NM. These results suggest that the regulation of astrocytic processes by neuronal activity is bidirectional: A loss of neuronal activity results in astrocytic process growth, while the return of neuronal activity causes a retraction of these reactive astrocytic processes. (Supported by PHS grant DC00393.)

344.9

NEURONAL ACTIVITY REGULATES ASTROCYTIC PROCESSES IN A BRAIN SLICE PREPARATION OF THE AVIAN AUDITORY SYSTEM. R.L. Hyson, K.S. Canady, E.W Rubel, Hearing Development Laboratories, Univ. of Washington, Seattle, WA 98195.

Afferent activity regulates the structure and metabolic activity of both neurons and glial cells in the second-order auditory nucleus, nucleus magnocellularis (NM) of the chick. Within hours of eliminating afferent activity, NM neurons have altered metabolic activities, and glial cell processes proliferate. Previous evidence from an *in vitro* brain slice preparation indicates that activity-dependent regulation of NM *neurons* requires Ca^{+2}-dependent release of some "trophic substance" from presynaptic auditory nerve terminals. In the present experiment, we determined whether activity-dependent regulation of glial processes has similar requirements.

Brain stem slices containing portions of the auditory nerve and NM bilaterally were maintained *in vitro*. In different groups of slices, NM on one side of the slice was unstimulated while NM on the other side was either: 1) Orthodromically stimulated, via electrical activation of the ipsilateral auditory nerve, 2) Antidromically stimulated, via electrical activation of NM neuron axons, or 3) Orthodromically stimulated in a medium containing low Ca^{+2} and high Mg^{+2} concentrations. Control slices were unstimulated on both sides. After receiving one of these treatments for 3 hrs, the slice was fixed, sectioned and processed using GFAP immunocytochemistry. The amount of immunoreactivity was compared in NM on the two sides of the same tissue sections. Just as *in vivo*, less GFAP immunoreactivity was observed on the stimulated side of the slice. This difference occurred under all three stimulation conditions. Thus, GFAP expression can be regulated *in vitro* by neuronal activity. Unlike neurons, the glial cells respond to local changes in neuronal activity without requiring Ca^{+2}-dependent synaptic release of neurotransmitter or trophic substances. This suggests that glial cell processes respond to local changes in the ionic environment resulting from altered neuronal activity. (Supported by PHS grant DC00393)

344.10

Additive Functional Subcortical Changes Contribute to Cortical Alterations Following Sensory Training. CL Hand, RL Craik, KG Gallo*, & PJ Hand. Idaho St. U., Pocatello, ID 83209, Beaver Coll., Glenside, PA 19038, & Univ. of PA, Phila., PA 19104.

To complete analysis of functional CNS changes resulting from sensory enrichment training of a spared rat vibrissa, analysis of subcortical vibrissal relays was undertaken. Bilateral vibrissa sparing/unilateral associatively-paired (AP) training was undertaken in 10 rats. Subtotal vibrissa deafferentation spared bilateral C3 vibrissae (SC3) before postnatal day 3. AP training (associatively pairing vibrissa stroking with sugar water) of L or R SC3 (SC3/AP) was 5 min/ day x 60 days. Behavioral and 2DG metabolic analyses previously revealed significant alterations in exploratory behavior and an increase in SC3/AP cortical area of 34.9%.

Present analysis of functional SC3 representations within subcortical relays reveals additive subcortical contributions to the functional plasticity observed in cortex. Areal increases for AP trained SC3 representations (compared to controls) within trigeminal brainstem complex (principalis, oralis, interpolaris & caudalis) averaged 10.8%. In VBm thalamus, areal increases for AP/SC3 averaged 22.0%. While functional area was altered along the pathway, the absolute level of functional activity within the altered area remained relatively unchanged. Alterations in cortical area following vibrissal training are partly the result of a cortical phenomenon and partly an additive effect of plasticity within the subcortical relays. NS22283-04.

344.11

ELECTRON MICROSCOPIC ANALYSIS OF THE RAT INFRAORBITAL NERVE ONE WEEK FOLLOWING AXOTOMY AT BIRTH. J. Golden, D.S. Zahm and M.F. Jacquin. St. Louis Univ. Sch. Med., St. Louis, MO 63104.

A great deal is known about the central consequences of infraorbital nerve injury at birth in rodents. However, little is known regarding the short-term effects upon the organization of the infraorbital nerve. This information is fundamental to our understanding of injury responses in the developing trigeminal system. As a first step towards filling this void, 3 newborn rats were subjected to left infraorbital nerve section. They were perfused one week later and the 3 left infraorbital nerves were removed at a point proximal to the lesion, processed for EM analysis and counted at a final magnification of 4300X. The 3 nerves contained 16, 18, and 24 fascicles. These values do not differ significantly from the normal adult, but they are higher than the 8-15 fascicles observed in the newborn (Renehan and Rhoades, Brain Res. 322:369, '84). Axon counts revealed 594, 913, and 715 myelinated, 6,717, 9,317, and 7,852 unmyelinated, and 117, 207, and 111, degenerating myelinated axons, respectively. In total there were 7428, 10,437, and 8,678 axons in the 3 nerves. These totals are well below those reported for both the normal adult (19,740 myelinated, 13,319, unmyelinated) and newborn infraorbital nerves (168 myelinated, 42,051 unmyelinated). In an adult nerve counted after the same lesion at birth (Mooney et al., Soc. Neurosci. Abstr. 9:, '83), 5,260 myelinated and 2,747 unmyelinated fibers were observed. Most of the ganglion cell death occurs during the first postlesion day (Henderson and Jacquin, this volume) and cortical responses to peripheral stimuli cannot be recorded until 7 days after this lesion (Waite and Cragg, Proc. Roy. Soc. Lond. B 214:191, '82). The present data suggest that degeneration and remyelination are incomplete by postnatal day seven. Support: DE07734, DE07662, NS23805.

344.12

NEONATAL INFRAORBITAL NERVE SECTION PRODUCES INCREASED CHOLECYSTOKININ (CCK) LEVELS IN RAT MEDULLA. M.C. Beinfeld, R.W. Rhoades, C.A. Bennett-Clarke, N.L. Chiaia & M.F. Jacquin. Dept. of Pharmacology and Dept. of Anat. & Neurobiol., St. Louis Univ. Sch. Med., St. Louis, MO 63104., Dept. of Anat., Med. Coll. of Ohio, Toledo, OH 43699.

Infraorbital nerve section at birth produces a profound deafferentation of the trigeminal brainstem complex that spares the innervation of laminae I and II of the medullary dorsal horn. Enfiejian et al. (Somatosens. Mot. Res. 6:537, '89) and Rhoades et al. (J. Neurosci. 8:2234, '88) have also demonstrated normal levels of substance P in the deafferented medulla by radioimmunoassay (RIA) and immunohisto- chemistry. Here, CCK-specific RIA and immunohistochemical methods were used to assess CCK levels in the medullary dorsal horn in adult rats subjected to left infraorbital nerve cut at birth. RIA showed that the left medulla contained 31% higher levels of CCK than the control right medulla (1.27 $\pm$ 0.19 vs. 0.97 $\pm$ 0.11 ng/mg protein). This increase was observed in 8 of 10 animals and was statistically reliable ($N = 10$: paired t-test, $p < .05$). Immunohistochemistry revealed a dense band of CCK-like staining in laminae I and II ipsi- and contralateral to the lesion ($N = 7$). Densitometric analysis showed a 7.5 $\pm$ 2.8% increase in immunoreactivity from sections on the left side ($P < .01$). These results indicate that CCK immunoreactivity, most probably of primary afferent origin (Clements and Beitz, Neurosci. 20:427, '87), is selectively preserved after neonatal nerve transection. This finding differs from that we have previously obtained for SP in that CCK increased to significantly supranormal levels. Thus, CCK-containing primary afferents may respond differently to neonatal nerve damage than other V ganglion cells. Support: DE07734, DE07662, BNS8517357, NS18667.

344.13

EARLY POSTNATAL DEVELOPMENT OF THE RAT CORTICOTRIGEMINAL TRACT. M.R. Wiegand & W.E. Renehan. Anatomical Sciences & Neurobiology, Univ. of Louisville School of Medicine, Louisville, KY 40292.

The carbocyanine dye, dil, was used to study the early postnatal development of the corticotrigeminal tract in the rat. Dil was placed in the presumptive barrel cortex of fixed brain tissue in neonatal rat pups on postnatal days (PND) 0-14. Descending corticotrigeminal fibers reached the level of the pyramidal decussation by PND 0, at which time sparse label could be seen along the medial aspect of ipsilateral principalis. Subsequent corticotrigeminal development proceeded in a rostral to caudal, ipsilateral to contralateral sequence. On PND 1, corticotrigeminal axons were labeled in all ipsilateral and the medial portion of rostral contralateral subnuclei. The density of the ipsilateral label began to decrease in all subnuclei with the exception of principalis by PND 2-3, with a concomitant increase in contralateral projections. Fibers were observed crossing to contralateral subnuclei at all levels by this age. By PND 4-5, contralateral corticotrigeminal projection morphology approached that of the adult, although persistent ipsilateral projections remained, especially in principalis. The adult-like pattern of corticotrigeminal fiber label was apparent by PND 6.

Rat corticotrigeminal development follows a rostral to caudal, ipsilateral to contralateral sequence that appears to evolve after the establishment of previously documented afferent system connections. This pattern contrasts with the caudal to rostral developmental sequence described in the cat (Tolbert et al., JCN, 1984). Support: NIH DEO7734

344.14

THE ABILITY OF AXOTOMIZED AFFERENT FIBERS IN SURAL NERVE TO MODULATE THE FLEXION REFLEX. J.X. Bao. Dept. of Neuroscience, University of Florida, Gainesville, FL 32610.

Intracellular studies have shown that axotomized myelinated afferent fibers in the sural nerve elicit apparently normal postsynaptic potentials in triceps surae motoneurons (Nishimura et al., Soc. Neurosci. Abstr. 15: 920, 1989). Unmyelinated fibers in the normal sural nerve facilitate the flexion reflex (Wall et al., J. Physiol. 356: 443-458, 1984). The goal of this study was to determine whether there was still this facilitative effect of the unmyelinated fibers after axotomy.

In an initial procedure,the sural nerve was ligated and capped with Gore-Tex tube. After two to three weeks the animals were decerebrated and spinalized. The magnitude of the flexion reflex was recorded in filaments of biceps femoris and semitendinous nerve to pinches of the toes or electrical stimuli of sural nerve before and after 20 seconds high intensity C fiber conditioning stimulation (0.5ms, 4mA, 1 HZ).

The normal sural nerve facilitated the flexion reflex for approximately 30 seconds to 2 minutes (23/26 flexor motoneurons). The facilitation effect not only existed in the sural nerve: the common peroneal nerve could produce a similar action. After axotomy, no flexor motoneurons (11 motoneurons) showed this facilitation effect, in spite of the fact that the normal unmyelinated fibers in the common peroneal nerve could still induce this facilitation. This suggests that the facilitative ability of the unmyelinated fibers in the sural nerve depended on the intact target innervation, although the myelinated fibers in the sural nerve did not have this target dependency. Acknowledgement. This work is supported by a NIH NS15913 grant to Dr. J.B. Munson and a grant from the state of Florida. The advice from Drs. J.B. Munson, R.P. Johnson, and F.J. Thompson are highly appreciated.

344.15

THE EFFECTS OF COLD STRESS ON THE INDUCTION OF C-FOS IN DEVELOPING RATS. M.P. Joyce and G.A. Barr. Biopsychology Doctoral Program, Dept. of Psychology, Hunter College, CUNY, New York, N.Y. 10021

As an animal matures, the nature of the behavioral and physiological responses to cold temperature changes. Young rat pups appear to rely predominantly on behavioral mechanisms, while internal thermoregulatory mechanisms predominate in older rats. The neural response to cold is mediated by several hypothalamic and extrahypothalamic nuclei. The response of these sites to decreased environmental temperatures changes as the animal develops. Recently, the induction of the oncogene c-fos has been shown to be a reliable indicator of cellular depolarization. The present study uses fos protein as a marker to examine changes in the neural response to cold in developing rats. Rats, 1-60 days of age, were exposed to an environmental temperature of 15°C for one hour and then sacrificed immediately or 1, 2, or 3 hours after removal from the cold. The biotin-avidin HRP method was used to label fos-like immunoreactivity. Fos-like nuclear labeling was observed in the POAH, posterior hypothalamus, PVN of the thalamus and hypothalamus, dorsomedial nucleus of the hypothalamus, lateral septal nuclei, nucleus of the diagonal band and the lateral habenula. In rats 28 days and older, fos-like labeling was observed in animals that were sacrificed immediately after removal from the cold. In younger pups, labeling was not apparent until 1 hour after removal from the cold.

344.16

DEVELOPMENT OF NERVES AND TEETH IN THE RAT MANDIBLE. C.S. Johansson* and C. Hildebrand. Dept. of Cell Biology, Faculty of Health Sciences, University of Linköping, 581 85 Linköping, Sweden.

The aim of this study is to provide a description of the anatomy of the inferior alveolar nerve (IAN) in the rat mandible and of the structural development of this nerve and its dental targets. The first signs of mineralization occur in the incisor and molar 1. Molars 2 and 3 follow later. The incisor erupts during the first week after birth and the first and second molars erupt by 3 weeks, when the pups are being weaned. Shortly after entrance in the mandibular canal the IAN splits into the mental nerve and a common dental branch. The latter emits separate branches to each molar root and to the incisor. The length of the IAN increases 3 times between birth and adulthood. The total number of IAN axons is about 5,000 neonatally and reaches a maximum of 12,500 9 days after birth. From 2 weeks on the number of axons is about 9,000. Myelination begins at birth, and the adult proportion of myelinated axons is established by 3 weeks. From a qualitative point of view the fine structure of the IAN undergoes a rapid development after birth, particularly during the first week. Signs of myelin sheath remodelling occur at the end of the first postnatal week. In the adult rat the size distribution of myelinated IAN fibres is bimodal and the largest fibres reach a diameter of 11 µm. Internodal length (L) increases slowly with increasing fibre diameter (D), reaching a maximal L-value of about 750 µm at D values around 11 µm.

344.17

EFFECTS OF EARLY POSTNATAL RECEPTOR DAMAGE ON DENDRITIC DEVELOPMENT IN GUSTATORY RECIPIENT ZONES OF THE ROSTRAL NUCLEUS TRACTUS SOLITARIUS (NTS) IN RAT. *P.S. Lasiter and D.L. Kachele. Florida Atlantic University, Boca Raton, FL. 33431 and Ohio State University, Columbus, OH, 43210.*

We have previously shown that damage induced to lingual gustatory receptors of the anterior tongue at postnatal day 2 (P2) specifically alters the developmental expansion of chorda tympani and greater superficial petrosal nerve (CT/GSP) axons within the rostral nucleus tractus solitarius (NTS). Receptor damage also decreases the planar distance between neurons in the CT/GSP terminal field that project axons to the second-order gustatory relay in the caudal parabrachial nucleus (PBN). These findings led us to speculate that, in addition to presynaptic effects, receptor damage may alter normal dendritic development in the CT/GSP terminal field. To examine this question Golgi impregnation studies were performed following unilateral receptor damage at P2. First, planar length of first- and second-order dendrites of multipolar neurons belonging to morphological classes associated with PBN projection neurons are significantly decreased following receptor damage. Second, the mean number of second-order dendritic branches/neuron is significantly reduced ipsilateral to damage, as compared to neurons contralateral to damage. These results confirm that early receptor damage specifically alters the development of both pre- and postsynaptic structures in the CT/GSP terminal zone. Supported by NIDCD Grant No. DC00732 to P.S.L.

344.18

ASSESSMENT OF SPIRAL GANGLION CELL DEVELOPMENT IN THE POSTNATAL HAMSTER. D.D. Simmons[1,2], L.K. Manson-Gieseke[1,2], M.S. Rogers[1], and P. Sahgal[1] [1]Natural Science Division, Pepperdine University, Malibu, CA and [2]Dept. of Biology, UCLA, Los Angeles, CA.

The postnatal development of spiral ganglion cells (SGCs) in the hamster was studied from birth (postnatal day 0) until the young adult stage (> PND 21). Cochleae were processed using standard light and electron microscopic techniques and data were limited to the modiolar portion of the cochlear nerve and the basal 40% of the spiral ganglion. In the young adult hamster cochlea, two types of SGCs were easily discernible. The majority (92%) of SGCs had cytoplasmic-to-nuclear density (CND) ratios greater than 1, whereas the remaining 8% had CND ratios less than or equal to 1. The darkly staining SGCs were always found in the lower 30 percentile of cell areas measured, but there were no differences in cytoplasmic-to-nuclear area (CNA) ratios between the two cell types.

From PND 0 to 3, SGCs exhibited roughly equal CND and CNA ratios, cell areas between 34 - 53 μm^2, multiple nucleoli and no evidence of myelination. By PND 3, the cochlear nerve fibers exhibited several layers of myelin lamellae. From PND 3 to 7, SGCs increased in cell size by almost 65%. By PND 7, nearly all of the SGCs were myelinated and the number of cells with multiple nucleoli was reduced drastically. By PND 10, there were clearly two cell types present as in the young adult animal. Few morphological changes occurred after PND 13 in either SGCs or their nerve fibers. These results are discussed in relation to the morphological maturation of innervation patterns in the organ of Corti.

This research was supported by grants [1]BNS 8719610 (NSF), and a grant from the Ralph M. Parsons' Foundation.

SENSORY SYSTEMS—DEVELOPMENT AND PLASTICITY III

345.1

MIGRATION OF CELLS FROM THE OLFACTORY EPITHELIUM OF ADULT INTACT RODENTS. A.G. Monti Graziadei, Dept. of Biological Science, Florida State Univ., Tallahassee, FL 32306.

Previous studies of partial and total olfactory bulb removal in adult rodents have shown that cells can exit from the olfactory epithelial compartment, migrate along the olfactory nerve and reach, intracranially, the nerve layer of the olfactory bulb. It has also been reported that in adult unoperated mice, which had received one injection of tritiated thymidine from three days to 24 hours before sacrifice, clusters of autoradiographically labeled cells were present in the lamina propria of the olfactory mucosa.

In order to ascertain if cell migration from the olfactory epithelium occurs in normal rodents, 5 weeks old rats and 4-5 months old mice, which did not undergo any sort of experimental manipulation, were sacrificed by perfusion with Bouin's fixative. The heads, embedded in paraffin, were serially sectioned and stained with hematoxylin. Migrating cells (MC) were present in both animal species, but differences were observed in regard to the areas of olfactory epithelium from which they migrated out. In rats, the MC formed cords originating from the olfactory epithelium covering the recesses of the olfactory cavity. The cords reached the nerve layer of the olfactory bulb. In mice, clusters of cells migrated out from the olfactory epithelium of the nasal septum and turbinates. These results suggest that the mechanism regulating cell migration in normal animals may be species-specific. NIH NS20699.

345.2

TIME COURSE OF UNILATERAL OLFACTORY DEPRIVATION EFFECTS ON OLFACTORY BULB FUNCTION. D.A. Wilson. Developmental Psychobiology Laboratory, Dept. Psychology, U. of Oklahoma and J. Wood, Dept. Anatomical Sciences, Oklahoma U. Health Sciences Center.

Olfactory deprivation effects on olfactory bulb neuroanatomy are dependent on the age at which the deprivation occurs. The present study examined the age-dependent affects of deprivation on olfactory bulb physiology and neurochemistry.

Rat pups were cold anesthetized at PN1 or anesthetized with ketamine/xylazine on PN20 and had a single naris cauterized. Control pups were cauterized on the top of the snout. Ten, 20 or 40 days later, rats were anesthetized with urethane and evoked responses to single and paired shocks of either the lateral olfactory tract (LOT) or olfactory nerve (ON) were examined. The results suggest that paired-pulse inhibition induced by stimulation of the LOT is enhanced by early deprivation but not by deprivation occurring later. On the other hand, preliminary results suggest that inhibition induced by paired-pulse stimulation of the ON is enhanced after 40 days of deprivation, regardless of the age at onset. The relationship between these functional effects and levels of olfactory bulb dopamine will be presented.

345.3

UNILATERAL NARIS CLOSURE AND PROTEIN SYNTHESIS WITHIN THE OLFACTORY MUCOSA. J.S. Stewart, D.L. Korol, and P.C. Brunjes. University of Virginia, Charlottesville, VA 22903.

Blocking airflow through one side of the nasal cavity during early life produces dramatic changes in olfactory bulb growth including enhanced cell death and reductions in laminar volume. These structural changes appear to follow a long chain of cellular events, which include rapid (within 24 hours) alterations in glucose metabolism and protein synthesis. Structural changes have also been reported in the olfactory mucosa (Farbman, et al., J. Neurosci. *8*, 1988), but these occur long after widespread alterations are seen in the bulb. In the present study we sought to determine if naris closure rapidly alters the cellular function of the olfactory receptor sheet. Rat pups underwent sham surgery or unilateral naris closure on postnatal Day 1. Twenty-four hrs later, rates of protein synthesis were examined by administering ^{3}H-leucine (i.c., 30 min pulse), and processing tissue for standard emulsion autoradiography. Grain densities were determined at three dorsal/ventral locations on the caudal portion of the nasal septum. No left/right differences were found at any location. Therefore, the rapid protein synthetic changes occurring after naris closure in the bulb do not result from similar cellular changes in the mucosa.

Supported by DC00338, HD18411, and HD07323.

345.4

UNILATERAL NARIS CLOSURE AND PROTEIN SYNTHESIS WITHIN THE OLFACTORY BULB. D.L. Korol, A. Rao, O. Steward and P.C. Brunjes. Univ. of Va., Charlottesville, VA 22903.

Closure of one external naris on postnatal day (P) 1 in the rat leads to a constellation of changes resulting in decreased ipsilateral olfactory bulb size. One of the earliest changes is a rapid reduction in protein synthesis across all bulb laminae (Korol & Brunjes, Dev. Brain Res. *52*, 1990). The present study extends these findings by examining which protein classes are altered. We evaluated the extent of incorporation of ^{35}S-methionine into protein. Whole olfactory bulbs were removed 24 or 48 hours after occlusion on P1 and incubated with label in vitro. SDS-PAGE gels and their fluorograms were prepared for 1-d analysis, and used to examine the extent of amino acid incorporation into different protein classes. While the pattern of protein separation seen with Coomassie blue staining appeared similar for control and deprived bulbs, the amount of synthesis was reduced in deprived bulbs. Initial examination suggested that the effects were not limited to specific classes of proteins: decreases in precursor incorporation appeared across all molecular weight bands. These data suggest that the effects of deprivation on protein synthesis reflect general changes in "housekeeping" events. Perhaps these are primary events in the cascade of changes following deprivation.

Supported by DC 00228 and HD 07323.

345.5

A MORPHOLOGICAL AND QUANTITATIVE INVESTIGATION OF OLFACTORY BULB DEVELOPMENT IN THE CLAWED FROG, *XENOPUS LAEVIS*. C.A. Byrd and G.D. Burd. Depts. of Molecular & Cellular Biology and Anatomy, University of Arizona, Tucson, AZ 85721.

We analyzed the larval development of the olfactory bulb (OB) from stage 29 to 58. The axons of olfactory receptor cells (ORC) begin to contact the neural tube at stage 29 (Klein and Graziadei, 1983). At this stage, the neural tube only contained a few differentiating cells. By stage 33, ORC axons were visible at the surface of the neural tube, and mitral cells were present immediately below the axons. Glomerular neuropil was first observed at stage 39, and individual glomeruli were evident by stage 45. Also at stage 45, the neuropil in the mitral cell/external plexiform layer began to develop. By stage 48, the accessory olfactory bulb (AOB) was distinct, and all normal adult layers were present in the main olfactory bulb (MOB).

We also examined the quantitative relationship between the ORC axons and the neurons in the mitral cell/external plexiform layer (MCL/EPL) of the MOB and AOB of tadpoles between stages 50 to 58. The number of ORC axons was obtained from EM montages, and the number of neurons in the MCL/EPL was determined from serial sections through the OB. The number of mitral cells was correlated with the number of ORC axons throughout larval development (n=11, r=0.9935), and the ratio of ORC axons to mitral cells was 4.4 ± 0.15. In conclusion, even though the OB continues to grow in size and neuron number, the relationship between the mitral cells and the sensory afferents remains constant.

Supported by BRSG and NIDCD #DC00446.

345.6

CORRELATED FIRING IN CLOSE AND DISTANT PAIRS OF MITRAL CELLS IN MOUSE OLFACTORY BULB. M. E. Lickey. Dept. of Anat. and Neurobiol., Wash. Univ. Sch. of Med., St. Louis, MO 63110

Neighboring sensory neurons, e.g. retinal ganglion cells, show positive temporal correlation of firing during periods of spontaneous activity. In brain development, correlated firing may be a cue for the formation of functional modules in the cortex, e.g. ocular dominance columns. Here I ask whether correlated firing can possibly help specify the morphology and connections of olfactory glomeruli. Using glass micropipets I recorded spontaneous extracellular spikes simultaneously from pairs of olfactory mitral cells that were either near neighbors (<100 microns apart) or far apart (>700 microns). A correlation coefficient was calculated for each pair based on spike frequencies during 20 to 50 activity samples spanning 3 to 30 minutes. While only a minority of pairs was positively correlated, near neighbors were more often positively correlated than widely separated cells. These results are consistent with the possibility that correlated firing of olfactory afferents is a cue for the formation of glomeruli. (USPHS NS 11699 to D. Purves and McDonnell Center for Cellular Neurobiology)

345.7

RECEPTIVE FIELD PROPERTIES OF TRIGEMINAL GANGLION CELLS IN FETAL RATS. N.L. Chiaia, G.J. Macdonald* and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

The peripheral and central connections of trigeminal (V) ganglion cells guide the aggregation of neurons and axons characteristic of the rodent's central V neuraxis. As yet, however, no one has evaluated the peripheral projections of individual V ganglion cells at the time when central patterns corresponding to the periphery are beginning to develop. We mapped the receptive fields of single V ganglion cells on embryonic (E-) days 18 (44 cells), 19 (40 cells), and 20 (53 cells). The data from the 3 ages are generally the same so they will be considered together. The receptive fields of V ganglion cells in fetal rats E-18 and older were no larger than those in mature animals. Specifically, we tested 44 cells that responded to stimulation of vibrissa follicles and 95.4% were discharged by indentation of only a single follicle. In adult rats, all vibrissa-sensitive V ganglion cells respond to deflection of only a single whisker. Trigeminal ganglion cells from fetal rats differed from those in adult animals in that nearly all (97.0%) gave rapidly adapting responses. Only 9 of the V ganglion cells that we recorded had any spontaneous activity (invariably <0.1 spikes/s) and they were all recorded from E-18 fetuses. These results demonstrate that individual V ganglion cells have tightly focused peripheral projections well before central patterns characteristic of the mature V neuraxis can be detected. Supported by BNS 85 17537, DE 07734, DE 08971, and the State of Ohio Research Challenge.

345.8

THALAMIC AND CORTICAL REORGANIZATION AFTER CHRONIC TRANSECTION OF THE MEDIAN AND ULNAR NERVES IN ADULT MONKEYS. P.E. Garraghty, R.D. Clower*, E.A. LaChica, and J.H. Kaas. Dept. Psychology, Vanderbilt Univ., Nashville, TN 37240.

Plastic changes are reliably observed in somatosensory cortex of adult monkeys after peripheral nerve manipulations. Questions remain, however, regarding: 1) the cortical distance across which somatotopic map reorganization can occur, 2) the extent to which earlier processing stations contribute to the reorganization, and 3) the characterization of the mechanism(s) underlying the reorganization. We have begun addressing these questions by studying the effects of chronic denervation of the entire volar surface of the hand in adult squirrel monkeys on the somatotopic maps in the ventroposterior lateral nucleus (VPL) of the thalamus and cortical areas 3b and 1. We have also employed antibodies against gamma-aminobutryic acid (GABA) to determine the role, if any, of this transmitter system in the reorganization. Within two months of the transections, we found: 1) that the entire expanse of deprived cortex had regained somatotopically organized responsivity to cutaneous stimulation of the dorsal surface of the hand and digits; 2) that a parallel reorganization was present in VPL; and 3) that staining for GABA was substantially decreased throughout the deprived zone of cortex. Thus, under these conditions, the cortical distance limit for injury-induced reorganization is no less than 3-3.5 mm. Finally, the somatotopic reorganization evident at the level of cortex is accompanied by changes in thalamic somatotopy and cortical GABAergic activity. (Supported by N.I.H. NS16446.)

345.9

NEURON MORPHOLOGY IN SOLITARY NUCLEUS DURING FUNCTIONAL DEVELOPMENT OF TASTE PATHWAYS. C. M. Mistretta and M. Womble. School of Dentistry, Univ. of Michigan, Ann Arbor, MI 48109.

To learn whether postsynaptic neuron morphology alters in association with developmental changes in taste response and receptive field properties, we are studying a functionally defined region of nucleus of solitary tract (NST) in sheep. Age groups include fetuses at 100, 110 and 130 days of gestation (term=147 days), perinatal animals, and postnatal lambs aged 30 to 50 days. Neural recordings identify the NST area with high frequency responses to NaCl and receptive fields on tongue tip, and an electrolytic lesion is made. Neurons are reconstructed from Golgi Cox preparations in an area around the lesion. In 100 day fetuses neurons are too immature to distinguish among types, but by 110 days elongate, multipolar and tufted cells are evident. Dendritic length and total branch points increase between 100 to 110 days, and subsequently. There is, however, an apparent dendritic reorientation in postnatal lambs. Neurons have large numbers of spines across all age groups. Thus, NST neurons acquire basic morphological features when peripheral responses to NaCl are still weak and in advance of a period of major afferent convergence. Subsequent changes in dendritic extent and orientation take place in association with functional alterations. (NIH Grant DC00456)

345.10

EFFECTS OF NEONATAL INFRAORBITAL NERVE SECTION ON THE CORTICOTRIGEMINAL PROJECTION (CTP) IN RAT. W.E. Renehan, M.R. Wiegand, S.S. Stansel & M.F. Jacquin. Anatomical Sciences & Neurobiology, University of Louisville School of Medicine, Louisville, KY 40292, and Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

The normal adult CTP is robust in contralateral trigeminal brainstem subnuclei caudalis, interpolaris and principalis and relatively weak in contralateral oralis and all ipsilateral subnuclei. Few CTP fibers terminate in the substantia gelatinosa of caudalis, but the magnocellular portion of this subnucleus exhibits a dense projection that tends to surround each vibrissal "barrelette." In the present study, anterograde WGA-HRP tracing methods were used to study the CTP in adult rats subjected to unilateral infraorbital nerve section at birth. Adult animals received WGA-HRP injections in the posteromedial barrel subfield of the somatosensory cortex contralateral or ipsilateral to the lesioned infraorbital nerve.

Cortical injections contralateral to the lesion revealed the following changes: 1) An increase in the density of the CTP to the substantia gelatinosa ipsilateral to the lesion. 2) Absence of a vibrissae-related pattern in the trigeminal brainstem complex on the same side as the lesion. 3) An increase in the density of the projection to rostral trigeminal subnuclei on the side opposite the lesion.

Injections ipsilateral to the lesion revealed a deafferentation-induced maintenance of a substantial ipsilateral CTP (Wiegand and Renehan, this volume). These data suggest that both afferent instructions and target factors play a role in the development of the corticotrigeminal projection pattern. They also are consistent with a similar study in kittens (Westrum et.al. '83, Soc. Neurosci. Abs.). Support DE07734 and DE07662

345.11

NEUROGENIC PERIOD OF LONG ASCENDING PROPRIOSPINAL NEURONS (LAPNs) IN THE LUMBAR SPINAL CORD OF THE RAT. K.N. Nandi, D.S. Knight* and J.A. Beal. Dept. of Cellular Biology & Anatomy, LSU Medical Center, Shreveport, LA 71130.

The purpose of this study was to determine the neurogenic period of LAPNs in the rat which project from the upper lumbar spinal cord to the cervical enlargement. To determine neuronal birthdates, tritiated thymidine was administered to fetal rats on embryonic (E) days E12 thru E16 via i.p. injection of the dam. To label LAPNs in the lumbar cord, using 40 to 50 day old pups of tritiated thymidine treated dams, Fluoro-Gold (FG) was injected via a micropipette into the cervical (C6) gray matter. FG labeled LAPNs were observed in all laminae of the gray matter on both the side ipsilateral and the side contralateral to the injection site. LAPNs undergoing mitosis were doubled labeled, i.e. labeled with both FG and tritiated thymidine. On day E12, all LAPNs exhibited tritiated thymidine label in their nuclei. Less than 5% of LAPNs were found still proliferating late on day E15. These late proliferating LAPNs were confined mainly to laminae I-III of the dorsal horn on both the ipsi- and contra-lateral sides. Results show that in rats, LAPNs continue to proliferate throughout most of the neurogenic period for spinal neurons (E12-E16) and beyond the neurogenic period of long projection neurons of the ascending tracts (Nandi, et al., 1990). Supported by NSF grant #BNS-8908601.

345.12

DEVELOPMENT OF APICAL, BASAL, AND MIXED DENDRITES OF FUSIFORM CELLS IN THE DORSAL COCHLEAR NUCLEUS OF THE HAMSTER. L. Schweitzer. Anatomical Sciences and Neurobiology, University of Louisville School of Medicine, Louisville, KY 40222.

Fusiform cells in the dorsal cochlear nucleus of the adult hamster have apical and basal dendrites which emanate from opposite poles of the cell body, are located in separate layers, receive different inputs, and have distinct appearances. The development of the dendrites was assessed using morphometric analyses of Golgi-impregnated brains from hamsters of various ages. In addition, a third type of dendrite that exits the cell laterally was analyzed. The distal branches of the laterally-extending dendrites end either near the apical dendrites, near the basal dendrites, or near both the apical and basal dendrites. Branches of laterally-extending dendrites that end near apical dendrites are qualitatively and quantitatively identical to the other apical dendrites (that is they are long dendrites with many short, spine-laden branches) and the converse is true of the branches ending near basal dendrites. This is also true for the distal portions of **mixed** laterally-extending dendrites that split and extend both apically and basally. That is, branches near apical dendrites have apical-like features and branches near basal dendrites have basal-like features. While it is possible that signals originating in the cell body could be transported down a common dendritic trunk and direct differential maturation of the apical and basal dendrites, it seems more likely that during development the environment surrounding these disparate distal branches determines their length, branching pattern, and appearance. NS-20162

345.13

TIME ANALYSIS OF RECEPTIVE FIELD(RF) REORGANIZATTION IN SI OF SPINAL KITTENS. C.Chau and P. McKinley. School of Physical and Occupational Therapy, McGill Univ.,Montreal, Quebec, H3G 1Y5.

McKinley et al (J.Neuroscience,in press,1990) showed that the reorganization of primary somatosensory cortex after T12 spinal cord transection at 2 weeks of age is robust. This study addresses the sequential nature of the re-shaping of the RF representation in this age group. Somatosensory cortex is mapped at either 3,6 or 9 weeks post-cordotomy (multiunit recording). The % non-cutaneous response among the three groups are comparable suggesting that somatotopically inappropriate RF representation is already present at 3 weeks post-cordotomy. As time progresses, there is an increase in representation of rostral body parts in hindlimb cortex suggesting that the more inappropriate the input, the longer it takes to drive a cortical area. Finally, the evolution of RF characteristics suggest that RF shaping is complex in the trunk. The final appearance of rostral-caudal RF orientation may result from an initial amalgamation of narrow dermatomal strips running dorsal-ventrally followed by a subsequent lateral inhibition (in a dorsal-ventral direction) of the amalgamated RF. sponsored by MRC.

345.14

A MODEL OF DORSAL COLUMN FUNCTIONAL INTEGRITY IN THE RAT. J.P. Vicedomini, J.G. Broton and R.P. Yezierski, The Miami Project to Cure Paralysis, University of Miami School of Medicine, Miami, FL

Cord dorsum potentials evoked by peripheral (tibial N.) or dorsal column (DC) stimulation were studied in the lumbosacral (LS) cord and dorsal column nuclei (DCN) in urethane anesthetized rats. Orthodromic and antidromic responses produced by applying stimuli at various voltages and repetition rates were analyzed. Antidromic responses were obtained from the LS cord and tibial N. following cervical cord (C1) stimulation. Reversible (DC lidocaine) or irreversible (mechanical) lesions were made to identify pathways responsible for orthodromic or antidromic responses. Orthodromically evoked LS and DCN potentials showed: (1) decreases in latency with increasing stimulus intensity paralleled by increases in response strength and DCN response duration; and (2) greater amplitude attenuation evoked by high frequency stimulation in DCN vs. LS responses. Antidromic LS or tibial N. potentials: (1) had nearly constant latencies at all stimulus intensities tested, but increased in amplitude and strength with greater stimulus intensities; and (2) exhibited different sensitivities to high frequency stimulation, with tibial N. potentials following repetition rates $\geq$500 Hz, and LS potentials attenuating and failing at about 200Hz. Lesioning techniques showed that orthodromic DCN responses were abolished by DC plus dorsolateral funiculus transections; lesions confined to the DCs were sufficient to abolish antidromic tibial N. responses. These electrophysiological results provide a framework for the development of criteria useful in the assessment of DC functional integrity.

Supported by The Miami Project to Cure Paralysis.

345.15

ELECTROPHYSIOLOGICAL CONSEQUENCES OF DORSAL COLUMN DEMYELINATION. R.P. Yezierski, J.G. Broton, R.M. Devon* and J.P. Vicedomini, The Miami Project to Cure Paralysis, University of Miami School of Medicine, Miami, FL, and *Department of Oral Biology, University of Saskatchewan, Saskatoon.

Demyelination of central axons occurs clinically, and in animal models of spinal cord injury (SCI). Demyelinated axons that survive SCI display conduction abnormalities. Following demyelination, there can be spontaneous remyelination by endogenous oligodendrocytes and invading Schwann cells which may lead to functional restitution. The present research combines electrophysiological and anatomical approaches to determine if recovery of axonal conduction occurs within a time course related to that of remyelination. Adult male rats (n=12) received dorsal column (DC) lysolecithin injections (0.2μl, 1%, w/v) and were used in acute electrophysiological experiments 5, 10, 15, 25 and 50 days post-injection. DC conduction was evaluated from the tibial N. and lumbosacral enlargement, below thoracic DC lesions, to the dorsal column nuclei (DCN). Light and electron microscopic observations were made within the lesion zone at each survival time. DCN evoked response latencies were increased and their amplitudes decreased 5 days post-injection. Partial recovery of various response measures was observed as early as 10 days post-injection; however, conduction abnormalities persisted even at 50 days post-injection. Light microscopy showed that many demyelinated axons remained in continuity through the lesion 5-10 days post-lysolecithin. Electron microscopy demonstrated remyelination by endogenous oligodendrocytes and invading Schwann cells at 15 days post-injection. The time course and extent of remyelination paralleled the partial recovery of electrophysiological measures.

Supported by The Miami Project to Cure Paralysis

345.16

THE MATURATION OF THE LATERAL SPINOTHALAMIC AND TRIGEMINOTHALAMIC TRACTS IN RATS. D.Y. Miya and G.A. Barr. Biopsychology Doctoral Program, Dept. of Psychology, Hunter College, CUNY, New York, N.Y. 10021

In order to identify the neurons of origin and to study the ontogeny of both the lateral spinothalamic tract (LSTT) and the trigeminothalamic tract, two retrograde labels, 5% WGA-HRP and green fluorescent latex microspheres were stereotaxically injected into neonatal rats. 1 and 10 day old rat pups were injected with either tracer into the ventral posterior lateral nucleus (VPL) of the ventrobasal complex (VB) of the thalamus unilaterally. Rats were sacrificed at several different times following injection (24, 48 & 72 hours). Labeling was found to be most intense at the 24 hour survival period. For the 1 and 10 day olds, both methods yielded densely labeled cells in the ventromedial aspect of the contralateral dorsal horn of the lumbar enlargement. Smaller cells were labeled laterally in more superficial layers of the dorsal horn (lamina IV). Labeled cells were not seen in the cervical or thoracic segments. Although the pattern and quantity of cells was the same in both age groups, the cells in the 10 day olds appeared more densely labeled. The trigeminothalamic tract and medial lemniscal tract were present also in both ages . In all animals, the ipsilateral medial lemniscus and the lateral most aspect of the contralateral principal sensory trigeminal nucleus were densely labeled following VPL injections. Results demonstrate that both sensory systems, the trigeminothalamic tract and LSTT are present at birth in the rat.

345.17

EFFECTS OF NEONATAL SEROTONIN REDUCTION ON THE DEVELOPMENT OF LAYER IV GRANULE CELLS OF RAT PRIMARY SOMATOSENSORY CORTEX. J.A. Daughtery[1], N.L Chiaia[2], C. Bennett-Clarke[2], R.W. Rhoades[2] & J.H. Haring[1]. St. Louis Univ. Sch. Med., St. Louis, MO 63104 and Med. Coll. of Ohio, Toledo, OH 43699.

A dense, discontinuous serotonergic (5HT) projection to rat SmI present during the first 3 postnatal wks (D'Amato et al., PNAS 84:4322, '87), assumes the pattern of the SmI barrel fields (Rhoades et al., J.Comp.Neurol. 293:190, '90). We have reported alterations in layer V pyramidal cells & cytochrome oxidase (CO) patches in SmI of rats treated with p-chloroamphetamine (PCA) at birth (Daugherty & Haring, Soc. Neurosci.Abstr. 15:1050, '89). The purpose of this study was to determine the impact of 5HT reduction on the development of layer IV granule cells in SmI.

Neonatal rats received PCA injections (10mg/kg, SC) on either PND 0 & 1 or PND 3 & 4. Other neonatal rats had injections of 5,7 DHT into the raphe nuclei. The barrel fields were studied in all treatment categories at various postnatal times in Nissl-stained tangential sections. The morphology of layer IV granule cells was studied in rats injected on PND 0 & 1 with a combined cytochrome oxidase - Golgi method. The reduction of 5HT did not alter aggregation of layer IV granule cells into barrels. In each category, barrels were present at the earliest time studied. By contrast, qualitative assessments of granule cell morphology in PCA-treated rats suggest that the development of these neurons is altered by a postnatal reduction of 5HT. Quantative analyses will reveal the specific changes in these granule cells. These data suggest that the early 5HT innervation of SmI does not function to organize the granule cells of the barrel fields. However the subtle morphological changes imply a role for 5HT in cell differentiation & perhaps synaptogenic interactions between layer IV granule cells and the developing thalamocortical axons to SmI. Support: NS25752 and DE07734.

345.18

SOURCE OF THE TRANSIENT SEROTONINERGIC IMMUNOREACTIVITY IN THE DEVELOPING SOMATOSENSORY AND VISUAL CORTICES IN THE RAT. C.A. Bennett-Clarke, N.L. Chiaia, G.J. Macdonald and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

The visual and somatosensory cortices (area 17 and S-I, respectively) contain dense serotonin (5-HT) immunoreactivity (IR) during the first 2 weeks of life (D'Amato et al. P.N.A.S., 84:4322, 1987; Rhoades et al. J.Comp. Neurol.,293:190, 1990). To determine the origin of these projections we carried out 4 experiments. At ages (P-5 to P-7) when the cortical pattern of 5HT-IR was well established, rats received either electrolytic lesions of the thalamus or the medial forebrain bundle (MFB), or injection of the neurotoxin 5,7-DHT into either nucleus raphe dorsalis (NRD) or the cerebral cortex. Rats were killed 2 days after these treatments. Neither destruction of the thalamus nor injection of 5,7-DHT into cortex had significant effects upon the dense 5-HT-IR in area 17 and S-I. Transection of the MFB markedly reduced the density of 5-HT-IR in the ipsilateral cortex and injection of 5,7-DHT into NRD reduced 5-HT-IR in both cortices. Additional pups (P-6) were treated with fluoxitene, a specific inhibitor of 5-HT. This had no significant effect on the 5-HT-IR. These results indicate the dense 5-HT-IR in developing S-I and area 17 arises from NRD and is contained in the axons of these cells. Supported by EY 04170, DE 07734, and funds from the State of Ohio Research Challenge.

TRANSPLANTATION: TRANSMITTER EXPRESSION

346.1

EXPRESSION OF GLUTAMIC ACID DECARBOXYLASE (GAD) mRNA DURING THE DEVELOPMENT OF GRAFTED FETAL THALAMIC NEURONS IN THE EXCITATOXICALLY LESIONED ADULT THALAMUS. F. Nothias[1], P. Salin[2], M. Peschanski[1] and M.F. Chesselet[2]; [1] INSERM U.161, 2 rue d'Alésia 75014 Paris; [2] Dept. of Pharmacology, U. of Pennsylvania ,PA 19104

Homotypic fetal neurons (E15), dissected from dorsolateral diencephalon containing both primordia of ventrobasal complex (VB) and reticular thalamic nucleus (RT), were implanted as cell suspension into the kainic-acid-lesioned adult rat thalamus. GAD mRNA was detected by *in-situ* hybridization histochemistry using ^{35}S cRNA probes (A. Tobin) and emulsion autoradiography. Compared to the contralateral intact adult RT, very low labeling, but higher than the background signal obtained with sense-GAD RNA, was seen in the transplant at 7 days post-grafting (pg; no labeling was observed in the lesioned area). The hybridization signal increased during the second week pg and at 10 days pg, individual labeled cells were well distinguished, intermingled with unlabeled neurons and scatered in the whole transplant. At the third week pg, when the transplant became morphologically mature (Nothias et al., Neurosci. 33;605-616 '90), and later (4 months) the level of labeling in individual grafted neurons was comparable to that in the intact adult RT. The results show that GAD gene expression in RT neurons is preserved when they are removed from their normal embryonic environment. In addition, preliminary results showed that the time course of GAD gene expression in fetal grafted RT neurons is comparable to that occurring during the normal ontogeny of the RT nucleus. Supported by NATO grant #.268-89.

346.2

GLUTAMATE AND ASPARTATE IN FETAL RAT FRONTAL CORTICAL GRAFTS AS DETERMINED BY IN VIVO MICRODIALYSIS. T.J. Tevler, B.W. Chopko, B.A. Donzanti, T.J. Voneida. Dept. of Neurobiology, NE Ohio Univ. College of Medicine, Rootstown, OH 44272.

Fragments of frontal cortical tissue obtained from 15 day fetal rats were transplanted into 3 adult rats immediately after the production of a transcortical lesion cavity in the rostral sensorimotor cortex. After a postoperative (PO) survival of 2 weeks (PO2W), 7 months (PO7M), or 8 months (PO8M), the hosts were anesthetized and prepared for in vivo microdialysis of the graft and of contralateral sensorimotor cortex. Basal levels of aspartate (asp) and glutamate (glu) and the response to a 30 minute infusion of 80 mM high-K were assessed with HPLC and electrochemical detection. In the PO2W graft, high-K resulted in bilateral increases in both asp and glu. The increase in graft asp was large (423% baseline) and sustained until 2 hours after washout of the high-K. Contralateral asp increased to a lesser extent (168%) and dropped below baseline within 1 hour after washout of high-K. The magnitude and pattern of glu levels in the PO2W rat were similar in both graft and contralateral cortex. In the PO7M graft, glu and asp levels fluctuated widely, with high-K treatment resulting in increases of both. In the PO8M graft, high-K resulted in a large and sustained increase in both asp (338%) and glu (281%). In contralateral PO8M cortex, high-K resulted in only a small increase in asp and a small decrease in glu. Fetal frontal cortical grafts release asp and glu in response to stimulation with high-K. Dissimilarities between graft and contralateral cortex suggest the possibility of differences in amino acid release, utilization, or removal. (Supported by OBR Research Challenge Grant.)

346.3

DOPAC IN FETAL RAT FRONTAL CORTICAL GRAFTS AS DETERMINED BY IN VIVO MICRODIALYSIS. B.W. Chopko, B.A. Donzanti, T.J. Voneida. Dept. of Neurobiology, NE Ohio Univ. College of Medicine, Rootstown, OH 44272.

Fragments of frontal cortical tissue obtained from fifteen day fetal rats were transplanted into 3 adult rats immediately after the production of a transcortical lesion cavity in the rostral sensorimotor cortex. After a postoperative (PO) survival of 2 weeks (PO2W), 7 months (PO7M), or 8 months (PO8M), the hosts were anesthetized and prepared for in vivo microdialysis of the graft and of contralateral sensorimotor cortex. Basal levels of DOPAC and the response to a 30 minute infusion of 80 mM high-K were assessed with HPLC and electrochemical detection. In the PO2W graft, the response to high-K was an increase in DOPAC levels to 267% baseline; DOPAC gradually decreased to 164% baseline by 4 hours after washout of the high-K. In the contralateral PO2W cortex, DOPAC levels diminished to 35% baseline after high-K treatment, and remained low thereafter. In the PO7M and PO8M grafts, the averaged response to high-K was a decrease in DOPAC to 58% baseline; baseline was reestablished within 1.5 hours after washout of high-K. In the PO8M contralateral cortex, DOPAC levels were undetectable throughout the experiment. In all grafts, dopamine levels were not consistently detectable. Fetal rat frontal cortical grafts appear to possess the ability to synthesize and metabolize dopamine. The observed differences between young and old grafts may reflect maturation of graft dopamine metabolism. (Supported by OBR Research Challenge Grant.)

346.4

INCREASED OPIOID PEPTIDE AND CATECHOLAMINE PRODUCTION IN HUMAN ADRENAL MEDULLARY EXPLANTS. J. Sagen and G.D. Pappas. Dept. Anat. and Cell Biol., Univ. II at Chicago, Chicago, IL 60612.

Our laboratory has been concerned with the potential for adrenal medullary transplants in CNS pain modulatory regions to reduce nociception by providing a local source of both opioid peptides and catecholamines, substances which independently, and perhaps synergistically, reduce pain sensitivity. Rodent studies in our laboratory have indicated that adrenal medullary transplants in the spinal cord subarachnoid space reduce pain sensitivity as assessed by both acute and chronic analgesiometric tests. Furthermore, recent work has indicated that rat adrenal medullary tissue maintained in culture may be a better source of graft tissue for reducing nociception. The purpose of the present study was to determine whether human adrenal medullary explants may be a viable source of graft tissue for pain reduction. Human adrenal glands were obtained from the Regional Organ Donor Branch of Illinois, and medullary tissue was dissected from cortical tissue and placed in explant culture for various time periods, ranging from 0 - 45 days. Basal opioid peptide and catecholamine release, and release following nicotinic stimulation was determined at several time intervals following placement in culture using RIA and HPLC, respectively. Explanted adrenal medullary tissue was also analyzed morphologically using immunocytochemistry and electron microscopy at various intervals. Results indicate that human adrenal medullary explants can survive in culture for at least 45 days. At early time points (0 - 3 days), the number of cells staining for tyrosine hydroxylase is small, but the number of stained cells increases dramatically after 7 days in culture. Cuboidally shaped catecholamine producing cells are found throughout the time course of the study, although the cells tend to become more pleomorphic following longer culture times. In addition, both met-enkephalin and catecholamine levels are significantly increased from 7 - 21 days in culture. These results indicate that human adrenal medullary explants may be a good source of graft tissue for the reduction of clinical pain. (Supported by NIH grants NS25054 and NS28931)

346.5

RELEASE OF ENDOGENOUS OPIOIDS FROM RAT BRAIN SLICES CONTAINING BOVINE CHROMAFFIN CELL TRANSPLANTS. Ortega, J.D.; Sagen, J.; Dept. Anat. and Cell Biol. Univ. Il at Chicago, Chicago Il, 60612.

Work in our laboratory has indicated that the transplantation of adrenal medullary chromaffin cells into CNS pain modulatory regions can reduce pain sensitivity by locally increasing opioid peptide levels. The analgesia produced by the transplanted cells is increased by nicotine, most likely via the stimulation of nicotinic receptors on the transplanted chromaffin cells. Previous studies have demonstrated that isolated bovine chromaffin cells survive for prolonged periods when transplanted into the rat periaqueductal gray (PAG), and continue to stain immunocytochemically for met-enkephalin, as well as catecholamine synthetic enzymes. The purpose of the present study was to assess whether transplanted bovine chromaffin cells release opioid peptides pharmacologically in response to nicotinic stimulation, as they do in situ. Adult male rats were stereotaxically injected with either 2μl of a bovine chromaffin cell suspension or equal volumes of vehicle. Following a 4-12 week period, brain slices (300 μm) containing the transplant and PAG were sectioned using a vibratome, and superfused continuously (2-3 ml/min) with oxygenated artificial CSF maintained at physiological temperature. Following a one hour stabilization period, perfusion samples before and after stimulation with several doses of nicotine (15-120 μM) were collected and analyzed for opioid peptide content using RIA. Each stimulation period was followed by a 10 min washout phase with artificial CSF. A dose-related increase in met-enkephalin release following nicotine administration was observed from slices with bovine chromaffin cell transplants. At pharmacologic concentrations, nicotine produced 20-40 fold increases in met-enkephalin levels. Met-enkephalin release returned to basal levels following each washout. At the highest nicotine doses, met-enkephalin levels were decreased, suggesting a depolarization block. Similar responses are found in cultured bovine chromaffin cells. Results of this study indicate that bovine chromaffin cell xenografts are pharmacologically responsive, and can release high levels of opioid peptides. (Supported by NIH grants NS25054)

346.6

POTASSIUM-EVOKED OVERFLOW AND DIFFUSION OF DOPAMINE IN DOPAMINE DENERVATED STRIATUM REINNERVATED WITH GRAFTS OF FETAL HUMAN VENTRAL MESENCEPHALIC TISSUE. G. A. Gerhardt[1,2], I. Stromberg[3], C. van Horne[2], M.N. Friedemann[1,2] and B.J. Hoffer[1,2]. Depts. of Psychiatry[1] & Pharmacology[2], Univ. of Colorado H.S.C., Denver, CO 80262 & Dept. of Histology[3], Karolinska Inst., Stockholm, Sweden.

The mechanisms by which grafts of fetal ventral mesencephalic tissue reinnervate and improve the functional properties of dopamine (DA)-denervated host striatum remain unclear. In order to explore the dynamic properties of graft derived DA-containing nerve endings, we employed the technique of high-speed chronoamperometry to study potassium (K^+)-evoked overflow of DA and diffusion of DA in graft- reinnervated brain tissue. Human fetal mesencephalic fragments (1st trimester) were transplanted into the ventricle adjacent to the lesioned striatum of unilateral 6-OHDA-lesioned Sprague-Dawley rats; rats were treated with cyclosporin-A daily. Lesioned non- grafted rats, which rotated more than 500 turns/hour following apomorphine injection (0.05 mg/kg), exhibited greatly decreased K^+-evoked overflow of DA as compared to control. Locally-applied DA signals had temporal dynamics that were slower compared to control striatum. In contrast, lesioned/grafted animals had reduced rotations compared to pre-grafting rates and showed increased K^+-evoked overflow of DA in areas within the graft. In addition, locally-applied DA signals had temporal dynamics analogous to control striatum. However, in areas at the periphery of the graft, adjacent to the host lesioned striatum, K^+- evoked responses were diminished in amplitude and exhibited longer time dynamics as compared to control. In addition, the diffusion properties of locally-applied DA in these brain regions were similar to that of 6-OHDA-lesioned striatum. These data suggest that there are areas at the periphery of DA- containing brain grafts where DA can more readily diffuse, and also suggest that small brain grafts may influence a relatively larger volume of the host brain because of changes in the host brain. Supported by USPHS AG06434, AG00441 and NS09199.

346.7

FETAL DOPAMINE CELL IMPLANTS INTO INTACT STRIATUM: EFFECT ON TRANSPLANT SURVIVAL. J.X. Qi*, P. Patino, C. Hutt*, E. Kriek, and C.R. Freed. Depts. of Med. and Pharm., Univ. of Colorado Health Sci. Ctr., Denver, CO 80262.

As fetal neural implants become a therapeutic option for human Parkinson's disease, there is concern that L-dopa therapy may inhibit transplant growth. Since the intact striatum is a dopamine rich environment which mimics maximal dopamine replacement, we have compared the survival, growth and behavioral effects of fetal mesencephalic dopamine cells implanted into intact and lesioned striatum. Male Sprague-Dawley rats 250-350 g were implanted unilaterally in striatum with a coarse suspension of fetal mesencephalic dopamine cells from embryonic day 15-16 fetuses (n=14). The tissue was allowed to grow in situ for four weeks, then half of the rats were lesioned with 6-hydroxydopamine (6-OHDA) in the nigrostriatal bundle. Another group not transplanted with fetal tissue was also lesioned with 6-OHDA. Results showed that animals transplanted then lesioned circled less after methamphetamine 5 mg/kg (METH) than animals with lesion alone. Unlesioned, transplanted animals did not circle after METH. After sacrifice, tyrosine hydroxylase (TH) positive cells were found in rats which were transplanted then lesioned but not in transplanted, unlesioned animals. Thus, intact striatal dopamine prevents the expression of graft TH activity but fetal cell implants can express TH after 6-OHDA lesion.

346.8

DEVELOPMENT OF EMBRYONIC DOPAMINERGIC NEURONS TRANSPLANTED TO THE STRIATUM OF RAT PUPS AND EFFECTS ON THE POST-LESION INCREASE OF STRIATAL ENKEPHALINERGIC IMMUNOSTAINING AND SEROTONINERGIC SPROUTING. J.P. Herman, *M. Manier**, N. Abrous*, *C.Feuerstein**, and M. Le Moal. Lab. Psychobiologie Comportements Adaptatifs INSERM. U259, Univ. Bordeaux II. 33077 Bordeaux Cedex-France. *INSERM U318, CHU Grenoble 38043 Grenoble, France.*

The ascending dopaminergic (DA) pathway of rat pups (PD3) was unilaterally destroyed by 6-OHDA (6μg) injected into the lateral hypothalamus. Three days later a cell suspension containing embryonic DA neurons was implanted into the denervated striatum. Tyrosine hydroxylase (TH), met-enkephalin (ME) and serotonin (5HT) immunohistochemistry were performed nine months later and quantified by image analyzer (Peretti-Renucci et al., J. Neurosci. Res., 1990).

Transplanted TH+ neurons developed and reinnervated the host striatum. In comparison to the development observed after adult-stage transplantation (Abrous et al., J. Comp. Neurol., 1988) several differences could, however, be noted. 1) Survival of transplanted DA neurons was lower, 2) Surviving cells migrated over long distances and did not form a compact graft tissue disorganizing the host parenchyma as seen in adult hosts, 3) Preferential migration routes could be distinguished, e.g., under the corpus callosum and towards the subependymal zone.

The lesion of the ascending DA pathways provoked an increase in ME immunostaining in the denervated striatum. This increase was inhomogeneous, more pronounced in the dorsal and lateral aspects of the striatum. The presence of the graft totally reversed this increase in the zones where, and only where, a TH+ reinnervation was present. Lesion-induced sprouting of striatal 5HT afferents was untouched by the grafts. These results suggest that morphological reorganizations induced by DA lesion are less easily reversed by intracerebral neural grafts than physiological types of modifications.

346.9

FETAL STRIATAL, CORTICAL OR VENTRAL MESENCEPHALIC TISSUE GRAFTS INTO INTACT STRIATUM. S.Y. Lu, M.T. Shipley, A.B. Norman, E.M. Zubrycki and P.R. Sanberg. Div. of Neuroscience, Depts. Psychiatry, Physiology and Anatomy. Univ. of Cincinnati College of Medicine, Cincinnati, OH 45267-0559

Intrastriatal grafting of fetal striatal (STR), cortical (CTX) or ventral mesencephalic (VM) tissue into the normal striatum produces behavioral deficits. We examined the cellular elements of the graft and their conectivity with the host using histochemistry for cytochrome oxidase (CO) and AChE, and immunocytochemistry for glial fibrillary acidic protein(GFAP), OX-42, tyrosine hydroxylase(TH), dopamine-B-decarboxylase(DBH) and 5-HT 5-15 months after transplantation. CO staining showed that the grafts were metabolically active. AChE staining within the STR grafts was patchy and contained AChE positive neurons and fibers. The CTX grafts exhibited AChE terminals with similar appearance to that of the host cortex. The VM grafts showed light AChE staining. 5-HT, TH and DBH immunoreactive(-IR) fibers were found in the STR and CTX grafts which presumably originated from the host brain. The density of these fibers varied greatly for different grafts. In one of four CTX grafts, many TH-IR neurons were found. The VM grafts developed TH-IR, 5-HT-IR, DBH-IR neurons and fibers. Fibers crossed the transplant and host border. GFAP and OX-42 stainings showed intermittent heavy staining along the border of the graft and the host, OX-42 stained macrophages were found in many grafts. Intrastriatal grafts in the intact normal brain were innervated by afferents from the host, and neurons in the graft can innervate host tissue. The glial barrier may be a factor limiting the integration between the graft and the host. The TH-IR neurons found in the CTX graft may be due to induced expression of TH as reported in previous studies. Supported by NINDS, HDSA, TSA.

346.10

CEREBELLAR SEROTONIN FIBER INNERVATION IN NORMAL AND *pcd* MUTANT MICE AND HOST AFFERENTS INTO INTRA-PARENCHYMAL CEREBELLAR GRAFTS. L.C. Triarhou, W.C. Low and B. Ghetti. Dept. of Pathol., Physiol.-Biophys., and Prog. in Med. Neurobiol., Indiana Univ. Sch. Med., Indianapolis, IN 46202.

One aspect of integration of implanted neurons into the neuronal circuitry of a defective host brain is the re-establishment of a host-to-graft afferent innervation. We addressed this issue by using the adult cerebellum (CB) of *pcd* mutant mice, which lack virtually all Purkinje cells (Pc) after P45. Pc constitute one of the CB cell types being innervated by axons of raphé serotonin (5HT) neurons. In normal mice, varicose 5HT-immunoreactive (ir) fibers are distributed to all CB folia with an anteroposterior (AP) density gradient. Following Pc loss in *pcd* mice, CB 5HT concentration is increased due to the accompanying parenchymal atrophy; 5HT-ir fiber density is higher than normal, but the AP gradient is preserved. Grafts of E12 CB cell suspensions were implanted into the CB of *pcd* mutants. The state of grafted Pc and host 5HT axons was monitored by calbindin and 5HT immunocytochemistry. Numerous host-derived 5HT-ir axons are present in the graft within 5 days; at 30 days the overall 5HT fiber density in the graft is somewhat higher than the surrounding host CB tissue. These findings offer an anatomical correlate of the biochemical state of 5HT in *pcd* mice and provide evidence for afferent innervation of donor CB tissue by 5HT fibers of host origin. It remains to be established whether this effect is related to a selective attraction of host fibers by grafted neurons or to a non-specific invasive property of 5HT systems. (PHS RO1-NS14426).

346.11

AN ULTRASTRUCTURAL IMMUNOCYTOCHEMICAL ANALYSIS OF SEROTONIN (5-HT) NEURONS GRAFTED TO ADULT RAT HIPPOCAMPUS. G. Chazal, A. Daszuta*, S. Garcia*, S. Oleskevich and L. Descarries. INSERM U6 and CNRS LNF 4, Marseille, France, and CRSN, Université de Montréal, Montréal (Québec), Canada.

5-HT-immunocytochemistry was used at the EM level to examine the ultrastructural features of 5-HT neurons that had reinnervated adult rat hippocampus, 4 to 5 months after a 5-HT denervation (5,7-DHT) and subsequent graft of embryonic raphe cells. Immunostained cell bodies and dendrites in the core of grafts were compared to their in situ counterparts in the nucleus raphe dorsalis, and axon terminals (varicosities) in a CA3 and a dentate gyrus (DG) outgrowth zone to the normal 5-HT innervation of hippocampus (Oleskevich et al., *Soc. Neurosci. Abstr.*, this volume). The shape, size and synaptic investment of grafted somata and their dendrites resembled those of in situ raphe neurons. Some of these dendrites contained clusters of small clear vesicles, and a few others were contacted by immunostained varicosities. In both the core and two outgrowth zones, the varicosities (n = 500) were also indistinguishable from their normal counterparts, with respect to shape, size, content, and frequency of synaptic contacts and juxtaposed elements. In single sections, only 7.4% of these profiles exhibited a junctional complex, yielding a stereologically extrapolated synaptic incidence of 16% for whole varicosities. These contacts were asymmetrical and mostly made on dendritic shafts, except in the molecular layer of DG where a few axo-spinous synapses were also seen. The microenvironment of immunostained varicosities was similar in the neuropil layers of both CA3 and DG, comprising mostly other axonal varicosities, neurites and dendritic shafts. Thus, in spite of their transplantation and growth into an abnormal milieu, and the fact that they hyperinnervated the host tissue, grafted embryonic 5-HT neurons appeared committed to express a particular set of intrinsic and relational features resembling their normal characteristics.

346.12

RESTORATION OF STRIATAL SP-mRNA EXPRESSION BY DOPAMINERGIC FETAL SUBSTANTIA NIGRA GRAFTS. B.A. Flumerfelt, I. Mendez , K. Elisevich and C.C.G. Naus. Depts. of Anatomy and Clinical Neurological Sciences, University of Western Ontario, London, Canada, N6A 5C1.

Evidence for survival and growth of fetal nigral grafts and reversal of behavioural and biochemical deficits in the dopamine (DA) depleted striatum is well documented. Restoration of a synaptic DA input on host neurons has also been demonstrated. One of the postsynaptic targets of graft-derived DA terminals is the substance P (SP) neuronal population within the striatum (Mendez et al., Restor. Neurol. and Neurosci. Suppl., 1989). In this study, the SP-mRNA expression in the striatum was analysed before and after the grafting procedure. Fetal nigral cell suspensions were stereotaxically implanted into the deafferented neostriatum of Wistar rats two weeks after inducing an ipsilateral nigral lesion with 6-hydroxydopamine. Striatal SP-mRNA expression was assessed using an SP ^{35}S-radiolabelled antisense oligonucleotide probe. DA depletion was accompanied by a corrresponding decrease in SP-mRNA in the striatum ipsilateral to the lesion. Restoration of SP-mRNA levels was observed in grafted animals that exhibited normalization of rotational behaviour and viable grafts as demonstrated by TH immunocytochemistry. This study thus suggests that reestablishment of the DA input on host SP neurons has a direct effect on the gene expression for SP production.

Supported by the M.R.C. and the Upjohn London Neurosciences Program

346.13

VASOPRESSIN-NEUROPHYSIN (VP-NP) AND CORTICOTROPIN-RELEASING FACTOR (CRF) ARE CO-LOCALIZED IN PARVICELLULAR NEURONS OF THE TRANSPLANTED PARAVENTRICULAR NUCLEUS. S.J. Wiegand, J.A. Olschowka and D.M. Gash. Dept. of Neurobiology and Anatomy, University of Rochester Medical Center, Rochester, NY 14642.

Grafts of the fetal paraventricular nucleus (PVN) characteristically contain neurons immunoreactive for a number of neuropeptides, including CRF and VP. The parvicellular VP-immunoreactive (VPir) neurons in PVN grafts are morphologically indistinguishable from the CRFir neurons, and are similarly distributed within the transplanted tissue. The objective of the present study was to determine the extent to which CRF and VP might be co-localized within cells of the transplanted PVN.

Blocks of tissue containing the developing PVN were obtained from normal Long-Evans rat fetuses (E15-17) and transplanted to the periventricular hypothalamus of adult, male, Long-Evans or VP-deficient Brattleboro rats. Developing neocortex or SCN served as control tissues. Animals were sacrificed 6-12 weeks after transplantation and the grafts were stained using a double-immunofluorescence procedure employing a mouse monoclonal antibody against VP-NP and a rabbit polyclonal antiserum against CRF. Alternate series of sections were double-stained for CRF and oxytocin(OT)-NP. CRF and VP-NP were co-localized in 70-90% of immunoreactive parvicellular neurons, with the remaining cells containing one or the other peptide. A relatively small number of magnocellular neurons were found to contain OT- or VP-NP, but these cells did not stain for CRF. Nor were CRF and VP-NP co-localized in the cells of cortical or SCN grafts. The extensive co-localization of VP-NP and CRF in the PVN grafts was particularly striking, and somewhat unexpected, as the graft recipients were neither adrenalectomized nor injected with colchicine prior to sacrifice. Consequently, neurons in the medial parvicellular PVN of Long-Evans host animals stained only moderately for CRF, and not at all for VP-NP. This strongly suggests a differential regulation of peptide expression in the grafted neurons and the homologous population of cells in the host brain, notwithstanding their exposure to identical titers of circulating glucocorticoids.

NEURONAL DEATH: DEAFFERENTATION AND PRENATAL STUDIES

347.1

AXON NUMBERS IN DEEP VIBRISSAL NERVES OF NEONATAL AND ADULT RATS: A QUANTITATIVE ELECTRON MICROSCOPIC STUDY. X. Huang, R.S. Crissman, M.F. Jacquin, W.E. Renehan, B.G. Klein, and R.W. Rhoades. Dept. of Anatomy, Medical College of Ohio, Toledo, OH 43699.

It has been suggested on the basis of light microscopic data that the deep nerves innervating the vibrissa follicles in newborn rats contain only 70% of the fibers that they will in adulthood (Munger, B.L. and F.L. Rice, J. Comp. Neurol., 252:404, 1986). This result is surprising since axon number decreases postnatally in several peripheral nerves (e.g. Renehan, W.E. and R.W. Rhoades, Brain Res., 322:369, 1984; Jenq, C-B. et al., J. Comp. Neurol., 244:445, 1986). We attempted to resolve this issue by using the electron microscope to count axons in the deep vibrissal nerves supplying the C-1 and C-4 follicles in both newborn and adult rats. In newborns, the average number of fibers supplying the C-1 follicle (N=5) was 287 ± 30.9 (s.d.); for adults, the average number was 324 ± 30.5 ($p>0.5$). In neonates, nerves to the C-4 follicle (N=6) contained 236 ± 28.4 axons; for adult follicles (N=4), this value was 217 ± 29.4 ($p>0.10$). Almost all of the axons in the neonates were unmyelinated. Thus, deep vibrissal nerve fiber number does not increase with postnatal age. However, adult numbers of axons exist in these infraorbital (ION) branches when the parent nerve still contains about 25% more fibers than will be the case in adulthood. Thus, if axonal loss occurs in vibrissal nerves, it is complete prior to that for the ION as a whole. DE 07734, BNS 85 17537.

347.2

SEGMENT-SPECIFIC DEATH OF THORACIC INTERNEURONS IN THE MOTH, *MANDUCA SEXTA*. T.M. Amos *, K.A. Mesce * and S.E. Fahrbach.^ *Graduate Program in Neuroscience and Dept. of Entomology, Univ. of Minnesota, St. Paul, MN 55108 and ^ Neuroscience Program and Dept. of Entomology, Univ. of Illinois, Urbana, Ill. 61801.

During metamorphosis of the holometabolous insect, *Manduca sexta*, the larval meso- and metathoracic ganglia fuse with the first two abdominal ganglia (A1 and A2) to form the adult pterothoracic ganglion. A pair of identified intersegmental interneurons is located in both A1 and A2 of the pterothoracic ganglion. These are the only interneurons present in the pterothoracic ganglion that project posteriorly through more than two ganglia. These neurons may be involved in generating ecdysis behavior since, in the adult, intracellular stimulation of the neurons in A2 produces the adult-specific phase of ecdysis.

Our studies show that these intersegmental interneurons are conserved larval interneurons. Pairs of intersegmental interneurons analogous to those in the adult are present in the larval thoracic ganglia as well as the first two abdominal ganglia. We present evidence suggesting that these larval thoracic interneurons die in a segment specific fashion during the first few days after pupal ecdysis. The thoracic neurons can no longer be backfilled with cobalt at the same time that light microscopic sections of thoracic ganglia show a small number of degenerating neurons, some of which are located in positions similar to the larval thoracic intersegmental interneurons.

Preliminary results suggest that new, adult-specific neurons may regulate this death. We have found, in 20% of animals treated with hydroxyurea (a DNA synthesis inhibitor which selectively ablates adult-specific neurons), that the thoracic intersegmental interneurons appear to be saved.

347.3

DUAL FATE OF SUBPLATE NEURONS IN THE RODENT. T.U. Woo, J.M. Beale* and B.L. Finlay. Biopsychology Laboratory, Cornell University, Ithaca, NY 14853.

While subplate neurons (layer VIb or VII) are lost during the development of the cat cortex, in rodents the subplate persists to adulthood, at least in part. Using tritiated thymidine, we traced the developmental course of the suplate in the golden hamster, comparing the amount of cell loss in this earliest generated layer with layer VI and LGN.

Tritiated thymidine injections in pregnant hamsters on embryonic day 10 (E10) and E11 labeled cells of the subplate, layer VI, and LGN. Animals were examined prior to cell death and at adulthood. Absolute number of silver grains was counted. At all criteria, a greater proportion of cell loss occurred in the subplate, as compared with layer VI and LGN. However, a substantial population of subplate cells remains, forming an intact layer in adult animals.

Since cats and hamsters differ in timing of neurogenesis, axonal innervation of cortex, and fate of subplate cells, comparative information can constrain our hypotheses on the function of this developmentally transitory population.

Supported by NIH Grant R01 NS19245.

347.4

CELL DEATH DURING THE EARLIEST STAGES OF SPINAL CORD DEVELOPMENT IN THE CHICK EMBRYO. S. Homma, H. Yaginuma and R. W. Oppenheim. Dept. of Neurobiology and Anatomy, Bowman Gray School of Medicine, Winston-Salem, NC 27103

Following neural tube closure, degenerating cells were observed in the neuroepithelium (NE) in virtually all areas of the presumptive spinal cord, including roof and floor plate regions. We have quantified the extent as well as the spatio-temporal distribution of dying cells in the NE of the brachial spinal cord beginning at stage (St) 15 (embryonic day 2, 50-55 hours of incubation). Brachial neural tubes were embedded in plastic, sectioned transversely at 2 µm and all pyknotic cells were counted in every fourth section through one segment. The number of dying cells was highest at St. 17 and 18 (approximately 60 dying cells per segment). Before St. 15 and after St. 22, dying cells were virtually absent. Although dying cells were most frequently observed in the floor plate, some were also found in most other regions of the neural tube. Debris from dying cells was phagocytized by adjacent NE cells. The period of cell death in the floor plate coincides with the projection of commissural axons across the ventral midline and thus may be associated with pathway formation.

347.5

DEVELOPMENTAL NEURON DEATH IN TARGET-SPECIFIC SYMPATHETIC GANGLION NEURONS. L.L. Wright and A.F. Elshaar. Dept. Anatomy, Boston Univ. Sch. Med., Boston, MA 02118.

Since target tissues are known to play a large role in regulating developmental neuron death, we compared the time course and magnitude of neuron death in rat superior cervical ganglion (SCG) neurons innervating the submandibular gland with those projecting to the eye in the rat. These target-specific subpopulations of SCG neurons were identified with retrogradely transported fluorogold, and the number of fluorogold labelled neurons, pyknotic neurons, and pyknotic-fluorogold-labelled neurons were tallied directly from the microscope at P3,5, and 8, during the normal period of neuron death. Results are expressed as the percentage of fluorogold-labelled neurons that are pyknotic.

In females, the peak neuron degeneration among SMG-projecting neurons occurred at P5, with significantly less degeneration at P8. Neuron degeneration in eye-projecting neurons remained constant over the 3 ages. In males, the peak degeneration in SMG-projecting neurons occurred at P3, while the peak degeneration in eye-projecting neurons occurred on P8. In both males and females a significantly greater percentage of eye-projecting neurons than SMG-projecting neurons were degenerating on P8. Cumulative percent loss of SCG neurons was statistically greater in females than in males in the SMG-projecting population, but not in the eye-projecting population.

These data indicate that there are differences in the magnitude and time course of neuron death between males and females, and between SCG neurons innervating different target tissues.

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347.6

INVOLVEMENT OF AFFERENT SYNAPTIC INPUT IN TROCHLEAR MOTOR NEURON DEATH DURING DEVELOPMENT. S. Hirano*, K. Kumaresan* and G.S. Sohal. Department of Anatomy, Medical College of Georgia, Augusta, GA 30912.

About half of the trochlear motor neurons die during normal development in duck embryos. In order to understand the nature of factors controlling survival or death of neurons we have begun to examine the involvement of afferent synaptic input in cell death. Brains of duck embryos before, during, and after the period of trochlear motor neuron death were processed for electron microscopy. Central afferent synapses on the trochlear motor neuron soma began to form before the onset of neuron death. This finding suggests that afferent input may be involved in neuron survival. This possibility was examined by decreasing input to the trochlear motor neurons. Deafferentation was achieved by ablation of portions of medulla and pons giving rise to the medial longitudinal fasciculus, a major source of afferents to the trochlear motor neurons, before motor neuron death begins. Our preliminary results indicate that there is a significant decrease in the number of surviving trochlear motor neurons. Further, the neurons were considerably smaller in size. The decrease in neuron survival occurred even though the target muscle was not manipulated. These observations suggest that afferent input may be important in the control of neuron numbers during development. (Supported by NIH Grant HD17800).

347.7

TIME COURSE OF POSTSYNAPTIC CELL DEATH FOLLOWING INFRAORBITAL NERVE SECTION AT BIRTH IN RAT. T.A. Henderson and M.F. Jacquin. Dept. of Anatomy & Neurobiology, St. Louis University School of Medicine, St. Louis, MO 63104.

It is well established that afferent inputs are necessary for the normal development of sensory systems. Neonatal deafferentation produces extensive transneuronal cell loss whithin 2 days in the gerbil auditory system (Hashisaki and Rubel, JCN 283:465, '89), yet no cell loss was detected at postnatal day (PND) 5 in the mouse lateral geniculate following enucleation at birth (Heumann and Rabinowicz, Exp. Brain Res. 38:75, '80). The "acute" effect of neonatal deafferentation upon cell numbers in the trigeminal (V) system is unknown. As a preliminary step in addressing that issue, 6 rats sustained left infraorbital nerve sections at birth and were sacrificed on either PND 1, 3, or 5. Brainstems and V ganglia were sectioned in paraffin and stained with cresyl violet. In each rat, all neuronal profiles containing nucleoli were counted in 5 sections through each of V brainstem subnuclei principalis and interpolaris. The average cell loss in principalis by PND 1, 3, and 5 was 26.1, 23.9, and 30.9%, respectively. Corresponding values in interpolaris were 9.2, 30.9, and 31.6%, respectively. In V ganglia ipsilateral to the lesion, a 28.3% mean cell loss was observed by PND 1. Thus, deafferentation-induced cell death in the V brainstem complex occurs very rapidly. Inasmuch as a 30-40% cell loss has been reported in adult rats following the same lesion at birth (Choy et al., Soc. Neurosci. Abstr. 15:1332, '89), the present data suggest that the majority of V brainstem cells which will die do so during the first few postnatal days. Support: DE07734, DE07662.

347.8

DEAFFERENTATION OF CLARKE'S NEURONS PREVENTS RETROGRADE CELL DEATH AFTER AXOTOMY. C.A. Sanner, and M.E. Goldberger, Medical College of Pennsylvania, Philadelphia, PA. 19129

Factors determining the amount of cell death in Clarke's Nucleus (CN) after axotomy were assessed in cat L3 spinal cord. Distance of lesion from the cell body effects cell death since T9 hemisection resulted in a greater cell loss than C2 animals. Age was also a factor since greater cell loss was seen after neonatal than after adult T9 hemisection. Afferents may also be a factor. L1-S2 dorsal rhizotomy 6 months prior to T9 hemisection resulted in total cell survival. Thus neurons that would have undergone retrograde cell death after axotomy were protected by the prior deafferentation. Though the cell population was composed almost entirely of smaller cells they stained positively for MAP2. This suggests that since total cell number is normal, the larger cells are most likely shrinking. Cell survival after combined lesions implies that there is an intrinsic balance between the influence of the target and that of the afferents upon CN neurons. Removal of one of these influences creates an imbalance while removal of both may create a new homeostasis that allows cell survival.

(Supported by grants NS24707 and NS15529)

347.9

UNILATERAL OLFACTORY DEPRIVATION INCREASES CELL DEATH AMONG PRENATALLY PROLIFERATING GRANULE CELLS IN THE MOUSE MAIN OLFACTORY BULB. K.T. Spence*, L.C. Skeen*, and B. Wolfson, University of Delaware, Newark, DE 19706.

Olfactory bulb granule cells (GCs) are deleteriously affected by olfactory deprivation (Skeen, et al., Neurosci. Lett., 54:301, 1985), however, the degree to which deprivation affects the prenatally produced subset of these neurons is unknown.

Pregnant dams were injected with ^{3}H-thymidine on embryonic days 13 through 16 to radiolabel the earliest-produced GCs. Pups from these dams underwent unilateral naris occlusion on postnatal (P) day1 and were sacrificed on P15, P30 and P45 to compare the number of radiolabeled GCs in deprived and nondeprived olfactory bulbs (OBs).

Prenatal exposure to ^{3}H-thymidine radiolabeled light and dark GCs, and both subtypes were affected by deprivation. There are 19% fewer light GCs (ANOVA, $p < .005$) and 26% fewer dark GCs (ANOVA, $p < .003$) in deprived versus nondeprived OBs. Deprivation-related death is apparent by P15 in light GCs and by P30 in dark GCs. These results indicate that among GCs early proliferation provides no insulation against the effects of early olfactory deprivation. The finding that dark GCs are affected by deprivation later than light GCs may reflect differences in the efferent connections of these cells.

347.10

NONGENETIC REGULATION OF PROLIFERATION AND CELL DEATH IN THE OLFACTORY EPITHELIUM (OE). V.McM. Carr and A.I.Farbman. Dept. Neurobiol.& Physiol., Northwestern Univ., Evanston, IL 60208.

Continued genesis and replacement of olfactory sensory neurons (SNs) occur throughout a vertebrate's life. Until recently SN longevity was thought to be genetically controlled, but recent studies now suggest that influences other than genetic may be important. To assess the role of target deprivation, quantitative [^{3}H]-TdR/autoradiographic analysis of both SN genesis and degeneration was carried out in unilaterally bulbectomized (OB-Xd) rats.

Results show that [^{3}H]-TdR incorporation was 3-4 times greater on the OB-Xd side than contralaterally at 7-11 days post-op following a 1-day exposure to [^{3}H]-TdR (2μCi/gm b.wt., 3x/24 hr.) ($p << .001$). Simultaneous analysis of degenerative activity shows not only OB-X-enhanced degeneration but also that a greater proportion of newly formed cells are dying precociously, especially in the basal and mid regions of the OE. These data indicate that proliferative activity and SN longevity may be environmentally controlled.

347.11

IN VIVO LESIONING AND DiI MARKING OF THE ENTORHINAL CORTEX IN THE FETAL MOUSE. D.C. Snyder, E. Bui*, B. W. Coltman*, and C.F. Ide Dept. of Cell and Molecular Biology, Tulane University, New Orleans, LA 70118.

We identified, using in vivo implantation of DiI crystals, the presumptive entorhinal cortex (EC) in the E15,16 mouse fetus. By term (E19), a prominent dye marked projection of the angular bundle into the CA1 region of the hippocampus was clearly evident. We then lesioned the lateral EC at E15,16 using suction or electrocautery methods; in some cases, DiI was placed in the lesion. Embryos were allowed to develop to term (E19) and then analyzed histologically. The consequences of the lesion were 1) removal of angular bundle innervation of CA1 during fetal development, 2) cell death anterior to the lesion site, presumably of cells which project into the lesioned area, and 3) little apparent cell death posterior to the lesion site, including CA1. Therefore, prenatal disruption of major cortical tract innervation of the developing hippocampus has little effect on its basic structure at birth.

LONG-TERM POTENTIATION IV

348.1

LOW FREQUENCY DEPRESSION IS MODULATED BY LTP IN THE IN VIVO DENTATE GYRUS. P.C. Rinaldi and V.B. Nguyen*. Div. of Neurosurgery, School of Medicine, Univ. of California, Irvine, CA 92717.

Low frequency stimulation (LFS) of the medial perforant path (mpp) projecting from the entorhinal cortex to dentate gyrus of the hippocampal formation results in habituation-like decrements in response called low frequency depression (LFD). In contrast, brief trains of high frequency stimulation (HFS) of mpp produce a lasting increase in response known as long-term potentiation (LTP). Both phenomena have been implicated in information processing and memory/learning. This work assessed their interaction, specifically, whether LFD is modified in a potentiated network. Population spike and excitatory post-synaptic potentials produced by bipolar stimulation of mpp were monitored in 12 rats under urethane anesthesia and were recorded from the s. granulosum of the dentate gyrus by microelectrodes. After establishing a 20 minute baseline with test stimulation (1/30 seconds), a 40 trial LFS series was delivered (0.5 Hz) to assess LFD. Following recovery, brief trains of HFS were employed to induce LTP. Post-LTP (30 minutes) a second LFS series was delivered for LFD assessment.

Comparison of computer averages of the population spike potential indicated that the relative amount of depression post-LTP was significantly less than pre-LTP ($t=4.43$, df 7, $p<0.005$). Analysis of population EPSP slope and spike amplitude showed that changes were located in the latter. Results suggest that changes in the potentiated network can significantly modulate the outcome of LFD and thus have the potential to modify processing or memory capabilities of the network. (Supported by NIH grant NS22980-01A1 to PCR.)

348.2

LTP-INDUCED EPSP/SPIKE DISSOCIATION IN THE DENTATE GYRUS IS BLOCKED BY LOCAL INFUSION OF BICUCULLINE. R.A.Tomasulo, W.B Levy, and O.Steward. Depts. of Neuroscience and Neurosurgery, Univ. of VA, Charlottesville, VA 22908.

The induction of long-term potentiation (LTP) in the dentate gyrus is accompanied by a change in the EPSP/spike (E-S) relationship. After inducing LTP, a given pEPSP evokes a larger population spike than previously. This phenomenon, termed the E-S dissociation, is seen in field potential studies as a shift to the left of the E-S curve, in which population spike amplitude is plotted against EPSP slope at various stimulus intensities. One possible cause of the E-S dissociation is a reduction in feed-forward (FF) inhibition. This hypothesis predicts that removing FF inhibition before inducing LTP will prevent the E-S curve shift with LTP. We blocked $GABA_a$ neurotransmission in a circumscribed area of the dentate gyrus (DG) of urethane-anaesthetized rats by inserting a micropipette filled with 8mM bicuculline methiodide in saline, as previously described (Steward, Tomasulo, Levy. Brain Res. 1990 in press). Effective blockade of inhibition was indicated by multiple population spikes after a single perforant path stimulus, a lowered spike threshold, and absence of paired pulse inhibition. We recorded E-S curves from this electrode and a control electrode, containing saline only, before and after inducing LTP in the perforant path with 8-pulse, 400 Hz trains. In 6 of 7 experiments, the bicuculline prevented the left shift, and, unexpectedly, revealed a shift to the right, so that a given EPSP evoked a 10-20% smaller population spike after inducing LTP. The control electrodes showed the expected left shift. We conclude that EPSP/spike dissociation in the DG reflects a reduction in $GABA_a$-mediated FF inhibition. The cause of the right shift remains to be determined. Supported by BNS8818766 to OS, NS15488 and MH0622 to WBL, and training grant NS07199 to RT.

348.3

PHENCYCLIDINE BLOCKS THE INDUCTION OF LONG-TERM DEPRESSION IN THE HIPPOCAMPAL DENTATE GYRUS IN VIVO. N. L Desmond, C. M. Colbert, D. X. Zhang, and W. B Levy. Dept. Neurosurgery, Univ. of Virginia Health Sciences Center, Charlottesville, VA 22908.

Long-term depression (LTD) of inactive synapses occurs in the entorhinal cortical (EC)-dentate gyrus (DG) system when LTP occurs at nearby, active synapses[1]. Given that NMDA receptor antagonists block the induction of LTP and that strong postsynaptic depolarization is the permissive event for induction of both LTP and LTD in this system[1], we assessed the effect of phencyclidine (PCP), a noncompetitive NMDA receptor antagonist, on the induction of LTD in the EC-DG system. Adult, Sprague-Dawley rats were anesthetized with urethane. Recording pipets were placed bilaterally in the DG; stimulating electrodes were positioned bilaterally in the angular bundles. Either PCP (6 mg/kg body weight) or normal saline was administered i.v. via a lateral tail vein. Compared to the saline control group (N=5), PCP blocked the induction of both LTD of the converging contralateral EC-DG response and LTP of the ipsilateral response (N=5). Compared to the immediately preceding baseline, conditioning stimulation depressed the converging contralateral response 34.2% (SEM 1.0%) for the saline group but only 1.8% (SEM 0.5%) for the PCP-treated group ($t=6.32$, $p<0.01$). The unconditioned ipsilateral response was unchanged in both groups of animals. Activation of NMDA receptors is thus required for the induction of LTD in the EC-DG system. In addition, subsequent conditioning stimulation at supramaximal intensity in the presence of PCP did not consistently induce LTP or LTD. Therefore, brief but massive postsynaptic depolarization alone is probably not sufficient to induce LTD or LTP in the EC-DG system.

[1] Levy & Steward. Brain Res. 175 (1979) 233. Supported by NIH NS26645 to NLD and NIH NS15488 and NIMH RSDA MH00622 to WBL.

348.4

KETAMINE BLOCKS LTP AT LEC-DG SYNAPSES. D.X. Zhang and W.B Levy, Dept. Neurosurgery, Hlth. Sci. Ctr., Univ. of VA., Charlottesville, VA 22908.

The induction of long-term potentiation (LTP) is often controlled by the NMDA receptor, e.g., NMDA receptor antagonists block the induction of LTP at medial entorhinal cortical (MEC) synapses on dentate gyrus (DG) granule cells. Some forms of LTP, however, do not require activation of the NMDA receptor, e.g., the DG-CA3 mossy fiber synapses. Whether or not the lateral (L) EC-DG synapses behave like the MEC-DG synapses or the DG-CA3 synapses is not known. In the region of LEC synapses there are NMDA receptors similar to the region of MEC-DG synapses. However, the region of LEC-DG synapses has a chemical similarity to the DG-CA3 synapses, including the presence of zinc and dynorphin. To elucidate the role of NMDA receptors in LTP of the LEC-DG synapses, we used ketamine in an LTP paradigm. The experiment used urethane-anesthetized albino rats with two stimulating electrodes (one in the LEC and the other in the MEC) and one DG recording electrode. The response to each stimulating electrode was mapped through the DG to ascertain the suitability of the stimulating electrode placements. Responses were tested alternately every 30 sec. Once a baseline was established, 70mg/kg ketamine was administered i.p. over 1 hr. Brief, high-frequency trains were delivered separately to the LEC and then to the MEC electrodes. After 1 hr testing, these trains were repeated, and finally the LEC was conditioned two more times. Each set of conditioning trains was separated by at least 30 min. In all the animals in which ketamine blocked LTP of the MEC-DG response, it also blocked induction of LTP of the LEC-DG response. Thus, even though the LEC-DG synapses may biochemically resemble the DG-CA3 mossy fiber synapses, LEC-DG synapses probably depend on activation of NMDA receptors for the induction of LTP.

Supported by NIH NS15488 and NIMH RSDA MH00622 to WBL.

348.5

KETAMINE BUT NOT MK-801 INHIBITS THE INDUCTION OF LONG-TERM POTENTIATION (LTP) IN THE DENTATE GYRUS *IN VIVO*. S. Maren, T. J. Shors, M. Baudry, and R. F. Thompson. Neural, Informational, and Behavioral Sciences, University of Southern California, Los Angeles, CA 90089.

Activation of the *N*-methyl-*D*-aspartate (NMDA) receptor is essential for LTP induction in the dentate gyrus and hippocampal CA1 region. The present study examined the effects of the noncompetitive NMDA antagonists, ketamine and MK-801, on LTP induction in the perforant path-granule cell pathway of anesthetized rats. Dentate hilar field potentials evoked by perforant path stimulation were recorded in nembutal anesthetized Long-Evans rats. Input/output functions were generated immediately before drug injection and 30 minutes following high frequency perforant path stimulation (theta rhythm: 10 30 ms 400 Hz bursts at 5 Hz). LTP was quantified by calculating the percent change in population spike (PS) amplitude and excitatory postsynaptic potential (EPSP) slope for responses having an initial PS amplitude of approximately 6 mV. Fifteen minutes prior to delivery of the perforant path tetanus, animals were injected intraperitoneally with either saline (S; 1 mg/kg; *N*=6), ketamine (K; 30 mg/kg; *N*=6), or MK-801 (M; 1 mg/kg; *N*=10). The development of LTP in animals treated with ketamine was significantly attenuated (LSD, $p<.01$) relative to those injected with either saline or MK-801 (mean changes in PS amplitude ± SEM: S=121 ± 32%, K=-1 ± 20%, M=71 ± 20%; and EPSP slope ± SEM: S=23 ± 6%, K=-10 ± 3%, M=18 ± 7%). These results are thus consistent with those of Abraham and Mason (Brain Research, 462, 1988) showing that MK-801 did not prevent LTP induction when administered 30 minutes before delivery of the tetanus. The contrasting modulation of LTP induction by ketamine and MK-801 may be related to the differential use-dependency of the two drugs as the pharmacological actions of MK-801, but not ketamine, are use-dependent *in vitro*. Supported by NIH(AG05142, AG05500) and the McKnight Foundation.

348.6

LONG-TERM POTENTIATION (LTP) INDUCES NOVEL POLYPEPTIDE IN AREA CA1 OF RAT HIPPOCAMPUS. T.J. Shors, N. Uenishi, N.R. Nichols, C.E. Finch, R.F. Thompson. Neurosciences Program, Univ. of Southern Calif., LA, CA 90089

In vitro induction of LTP in hippocampal slices, followed by [35S]methionine labeling and two-dimensional gel electrophoresis was used to identify changes in protein synthetic patterns. We found a polypeptide in the CA1 region of the hippocampus that has a molecular weight of 48kD and an isoelectric point of 6.8. The rate of synthesis was increased 1-3 hours following the induction of LTP (56% increase in EPSP slope) using high-frequency (100 Hz) stimulation to the Schaffer collaterals. Relative to unstimulated slices, protein radioactive image was increased in 7/10 experiments using pooled slices; no change occurred in 3 experiments. Low-frequency stimulation at 1 Hz did not induce a change in 4/6 experiments. The relative increase was evident only when CA1 was separated from the dentate gyrus. The induction occurred in dentate samples regardless of stimulation (12/13). This 48kD polypeptide does not appear to be any of those previously associated with the induction of LTP (i.e. phosphorylated proteins) since it is more basic and was detected during the maintenance phase. [supported by NIH (AG05142, AG05500, AG07909) and McKnight Foundation]

348.7

LONG TERM POTENTIATION AND DEPRESSION IN THE RAT VISUAL CORTICAL-CALLOSAL SLICE PREPARATION. R.L.Berry,B.W.Chopko & T.J.Teyler. Neurobiology Dept, NE Ohio College of Medicine, Rootstown, OH 44272

In order to study interactive long term potentiation (LTP) and depression (LTD) in the rat visual cortex, we have developed a slice preparation in which both geniculate (GC) and callosal (CC) afferents to rat visual cortex are preserved. Slices were obtained from 16-25d rats and maintained in a perfusion system. A recording electrode was placed in layer II/III of OC1 (area 17), OC2 (area 18) or at the OC1/2 border (location verified by AChE histology). Stimulation of the CC pathway was followed (60 ms delay) by stimulation of the GC pathway. Field potentials were recorded for a 30 min baseline period, tetanic stimulation (20 pulses/5 s at 100 hz--10 min) given to the CC pathway alone, and field potentials recorded for a further 70 min.

The incidence of LTP and LTD of the field potential response to CC and GC test stimulation is shown below. LTP or LTD to at least one pathway was seen in 7/11 slices. The three slices that showed GC LTP did not show either LTP or LTD to CC stimulation. These results show that tetanic stimulation of the callosal projection to visual cortex can result in novel forms of LTP and LTD.

	LTP	LTD	No change
CC	1/11	3/11	7/11
GC	3/11	2/11	6/11

Supported by the ONR.

348.8

NEOCORTICAL SYNAPTIC LONG-TERM DEPRESSION INDUCED BY TETANIC STIMULATION IN THE PRESENCE OF AN NMDA RECEPTOR ANTAGONIST. V.A.Aroniadou and T.J.Teyler, Dept. of Neurobiology, Northeastern Ohio College of Med., Rootstown, OH 44272.

In visual cortical slices of young rats, stimulation (3-5 V) of the subjacent white matter evoked a field potential consisting of two components (peak latency 5-7 ms and 13-16 ms, respectively) in layer II/III of area 17. The response was reversibly abolished by perfusion with Ca++ free medium indicating that it consisted of synaptic activity alone.The second component was of lower amplitude, was probably polysynaptic (it did not follow stimulations above 0.2 Hz), and was reversibly blocked by addition of the NMDA receptor antagonist DL-2-amino-5-phosphonovalerate (APV; 50 uM) to the medium.

Tetanic stimulation (200 ms trains at 100 Hz every 5 sec for 10 min) in normal medium induced a 200 to 460 % potentiation of the first component (n=9). The second component was either unaltered, reduced, or it fused with the first component which had a greater duration in the potentiated response. Long-term potentiation (LTP) started developing ~ 15 min post-tetanus, reached a peak at ~80 min, and remained at the peak level throughout the observation period (3 hours). Tetanization in the presence of APV resulted in long-term depression (LTD; n=4) or no change (n=2) after return to the normal medium. LTD started developing ~15 min post-tetanus reaching peak level (150-310%) at ~45 min. It appears that mechanisms activated during LTP-inducing tetanic stimulation may result in long-term depression if allowed to operate without concurrent activation of NMDA receptors (supported by the ONR).

348.9

EFFECTS OF DISINHIBITION ON LTP INDUCTION IN SLICES OF VISUAL CORTEX. W.A. Press* and M.F. Bear. Center for Neural Science, Brown University, Providence, RI 02912.

One interest of this lab is the role of excitatory amino acid (EAA) receptors in the mechanisms of synaptic plasticity in the kitten visual cortex. In this study we have approached this question using visual cortical slices *in vitro*. Extracellular field potentials (FP's) as evoked by single shock stimuli applied to the white matter/layer VI border were recorded in layer III. These FP's usually were comprised of two components: a short latency (2.7 ± 0.2 ms) nonsynaptic response and an early (6.8 ± 0.3 ms) synaptic response. The synaptic response depended entirely on the activation of non-NMDA EAA receptors. Conditioning the inputs with patterned high frequency stimuli resulted in a long-term potentiation of the early synaptic responses in 2 out of 25 (8%) attempts. The presence of bicuculline methiodide (BMI) in concentrations greater than 0.6 μM revealed an additional late (12.4 ± 1.6 ms) component of the FP which could be blocked by 100 μM AP5. When disinhibited by 0.8-1.2 μM BMI, LTP was evoked in 3 out of 8 (37.5%) attempts. The induction of LTP could be blocked by AP5. Increasing [BMI] further usually led to epileptiform activity. In an effort to produce strong local disinhibition without seizures, the recording electrode was filled with concentrated BMI. Our results to date, using cortical slices from rats, suggest that LTP can be readily elicited using this method. It is apparent that layer III of neocortex can exhibit synaptic potentiation as robust as that seen in hippocampus provided that GABAergic inhibition is reduced. (Supported by ONR Young Investigator Award N00014-88-K-0756)

348.10

DIFFERENT VOLTAGE-DEPENDENT THRESHOLDS FOR THE INDUCTION OF LONG-TERM DEPRESSION AND LONG-TERM POTENTIATION IN SLICES OF RAT VISUAL CORTEX. A. Artola,* S. Bröcher* and W. Singer (SPON: European Neuroscience Association) Max Planck Institute for Brain Research, 6000 Frankfurt/M, F.R.G.

We provide evidence for long-term depression (LTD) of synaptic responses (p.s.p.s) to white matter stimulation in layer II-III cells. In normal medium, low intensity tetani (tet., 50Hz) induce no modifications. However, when postsynaptic activation is raised during tet. by application of bicuculline (BIC), either LTD or long-term potentiation (LTP) are obtained depending on BIC concentration. LTD (81.7±4.5% of control, n=7, mean±s.e.m.) occurs with 0.1-0.2μM BIC and LTP with 0.3μM BIC. Initial slopes of p.s.p.s change accordingly. LTD occurs also in normal medium (67.2±2.6%, n=13) if postsynaptic activation is increased by raising intensity of tet.. In that case LTD is prevented by hyperpolarizing (-40mV) the cell during tet. while LTP is induced with depolarization (+20mV). LTD can be reversed by stimulation conditions inducing LTP and vice versa. During LTD, a second, non-tetanized, intracortical pathway remains unchanged. Addition of the NMDA receptor-blocker APV (25μM) prevents induction of LTP but not of LTD. This suggests that 1) LTD and LTP both depend on postsynaptic activation and have different voltage-dependent thresholds, 2) LTP but not LTD requires NMDA receptor activation, 3) modified synapses are on the recorded neuron and 4) LTD is selective for the tetanized pathway.

348.11

LONG-TERM DEPRESSION (LTD) OF ELECTROTONIC AND CHEMICAL COMPONENTS OF MIXED SYNAPSES ON THE MAUTHNER (M-) CELL. X.-D. Yang and D.S. Faber. Neurobiology. Lab, Dept. of Physiology, School of Medicine, SUNY at Buffalo, Buffalo, NY 14214.

LTP and LTD are synaptic phenomena often related to learning and memory. Previous work showed that suprathreshold tetanization of the 8th nerve potentiates both electrotonic coupling and glutaminergic chemical response at the mixed synapses between the nerve and the M-cell in goldfish. We report here that when weak subthreshold tetanization is paired with an antidromic (AD) spike, there can be long-term and reversible depressions of both synaptic responses. *In vivo* intracellular recordings were obtained from the M-cell lateral dendrite. The cell was stimulated antidromically via spinal cord, and orthodromically via 8th nerve. The depression protocol consisted of 210 trains of 5 pulses at 500 Hz with 2 sec intervals and paired with one AD spike at the beginning of each train. Both electrotonic and chemical responses could show depressions up to 50% (n=5), the longest lasted 45 min. The depression could be reversed by suprathreshold tetanization, which, if applied repeatedly, could potentiate the reponses further, but eventually reached a limit. Also, additional depression protocols could depress the synaptic reponses only to a certain minimum. Since AD spike is small on the dendrite, where the synapses are located, and produces a powerful collateral inhibition of the M-cell, we suggest that low level activation of the excitatory synapses and the simultaneous postsynaptic inhibition together caused the depression, though the mechanism is unknown. Also, synaptic efficacy has a finite range, beyond which usual methods for LTP or LTD cannot induce further changes.

348.12

A ROLE FOR INTRACELLULAR CALCIUM IN INITIATING CEREBELLAR LONG-TERM DEPRESSION. A. Konnerth, J. Dreessen & G.J. Augustine. MPI for Biophysical Chemistry, Goettingen, FRG.

Thin-slice patch clamp and digital imaging methods were used to examine long-term depression (LTD) of synaptic transmission between parallel fibers (PF) and Purkinje cells in the rat cerebellum. Brief activation of the climbing fibers (CF) innervating the Purkinje cells caused a prompt and transient (< 5 min duration) elimination of synaptic currents (EPSCs) evoked by PF stimulation. This was followed by recovery of EPSC magnitude to its initial level (or even larger) and then by a pronounced depression of EPSCs which reached a maximum in 10-60 min and lasted up to 3 hours. This final phase apparently corresponds to LTD. We examined whether this LTD is caused by a rise in Ca concentration ($[Ca]_i$) in the Purkinje cell. CF stimulation produced a large and transient (10-100 s long) rise in $[Ca]_i$. This rise was most prominent in the dendrites and apparently was due to activation of voltage-gated Ca channels. Direct depolarization of Purkinje cells opened Ca channels, raised $[Ca]_i$, and caused changes in EPSC amplitude much like those produced by CF stimulation. The long-lasting depression caused by depolarization was blocked by intracellular application of EGTA (10 mM), indicating that the synaptic effects of depolarization were caused by changes in $[Ca]_i$. We conclude that CF stimulation initiates LTD of the PF synapse by transiently raising $[Ca]_i$ in the postsynaptic Purkinje cell.

348.13

ACTIVATION OF Ca^{2+}/CALMODULIN-DEPENDENT PROTEIN KINASE II IN CEREBELLAR GRANULE CELLS BY N-METHYL-D-ASPARTATE RECEPTOR ACTIVATION. [1,2]K. Fukunaga, [2]E. Miyamoto* and [1]T.R. Soderling, [1]Dept. of Mol. Physiol., Vanderbilt Univ. Med. Sch., Nashville, TN 37232-0615, USA, [2]Dept. of Pharmacol., Kumamoto Univ. Med. Sch., Kumamoto 860, Japan

Recent studies have strongly suggested the involvement of protein kinase C or CaM kinase II in the induction of long-term potentiation (LTP) in the CA1 region of hippocampus. Furthermore, during the induction of LTP, a protein kinase has been reported to be converted to a Ca^{2+}-independent form that is constitutively active. We have investigated generation of the Ca^{2+}-independent form of CaM kinase II by the activation of the NMDA receptor in cerebellar granule cells. Glutamate did produced an elevation of Ca^{2+}-independent CaM kinase II activity when granule cells were incubated in Mg^{2+}-free buffer, and this response was potentiated by 1μM glycine. This response required extracellular Ca^{2+} and was blocked by specific antagonists of the NMDA receptor. Phosphopeptide mapping of the ^{32}P-labeled 58-60kDa subunit of CaM kinase II revealed a correlation between generation of Ca^{2+}-independent activity and ^{32}P-incorporation into peptide CB1 which contains Thr 286/287. These results indicate that the excitatory neurotransmitter glutamate acting through the NMDA receptor can stimulate autophosphorylation of CaM kinase II to generate its Ca^{2+}-independent form.

349.1

EFFECTS OF AGING ON THE MESOSTRIATAL DOPAMINE SYSTEM: A BEHAVIORAL AND NEUROBIOLOGICAL STUDY. R. Burwell[1], L. Thai[2], K. Stenvers[2], and M. Gallagher[1]. [1]Department of Psychology, [2]Curriculum in Neurobiology, University of North Carolina, Chapel Hill, NC 27599.

Twenty male Long-Evans rats completed a longitudinal study of reaction-time (RT) performance. Subjects were tested every six weeks from 14.5-25 mo of age. On average, RT latencies increased significantly with age. Some rats, however, maintained a stable performance throughout the study. At 25 mo of age, other behavioral tests were conducted. Compared to young rats tested at the same time, the 25 mo rats were impaired in learning a spatial task in the Morris water maze. Again, however, some aged rats learned this task as readily as the younger animals. Although no correlation was found between RT latencies at 25 mo and spatial learning performance within the aged group, a possible biomarker for the emergence of learning impairment was identified in the RT task. Compared to rats that maintained their learning ability at 25 mo of age, rats that were impaired in the spatial learning task were significantly less accurate on their RT performance at every assessment. Thus, even at 14.5 mo of age, RT accuracy was significantly related to the emergence of learning impairment at 25 mo.

Quantitative autoradiography in brain slices from these animals is currently being used to assess the integrity of pre- and post-synaptic neurons for the dopaminergic (DA) system. The status of markers for the mesostriatal DA system will be discussed in relation to RT performance and other behavioral data, i.e. spatial learning, spontaneous alternation, and locomotor activity.

Supported by NSF predoctoral Fellowship to RB, a NIMH RSDA to MG (KO2-MH00406, and grants NIMH MH39180 and NSF BNS 87-19881.

349.2

NORADRENERGIC, CHOLINERGIC AND OPIOID CORRELATES OF SPATIAL MEMORY IMPAIRMENT IN AGED RATS. T. D. Smith[1], M. Gallagher[2] and F. M. Leslie[1]. [1]Dept. of Pharmacology, University of California, Irvine, Irvine, CA. 92717 and [2]Dept. of Psychology, University of North Carolina at Chapel Hill, Chapel Hill, NC. 27599.

Spatial memory performance in young (6 mo) and aged (24 mo) male Long-Evans rats was assessed in the Morris water maze. A subpopulation of aged rats performed within the range of young rats and was designated the memory unimpaired (MU) group. The remaining aged rats displayed spatial learning deficits and were designated memory impaired (MI). Quantitative autoradiography in brain slices from these animals was used to assess the integrity of pre- and post-synaptic neurons for cholinergic, noradrenergic and opioid neurotransmitter systems. [^{3}H]Hemicholinium binding to cholinergic pre-synaptic reuptake sites and [^{3}H]pirenzepine binding to cholinergic M1 receptors decreased with age in olfactory tubercle and deep layers of frontal motor cortex, respectively. [^{3}H]Pirenzepine displayed an increase in binding density in aged MU as compared to both young and MI rats in the superficial layers of frontal cortex and in olfactory tubercle. [^{3}H]Desmethylimipramine labeling of noradrenergic pre-synaptic reuptake sites showed an increase with age in frontal and anterior cingulate cortices, and a memory-related increase in the aged MU rats in insular cortex and dentate gyrus. [^{3}H]Idazoxan binding to noradrenergic alpha-2 receptors was densest in the aged MU and least dense in MI rats in many limbic regions, as well as in frontal cortex. This same memory-related pattern of labeling was also observed with [^{3}H]DADLE binding to delta opioid receptors. In contrast, [^{3}H]diprenorphine binding to kappa opioid receptors was increased in the aged MI subpopulation in various limbic regions. [^{3}H]DAGO binding to mu opioid receptors displayed mainly age-related, but not memory-related changes. These results suggest that selective cholinergic, noradrenergic and opioid receptor radioligands not only show age-related changes in binding densities but distinguish between memory impaired and unimpaired aged rats.

Supported by PMA advanced predoctoral fellowship, AFAR grant 12855, MH 39180 and RSDA K02-MH 00406.

349.3

HIPPOCAMPAL CHOLINE ACETYLTRANSFERASE ACTIVITY CORRELATES WITH SPATIAL LEARNING DEFICITS IN AGED RATS. G. L. Dunbar[1], R. J. Rylett[2], and L. R. Williams[3]. [1]Dept. of Psychol., Central Michigan Univ., Mt. Pleasant, MI 48859 (USA); [2]Dept. of Physiol., Univ. of Western Ontario, London, Ontario, Canada N6A 5C1; [3]CNS Diseases Res., The Upjohn Co., Kalamazoo, MI 49001 (USA). Since 24-month old rats show significant decreases in choline acetyltransferase (ChAT) and high-affinity choline uptake (HACU) when compared with 4-month old rats (Williams & Rylett, J. Neurochem., in press), we investigated whether these reduced levels correlated with age-related learning deficits. Eighteen male, Fisher rats (24 months old) were tested for 10 days on a Morris water maze task. After behavioral testing, levels for ChAT and HACU were made in the hippocampus, frontal cortex, and striatum. Stepwise regressions for predicting total time and distance swum in the maze from all six biochemical measures showed only hippocampal ChAT as a significant predictor (both $ps < .02$). These results indicate that lower levels of hippocampal ChAT correlate with age-related learning deficits.

349.4

COMPLEX MAZE LEARNING IN AGED RATS IS RELATED TO BLOOD GLUCOSE LEVELS AND INSULIN RESPONSE BUT IS UNRESPONSIVE TO GLUCOSE TREATMENT. *J. Long[1], B. Davis[2], P. Garofalo*[1], E. Spangler*[1], D. Ingram[1].* [1]Gerontol. Res. Ctr., NIA, NIH, Baltimore, MD 21224; [2]Dept. Neurobiol. Anat., U. Roch. Sch. Med., Rochester, NY 14642.

In aged rats and humans, glucose regulation has been correlated with memory performance, and glucose treatment can result in improved performance (Gold P., & Stone W., *Neurobiol. Aging*, 9:709, 1988). We tested this glucose hypothesis with rats in a 14-unit T-maze that has provided robust evidence of age-related performance decline (Ingram D., *Neurobiol. Aging*, 9:475, 1988). Aged (24-25 mo) and young (6-7 mo) male F-344 rats were pretrained for 1-way active avoidance to a criterion (8 avoidances/10 trials) before receiving complex maze training (4 daily trials over 5 days) with the contingency of moving through each of 5 segments within 10 sec to avoid footshock (0.8 mA). Ten min before daily training, aged rats received either saline (n=7) or glucose in doses of 10 (n=7), 100 (n=6), or 500 (n=10) mg/kg i.p., while young rats received saline. Significant ($ps<.05$) age-related increases in errors, runtime, shock frequency and duration were observed, but the only significant ($ps<.05$) glucose effect was to reduce runtime and shock duration at the highest dose. About 4-6 wks later, all rats were fasted for 12-hr; injected i.p. with glucose (150 mg/kg); and bled at 0, 30, 60, and 120 min post-injection to obtain estimates of blood glucose and insulin levels. Significant correlations ($ps<.05$) were observed between maze errors and baseline glucose levels, $r(21)= -.62$, and peak glucose response, $r(19)= .49$. However, within the aged group, significant correlations ($ps<0.01$) with maze errors emerged only for baseline glucose, $r(13)= -.69$, and peak insulin response, $(r(13)= .65)$. Thus, regulation of insulin, but not glucose, appeared related to learning abilities among aged rats.

349.5

MEMORY AND SENSORY-MOTOR SKILLS IN AGING RATS: NEURAL CORRELATES AND DIET RESTRICTION. D. S. Olton and A. L. Markowska Dept. of Psychology, The Johns Hopkins University, Baltimore, MD 21218.

The cognitive effects of aging were examined by testing rats in a battery of cognitive and sensory-motor tests. Two strains (Fisher-344, and a cross between Brown-Norway and Fisher-344) were tested at 10-12 mo, and 23-25 mo of age, with a few rats at 30 mo and 36 mo of age. The behavioral tests in a Morris water maze included spatial navigation, discrimination reversal, cue learning, and recent memory as assessed with a delayed match-to-sample task. Sensory-motor tests provided measures of different skills. The experiments were designed to answer questions about age-related impairments in different behavioral domains, and the effects of diet restriction: life-long, only during development, or only at the time of testing.

349.6

RELATIONSHIP OF AGE-RELATED BEHAVIORAL DEFICITS TO NEUROCHEMICAL MARKERS. G.L. Wenk, A.L. Markowska, L. Gorman, V.E. Koliatsos, D.L. Price, and D.S. Olton. Depts. of Psychology and Pathology, The Johns Hopkins University, Baltimore, MD 21218.

For rats of different ages, strains, and diets, correlations among different measures of behavior and brain function were determined. Fisher-344 (ages 4, 12 & 24 mo) and a cross between the Brown-Norway and Fisher-344 (ages 4, 12, 24, 30 & 36 mo) were given cognitive and sensorimotor tests, followed by neurochemical and immuno-cytochemical measurements of markers for various neurotransmitter systems in basal forebrain, locus coeruleus, neocortex, hippocampus and caudate/putamen. The variance of some of the behavioral and neurochemical measures was larger in the older rats than in the younger rats. The pattern of correlations among the different measures provides information about the differential effects of aging on selected neural systems. Supported by NIH NS 20471 and AG 05146.

349.7

AGE-RELATED ALTERATIONS IN MOTOR LEARNING ARE CORRELATED TO LOSS OF CEREBELLAR NORADRENERGIC FUNCTION. P.C. Bickford-Wimer, C. Heron and R. de la Garza Vet.Admin. Med. Ctr; and Dept. Pharm. UCHSC, Denver, CO 80262.

Motor learning is fundamental to normal execution of movement because continual adaptation to change is required. Age-related declines in motor function are well documented in the literature for both humans and animals. Alterations in the ability to learn new motor patterns, however, has not been studied extensively. In humans there is a decline in mirror tracking proficiency with advanced age suggesting that motor learning is altered. We investigated age-related alterations in the ability of F344 rats to learn novel motor skills. The behavioral paradigm was designed after that of Watson and McElligot (Br. Res.**296**:129-138, 1984). Performance on this task is dependent on cerebellar NE. Because we have previously demonstrated age-related changes in cerebellar noradrenergic function, it was our hypothesis that alterations in motor learning would correlate with this deficit. The animals were trained to run back and forth on pegs for a water reward. Two weeks after training the animals were tested on a novel peg pattern. Three groups of rats were tested: young, young treated with 6-hydroxydopamine (6OHDA) and 18-20 month old rats. Both the young 6OHDA lesioned and aged rats showed impairments in the acquisition of the novel locomotor task. Subsequent to behavioral testing, extracellular recording from cerebellar Purkinje cells demonstrated a correlation between performance and the ability of NE to modulate GABAergic transmission ($r=.78$; $p<0.05$). This supports our hypothesis of a role for cerebellar noradrenergic function in motor learning deficits associated with aging. (VAMRS and AG 04418).

349.8

AGE-RELATED DIFFERENCES IN LONG-TERM RETENTION OF DELAY CLASSICAL CONDITIONING IN RABBITS. J. M. Coffin & D. S. Woodruff-Pak. Department of Psychology, Temple University, Philadelphia, PA 19122

Age differences in acquisition of the NM/eyeblink response in rabbits in the delay paradigm (400 msec CS-US interval) are significant, but they are not as large as age differences in the trace paradigm. In the delay task older (O) rabbits perform as well as young (Y) by the 4th training day. We present here data from a 12-month and 18-month retest in Y and O rabbits on retention in the delay task. Y rabbits were 7 months old at acquisition and 19 and 25 months old at the 12- and 18-month follow-ups. O rabbits were 36 months old at acquisition and 48 and 54 months old at the follow-ups. An 85 dB, 1 KHz tone CS was paired with a 3 psi airpuff US in acquisition and subsequent retests. Daily sessions of 90 trials were run until rabbits achieved a criterion of 8 CRs in 9 trials. A test of rabbits' hearing sensitivity, assessed at the 18-month follow-up by reducing the tone by 5 dB intervals until no CRs were present, revealed no age differences. Rabbits retrained following 12- and 18-month intervals showed no significant age differences. Mean trials to relearning at 12 months was 146 for Y and 161 for O rabbits. At the 18-month follow-up, O rabbits relearned in a mean of 55 trials, while Y rabbits took 90 trials. Age differences exist in acquisition, but there are no age effects on retention. (Supported by an Alzheimer's Association/NJAHCF Research Grant).

349.9

AGE DIFFERENCES IN A 750 MSEC CS-US DELAY CLASSICAL CONDITIONING PARADIGM. D K. Sasse and D. S. Woodruff-Pak. Department of Psychology, Temple University, Philadelphia, PA. 19122.

Small but statistically significant age differences in eyeblink classical conditioning have been demonstrated in the delay paradigm with a 400 msec CS-US interval. Large age differences have been demonstrated in the trace paradigm when the CS and US were offset by 750 msec. The major aim of this study was to test for age differences in classical conditioning in a 750 msec *delay* paradigm. We also compared this long 750 msec delay interval with data collected in a 750 msec trace paradigm (Woodruff-Pak, Lavond, Logan, & Thompson, 1987). Criterion for learning was 8 CRs out of 9 consecutive trials. In the 750 msec delay paradigm, significant age differences were found in the rate of acquisition between 51-month-old (796 trials), 24-month-old (770 trials), and 4-month-old rabbits (535 trials) ($F=5.00$; $p<.05$). No significant differences were found between the trace and delay paradigms. The interaction between age and conditioning paradigm (trace versus delay) was not statistically significant. A hearing test (measuring CRs/block as the tone CS was systematically decreased at 5 dB intervals) revealed no age differences in hearing. Differences in rate of conditioning were not due to diminished hearing capacity in aged rabbits. Results suggest that large age differences in longer CS-US intervals may result from the long time period between CS onset and US onset. (Supported by NIH RR07115).

349.10

HUMAN ASSOCIATIVE LEARNING: ANALYSIS OF TRACE AND DELAY EYE-BLINK CONDITIONING . R. A. Deyo, I. D. E. Gabrieli and I. F. Disterhoft. Dept. of Psychology Northwestern Univ. Evanston, IL 60208.

In rabbits, acquisition of Trace and Delay eye-blink conditioning tasks involve unique combinations of neural systems. Acquisition of Trace conditioning, but not Delay conditioning, is blocked by hippocampal lesions. Analysis of Trace and Delay conditioning may reveal similar organization of associative learning in the human brain. There are, however, few studies of trace conditioning in human subjects, and no study of long-term retention of conditioned eye-blink responses. The purpose of the present study was to compare and evaluate acquisition and retention of delay and trace eye-blink conditioning tasks using healthy subjects.

In the first experiment, 24 college students (age = 18-22) received either Delay or Trace conditioning that consisted of 50 acquisition trials. Trace conditioned subjects were trained using a paradigm employing a 100ms tone CS, a 500ms trace interval and a 100ms corneal air puff (UCS). Delay conditioned subjects were trained using a paradigm employing a 600ms tone CS that overlapped and coterminated with the UCS. Analysis of the percentage of conditioned responses (CRs) for each group indicated that Trace conditioned subjects made more conditioned responses than subjects in the Delay conditioned group. There were no group differences in CR amplitude or latency or in the size of the unconditioned response amplitude.

In Experiment 2, trace and delay conditioned subjects were trained using the parameters described above. All subjects received 50 trials on Day 1 and were retested 24 h, 7 or 28 days later. Analysis of the percentage of CRs indicated that conditioning on both tasks is preserved for long periods.

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349.11

CYTOARCHITECTURAL CHANGES IN THE FRONTOPARIETAL CORTEX OF AGED AND BASAL FOREBRAIN LESIONED RATS. C.L. Wellman and D.R. Sengelaub. Program in Neural Science and Dept. of Psychology, Indiana University, Bloomington, IN 47405.

Alzheimer's disease (AD) is characterized by deficits in learning and memory and decreased cortical cholinergic activity. Lesions of basal forebrain nuclei have been used to model the behavioral and pharmacological deficits of AD. Because AD typically occurs in aged populations, the changes seen in AD could be due to neuropathology specific to AD, age, or an interaction. We are using basal forebrain lesions in rats to determine the contribution of these factors. Cellular changes in the frontoparietal cortex of 13-month-old intact (n=3) and basal forebrain lesioned (n=4) adult rats and intact, 22-month-old (n=5) aged rats were assessed in frozen sectioned, cresylecht violet stained material. Lesions did not affect neuron number, but significantly reduced soma size in particular cortical laminae, resulting in decreased laminar thickness relative to intact adult rats. These effects were most pronounced in lamina II-III, where soma size was decreased by about 42%. In aged rats, neuronal loss was observed in several laminae and was most pronounced in laminae II-III and VI, averaging about 22%. Soma size in aged rats was decreased about 31% relative to intact adults, but this atrophy was not reflected in changes in laminar thickness. Decreases in soma size were seen in both superficial and deep laminae. Thus, the patterns of regressive change in the cortex differ in basal forebrain lesioned adults and intact aged rats. Basal forebrain lesions in aged rats should thus result in more extensive cortical neuronal changes, and these changes should correspond to more severe behavioral deficits than are seen after lesions of young adult rats. Basal forebrain lesions in aged rats may better model the neural and behavioral changes of AD.

349.12

LIFE-LONG FOOD RESTRICTION AND SPATIAL MEMORY: A LONGITUDINAL AND CROSS-SECTIONAL STUDY IN THE RAT.

M. Gyger*, D. Muller and E.M. Rouiller. Nestlé Research Center, Nestec Ltd., Vers-chez-les-Blanc, CH-1000 Lausanne 26, Switzerland; Dept Pharmacology, Centre Médical Universitaire, CH-1206 Genève, Switzerland; Dept Physiology, University of Fribourg, CH-1700 Fribourg, Switzerland.

Food restriction extends life-span and slows down age-related physiological deteriorations. Cognitive abilities seem also affected. We tested rats either repeatedly during their life (longitudinal study) or once at 24 months of age (cross-sectional study). Three food conditions were compared: ad libitum, 70 % restriction and a two-day-a-week fasting schedule. Spatial memory was assessed using the Morris water maze.

No effect of food restriction is found during the learning (at 6 months) and the relearning of the task (at 12, 19 and 24 months). A retrieval test (the platform is removed) indicates that the searching pattern of one group only, the 70 % restricted rats, is still focussed on the platform location past 12 months of age. A reversal test (the platform is moved to the quadrant of the maze diagonally opposite to the previous location) shows that the 70 % restricted rats perform better than ad libitum sujects during the first four trial block.

Hippocampal function was assessed by LTP of slices taken from 25 month old ad libitum and 70 % restricted rats. The results of the cross-sectional study currently in progress will be discussed in relation to the data of the longitudinal study.

349.13

EFFECTS OF AGE AND COGNITIVE STATUS ON RAT BRAIN INSULIN-LIKE GROWTH FACTOR-I BINDING SITE LEVELS. D. Seto, D. Diorio, W. Rowe, M.J. Meaney, R. Quirion and J.-G. Chabot. Douglas Hospital Research Centre, Department of Psychiatry, McGill University, Verdun, Quebec, Canada.

In recent years insulin-like growth factor-I (IGF-I) gene, protein expression and specific binding sites have been demonstrated in the mammalian (CNS). Additionally, some results have suggested that IGF-I may act as neurotrophic and/or neuromodulatory substance in the brain. During brain aging the densities of several neurotransmitter receptors are affected. In the present study, the levels of [^{125}I] IGF-I CNS binding sites in young adult (3, 6 and 9 month-old) and 24 month-old cognitively-unimpaired and cognitively-impaired Long Evans rats were determined. We observed that the densities of [^{125}I] IGF-I binding sites were similar in young and aged, cognitively-unimpaired rats. In the aged, cognitively-impaired animals receptor densities were similar, except in the CA1 region of the hippocampus where receptor density was slightly decreased. There was considerable (~50%) neuron loss in the CA1 region of the hippocampus in the aged, cognitively-impaired animals compared with both young and aged, cognitively-unimpaired animals, however, the same was true for the CA3 region where there was no change in IGF-I receptor density. Thus, the brain densities of IGF-I sites appear to be maintained throughout aging, with the possible exception of the CA1 area of the hippocampus.

349.14

SYMPATHETIC AND CHOLINERGIC RESPONSES TO NERVE GROWTH FACTOR (NGF) INFUSION IN YOUNG AND AGED RATS. R.M. Booze, M.L. Smith, R.D. Guarino*. Department of Physiology and Pharmacology, Bowman Gray School of Medicine, Wake Forest University, Winston-Salem, NC 27103.

Chronic infusion of NGF results in increased cholinergic cell survival in the medial septal nucleus after fimbria/fornix lesions. NGF administration also results in the proliferation of sympathetic axons which innervate blood vessels (Saffran, Brain Res. 1989). We investigated 1) whether these cholinergic and sympathetic responses occurred following NGF infusion and 2) whether the responses were influenced by aging.

Bilateral electrolytic and sham lesions of the fimbria/fornix were performed on young (3-4 months, n=12) and aged (22 month, n=12) male Fischer-344 rats. Vehicle (aCSF), 7S-NGF, and NGF antibodies were delivered by a subcutaneous Alzet osmotic mini-pump and a unilateral cannula placed in the dentate hilus of the dorsal hippocampus. Four weeks after surgery, serial cryostat sections through the septum and the dorsal hippocampus were collected for catecholamine histoflourescence, as well as for Nissl and AChE staining. NGF-infused young and aged brains displayed proliferation of the sympathetic innervation of cerebral blood vessels; however, only the aged brains showed sprouting into the surrounding parenchyma, most prominently in the forebrain contralateral to the infusion site. Lesioned animals of both ages displayed hippocampal sympathetic sprouting, although sprouting was less in aged animals. Infusion of NGF antibodies resulted in attenuated innervation of blood vessels in all rats, suggesting that the above effects are mediated by NGF. NGF-infused young and old brains showed a hypertrophy of cholinergic cell bodies in the medial septum.

These results confirm the responsiveness of sympathetic innervation and cholinergic neurons to NGF infusion in young and aged rats. Extrapolation of these data to aged humans suggests infusion of NGF as a cholinergic enhancing agent would induce a significant sympathetic response. (Supported by the American Federation for Aging Research and NIH grant RR-05404.)

349.15

NIMODIPINE MODULATION OF HIPPOCAMPAL NEUROPHYSIOLOGY *IN VIVO* AND *IN VITRO*. M. Mazzanti, O. Thibault, D.S. Kerr, and P.W. Landfield. Department of Physiology & Pharmacology, Bowman Gray Sch. of Med., Wake Forest Univ., Winston-Salem, NC 27103.

Nimodipine, a dihydropyridine (DHP) that blocks L-type Calcium (Ca) channels, appears to exert significant actions on central nervous system function. In aged animals, learning, motor behaviors and recovery of function have been shown to be improved by nimodipine (Schuurman *et al.*, Neurosci Res Comm, 1987; Gispen *et al.* In: The Calcium Channel, Springer, 1988; Deyo *et al.*, Science, 1989; for review, see Scriabine *et al.* FASEB J, 1989). Such findings are consistent with growing evidence that Ca currents are increased in aged rat hippocampal neurons (e.g., Landfield, In: The Calcium Channel, Springer, 1988). Studies on the actions of DHP's indicate clearly that these agents affect Ca currents in isolated neurons, but much less is known about DHP effects on the physiology of relatively intact brain systems.

In the present study, we examined the effects of nimodipine (40 mg/kg, p.o.) on hippocampal frequency potentiation (FP) of the extracellular population spike, in intact, anesthetized rats. Frequency potentiation is impaired consistently in aged rats, apparently due to excess Ca, and this impairment is correlated with impaired memory functions. Thirty minutes after nimodipine administration, FP during a 4 min train of 7 Hz stimulation was significantly greater in nimodipine animals, in comparison to vehicle controls. In particular, the spike was larger during the last 2 min of the train.

Intracellular recordings in hippocampal slices were also conducted, to analyze the possible mechanisms underlying the nimodipine effects on spike FP. Nimodipine decreased, and BAY K 8644 (a DHP agonist) increased, the calcium-dependent, potassium-mediated afterhyperpolarization (AHP) in these neurons. This AHP is also increased in aged neurons.

Thus, the AHP may be modulated through L-type channels, and nimodipine may strengthen spike FP by blocking inhibitory effects of the AHP.

349.16

AGE-DEPENDENT CHANGES IN HIPPOCAMPAL SLOW WAVE PERIODICITY IN THE ANESTHETIZED RABBIT. S.D. Berry and L.J. Dreshfield. Psychology Dept., Miami Univ., Oxford, OH 45056.

Extracellular unit activity and slow waves were recorded from young (6-11 mos.) and aged (48-50 mos) New Zealand white rabbits. Animals were anesthetized (Ketamine 50 mg/kg; Xylazine, 10 mg/kg; i.m.) and maintained in a stereotaxic headholder throughout recordings. Stainless steel microelectrodes (3-5 um. tip) were lowered into area CA1 of the dorsal hippocampus until cellular activity could be isolated in the pyramidal cell layer. Several minutes of free-running activity were recorded on a VCR-based instrumentation recorder (Vetter, Model 420C). At the end of recording, animals were euthanized with T-61 (Hoechst-Roussel) and electrode sites were marked with dc current. Slow wave records were filtered (0.5-25 Hz) and digitized at 100 Hz. Autocorrelations of 5 sec. samples indicated that aged rabbits had significant periodicity at approximately 3-4 Hz, whereas slow waves from younger animals were dominated by 2-2.5 Hz activity. Treatment with scopolamine hydrobromide (1.5 mg/kg, subcutaneous) was observed to shift the slow wave periodicity in younger rabbits toward the 3-4 Hz period seen in older subjects. Results indicated differences in hippocampal slow wave activity between young and old rabbits, and suggested a cholinergic basis for age-related differences. (Supported by research grant # AGO7014, NIH)

349.17

INTRACELLULAR RESPONSES OF NEOSTRIATAL NEURONS IN AGED RATS: EFFECTS OF N-METHYL-D-ASPARTATE. M. S. Levine and C. Cepeda. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

Previous studies from our laboratory have demonstrated decreased excitation in neostriatal neurons in aged rats and cats. Results obtained from intracellular recording experiments in in vivo and in vitro preparations indicated that a population of cells were less capable of generating action potentials and displayed higher thresholds to evoke EPSPs by extracellular stimulation. The present study was designed to assess the responsiveness of aged neostriatal neurons in slices to application of excitatory and inhibitory amino acids by combining intracellular recordings with iontophoresis. Slices (400 μm) were obtained by standard methods from young (3 month) and aged (>24 month) Fisher 344 rats. Intracellular recordings were obtained with K-Acetate filled microelectrodes (60-120 MΩ). A five-barrelled pipette containing NMDA, AP-5, GABA, glutamate and saline was positioned close to the recording microelectrode (100-200 μm). In all young neurons (N=10), NMDA produced robust depolarizations accompanied by repetitive firing. In aged neurons NMDA produced less consistent responses. In about 50% of the tested cells (N=12) to date, NMDA depolarized the membrane but action potentials were not generated. The specific NMDA antagonist AP-5 blocked NMDA responses in cells obtained from young rats. However, antagonism of NMDA responses by AP-5 required higher currents in aged cells. In both aged and young cells, GABA decreased the amplitude of the EPSP, depolarized the membrane and greatly increased ionic conductance. The decrease in NMDA responsiveness in aged neostriatal neurons may account, in part, for the decreased ability of these cells to produce excitatory responses. Supported by USPHS AG 7462.

349.18

INTRACELLULAR RESPONSES OF NEOSTRIATAL NEURONS IN AGED RATS: DECREASED MODULATION BY DOPAMINE. M. Bertolucci-D'Angio, C. Cepeda, D. Birt, N.A. Buchwald and M.S.Levine. Mental Retardation Research Center, UCLA, Los Angeles, CA 90024.

Evidence indicates that alterations in neostriatal (NS) dopaminergic function occur during aging. We have demonstrated that in aged cats the ability of dopamine (DA) to modulate evoked cortical neuronal responses decreases. The present study used intracellular recording to assess DA's ability to modulate responses of NS neurons. Recordings were obtained from cells in slices of young (3 months) and aged (>24 months) Fisher 344 rats. DA was bath applied at a series of ascending concentrations. The average resting membrane potential (RMP) and action potential amplitudes (AP) were similar in neurons recorded in each age group (67±3.2 (Mean±SD) vs 65±9.1 mV for RMP and 60±5.1 vs 62±11 mv AP for aged and young groups, respectively). Input resistance was slightly higher in neurons in aged rats (29±8.2 vs 25±9.2 MΩ). DA induced a dose-dependent depolarization of the RMP (6.2±2.4 mV at 10 μM to 10±4.6 mV at 100 μM) in both groups. As described previously, in virtually all neurons in young rats DA reduced the amplitude of a depolarizing postsynaptic potential evoked by local extracellular stimulation in a dose-dependent and reversible manner. The magnitude of the change increased from 28% (10 μM)to 46% (100 μM). To date, in neurons obtained from slices in aged rats, DA produced no effect on extracellularly evoked depolarizations or effects occurred only at the highest concentrations (100 μM). These results demonstrate that the ability of DA to modulate synaptic input to NS neurons is compromised during aging and are consistent with observations indicating a loss of DA receptors with age. Supported by USPHS AG 7462.

349.19

CHANGES IN ELECTROPHYSIOLOGICAL PROPERTIES OF AGED CAT LUMBAR MOTONEURONS FOLLOWING AXOTOMY. J.K. Engelhardt, J. Yamuy*, F.R. Morales and M.H. Chase. Brain Research Institute, Dept. of Physiology and Dept. of Anatomy & Cell Biology, UCLA Sch. of Med., Los Angeles, CA 90024.

The electrophysiological properties of normal adult motoneurons are dramatically altered following axotomy. The experiments reported here were undertaken to determine whether the aging process interferes with the ability of motoneurons to respond to axotomy. Selected hindlimb muscle nerves were cut in 5 cats (13-16 years old) and measures were taken to avoid reinnervation. After 4-6 weeks the animals were prepared for intracellular recording from identified hindlimb motoneurons. Various basic electrophysiological properties were measured and the data were analyzed within the framework of linear cable theory. Axotomized old motoneurons, when compared with unaxotomized control motoneurons from different nerves in the same animals, exhibited a 35% reduction in axonal conduction velocity, a 127% increase in cell input resistance and a 45% increase in membrane time constant. Estimates of total cell capacitance indicate that the increase in input resistance after axotomy is due to the combined effects of an increase in membrane resistance (reflected in the increase in membrane time constant) and a decrease in cell surface area, relative to the control, non-axotomized old motoneurons. We conclude that, in spite of the aging process, old motoneurons retain their ability to react to axotomy. (Supported by USPHS AGO 4307.)

349.20

EFFECT OF NORMAL AGING ON MEMBRANE PHOSPHOLIPID METABOLISM BY ^{31}P *IN VIVO* NMR SPECTROSCOPY. Panchalingam K, Pettegrew JW, Strychor S and Tretta M. Laboratory of Neurophysics, Dept. of Psychiatry, U. of Pittsburgh, Pittsburgh, Pa. 15261

^{31}P *in vivo* nuclear magnetic resonance studies (NMR) were performed on normal controls. ^{31}P NMR spectroscopy provides a direct, non-invasive assessment of phosphomonoesters (PME), phosphodiesters (PDE), high energy phosphates phosphocreatine and adenosine triphosphate (ATP). The measurements were made in the dorsal prefrontal cortex of normal subjects (N=29) between the ages of 12 and 81 years. All subjects were screened for and determined to be free of medical, neurological and psychiatric disorders. The results demonstrate that the PME levels decrease linearly with age ($p=0.01$, $r=0.52$) and the PDE levels increase with age. This indicate a decreased synthesis and increased breakdown of membrane phospholipids with aging in human cerebral cortex. These measurements are compared with those measured in Alzheimer's Disease (ADRDA-NINCDA criteria) patients and schizophrenia patients. In AD patients, the PME levels are elevated in the early stages of the disease and then decreased as the disease progresses. This would indicate an increased membrane phospholipid synthesis during the early stages of AD. The high energy phosphates are not altered in AD brains. In schizophrenics, the PME levels are decreased ($p=0.005$) and PDE levels are increased ($p=0.01$) indicating a abnormal breakdown of membrane phospholipids. There is no alteration in the high energy phosphates. These findings provide molecular insights into normal brain aging process and how it differs from AD and schizophrenia.

349.21

VIBRATION-INDUCED CEREBRAL BLOOD FLOW RESPONSES: EFFECTS OF NORMAL AGING. L.W. Tempel, J.S. Perlmutter. Departments of Neurology and Radiology, Washington Univ. Sch. of Med., St. Louis, MO 63110.

The purpose of this study was to determine whether vibration-induced cerebral blood flow (CBF) responses change with normal aging. We measured CBF with PET and bolus-administered $H_2{}^{15}O$ in 14 "young" (mean 23 yr;range 20-27) and 12 "elderly" (mean 58 yr;range 40-72) subjects. Mean global CBF did not significantly change from resting-state to vibration in young or elderly. Furthermore, mean CBF did not significantly correlate with age. Regional CBF respohses were identified in each individual by subtraction-image analysis. The responses in elderly and young subjects then were compared by construction of an image with each pixel representing a computed t statistic based upon regional values from the individual subtraction images from the 2 groups. The advantage of this approach is that it considers regional variance rather than assuming constant variance throughout the brain. No substantial differences between responses in young and elderly subjects were identified. In contrast, T images comparing rest and vibration scans show consistent responses in both young and elderly in contralateral primary sensorimotor area (PSA), supplementary motor area (SMA) and ipsilateral cerebellum. Since cerebellum was often not included within the limited axial range of our studies, we only calculated the linear regression between age and the magnitude of blood flow responses in PSA and SMA. There were no substantial relationships between age and responses to either left or right hand vibration (PSA slope=-.10 and .02,SMA slope=-.02 and -.01,respectively). We conclude that the blood flow responses to vibrotactile hand stimulation are both qualitatively and quantitatively similar in young and elderly normals.

NEUROTRANSMITTER AND NEUROMODULATOR DEVELOPMENT

350.1

LYSOPHOSPHATIDYLSERINE ENHANCES THE TRANSFER OF 22:6n3 TO PHOSPHATIDIC ACID IN BRAIN MICROSOMES. Z-Y Hu*, G. Y. Sun and P. Rhodes. Dept. Biochem. and Child Health, Univ. Missouri School of Medicine, Columbia, MO.

Phosphatidylserine (PS) in brain is known to contain a high proportion of docosahexaenoic acid (22:6n3) although exactly how this acyl group profile is attained is not understood. PS is important in many neuronal membrane functions. In this study, rat brain microsomes were incubated with ^{14}C-22:6n3, ATP, and CoA in the presence and absence of LPS in order to test whether some 22:6n3 may be incorporated into PS through the lysophospholipid: acyl-CoA acyltransferase route. Similar incubations were carried out with other lysophospholipids (LPI, LPC, LPA) for a comparision. Results indicated that in spite of an active uptake of labeled 22:6n3 by PC, PI and PA in the presence of their respective lysophospholipids, LPS was a poor substrate for this reaction. Surprisingly, LPS (5-20 μM) specifically enhanced the incorporation of labeled 22:6n3 into PA in microsomes by 2-3 fold. This effect of LPS is probably not due to an increase in LPA resulting from phospholipase A_2 action because incoporation activity was not affected by EGTA. On the other hand, addition of LPA to microsomes gave a corresponding increase in labeled PA, and LPS further increased this activity. Results seem to suggest that LPS enhanced PA synthesis by activating LPA acyltransferase through some kind of physical interaction with the enzyme.

350.2

FUNCTIONAL Na^+ CHANNELS AND ENDOGENOUS GABA PRECEDE FUNCTIONAL $GABA_A$ RECEPTORS IN EMBRYONIC RAT STRIATUM. M.L. Fiszman, G.D. Lange, N.S. Nadi, S.V. Smith, E.A. Novotny, and J.L. Barker. LNP and ICS, NINDS, NIH, Bethesda, MD, 20892, USA.

The development of excitability in embryonic CNS cells was examined by flow cytometry using oxonol, a fluorescent, voltage-sensitive, indicator dye. Cell suspensions were obtained from rat cerebral hemispheres at 13 days gestation (E13) and from striatum (STR) at E14, E15 and E17. As previously described (Fiszman *et al*, *Dev. Brain Res.* in press), cells stained with the supravital dye acridine orange (AO) fell into two populations. The high Forward Angle Light Scatter population (high FALS) was strongly stained with the dye and comprised 85% of scattering events. About 10% of the events collected scattered little light (low FALS) and accumulated less AO. Analysis of sorted material showed that the low FALS population contained flat, phase-dark cells which were neurofilament negative; subcellular fragments and phase-bright cells were neurofilament positive. Cell diameters ranged from 1 to 3μm. The high FALS population was 99% phase-bright, neurofilament-positive cells. Diameters ranged from 4 to 10μm. Exposure of E13 cells to veratridine depolarized the high FALS population. However, these cells did not respond to the $GABA_A$ agonist muscimol. In the E14 STR muscimol depolarized only the high FALS population. Responses to both muscimol and veratridine were well developed in both populations at E17. Detectable amounts of GABA, determined by HPLC, were found in E13 cortex and at all ages in STR. Our results suggest that Na^+ channels are functionally operative before $GABA_A$ receptors and that endogenous GABA is present before the appearance of the responses induced by $GABA_A$ agonists. The differences in responsiveness among populations during the development of the STR suggest heterogeneity in the distribution of Na^+ channels and $GABA_A$ receptors. This distribution becomes more homogeneous as development proceeds.

350.3

EXPRESSION OF GLUTAMATE-LIKE IMMUNOREACTIVITY IN DEVELOPING COCHLEAR AND VESTIBULAR GANGLIA OF THE QUAIL. M. Mujeeb, J. Represa and P. Bernd, Department of Anatomy and Cell Biology, SUNY Health Science Center, Brooklyn, NY 11203.

It is believed that glutamate and aspartate are the neurotransmitters released by cochlear and vestibular neurons in the adult. The purpose of the present study was to determine whether glutamate is present in developing cochlear and vestibular ganglia, and, if so, establish the timing of glutamate expression. Frozen sections (10 µm thick) of the temporal region of quail embryos aged 7, 9, 14, and 15 days were prepared and examined for glutamate-like immunoreactivity (Glu-LI). A mouse monoclonal anti-glutamate antibody (INCSTAR Corp.) was used at a dilution of 1:1000, in conjunction with an avidin-biotin immunoperoxidase technique. Glu-LI was detected in embryonic cochlear and vestibular ganglia at all ages examined. In all cases, processes were darkly stained, while neuronal cell bodies became more intensely immunoreactive as development proceeded. It remains to be determined when Glu-LI first appears in cochlear and vestibular ganglia; ages prior to 7 days have not yet been examined. This study demonstrates that the putative neurotransmitter, glutamate, is present in cochlear and vestibular neurons during development. Supported by a grant from the Deafness Research Foundation.

350.4

DEVELOPMENTAL CHANGES IN APPARENT NMDA-ANTAGONIST EFFICACY IN HIPPOCAMPAL CA_3 PYRAMIDAL CELLS. R.J.Brady and J.W.Swann, Wadsworth Ctr for Labs. & Res., NYS Dept. of Health, Albany, NY 12201.

Evidence exists for heterogeneity in the NMDA receptor population. We have shown a developmental change in the divalent cation sensitivity of the NMDA receptor-channel complex in the CA3 infrapyramidal zone (IPZ). In recent studies NMDA-induced depolarization of CA3 pyramidal cells was evoked by iontophoretic agonist application into the proximal portion of the basilar dendrites, the IPZ. Intracellular recordings of the responses were obtained under current-clamp in mature (PND>35) and immature (PND 10-15) hippocampal slices in which both synaptic activity and calcium currents were blocked. An iontophoretic dose-response relationship was constructed for each slice and the current needed to produce a response that is 50% of maximum calculated. The EAA-receptor antagonists kynurenic acid (KYN) or D(-)2-amino-5-phosphonovaleric acid (D(-)AP5) were then added to the bathing media at different concentrations. Dose-response curves were obtained under each condition to assess the shift. This effect can be quantified for a single antagonist concentration by calculating the ratio of the currents needed to produce a response that is 50% of maximum, A^1. In hippocampal slices taken from immature rats 100µM KYN yields an A^1 value of 2.41±0.05 while in adult tissue, 1.55±0.03. A similar observation was seen with D(-)AP5. The greater antagonist efficacy in immature tissue is a further indication of developmental change in the IPZ NMDA receptor population. Perhaps an antagonist-preferring form of the receptor-channel complex predominates in the immature tissue. Supported by grants NS23071 to RJB and NS18309 to JWS from NINDS-NIH.

350.5

HISTOLOGIC ALTERATIONS OF THE CEREBRAL CORTEX IN ADULT MICE PRENATALLY TREATED WITH DIAZEPAM. Márquez-Orozco A., Márquez-Orozco, M.C. and Alcantara-Ortigoza, M.A. Dept. of Embriology, School of Medicine, UNAM, 04510 México, D.F. MEXICO.

Prenatally administered diazepam accumulates in the fetal cerebral and cerebellar cortices and mesencephalon. Posnatally until adult age, these mice present alterations in locomotor activity, swimming pattern, and long-term memory. We investigate if histologic alterations in motor cortex occur in adult mice prenatally treated with diazepam. Single daily doses (2.7 mg/kg) of diazepam were i.p. administered to female CD-1 strain mice, between the 6th and 7th day of gestation. A control group received equivalent volumes of saline sol. The offsprings were wet-nursed by non-treated mice, weaned, and kept for 240 days. Their motor activity and swimming pattern, were periodically measured from the 6th day till the 8th month. Mice were deeply anesthetized, perfused with 10% formaline, and the brains were removed, fixed, and stained with current and a modified fast-Golgy thechniques. Forty µm sections were observed under a photonic microscope. The experimental animals showed atypically distributed and more aboudant glia cells. The dendritic patterns and vascular distribution differed from those of the control animals. Locomotor activity and swimming patterns were altered, which could indicate motor cortex impairment.

350.6

CHANGES IN CORTICAL MONOAMINE CONTENT AND NEURONAL RESPONSE IN RATS DURING EARLY ADULTHOOD. J. Goyer, A. Ferron and T.A. Reader, Departement de physiologie, Faculte de Medecine, Universite de Montreal, C.P. 6128 Succ. A, Montreal, P.Q., CANADA H3C 3J7.

Age-related alterations in the response of parietal cortex neurons to microiontophoretic applications of the three monoamines (MA) noradrenaline (NA), dopamine (DA) and serotonin (5-HT), and their agonists were investigated in relation to measurements of endogenous cortical MA levels. Single-unit cell recordings were made from 3, 6 and 12-month old male Sprague-Dawley rats under urethane anesthesia. Two response parameters were taken into account: the responsiveness (% of responsive cells), and the sensitivity, expressed as the charge (in nC) which caused a 50% reduction in the spontaneous firing rate. When compared with 3-month old rats, 6-month old rats showed a reduced sensitivity to MAs (NA 1944 vs 1502 nC; DA 2300 vs 1265 nC; 5-HT 2123 vs 970 nC) and HPLC assays showed very large increases in both NA and 5-HT content (NA 3.6 vs 1.8 ng/mg prot.; 5-HT 3.2 vs 1.3 ng/mg prot.), while DA content showed a small but significant decrease (.01 vs.06 ng/mg prot.). The observed MA sensitivity changes were paralleled by corresponding changes in the sensitivity to amidephrine (α1 agonist), quinpirole (D2), and mCPP (5-HT1B) and DOI (5-HT2). In 12-month old rats, both responsiveness and sensitivity to all three amines were decreased (NA 53% vs 89% responsiveness, 2407 vs 1944 nC; DA 55 vs 83%, 3300 vs 2300 nC; 5-HT 50 vs 80%, 2526 vs 2123 nC), while MA content remained unchanged (NA 3.4 ng/mg prot.; DA 0.02 ng/mg prot.; 5-HT 2.7 ng/mg prot.), as compared to the 6-month old rats. These results indicate that neuronal sensitivity to NA, DA and 5-HT may be regulated by factors other than neurotransmitter availability. Furthermore, major developmental modifications of the cortical MA systems may still occur in rats as late as the age of 12 months.

350.7

HEMICHOLINIUM-3 BINDING SITE REGULATION *IN VIVO* DURING POSTNATAL DEVELOPMENT OF THE RAT. H.K. Happe and L.C. Murrin, Dept. of Pharmacology, University of Nebraska Medical Center, Omaha, NE 68198

High affinity choline transport is the rate limiting step in the synthesis of acetylcholine. [^{3}H]Hemicholinium-3 (HC3) binds with high affinity and specificity to a site associated with the choline transporter and both choline transport and the apparent number of HC3 binding sites are regulated *in vivo* by drug treatments known to alter acetylcholine release and *in vitro* by depolarization. Previously we described the development of HC3 binding sites by autoradiography. Low levels are present during the early postnatal period, and then increase significantly by day 15 in striatum and by day 21 in cortex. In this study we examine the *in vivo* regulation of HC3 binding sites in adult rats and at postnatal days 5 and 15. Animals were administered drugs i.p. for 30-60 min. and striatal and cortical membrane fractions were prepared. HC3 binding was assayed at 10 nM [^{3}H]HC3. Blanks were generated with 10 µM cold HC3. Nicotine (5 mg/kg) increased HC3 binding sites in adult striatum and cortex by 10-20% and produced even greater increases at d15 and d5. Haloperidol (4 mg/kg) increased binding sites in striatum only, with the greater effect on d5 animals. Oxotremorine (1.1 mg/kg) greatly reduced binding in both adult cortex and striatum. The effects of oxotremorine in cortex were diminished at earlier ages. On the other hand atropine (20 mg/kg) did not significantly affect HC3 binding sites at any age. Pentobarbital (64 mg/kg) decreased HC3 binding in adult striatum but had no significant effect in cortex. The striatal effect of pentobarbital was more pronounced in day 5 animals. In summary, we report that acute nicotine treatment increases apparent HC3 binding site number, in addition to other drugs previously reported to alter HC3 binding. All drug treatments that were effective in adult animals were also effective as early as day 5, indicating that the small population of cholinergic terminals present during early postnatal development are regulated by similar muscarinic and nicotinic cholinergic, and dopaminergic mechanisms. It is not known if the changes in magnitude of drug effects are due to differences in drug metabolism or to developmental differences in regulation of cholinergic mechanisms. Supported by NS23975 and the McDonald Foundation.

350.8

KINESIN IS DEVELOPMENTALLY REGULATED IN RAT BRAIN. K.L. Moya, B. Tavitian and L. Di Giamberardino. S.H.F.J., C.E.A., INSERM U334, 91406 Orsay, France.

Kinesin mediates ATP-dependent movement along microtubules and is considered to be the "motor" for rapid anterograde axonal transport. We used a monoclonal antibody to the 120kD form of bovine brain kinesin to examine developing rat brain.

The antibody recognized a single protein (115kD, pI=6.5) on 2-D immunoblots of partially purified microtubule associated proteins. Quantification of 1-D immunoblots containing homogenates from E(mbryonic day)18, P(ostnatal day)0, P3, P7, P14, P21 and adult brains showed that relative levels of kinesin greatly decreased after P14. Partial subcellular fractionation revealed that soluble kinesin was more abundant than kinesin in the crude membrane fraction. Furthermore, the decrease of kinesin in the two fractions was markedly different. Soluble kinesin decreased sharply and constantly from E18 to P14, while levels in the particulate fraction remained similar from E18 to P14 then greatly diminished at P21. These results show that kinesin is more abundant in the brain early in development and that the subcellular localization appears to be differentially regulated.

350.9

FREE RADICAL GENERATION FROM DOPAMINE THROUGH THE ACTION OF MONOAMINE OXIDASE (MAO). F.F.Ahmad, D.L.Cowan* and A.Y.Sun.Dept. Pharmacology and Dept. Physics*, University of Missouri, Columbia, MO 65212.

It is known that monoamine oxidase (MAO) catalyzes the oxidative deamination of catecholamines to aldehydes according to the following equation:

$$R\text{-}CH_2\text{-}NH_2+O_2+H_2O \longrightarrow R\text{-}CHO+H_2O_2+NH_3.$$

Since free radical damage has been implicated in cellular degeneration in the brain, particularly the dopamine neurons in Parkinson's disease (PD), we used the ESR spin trapping technique to detect whether dopamine (DA) may serve as a substrate for free radical formation. Incubation of DA with mouse brain mitochondria resulted in the formation of free radicals. The spin adduct of 5,5-dimethyl pyrrolidine-N-oxide (DMPO) of the hydroxyl radical and an unidentified carbon center radical were detected. The free radical formation was demonstrated with purified MAO and was inhibited by either pargyline or Clorgyline (MAO inhibitors). It is possible that under certain conditions, the abnormal metabolism of dopamine may lead to free radical generation, resulting in cell death and tissue injury. (Supported by AA7585.)

350.10

DEVELOPMENTAL CHANGES OF SYNAPTIC TYROSINE KINASE AND pp60src IN RAT FOREBRAIN. S.B. Cudmore and J.W. Gurd Dept. of Biochemistry, Scarborough Campus, University of Toronto, Ontario, M1C 1A4.

The subcellular distribution of protein tyrosine kinase (PTK) and phosphotyrosinylated proteins in the developing rat forebrain has been studied. Subcellular fractions from rats ranging in postnatal age from 5 to 60 days were prepared and analyzed for i) PTK activity using polyglutamyltyrosine as substrate ii) endogenous PTK substrates using polyclonal antibodies specific to phosphotyrosine and iii) developmental changes in levels of pp60src. PTK activity was highest in homogenate of young brains (p10) and decreased 2.7-fold to adult levels by p60. Although PTK activity of all fractions underwent a generalized decrease during development, different fractions exhibited characteristic developmental profiles. PTK activity in P3 was maximal at p5 and decreased 44% to adult values by p60. Both cell and synaptosome soluble activity decreased 40-50% between p10 and p15 and an additional 20-25% by p60. PTK activity in the synaptosome particulate (SP) fraction exhibited a biphasic developmental profile with maximal levels occurring at p10 and p20 and then underwent a 50% decrease by p60. Endogenous substrates for PTK were identified by incubating individual fractions with 2 mM ATP in the presence of Na-O-Vanadate and probing nitrocellulose blots with antiphosphotyrosine antibodies. Several PTK substrates were identified in SP and P3, including proteins of Mr 180K, 120K, 100K, 85K, 68K, 62K, 54K, 52K and 42K. Two major p-tyr containing proteins (Mr 54/52 and 120K) were identified in S3 while the 54/52K protein was the only p-tyr containing protein detected in the synaptosome soluble. Changes in the levels of individual p-tyr containing proteins occurred during development. Western blotting using monoclonal antibody 327, specific for pp60src, showed that pp60src levels decreased throughout development in homogenate and P3. In contrast, pp60src in SP was highest between p15 and p25 before decreasing to adult levels. These results indicate that both the tyrosine phosphorylation of individual proteins and the levels of PTK are developmentally regulated and suggest that PTK may play a specific role in synaptogenesis. Supported by N.S.E.R.C.

LIMBIC SYSTEM II

351.1

EFFECT OF WATER TEMPERATURE ON SPATIAL LEARNING (MORRIS MAZE) IN INFANT RATS. K.L. ALTEMUS, N.J. LITTLE*, E.A. Foley* and C.R. Almli. Develop. Neuropsychobiol. Lab., Dept. Psych., Washington University, St. Louis, MO 63130.

Previous work has dated the emergence of object localization abilities in a Morris water maze task to postnatal day 18. This study determined the effect of water temperature (23°C vs 30°C) on the ontogeny of spatial abilities. Albino rats were tested in a water maze under proximal, distal, or random conditions for 3 consecutive days, with testing beginning on postnatal days 15, 16, 17, 18, or 19. Latencies to enter the escape quadrant and latencies to escape were recorded for each session of 12 1-min acquisition trials. Each daily session concluded with a 1-min probe trial, during which escape was impossible. Latencies to enter the quadrant previously containing the platform, and the total time within this quadrant, were recorded. Water temperature did not affect the age at which spatial behaviors emerged (postnatal day 18), but did enhance "performance" as early as postnatal day 19. (Conducted under NIH Guide for Care and Use of Laboratory Animals)

351.2

EFFECTS OF THE NMDA ANTAGONIST MK-801 ON BEHAVIOR, BODY WEIGHT AND DENTATE GRANULE CELL MORPHOLOGY IN DEVELOPING RAT PUPS. B.J. Claiborne & A.M. Felthauser*. Div. of Life Sciences, University of Texas, San Antonio, TX 78285.

We are interested in the relationship between neuronal activity and dendritic morphology in the hippocampus. Here we have examined the effects of MK-801, an antagonist of the NMDA subclass of glutamate receptors, on the concurrent dendritic growth and branch loss seen in developing dentate granule cells (Rihn & Claiborne, Dev. Brain Res., In Press). Rat pups were treated with MK-801 (1 mg/kg i.p.) or saline every 12 hours for 10 days, beginning on postnatal day 14. Animals (n=8) were sacrificed on PN day 25 and granule neurons (n=18) were filled with Lucifer Yellow in fixed hippocampal slices.

The MK-801-treated pups displayed marked behavioral effects beginning with sedation for the first two days, followed by hyperactivity for the remainder of the period and, on PN day 25, weighed 30% less than the littermate controls (41 ± 1.6 vs. 63 ± 3.3 gms; mean $\pm$ S.D.; $P < .001$). However, dentate molecular layer width, number of dendritic branches per granule cell, transverse dendritic spread and percentage of branches reaching the top of the layer were similar for the two groups. These data show that although MK-801 treatment affected both activity patterns and weight gain in the pups, it did not influence developmental changes normally seen in dentate granule cells during this period. (Supported by NSF)

351.3

ADRENAL HORMONE EFFECTS ON HIPPOCAMPAL EXCITATORY AMINO ACID RECEPTOR BINDING. A. S. Clark and C.W. Cotman, Department of Psychobiology, University of California, Irvine, CA 92717.

Synaptic transmission in the hippocampus is primarily mediated by excitatory amino acids (EAA). The hippocampus is also a major target for adrenal steroids. To define whether adrenal hormones might influence the plasticity of hippocampal neurons via modulation of EAA receptors, we studied the effects of acute adrenalectomy and hormone replacement on EAA binding in the rat hippocampus using quantitative autoradiography.

Adult male Sprague-Dawley rats were adrenalectomized (ADX) and given access to 0.9% saline. Corticosterone (CORT) was administered by injection (10 mg/day s.c.) or in the drinking water (25 µg/ml). Rats were killed after 5-7 days, the brain removed, frozen on dry ice and stored at -70 °C. Quantitative autoradiography of glutamate receptor subtypes (NMDA, kainate and AMPA) was conducted on 6 µm cryostat sections through the hippocampus. Binding to the NMDA receptor was highest in the stratum radiatum of CA1 in animals of all treatment groups (1900 fm/mg protein). No marked changes in NMDA binding were observed in any hippocampal area although a small (10-15%) decrease in NMDA binding in ADX rats relative to controls was sometimes present in CA3 radiatum. Kainate binding was highest in stratum lucidum of CA3 (2200 fm/mg protein). A small decrease in kainate binding after ADX was occasionally observed in CA3 radiatum. CORT replacement had no effect on EAA binding in any hippocampal region measured. Although adrenal hormones likely play some role in hippocampal function, their short-term manipulation appears to have a limited influence on the binding of EAA to their synaptic receptors. However, interactions between these two systems may modify events subsequent to receptor binding. Supported by the Pew Foundation.

351.4

MICRODIALYSIS OF SEPTOHIPPOCAMPAL LESIONED ANIMALS. D. Maysinger, D.G. Herrera and A.C. Cuello; Dept. of Pharmacology & Therapeutics, McGill University, 3655 Drummond St., Montreal, Quebec, H3G 1Y6, Canada.

Microdialysis probes (4mm) were implanted bilaterally into the hippocampi of adult male Wistar rats. Artificial cerebrospinal fluid (CSF) with and without KCl (100mM) was then delivered at a rate of 2ul/min into the hippocampi. Administration of K+ in high concentrations, but not CSF alone, led to behavioural alterations including manifestations typical of motor seizure. In some animals, the putative seizure reached stage 4 on the scale of Racine (Electroenceph. Clin. Neurophysiol. 32:281). Alterations were observed in neurotransmitter release and c-fos expression (assessed by immunostaining). Furthermore, animals bearing a unilateral fimbria-fornix lesion which underwent similar testing, showed comparable motor responses. Degenerative changes in the medial septum following fimbria-fornix lesion were assessed with ChAT and NGFr immunostaining. These alterations are being correlated with the changes in the release of various neurotransmitters into the extracellular space. Supported by M.R.C. (Canada) and Canadian Centers of Excellence Network for Neural Repair and Functional Recovery.

351.5

EFFECTS ON BASAL FOREBRAIN STRUCTURES FROM EXPERIMENTALLY INDUCED HYDROCEPHALUS. R.M. Kriebel, J.J. Stella*, K.E. Miller and J.P. McAllister. Depts Anatomy, Phila Col Osteopath Med, Temple Univ Sch Med, Phila PA 19131 and Searle/Monsanto Co., St. Louis MO 63198.

The neurological deficits found in infantile hydrocephalus have most often been explained by pathological changes in cerebral cortex. It has been the primary goal of our studies to provide a cellular basis for the residual neurological deficits observed even though surgical intervention may have relieved the effects of ventriculomegaly on the cerebral cortex. Our previous studies have shown decreases in ChAT labeled neurons and severe neuropil degeneration of the basal forebrain region, especially in septal nuclei. These studies suggested that basal forebrain as well as cortex should be considered in mechanistic explanation of neurological deficits seen with this disorder. In the present study we attempted to discern if on a macroscopic level specific regions within the basal forebrain were differentially affected in experimental hydrocephalus, and if ventriculoperitoneal (VP) shunt treatment reversed the effect. Kaolin injection induced hydrocephalus, aldehyde fixed brains were sectioned coronally with subsequent staining for cells and fibers. The basal forebrain was divided according to standard atlas designations and planimetrically analyzed with a Bioquant® system. Decrease in volume was observed in all areas of basal forebrain; these changes were only partially normalized in VP brains. NIH support HD21527.JPM

351.6

THE EARLIEST AREAL DIFFERENTIATION OF THE HUMAN CEREBRAL CORTEX: ENTORHINAL AREA. I. Kostović, Z. Petanjek and M. Judaš. Sect. of Neuroanatomy, Medical Faculty Zagreb, YU.

We have used Golgi and Nissl method to identify neurons and architectonic laminae expressing the early areal specialization in the cerebral cortex of human postmortem fetuses (10-13 weeks of gestation - w.g.). The first area-specific cells are large neurons ("organizers") with widely bifurcating apical dendrites, appearing at the outer margin of the cortical plate of the prospective entorhinal cortex (E.C.) of 10w.g. fetus. Concomitantly, multilaminated spread of the deep part of the cortical plate occurs. In a 10,5 w.g. fetus "organizer" neurons form magnocellular layer. At 13 w.g., characteristic fiber-rich dissecant and cell-dense principal laminae develop unevenly within the cortical plate of E.C., causing precocious subareal parcellation of E.C. In rostral entorhinal subareas cell islands of the future layer II develop at the subpial depths where "organizer" neurons reside. In caudal entorhinal subareas well developed lamina dissecans stops abruptly at the border of neocortex while lamina principalis interna continues into upper subplate zone. In conclusion, both area-specific neurons ("organizers") and fiber-rich (afferent) strata develop synchronously during the earliest areal differentiation of the cerebral cortex. The precocious lamination of the cortical plate is crucial event in the histogenesis of the entorhinal cortex. Supp. by U.S.-Yugoslav Joint Board JF 855.

351.7

DEVELOPMENT OF TH-POSITIVE AND DBH-POSITIVE FIBER INNERVATION OF THE RAT HIPPOCAMPUS. A.M. Moudy, D.D. Kunkel, A.T. Malouf & P.A. Schwartzkroin. Dept. Neurol. Surgery, Univ. Washington, Seattle, WA 98195.

Development of noradrenergic innervation of rat hippocampus (HC) by locus coeruleus (LC) was examined histochemically in the roller tube cultured slice preparation and in fixed tissue from rats 4 to 21 days postnatal. The presence of tyrosine hydroxylase (TH)- and dopamine beta-hydroxylase (DBH)-positive fibers was evaluated in sections of HC and LC. HC and LC slices (250 μm) were obtained from brains of 4-day-old pups with a Vibratome tissue slicer and co-cultured in the roller-tube slice preparation. Large, multipolar TH- and DBH-positive cells were visible within LC, and positively stained fibers could be seen traversing the space between LC and HC. In sections from 21-day-old rats, the highest density of stained fibers was found in str. radiatum (SR) of CA3, and in the hilus. Beaded fibers could be seen traveling from the fimbria through the pyramidal cell layer, branching extensively in SR. Heavy fiber densities in the infragranular region of dentate gyrus (DG) were oriented parallel to the granule cell layer, but very few could be found among the cell bodies in str. granulosum. Intermediate densities of fibers were found in CA1 SR, lacunosum and oriens, and in the molecular layer of DG. Other areas of HC showed only low-density innervation. Tissue from 10-day-old rats showed fibers in CA3, not as dense as in adult, but in a similar pattern. Innervation was more evenly distributed within the hilus, without parallel organization into a dense band. In 4-day-old tissue, fibers were much more sparsely distributed. Very few fibers were seen in either CA3 or CA2. In CA1, fibers were far less dense than in adult, but more dense than in either CA2 or CA3. DG showed very few positive fibers, almost none in the infragranular band. The subicular area had the most dense distribution pattern at 4 days.

Supported by NIH, NINDS fellowship NS08639, and grant NS15317.

351.8

SYNAPTIC DEVELOPMENT AND REORGANIZATION IN CA1 OF RAT HIPPOCAMPAL SLICE CULTURES. D.D. Kunkel, D.L. Schmiege, A.T. Malouf & P.A. Schwartzkroin. Dept. Neurological Surgery, Univ. Washington, Seattle, WA 98195.

Hippocampal slice cultures combine the advantages of dissociated cell culture and acutely prepared brain slice preparations. They provide an organotypic preparation for studying a developing system which has lost extrinsic afferents. We used conventional light and electron microscopic techniques to provide quantitative information on cellular and synaptic features in developing rat hippocampal slice cultures. Three subfields of CA1 were examined: strata oriens, pyramidale, and radiatum (SO, SP, SR). Rat hippocampal slices were prepared from 4-6-day-old rats, and cultured for 1-8 weeks using modifications of methods developed by Gähwiler. Hippocampal slice cultures maintained an intrinsic cellular and synaptic organization similar to that of the original hippocampal brain tissue. Observations on synaptic organization, density and differences in asymmetric and symmetric synaptic densities were made. Total synaptic densities increased in all subfields from 1 week (6/100 μm^2) to 3 weeks (14/100 μm^2); thereafter, the density slightly decreased (12.5/100 μm^2). In SO and SR, both symmetric and asymmetric synapses increased to their greatest density between 3 and 4 weeks; a gradual decrease was seen after this period. After 1 week of development, the proportion of asymmetric versus symmetric synapses in SR and SO was constant at 70% and 20%, respectively. In SP, asymmetric and symmetric synaptic density increased gradually to 5 weeks, and then leveled off. Approximately 50% asymmetric and 35% symmetric synapses were observed in SP.

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351.9

ANTIBODIES TO LUCIFER YELLOW AND CASCADE BLUE: LIGHT AND ELECTRON MICROSCOPIC OBSERVATIONS OF DYE-FILLED NEURONS IN RAT HIPPOCAMPUS. D.L. Schmiege, D.D. Kunkel, A.T. Malouf, H.E. Scharfman & P.A. Schwartzkroin. Dept. Neurological Surgery, Univ. Washington, Seattle, WA 98195.

Intracellular injection of fluorescent markers, such as Lucifer Yellow, has been an important tool for morphological analysis of physiologically identified neurons. Although fluorescent dye injections and other fluorescent tracing techniques are widely used, long-term visualization of cellular morphology, and particularly ultrastructure, is often impossible. As a means of enhancing morphological characterization, we have used the recently introduced antibodies to Lucifer Yellow (LY) and Cascade Blue (CB) (Molecular Probes; rabbit anti-LY or -CB). Pyramidal and non-pyramidal neurons, in acute hippocampal slices or in hippocampal slice cultures, were physiologically identified and filled with LY or CB. Immunocytochemical techniques, for both light (LM) and electron microscopy (EM), were utilized to prepare fluorescent dye-filled cells for more stable visual examination. Slices with LY- or CB-filled neurons were fixed, cryoprotected and sectioned; tissue was then exposed to rabbit anti-LY or -CB antibody (1:500 dilution), then goat anti-rabbit IgG conjugated with HRP. At the LM level, specific neurons were identified by an optically dense HRP/DAB reaction product which was localized within somata, dendrites and axons. Dendritic spines and the axonal plexus were clearly visible; non-specific background staining was virtually absent in these preparations. At the EM level, a fine electron-dense granular reaction product could be seen within the soma and cell processes. The evenly distributed granular reaction product did not obscure cellular details significantly, thus allowing characterization of subcellular components and synaptic specializations.

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351.10

ELECTROPHYSIOLOGICAL COMPARISON OF RAT PYRAMIDAL AND NON-PYRAMIDAL HIPPOCAMPAL NEURONS IN VITRO. J. R. Buchhalter and M. A. Dichter. Dept. of Neurology, Univ. of Pennsylvania and Graduate Hosp., Philadelphia, PA 19146.

Differences in electrophysiological properties between pyramidal and non-pyramidal neurons have been demonstrated in the hippocampal slice preparation. We have examined these characteristics in dissociated rat hippocampus.

Hippocampi were removed from E18-E19 embryos, incubated in a trypsin solution, dissociated by trituration and plated at a density of 600,000 viable cells in 35 mm Petri dishes containing poly-L-lysine coated glass coverslips. Cultures were maintained for 3-4 weeks in a calf serum supplemented media before intracellular recordings were made with 3.5 M potassium acetate containing pipets. The extracellular solution contained (in mM): 145 NaCl, 3 KCl, 2 $CaCl_2$, 1 $MgCl_2$, 10 HEPES, 8 glucose. Recordings were made at room temperature (22-25°C) or with the bath heated (29-36°C). No significant differences were noted between pyramidal and non-pyramidal neurons with regard to action potential (AP) amplitude, rate of rise and fall, duration at half maximal amplitude, accommodation to steady state depolarization or resting membrane potential and input resistance. Expected differences in AP duration, and rates of rise and fall were observed when room and elevated temperature recordings were compared, independent of morphology.

We conclude that the differences noted in the hippocampal slice preparation are not apparent in dissociated cell culture. This may be due to the absence in vitro of factors which control the development of ion channels in vivo. It is also possible that we have recorded from a population of non-pyramidal neurons with electrophysiological properties which overlap with those of pyramidal neurons. Partial overlap has been recorded in the slice preparation with non-pyramidal neurons from different layers (Lacaille J. et al, J Neurosci, 7:1779-93, 1987).

351.11

HIPPOCAMPECTOMY IN INFANT RHESUS MONKEYS FACILITATES OBJECT-REWARD ASSOCIATION LEARNING BUT NOT MEMORY FOR CONDITIONAL OBJECT-OBJECT ASSOCIATIONS. R. Killiany and H. Mahut, Psychol. Dept., Northeastern Univ., Boston, MA 02115

The performance of lyr old normal control (Gr.1, n=7), 5mos old normal control (Gr.2, n=4) and lyr old infants hippocampectomized at 2 mos of age (Gr.3, n=5) was compared to that of 6 young adults (Gr.4) on a task that, first, involved presentations of 4 object pairs and monkeys had to learn which pairings were rewarded and which were not. A given object appeared as a member of both rewarded and non-rewarded pairs. In the last stage, conditional retrieval, monkeys had to discriminate between three single objects depending on their past association with other objects (Saunders & Weiskrantz, 1989). We found that: 1. In learning associations, infants in groups 2 and 3 were as efficient as were adults while those in Gr.1 performed significantly worse than did those in the other three groups. 2. In the conditional retrieval stage, infants in groups 2 and 3 were significantly impaired compared to lyr old (Gr. 1) or adult (Gr. 4) monkeys.

Thus, though the hippocampal system may mediate object recognition memory at adult levels of efficiency as early as at 4.5 mos of age (Mahut & Killiany, Abstr. Soc. Neurosci., 1990), it is not sufficiently mature at 5 mos of age to mediate conditional retrieval at the same level of accuracy as that seen at either one or four years of age.

351.12

ONTOGENY OF OBJECT-REWARD ASSOCIATION LEARNING AND TRIAL-UNIQUE OBJECT RECOGNITION MEMORY - EFFECTS OF EARLY HIPPOCAMPECTOMY ON THE TWO CAPACITIES IN RHESUS MACAQUES. H. Mahut and R. Killiany, Psychol. Dept., Northeastern Univ., Boston, MA 02115

The present study examined the ontogenetic development of the two capacities and the effects upon them of hippocampectomy at 2 mos of age on : 1. Learning of a concurrent object discrimination task (COD) with repeated trials in which one member of each of the 8 pairs of objects remained the positive stimulus. 2. Delayed matching to sample recognition task (DMS) with 3 groups of infants: Gr. A (n=4) normal, 6.7 mos old; Gr. B (n=8) normal, 3 mos old and Gr. C (n=10) 3 mos old operated infants. Four normal, 3-4 yr old adults with identical training history constituted an age control group (Gr. D).

On the COD task, the only significant difference was found with infants in Gr. B that performed better than did young adults. On the DMS task, no significant differences were found among the three age groups of normal control monkeys. However, operated infants were significantly impaired compared to the other three groups of monkeys.

These findings support our earlier suggestion (Mahut and Moss, The Hippocampus, 4:241, 1986) that there might be complex developmental interactions between brain systems that mediate associative learning (cf 'habit', Hirsh 1974) and a maturing hippocampal system.

mRNA REGULATION: TRANSCRIPTION FACTORS

352.1

INDUCTION OF IMMEDIATE EARLY GENE mRNAs BY CYCLOHEXIMIDE IN MOUSE SKELETAL MUSCLE IN VIVO. S.R. Abu-Shakra*, A.J. Cole, D.W. Saffen*, J.M. Baraban, P.F. Worley, and D.B. Drachman. Depts. of Neurology and Neuroscience, Johns Hopkins University, Baltimore, MD 21205.

Recent studies have described a family of genes referred to as immediate early genes (IEG) that respond rapidly and transiently to growth factor stimulation in fibroblasts. The finding that several IEGs which encode transcription factors respond to nicotinic stimulation in PC12 cells (Bartel et al., Genes Dev., 1989, 3:304) raises the possibility that these transcription factor mRNAs play a role in skeletal muscle response to neuromuscular transmission. In initial studies, basal levels of several IEG mRNAs were undetectable by Northern blot analysis of total RNA from mouse gastrocnemius. Accordingly, we examined IEG mRNA levels after treatment with cycloheximide, a protein synthesis inhibitor that superinduces IEG mRNA levels in other cell types, by increasing IEG transcription and decreasing mRNA degradation. Four hrs. after cycloheximide treatment (40-80 mg/kg, i.p.), the following IEG mRNAs were readily detectable: zif/268, c-jun, and nur/77. Our results suggest that these known or putative transcription factors are expressed in myocytes and may play a role in regulating transcriptional responses to muscle stimulation.

352.2

COLOCALIZATION OF SPINAL OPIOID PEPTIDE mRNA AND FOS PROTEIN IN A RAT MODEL OF PERIPHERAL INFLAMMATION AND HYPERALGESIA.

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The relationship of dorsal horn Fos proteins and opioid peptide precursor mRNAs for preprodynorphin (PPD) and preproenkephalin (PPE) was examined following peripheral inflammation and hyperalgesia. Rats received an injection of carrageenan (6mg/150 µl saline) into one hindpaw and were sacrificed 3d later by perfusion with 4% paraformaldehyde. The L-5 segment of the spinal cord was processed for in situ hybridization histochemistry using oligonucleotide probes labeled with ^{35}S to identify PPD and PPE mRNA levels. The tissue was then processed using the PAP method with antisera against Fos-related nuclear proteins.

At 3d post-inflammation, a large ipsilateral increase in PPD mRNA and Fos proteins was detected in a subpopulation of dorsal horn neurons, while a small increase in PPE mRNA was noted. In laminae I & II, more than 80% of the neurons exhibiting increased PPD mRNA colocalized Fos proteins, while only half of the PPE neurons had Fos nuclear labeling. In laminae V-VI, about half of the neurons exhibiting either PPD or PPE mRNA exhibited Fos immunoreactive proteins.

These data demonstrate that a subpopulation of dorsal horn neurons colocalize either PPD or PPE mRNA and Fos proteins. The activation of nuclear proteins such as Fos, may play a role in the transcription of PPD and PPE. However, the number of neurons displaying increased Fos proteins is greater than the number of neurons colocalizing PPD or PPE. This suggests that other transcriptional events are also involved in the response to peripheral inflammation and hyperalgesia and are regulated by Fos-related nuclear proteins.

352.3

ANATOMIC DISTRIBUTION OF C-FOS, C-JUN AND NGFI-A mRNA FOLLOWING A KINDLING AFTERDISCHARGE (AD). D. A. Hosford, M. Simonato*, Z. Cao*, C. Shin, L. Butler*, and J. O. McNamara, Epilepsy Res. Lab, Duke and V.A. Med. Ctrs., Durham, NC 27705.

Kindling development requires the production of a focal AD after electrical stimulation. The mechanism by which brief, periodic ADs produce the longlasting kindling phenomenon is unknown. Immediate-early genes (IEGs) may transduce brief stimuli into long term changes in cell phenotype by regulating gene expression. Importantly, the IEGs c-fos, c-jun, and NGFI-A are induced by chemoconvulsant seizures (Morgan et al., 1987; Saffen et al., 1988). To test the hypothesis that IEGs contribute to kindling development, it is first necessary to define their anatomic distribution after an AD.

Male Sprague-Dawley rats were killed 15 min after a single AD (mean duration = 41 sec) produced by an electrode implanted in the right angular bundle, or after sham stimulation. [^{32}P]-labelled oligonucleotide probes (50-mers) for c-fos, c-jun and NGFI-A were allowed to hybridize to brain sections in situ. Optical densities of anatomic regions were measured using autoradiograms generated by apposition of film to the brain sections. C-fos mRNA was significantly induced ($p < .01$) in pyriform cortex, neocortex, dentate granule cell (DGC) layer, CA3, and CA1 pyramidal cell layers after a single AD. No significant induction was observed in basal ganglia, thalamus, or amygdaloid nuclei. The pattern of induction of c-jun and NGFI-A transcripts had an anatomic distribution similar to that for c-fos. The findings are noteworthy for 2 reasons. First, the breadth of this anatomic distribution is surprising, given the brief duration of the initial AD. Second, the similarity of the anatomic distribution to that of the N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptors suggests that an NMDA receptor-mediated mechanism during an AD may induce these IEGs. (NIH and V.A. grant support)

352.4

C-FOS EXPRESSION IN DIRECTIONALLY SELECTIVE VISUAL SYSTEM NEURONS. Ximena Rojas*, Josh Wallman and John Leah*. Dept. of Biology, City College of CUNY, New York, NY 10031 and Griffith University, Brisbane, QLD, Australia.

Immediate early genes, such as c-fos, are transiently expressed in brain cells just after very strong stimulation and produce proteins that control transcription of other genes. To explore further the role of these genes under physiological conditions, we have studied the expression of c-fos in electrophysiologically well characterized neurons of the chick accessory optic system (AOS) during biologically relevant visual stimulation.

Chicks that had had a translucent occluder over one eye for 3-11 days were presented with unidirectional whole-field visual motion for 2 hr, with the other eye covered. The protein product of c-fos was detected by immunocytochemical methods. In the rostral and caudodorsal parts of the nucleus of the basal optic root of the AOS, in which all neurons are upward-sensitive, we found intense and widespread labeling only when the contralateral eye viewed upward motion. Label was entirely lacking both if the eye viewed horizontal motion and in the corresponding structures on the ipsilateral side. Similar specificity was found in regions known from electrophysiology to respond to other motion directions. In the pretectal lentiform nucleus, only horizontal visual stimulation of the contralateral eye produced labeling. Thus we find c-fos expression only in those directionally selective neurons tuned to the motion direction of the visual stimuli.

352.5

REGULATION OF C-FOS EXPRESSION BY BRADYKININ BUT NOT BY MUSCARINIC RECEPTOR STIMULATION IN NG108-15 CELLS. J. Trejo, D. Goldstein, E.A. Martinson, H. Abe and J.H. Brown. Department of Pharmacology M-036, U.C San Diego, La Jolla, CA 92093

We recently demonstrated that muscarinic receptor (mAChR) stimulation by carbachol leads to increased expression of c-fos mRNA in 1321N1 astrocytoma cells. In these cells, m3 mAChR activation stimulates phospholipid turnover, increases intracellular calcium (Ca^{2+}) and activates protein kinase C (PKC). To determine whether c-fos is induced by activation of m4 mAChR that regulate adenylate cyclase rather than phospholipid hydrolysis, we studied NG108-15 cells. We found no increase in c-fos mRNA in serum-deprived NG108-15 cells stimulated with carbachol for up to 2 hrs. In contrast, bradykinin (BK), which stimulates inositol phosphate generation and increases Ca^{2+}, in NG108-15 cells, induces a 5-fold increases in c-fos mRNA within 30 min. In cells depleted of protein kinase C by overnight treatment with PMA, BK failed to increase c-fos mRNA above control levels suggesting that PKC is necessary for the BK-mediated c-fos induction. Forskolin and ionomycin also increase c-fos mRNA approximately 5-fold; further studies will determine whether BK interacts with these cAMP- and Ca^{2+}-dependent pathways. In addition, we will use NG108-15 cells transfected with the gene encoding an mAChR subtype (m1) that couples to phospholipid metabolism to ask whether carbachol can activate c-fos expression by the same pathway used by BK. Supported by GM 36927.

352.6

ANALYSIS OF c-*fos* AND c-*fos*-RELATED GENE FUNCTION IN THE MAMMALIAN NERVOUS SYSTEM. J.L.Sonnenberg*, R.Molinar-Rode, K.Schilling, T. Curran* and J.I.Morgan. Roche Institute of Molecular Biology, Roche Research Center, Nutley, NJ 07110.

Diverse forms of stimuli elicit a rapid and transient induction of so-called immediate-early genes, such as c-*fos* and c-*jun*, in the vertebrate CNS. Many of these genes encode transcription factors that are thought to mediate the long-term adaptive response of neurons to stimulation by altering the expression of other target genes (Trends in Neurosci. (1989) 12:459; Science (1989) 246:1622); Neuron (1989) 3:359). In the rodent CNS, the glutamate receptor plays a critical role in coupling excitatory stimuli to the transcriptional activation of c-*fos*, c-*jun* and *jun*-B, genes that encode proteins present in transcription factor AP-1. AP-1 is composed of heterodimeric protein complexes containing one Fos-like and one Jun-like protein. In the CNS following stimulation, the levels of the various Fos- and Jun-like proteins vary in a dynamic manner with time. We have investigated this phenomenon by isolating *fos*-related genes that are expressed following kainic acid treatment. The properties of these gene products have been assessed in gel shift, protein association and transfection assays. In addition we have analysed the coupling of the K-type glutamate receptor to c-*fos* both in vivo and in a neuronal cell line stably transfected with an inducible c-*fos*-β galactosidase fusion gene. Supported by the DFG (to KS, Schi 271/2-1).

352.7

NMDA INDUCTION OF C-FOS EXPRESSION IN INDIVIDUAL NEURONS ISOLATED FROM THE DENTATE GYRUS. L.S. Lerea and J.O. McNamara, Duke Univ. and V.A. Med. Cntr., Durham, NC, 27705

Seizures induce the dramatic and transient expression of the immediate early gene c-fos in discrete neuronal populations in rat brain. The mechanism by which seizures induce c-fos expression is unknown. Activation of the N-methyl-D-asparate (NMDA) receptor appears to be **necessary** for the full expression of seizure induced c-fos in dentate granule cells since NMDA receptor antagonists inhibit c-fos induction without inhibiting seizure induced granule cell firing (Labiner et al., this volume). We sought to determine whether NMDA receptor activation alone was **sufficient** to induce c-fos in dentate granule cells. The dentate gyrus was microdissected from the hippocampus of 4 day old rats and enzymatically dissociated to yield neuronal and non-neuronal cells. Neurons were identified immunocytochemically with a monoclonal antibody to neurofilament protein. c-fos mRNA expression was measured by in situ hybridization using ^{32}P-dATP incorporated into a 50 base pair oligonucleotide complementary to the coding region of rat c-fos (Curran, T., et al., Oncogene 2:74-87, 1987). NMDA treatment of dentate cells in vitro produced a dose and time dependent increase in c-fos mRNA localized to neuronal somata (10-15 fold compared with vehicle). Induction of c-fos was maximal at 30 uM NMDA and was blocked by the NMDA receptor antagonist APV (100 uM). Stimulation of dentate gyrus cells with NMDA also resulted in an increase in intracellular calcium as measured with the calcium sensitive dye fura-2. We conclude that NMDA receptor stimulation is **sufficient** to induce c-fos expression in neurons isolated from the dentate gyrus. Ongoing investigations are seeking to elucidate the mechanism(s) by which NMDA receptor activation triggers c-fos mRNA expression. Supported by NRSA GM12049.

352.8

DISSOCIATION OF NEURONAL ACTIVITY AND SEIZURE-INDUCED c-*fos* mRNA EXPRESSION BY NMDA RECEPTOR ANTAGONIST. D.M. Labiner, D.A. Hosford, C. Shin, Z. Cao', L.S. Butler', J.O. McNamara. VA and Duke Univerity Medical Center, Durham, NC 27705

Expression of c-*fos* has been used as a marker of neuronal activity in the mammalian nervous system, but the mechanisms regulating its expression in vivo are incompletely understood. We previously found that NMDA channel blockers decreased seizure-induced c-*fos* mRNA expression in the dentate granule cells (DGC) of hippocampus without affecting afterdischarge duration (ADD). This suggests that c-*fos* expression was decreased without decreasing neuronal activity. To test this suggestion, we used field potential recordings to measure the effect of the NMDA channel blocker, Mk-801, on DGC firing during kindled seizures.

Rats were kindled by stimulation of the dorsal hippocampus. DGC field potentials were recorded from a concentric bipolar electrode placed in the dentate hilus opposite the kindled hippocampus. Animals serving as their own controls were given either vehicle or Mk-801 (1.0 mg/kg, ip) 1 hr prior to receiving a stimulus-evoked seizure. Mk-801 did not inhibit ADD or clonic motor seizure duration. Unexpectedly, significantly **more** population spikes per seizure were recorded in each of the animals tested following Mk-801 (849 ± 80) compared to vehicle (297 ± 114; $p<0.05$). In animals sacrificed 30 min after the last seizure, in situ hybridization with a ^{32}P-oligonucleotide probe was used to measure c-*fos* mRNA in the DGC of the recorded hippocampus. The dramatic seizure-induced increase in c-*fos* mRNA in the DGC was attenuated by Mk-801 (50%, $p<0.01$).

An NMDA channel blocker decreased seizure-induced c-*fos* mRNA expression in the DGC while **increasing** the seizure-induced firing of these cells, thereby dissociating neuronal activity from c-*fos* expression. We suspect that an NMDA receptor mediated rise in $[Ca^{++}]_i$ is required for full induction of c-*fos*. These data warrant caution in use of c-*fos* expression as a marker of neuronal activity.

352.9

EFFECTS OF N-METHYL D-ASPARTATE (NMDA) ANTAGONIST MK801 ON CORTICAL NEURAL FOS EXPRESSION FOLLOWING VARIOUS STIMULI IN SERUM-FREE CULTURE. K.Hisanaga, S.M.Sagar, K.J.Hicks* and F.R.Sharp. Depts.of Neurology and Physiology, UCSF and VAMC, San Francisco, CA 94121

Expression of the c-fos proto-oncogene in cultured neurons derived from fetal rat cerebral cortex was examined using Northern blotting for c-fos mRNA and immunocytochemistry for Fos (and/or Fos related antigens). Induction of c-fos mRNA was observed after treatment with phorbol diester, dibutyryl-cyclic AMP, vasoactive intestinal peptide, basic fibroblast growth factor (bFGF), fetal calf serum, high K^+, Zn^{2+}, and l-glutamate (10 μM). Although all these stimulants increased Fos immuno-reactivity in some neurons (7-24%), the Fos induction by all of these stimulants was inhibited by pretreatment with 1 μM MK801. Moreover, MK801 inhibited the induction of Fos by kinate and quisqualate, agonists for other glutamate receptors. It is possible that some of these stimulants induced Fos in these neurons by NMDA receptor-mediated Ca^{2+} influx following glutamate release from other neurons in the culture. However, Zn^{2+} is known to act as a NMDA antagonist, and bFGF is believed to reduce glutamate induced— Ca^{2+} influx. The present results suggest that either Fos expression was affected by an unkown action of MK801, or that NMDA receptor might be associated with cortical neural c-fos expression via a non-glutamate as well as a glutamate mediated mechanism.

352.10

ADRENALECTOMY-INDUCED C-FOS EXPRESSION IN CRF CONTAINING NEURONS IN THE PARAVENTRICULAR NUCLEUS. E. Robbins and F. Baldino, Jr. Cephalon, Inc., West Chester, PA 19380.

Corticotropin-releasing factor (CRF) neurons in the paraventricular nucleus of the hypothalamus (PVN) have been shown to be uniquely sensitive to alterations in the levels of adrenal steroids. CRF and arginine vasopressin (AVP) are markedly elevated in adrenalectomized rats. It has been established that CRF-containing neurons within the parvocellular subdivisions of the PVN are primarily responsible for the elevated CRF levels, and that AVP mRNA can be co-localized within these same neurons in adrenalectomized animals. This phenotypic alteration is reversed by the administration of glucocorticoids. The purpose of this study was to investigate the role of c-fos in the plasticity of the CRF/AVP phenotype.

A c-fos cDNA (2200 bases) was used to generate a ^{35}S-labeled RNA probe for in situ hybridization histochemistry. Male Sprague-Dawley rats from Charles River Laboratores were adrenalectomized or sham-adrenalectomized under mild anesthesia and were sacrificed 4 days after surgery. Rats were kept in a temperature, humidity and light (12h on/off) controlled room and allowed food and water ad libitum. In situ hybridization was performed on 12um tissue sections according to previously published methods using 3.5ng probe per section with a detection time of 3 weeks after exposure to autoradiographic emulsion.

C-fos mRNA was detected within individual neurons in the medial parvocellular subunit of the PVN and was co-distributed with CRF-immunoreactive neurons. At the single neuron level, c-fos mRNA was co-localized with CRF-immunoreactive material. These data suggest an important role for early genes in the plasticity of neuronal phenotypes in the adult CNS.

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352.11

C-FOS AND C-JUN EXPRESSION IN THE RAT BRAIN FOLLOWING TRANSIENT ISCHEMIA. T. Wessel, H. Baker, T.H. Joh and B.T. Volpe. The Burke Rehabilitation Center, Cornell University Medical College, White Plains, NY 10605.

A variety of insults induce the early immediate genes c-fos and c-jun in the central nervous system. We examined the regional specificity and the time course of c-fos and c-jun expression in Wistar rats subjected to 20 minutes of forebrain ischemia by four- vessel occlusion. Transient ischemia typically produces severe neuronal loss in the CA1 region of the hippocampus and in the dorsolateral caudate nucleus. *In situ* hybridization with specific DNA probes revealed a striking temporal sequence of c-fos and c-jun expression 30 minutes after re-perfusion: the most prominent response occurred in the dentate gyrus, cerebellum, medial habenula and pyriform cortex; more moderate hybridization signals were seen in the medial amygdala, several hypothalamic nuclei and the perimamillary region as well as in the ependyma of the lateral and third ventricles. These brain regions displayed synchronous c-fos and c-jun induction which diminished to different degrees at 1, 2, 3 hours after re-perfusion. Specifically, the dentate gyrus was still clearly outlined 3 hours after re-perfusion, when hybridization signal over the cerebellum had vanished. Uniquely, CA1 though CA4 in the hippocampus showed a delayed response that was most prominent 1 hour after re-perfusion. Expression of these proto-oncogenes was not detected at 6 hours or 1 week post ischemia. Sham-operated animals did not demonstrate c-fos and c-jun induction. These results in the postischemic brain indicate that transcription of c-fos and c-jun occurs early, evolves over hours and decays at different rates in various brain regions. While the pattern of c-fos and c-jun induction does not correspond solely to areas of selective vulnerability, the delayed response of the hippocampus may reflect a transsynaptic effect that contributes to selective neuronal injury. Supported by MH 44043 and MH 40090.

352.12

C-FOS GENE EXPRESSION AND GLUTAMATE CONTENT ARE INCREASED WHILE GLUTAMINE CONTENT IS DECREASED IN THE BRAIN OF DEVELOPING RATS AFTER TRANSIENT HYPOXIA. D. Krainc, K.P. Gudehithlu, A-M. Duchemin, M. Hadjiconstantinou and N.H. Neff. Departments of Pharmacology and of Psychiatry, The Ohio State University College of Medicine Columbus, Ohio 43210 USA

We have studied the relationship between neonatal hypoxia and c-fos expression. Seven days old rats were exposed to various periods of hypoxia (8% O_2 balance N_2) and c-fos mRNA, glutamate and glutamine content were assayed in hippocampus, frontal cortex and striatum. We found 3 hr of hypoxia resulted in the expression of c-fos in the hippocampus, frontal cortex and striatum. c-Fos mRNA of hippocampus was increased immediately after exposure to hypoxia and then gradually declined to basal content by about 2 hr. In the frontal cortex and striatum, c-fos mRNA increased immediately after hypoxic exposure and continued to increase for about one hr after hypoxia. About 3 hr are required for c-fos mRNA to return to basal values in both brain regions. There were concomitant increases of glutamate and a decrease of glutamine when c-fos expression increased in the three regions of brain. Within 1 hr after hypoxia, glutamate and glutamine returned to control values. However, glutamate then decreased while glutamine increased. At 6 hr the substrates returned to control values. Our studies suggest that the temporal pattern of c-fos mRNA changes in neonatal rats exposed to hypoxia might be correlated with glutamate and glutamine content.

352.13

INDUCTION OF c-FOS mRNA IN CULTURED RAT ASTROCYTES BY CALCITONIN GENE-RELATED PEPTIDE

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Retrograde changes caused by peripheral nerve injury affect both neurons and their surrounding glial cells. The dramatic increase of the mRNA encoding the calcitonin gene-related peptide (CGRP) as early as 16 hours after axotomy represents one of the earliest molecular changes in facial motoneurons. CGRP has a variety of functions in the PNS and CNS. In order to gain information about its possible role in the cellular reactions accompanying neuronal regeneration its effect on astrocytes was studied *in vitro*. A strong and transient increase in c-fos mRNA was observed in cultured astrocytes after treatment with CGRP. Quantitative Northern blot analysis revealed an increase of c-fos mRNA within 15 min, a peak after 30 min and a subsequent decline. This CGRP effect was concentration dependent, half maximal stimulation of c-fos mRNA being obtained in the range of 100-300 nM. Calcitonin was found to mimic the action of CGRP on cultured astrocytes. It transiently induced c-fos gene expression with a similar time course as CGRP, but its effect was somewhat weaker. These data provide the first evidence for an effect of CGRP/calcitonin on astrocytes and demonstrate the usefulness of measuring c-fos gene expression for studying receptor-mediated transcriptional activation mechanisms.

352.14

IPR INDUCIBILITY OF C-FOS IS ONE DIFFERENCE BETWEEN EARLY AND LATE PASSAGE C6 GLIOMA CELLS. R.M. Gubits and H. Yu*. Dvsn. Ped. Neurol., Dept. Neurol., Columbia Univ., Coll. Phys. & Surg., NY, NY.

C6 glioma cells (Benda et al., Science 161:370,1968) are widely used as a model system for the study of glial cell function. Depending upon cell passage number and culturing conditions, C6 cells can express a predominantly astrocytic or oligodendroglial phenotype. During our studies on changes in gene expression after β-adrenergic receptor (**βAR**) activation, we noted a cell passage number-related difference in the response of C6 cells to treatment with 10 μM isoproterenol (**IPR**). A further comparison of early (**E**) and one population of late (**L**) passage C6 cells revealed the following: 1.) Morphological changes, 2.) Changes in generation time, and 3.) Numerous differences in gene expression, including: a.) IPR-inducible c-fos mRNA expression in E, but not L cells, b.) No difference between E and L cells in c-jun mRNA response to IPR treatment, c.) IPR-inducible preproenkephalin mRNA expression in both E and L cells, but from a basal level 5-fold higher in L cells, d.) IPR-inducible expression of NGF mRNA in E cells, compared with constitutive expression in L cells, e .) IPR-inducible changes in GFAP mRNA level in E cells, but undetectable GFAP mRNA expression in L cells, and f.) higher constitutive expression of tubulin mRNA in E than in L passage cells. Vernadakis et al., (Adv. in the Biosci. 61:371,1986) have suggested that the altered phenotype of late passage C6 cells may represent a model system for glial aging. Our results suggest that the aging process may include an altered response to βAR activation. (Supported by Giblin Foundation for Pediatric Neurological Research).

5HT RECEPTORS: BEHAVIOR AND PHARMACOLOGY

353.1

MODULATION OF 5-HT_2 RECEPTOR-INDUCED BEHAVIORAL MODELS VIA DIFFERENT 5HT_1 RECEPTOR SUBTYPES. R.A. Glennon*[2], N.A. Darmani[1], U. Pandey*[1] and B.R. Martin*[1]. Depts of Pharmacol/Toxicol[1]. and Medicinal Chemistry[2], MCV/VCU, Richmond, VA 23298.

Receptor binding studies have identified several types of 5-HT receptor sites (5-HT_1, 5-HT_2, 5-HT_3 and 5-HT_4). 5-HT_1 sites are heterogeneous and appear to consist of a number of subtypes of which the 5-HT_{1A} and 5-HT_{1B} sites are best studied. One of the most often used models of 5-HT_2 receptor function is the head-twitch response (HTR) in rodents. Recently we characterized the ear-scratch response (ESR) induced in mice as a behavioral model for 5-HT_2 -selective receptor agonists.

Intraperitoneal injection of DOI induced both HTR and ESR in a dose-dependent manner (0.6-5.0 mg/kg, i.p.). For drug interaction studies a dose of 2.5 mg/kg DOI was used to induce both behaviors in mice. The DOI-induced behaviors were observed immediatly after injection for 30 minutes. The non-selective 5-HT agonist 5-MeO DMT (2-8 mg/kg, i.p.) and the 5-HT_2 antagonists ketanserin (0.06-1.0, s.c.) and spiperone (0.02-0.5 mg/kg, i.p) pretreatment dose dependently reduced both behaviors. However pretreatment with a selective 5-HT_{1A} agonist 8-OH DPAT (0.5-2.5 mg/kg. i.p.) only inhibited the HTR and had no significant effect on ESR. The 5-HT_{1B}/5-HT_{1C} agonist TFMPP (0.6-1.2 mg/kg, i.p.) and the 5-HT_{1B} agonist RU 24969 (0.25 mg/kg, i.p.) pretreatment inhibited ESR but not HTR. These results suggest that the 5-HT_2 receptor-induced HTR and ESR are selectively modulated by 5-HT_{1A} and 5-HT_{1B} sites respectively. Supported by NIDA grants DA-01642, DA-02396 and DA-05274.

353.2

SINGLE DOSE 8-OH-DPAT PRETREATMENT DOES NOT ALTER THE 5-HT RELEASE-INHIBITORY RESPONSE TO SUBSEQUENT 8-OH-DPAT CHALLENGE AS DETERMINED BY *IN VIVO* MICRODIALYSIS. S. Hjorth, Dept. of Pharmacology, Univ. of Göteborg, Box 33 031, S-400 33 Göteborg, SWEDEN

Brain 5-HT_{1A} receptors and -mechanisms have been implicated in anxiety and depression. Whereas there is an abundance of data on the acute effects of 5-HT_{1A} receptor agonists in different experimental models, very little is known about their subacute and chronic modes of action. The present study attempted to test the hypothesis that 5-HT_{1A} autoreceptors are desensitised by single pretreatment with 5-HT_{1A} agonists (Kennett *et al.* EJP *138*, 53, 1987). Groups of rats were given a single injection of vehicle or the prototype 5-HT_{1A} agonist 8-OH-DPAT (0.025, 0.1 or 1.0 mg/kg, SC). About 24 h later an *in vivo* brain microdialysis probe was stereotaxically implanted (chloral hydrate anaesthesia) into the ventral hippocampus, which receives prominent 5-HT input from the brainstem raphé. Following a control period (2-3 h), to establish stable baseline output levels of 5-HT, the rats were challenged with 8-OH-DPAT at a sub-maximally effective, preferentially 5-HT_{1A} autoreceptor active, dose (0.025 mg/kg SC), and the measurements were continued for 2 h. The 8-OH-DPAT challenge decreased 5-HT release with a similar time-course in all of the pretreatment groups. Furthermore, there were no significant differences in the baseline output of 5-HT between the groups (60-70 fmoles/20 μl sample), neither was there any significant difference with regard to maximum release reduction (≈ 60 %), nor in the overall response to 8-OH-DPAT challenge (summed 5-HT output 0-2 h after drug). The data suggest that release-regulating 5-HT_{1A} autoreceptors remain responsive to agonist challenge 24 h after a single dose 8-OH-DPAT pretreatment.

353.3

ATTENUATED 5-HYDROXYTRYPTOPHAN (5-HTP) INDUCED RESPONSE SUPPRESSION FOLLOWING PRETREATMENT WITH A SELECTIVE 5-HT_2 RECEPTOR AGONIST (DOI) IN AN ANIMAL MODEL OF DEPRESSION. E.A. Engleman, J.N. Hingtgen, J.M. Murphy, F.C. Zhou & M.H. Aprison. Depts. Psychiat; Biochem; Psychol; & Anat; Prog. Med. Neurobiol.; Inst. Psychiat. Res.; Indiana U. Sch. Med. & Purdue Sch. Sci., Indianapolis, IN 46202

Integrating various types of neurobiological data with clinical data, Aprison and Hingtgen formulated the hypersensitive postsynaptic serotonin (5-HT) theory of depression. A behavioral model was developed in which rats exhibit a period of operant suppression after IP administration of D,L-5-HTP. Subsequent studies suggest that this effect may be mediated through 5-HT_2 receptor activation. Recently, IP injections of the 5-HT_2 agonist 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI) have been shown to down regulate 5-HT_2 type receptors (Buckholtz et al., Life Sci., 42:2439, 1988). In the current study, 24 hours after IP administration of 1.0 or 2.0 mg/kg DOI, mean 5-HTP (25 mg/kg) induced response suppression was reduced by 37 and 58%, respectively, from baseline 5-HTP suppression values ($p<0.05$). In a related experiment, IP injection of DOI alone in small doses (0.5 mg/kg) induced response suppression similar to that observed with 25 mg/kg D,L-5-HTP. These results provide further evidence of 5-HT_2 receptor involvement in 5-HTP induced behavioral suppression. (Indiana Dept. of Mental Health & AA03243).

353.4

MODIFICATION OF 8-OH-DPAT-INDUCED HYPOTHERMIA AFTER ACUTE OR SUBCHRONIC RITANSERIN TREATMENT. R. F. Kucharik*, S.M. White, J.A. Moyer.
Wyeth-Ayerst Research, CN 8000, Princeton, NJ 08543-8000.

The hypothermic response to 8-OH-DPAT is centrally mediated by 5-HT_{1A} receptors (Gudelsky, G.A., et al., Neuropharmacology 25:12, 1986) and is thought to be presynaptically mediated in the mouse (Goodwin et al., Neuropharmacology, 24:12, 1985). In this study the effects of acute and subchronic (3 days) administration of ritanserin, a potent and selective 5-HT_2 receptor antagonist, on 8-OH-DPAT-induced (0.5 mg/kg sc) hypothermia were studied in male CF-1 mice. Acute ritanserin (1 mg/kg ip) did not alter body temperature, but potentiated the hypothermic response elicited by 8-OH-DPAT. A higher dose of ritanserin (10 mg/kg ip) caused a significant decrease in body temperature which was further decreased by 8-OH-DPAT. Twenty-four hours after a single dose treatment with ritanserin (1,3, 10,30 mg/kg ip), the magnitude of 8-OH-DPAT-induced hypothermia was similar to controls in that ritanserin treatment neither potentiated nor attenuated 8-OH-DPAT-induced hypothermia. After subchronic administration of ritanserin (1,3,10,30 mg/kg ip), there was a significant attenuation of 8-OH-DPAT-induced hypothermia . The results of these studies suggest a possible interaction between 5-HT_{1A} and 5-HT_2 receptors. Additional studies will be conducted to characterize the selectivity of this interaction.

353.5

EFFECTS OF SEROTONERGIC AGENTS ON THE SPONTANEOUS ACTIVITY OF NEURONS OF THE RAT DEEP CEREBELLAR NUCLEI. P.A. Cumming-Hood, J.C. Strahlendorf and H.K. Strahlendorf. Depts. of Physiology and Neurology, Texas Tech University Health Sciences Center, Lubbock, TX 79430.

Superfusion and iontophoretic application of serotonin (5-HT) in the in-vitro cerebellar slice preparation from young adult male rats caused both excitation and inhibition of the spontaneous activity of neurons of the deep cerebellar nuclei (DCN). Selective agonists and antagonists for the 5-HT receptor subtypes known to exist in the DCN (5-HT_{1A}, 5-HT_{1B} and 5-HT_2) were used to determine which subtype(s) might be mediating the effects noted. Iontophoretic application of 8-hydroxy-2-N-dipropylaminotetralin (8-OH-DPAT), a 5-HT_{1A} agonist, mimicked the inhibitory effect of iontophoretically applied 5-HT. This response, as well as 5-HT-induced inhibition, was blocked by superfusion of spipirone, a 5-HT_{1A} antagonist. 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI), a 5-HT_2 agonist, caused inhibition of spontaneous activity when applied iontophoretically. Superfusion of ritanserin, a 5-HT_2 antagonist, attenuated this response. To test the hypothesis that the DOI-induced inhibition could be mediated through an interneuron, picrotoxin, a GABA (chloride channel) antagonist, was applied iontophoretically and found to attenuate the response. These results indicate that the inhibitory response to 5-HT is mediated, in part, by the 5-HT_{1A} receptor and that DOI-induced inhibition may be mediated via a GABAergic interneuron. Supported by NS 19296 and the Tx. Adv. Res. Prog. Grant 010674-020.

353.6

DISCRIMINATIVE STIMULUS EFFECTS OF THE 5-HT_1 AGONISTS RU 24969 AND mCPP IN THE PIGEON. J.B. Hogan[1]*, H.C. Holloway[2], and J.E. Barrett[2]. [1]Dept. of Psychology, Univ. of Maryland at College Park, College Park, MD 20742 and [2]Dept. of Psychiatry, Uniformed Services Univ. of the Health Sciences, Bethesda, Maryland 20814-4799

The present study attempts to characterize the discriminative stimulus effects of RU 24969, a 5-$HT_{1A/1B}$ agonist and mCPP, a 5-$HT_{1B/1C}$ agonist in the pigeon. White Carneau pigeons were trained to discriminate drug from saline in a 2 key operant paradigm, where responding on the right key was reinforced following drug administration, and responding on the left key was reinforced after saline administration. One group was trained to discriminate 1.7 mg/kg RU 24969 from saline, and a second group was trained to discriminate 3.0 mg/kg mCPP from saline. Generalization to the RU 24969 stimulus occurred with 8-OH-DPAT and with metergoline, but not with TFMPP, mCPP, pirenperone, or fenfluramine. Generalization to the mCPP stimulus occurred with TFMPP and fenfluramine, but not with RU 24969, 8-OH-DPAT, or pirenperone. In addition, the stimulus properties of RU 24969 were blocked by prior administration of the putative 5-HT antagonist NAN-190. In view of the apparent absence of 5-HT_{1B} receptors in pigeon brain (Waeber et al, N. Schmied., 1989 340: 486-494.) these findings suggest that pigeons respond to the stimulus effects of the 5-HT_{1A} component of RU 24969 and the 5-HT_{1C} component of mCPP.

353.7

EFFECT OF GEPIRONE ON SOCIO-PSYCHOLOGICALLY INDUCED STOMACH LESIONS OF MICE IN THE COMMUNICATION BOX. C. Hara and N. Ogawa. Dept. of Pharmacology, Ehime Univ. Sch. of Med., Ehime 791-02, JAPAN.

In the communication box, non-foot shocked mice (NFS) exposed to emotional responses of foot shocked mice (FS) 3-hr a day for 3 days (1.5 mA), developed stomach lesions with 100% incidence, and the stomach lesions were prevented by 5-HT1A related agents, bispirone (BSP), ipsapirone (IPS) and SM3997, similar to chlordiazepoxide (Hara, C., Jpn J. Pharmacol., 5(Suppl.):153P,1990). The present study examined effect of gepirone (GPR), a 5-HT1A partial agonist, on the stomach lesions. Male ICR mice (8 weeks old) were used. The experiment was started at 7:00 PM. GPR was daily given i.p. to the NFS mice for 3 days, 15 min before the experiment. The NFS mice were fasted after the experiment on the 2nd day. The FS mice were exchanged to naive mice daily. After terminating the experiment, the NFS mice were sacrificed by ether and inspected for the stomach lesions visually. GPR (0.1-1.0 mg) prevented the stomach lesions. The preventing effects of GPR, BSP and IPS seemed to be more potent than SM3994. Therefore, the preventing effect may be based on the anxiolytic action.

354.1

SEROTONIN AND THYROTROPIN RELEASING HORMONE INTERACTIONS ON RAT SPINAL MOTONEURONS *IN SITU*. D.A.Jackson and S.R.White. Dept. of VCAPP, Washington State Univ., Pullman, WA 99164.

Responses of ventral horn cells to serotonin (5HT) are enhanced by prior application of thyrotropin releasing hormone (TRH) in the spinal cord slice preparation (Kow and Pfaff, Soc. Neurosci. Abst., 1989, 15:365.8). In this study we used microiontophoretic techniques to investigate 5HT/TRH interactions on spinal motoneurons in urethane-anesthetized control rats and in rats with chronic sciatic axotomies to test whether damaged motoneurons are more responsive to TRH than are controls.

Both short-term (3-7 day) and long-term (10-19 day) sciatic axotomy significantly prolonged the enhancing effect of TRH (5 mg/kg, i.v.) on MSR amplitude compared to control animals. Furthermore, long-term axotomy had a significantly greater effect than short-term axotomy. Glutamate-evoked motoneuron firing was enhanced by microiontophoretically applied 5HT for all motoneurons tested (axotomized and control rats). However, TRH applied microiontophoretically, either prior to or simultaneously with the 5HT application, failed to enhance the 5HT response in motoneurons from control or from chonic axotomized animals. These results suggest that responses of motoneurons to TRH in the slice preparation may be altered compared to *in situ* motoneurons. In addition, prolongation of TRH effects on MSR amplitude in chronic sciatic axotomized rats does not appear to be mediated by a direct effect of TRH on the motoneurons.

354.2

RELATIONSHIP OF GABA NEURONS TO RETICULO- AND RAPHE-SPINAL NEURONS IN THE MEDULLARY RETICULAR FORMATION. *E. Rodriguez-Veiga, C. Holmes and B.E. Jones.* Fac. Veterinary Med., U. C. M., Madrid, Spain and Montreal Neurological Inst., McGill Univ., Montreal, Quebec, Canada H3A 2B4.

Multiple GABA neurons have been identified within the medullary reticular formation (Mugnaini & Oertel, 1985) but the projections of these cells have not been defined. In order to determine if GABA neurons, like reticular and serotonin raphe neurons, give rise to long projections to the spinal cord, wheat germ agglutinin conjugated to horseradish peroxidase (WGA-HRP) was injected into the cervical cord of the rat and the tissue processed for HRP histochemistry (with diaminobenzidine, DAB, and/or tetramethyl benzidine, TMB, enhanced with cobalt) and immunohistochemistry for glutamic acid decarboxylase (GAD) and serotonin by the peroxidase-antiperoxidase (PAP) technique (using DAB). Large numbers of large reticular neurons and serotonin raphe neurons were retrogradely labeled from the spinal cord. These neurons were commonly surrounded by prominent GAD-immunoreactive varicosities. Although GAD-immunoreactive neurons were interspersed among these cells, very few were retrogradely labeled from the cord. It was concluded that the GABA neurons in the medullary reticular formation lie in amongst reticular and serotonin raphe spinal projection neurons and may influence the spinal cord primarily by innervating these neurons.

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354.3

EFFECTS OF DOPAMINE DEPLETION ON GABA CONTENT IN THE PITUITARY PARS INTERMEDIA. H. Bouman, L. Bauce, B.A. MacVicar, Q.J. Pittman. Neuroscience Resesarch Group, University of Calgary, Calgary, Canada.

The pars intermedia (PI) of the rat pituitary consists of melanotrophs that receive colocalized inhibitory dopaminergic and GABAergic inputs descending through the stalk from the hypothalamus. In order to determine the extent of dopamine (DA) and GABA colocalization, we have tried to selectively deplete pituitary DA stores and evaluate the consequences of this intervention on GABA concentrations. Rats received either two intracerebral ventricular (ICV) administrations, 24 hours apart, of 6-OHDA or 6-OHDA was directly injected into the pituitary. HPLC measurements of pituitary and hypothalamic DA and GABA levels were made 1 week later. ICV injections did not alter pituitary DA levels, while intra-pituitary injections depleted 96% of pituitary DA, and 61% of hypothalamic DA. There were no changes in GABA levels in either brain area. Therefore pituitary DA can be depleted following intra-pituitary 6-OHDA injections, but this is not accompanied by an equivalent drop in GABA. DA and GABA may not be significantly colocalized in the rat PI. Alternatively, large quantities of GABA in pituicytes or nerve fibers of the posterior pituitary may obscure possible changes in GABA levels in nerve fibers innervating the PI. Supported by MRC. H. Bouman is a PMAC student

354.4

SIMULTANEOUS MEASUREMENT OF GABA AND 5-HT RELEASE IN DORSAL RAPHE OF FREELY-MOVING RATS: PHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES.
D.W. Gallager, T.D. Hernandez, G.M. Anderson* and M.J. During
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Dorsal raphe neurons exhibit significant subsensitvity to GABA, but not 5-HT, following amygdala kindling (Hernandez et al, Brain Res., 1990). Indeed, this long term change persists for at least 3 months after the last kindled seizure (Hernandez et al., Neurosci. Abstr., 1990). The mechanism by which kindling results in this persistent change in GABA sensitivity remains unclear. It is possible that subsensitivity is due to increased release of GABA in response to kindled seizures, since serotonergic neurons of the dorsal raphe also exhibit significant subsensitivity to GABA following chronic exposure to diazepam, which potentiates the actions of GABA. We have therefore developed a microdialysis system to quantitate both GABA and 5-HT release in the dorsal raphe of unrestrained, awake rats. Basal GABA levels ranged from 2-10 nM and 5-HT levels ranged from 0.5-5.0 nM in the dialysate (1 ul/min). Both GABA and 5-HT increased 10 to 20 fold with 60 mM K+ depolarization. During perfusion of this nucleus with the GABA uptake inhibitor, nipecotic acid, GABA levels increased approximately 8 fold, whereas 5-HT *decreased* to unmeasurable levels. We will present data on the characterization of basal and amygdala kindled seizure-induced release of several neurotransmitters (including GABA and 5-HT) and their interaction within dorsal raphe in response to pharmacological manipulations (K+ depolarization, nipecotic acid and the 5-HT uptake inhibitor, fluoxetine).

354.5

EVALUATION OF THE INTERACTIONS OF SEROTONERGIC LIGANDS WITH MU, KAPPA, & DELTA OPIATE BINDING SITES. P.J. Monroe[1], S.E. Perschke*[2], T. Crisp[2], & D.J. Smith[3], Nova Pharmaceutical Corporation[1], Baltimore, MD 21224, Northwestern Ohio Universities[2], Rootstown, Ohio 44272, and West Virginia University[3], Morgantown, WV 26506.

Serotonergic receptor antagonists, e.g. methysergide and ritanserin, attenuate the analgesic effect of spinally administered opiates, suggesting an interaction between opiate and serotonergic neuronal components (Crisp and Smith, 1989). However, spiroxatrine, a serotonergic agent with partial agonist properties, has been shown to interact directly with opiate binding sites (Leysen, et al., 1977). The present study was undertaken to determine if the effect of these drugs on opiate-induced analgesia could be explained by a direct effect on opiate receptors.

Fourteen serotonergic agents were evaluated for their abilities to interact with opiate receptor subtypes labelled by [^{3}H]-DAGO (mu), [^{3}H]-DADLE (delta), and [^{3}H]-U69593 (kappa). Consistent with previous results, spiroxatrine was found to interact equipotently with all three opiate subtypes, yielding Ki values of approx. 110 nM. Of the other compounds tested, only the 5HT2 selective agent, ritanserin, was found to interact with opiate sites, yielding Ki values of 10 (delta), 21 (mu), and 5 uM (kappa). These results suggest that spiroxatrine and ritanserin may act at opiate sites to inhibit opiate-induced analgesia.

354.6

INTERACTIONS OF ADRENERGIC LIGANDS WITH MU, KAPPA, AND DELTA OPIATE BINDING SITES. S.E. Perschke*[1], P.J. Monroe[1], and D.J. Smith[2]. Nova Pharmaceutical Corp.[1], Baltimore, MD 21224 and West Virginia Univ.[2], Morgantown, WV 26506.

Adrenergic neuronal systems appear to play a modulatory role in nociception in the spinal cord. Intrathecal adrenergic agonists produce analgesia which is inhibited by adrenergic antagonists. Futhermore, opiate-induced analgesia is sensitive to adrenergic antagonists, suggesting a noradrenergic-opiate neuronal interaction may also occur. On the other hand, adrenergic drugs may be capable of directly interacting at opiate sites. Thus, the present study was undertaken to examine the abilities of several adrenergic agents to interact with opiate receptor subtypes labelled by [^{3}H]-DAGO (mu), [^{3}H]-DADLE (delta), and [^{3}H]-U69593 (kappa).

Prazosin and WB4101 (Alpha-1) were equipotent in competing for all three opiate subtypes, yielding Ki values near 10 uM. Clonidine and yohimbine (alpha-2) were approximately 4 fold less potent. Phentolamine (non-selective) was most potent, competing for binding at all three sites with Ki values near 1 uM. Beta adrenergic agents were ineffective. Effective spinal concentrations of adrenergic agents used in studies of pain modulation fall in the range of 1 to 100 uM, assuming equal distribution within the injected compartment. Thus, it may be important to reconsider the involvement of adrenergic systems in spinal or opiate-induced analgesia.

354.7

A MU AGONIST BUT NOT A KAPPA AGONIST SUBSTITUTES FOR A DOPAMINE D2 AGONIST IN A QUINPIROLE VS SALINE DRUG DISCRIMINATION. D.V. Widzowski, S.C. Johnson and D.A. Cory-Slechta. Environ. Health Sci. Center and Program in Neuroscience, School of Med. and Dent., Univ. of Rochester, Rochester, N.Y. 14642.

Neurotransmitter systems in the CNS are thought to interact with one another in a number of ways, both postsynaptically and presynaptically. In vivo and synaptosomal studies have shown that mu opioid agonists enhance dopamine (DA) release, while kappa opioid agonists decrease it. If such interactions have functional properties, then mu agonists, but not kappa agonists would be expected to substitute for the D2 agonist quinpirole in a drug discrimination (DD) procedure. Rats were trained to discriminate between an I.P. injection of 0.050 mg/kg of the DA D2 agonist quinpirole HCl and saline in a standard two lever DD. Baseline quinpirole responding under training conditions was > 90% following quinpirole injections and < 10% for saline injections. Quinpirole responding was clearly mediated by the D2 receptor since haloperidol, but not the D1 antagonist SCH 23390 blocked drug-lever responding. Moreover, drug responding generalized to apomorphine (0.25 mg/kg) but not to the selective D1 agonist SKF 38393 (6 mg/kg). Morphine (3 mg/kg) also engendered quinpirole responding, with the effect dependent on preinjection time, increasing from 40% drug-lever responding at 15″ preinjection to 80% at 60″. This time course data parallels in vivo dialysis studies of the time course of morphine-induced DA release. Naloxone completely blocked the morphine effect. Haloperidol partially blocked the effect, suggesting its mediation via DA D2 receptors. In contrast, the kappa agonist U-50,488 did not engender quinpirole responding at any of the doses (2 and 4 mg/kg) nor preinjection times (15 to 60 minutes) tested. These results support a role for mu agonists in modulating the behavioral functions of dopaminergic systems and suggest that drug discrimination could be very useful in determining such interactions between neurotransmitter systems. This work was supported by NIH grant R01 ES05017.

354.8

CENTRAL ANGIOTENSIN II. OCCURRENCE IN CATECHOLAMINE NEURONS OF THE MEDULLA OBLONGATA AND INTERACTIONS WITH NEUROPEPTIDE Y RECEPTORS IN CARDIOVASCULAR AREAS. I.A. Aguirre, K. Fuxe, R. Coveñas*, D. Ganten*, M. Goldstein and L.F. Agnati*. Dept. Histology and Neurobiology. Karolinska Institut. 104 01 Stockholm. Sweden.

High densities of angiotensin II (ANG II) immunoreactive (IR) nerve terminal networks codistribute with high densities of noradrenaline (NA), adrenaline (A) and neuropeptide Y (NPY) nerve terminal networks in cardiovascular areas. We have therefore studied the possible coexistence of ANG II/tyroxine hydroxylase (TH) immunoreactivities (IR) in the medulla oblongata. Double immunolabelling techniques using a standard immunofluorescence protocol (Rabbit-ANG II antiserum Denise and mouse monoclonal TH antibodies) were employed to demonstrated ANG II/catecholamines costoring neurons. In the receptor autoradiographical experiments coronal cryostate sections from the region of the dorsal medulla were used together with the radioligand 125I-NPY 1-36 (0.5 nM) (Amersham, U.K.), which was found to label NPY receptors of the Y1 type. Incubation with ANG II (10 nM) was made (Peninsula Lab., U.S.A). Functional (cardiovascular) studies were also performed using intracisternal injections of ANG II (3 nmol/10 µl and 10 nmol/10 µl) and porcine NPY (pNPY) (Peninsula Lab., U.S.A.) (7.5 pmol/10 µl and 75 pmol/10 µl) in the α-chloralose anaesthetized rat. In the NA A1 area 20-30 % of the TH IR nerve cell bodies were ANG II IR and in the A C1 area 5-40 % of the TH IR nerve cell bodies displayed ANG II IR. The autoradiographical study showed that ANG II significantly increased the 125I-pNPY 1-36 binding within the dorsal strip (dorsal cardiovascular region of the nucleus tractus solitarius) with a trend for an increase of the 125I-pNPY 1-36 binding in the area postrema. A threshold dose of ANG II (3 nmol) not only counteracted the vasodepressor action of an ED50 dose of pNPY 1-36, but also led to the development of a highly significant vasopressor action of ANG II compared with the control group. These results can be explained in the basis of the existence of true ANG II/NPY Y1 receptor-receptor interaction, which may take place at the intrasynaptic level.

354.9

EFFECTS OF NEUROTENSIN (NT) ON MIDBRAIN DOPAMINE (DA) NEURONS: ARE THEY MEDIATED BY NT-DA COMPLEXATION? W.-X. Shi and B.S. Bunney. Depts. of Psychiatry and Pharmacology, Yale Univ. Sch. of Med., New Haven, CT 06510.

Effects of NT on midbrain DA cells were studied in brain slices containing the ventral tegmental area and the substantia nigra. The spontaneous activity of DA cells was recorded extracellularly. At low concentrations (0.2-10 nM), slice perfusion with NT attenuated the inhibition of DA cells induced by DA without a significant change in basal firing rate. At higher concentrations (>10 nM), NT consistently caused an increase in firing rate. At even higher concentrations (>100 nM), NT caused a cessation of activity. Whether this inhibitory effect of NT is due to depolarization inactivation or a toxic effect remains to be determined.

NT has been shown to bind with DA. To determine if the effects of NT are mediated by the formation of a NT-DA complex, several NT analogues were studied. NT (8-13) which is active in binding with both NT receptors and DA, mimicked the effects of native NT. Neuromedin N, a peptide that competes with NT for the same receptor and does not binds with DA, also mimicked the effects of NT on DA cells. However, NT (1-11), a N-terminal fragment of NT which is inactive in competing for NT receptors but binds with DA, was found to be ineffective. In addition, it was found that the excitatory effect of NT on DA cells was largely unchanged in the presence of DA receptor blockade by sulpiride. These results suggest that formation of a NT-DA complex may not account for the effects of NT on DA cells. When combined with the fact that there is a high density of NT receptors on DA cells, our data support the suggestion that the observed effects of NT on DA neurons are most likely to be mediated by an activation of NT receptors on DA cells.

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354.10

EFFECT OF AMFONELIC ACID ON INCREASED EXTRACELLULAR DOPAC IN THE NUCLEUS ACCUMBENS AND THE STRIATUM INDUCED BY TYPICAL AND ATYPICAL NEUROLEPTICS AND NEUROTENSIN. R. Rivest, F.B. Jolicoeur and C.A. Marsden[1*], Dept. of Psychiatry, Fac. of Med., Univ. of Sherbrooke, Canada JIH-5N4 and [1]Dept. of Physiol. and Pharmacol., Med. Sch., Queen's Medical Centre, Nottingham NG7 2UH, UK.

Many central effects produced by neurotensin (NT) resemble those of atypical neuroleptics (Jolicoeur et al., Hand. Neurochem., 8:95, 1985). A proposed model for discrimination between neuroleptics suggested that amfonelic acid potentiates the *ex vivo* increase of striatal DOPAC level induced by typical but not atypical neuroleptics (Waldmeir et al.,Biochem. Pharm. 34:39, 1985). In the present study, we have examined whether amfonelic acid can be used to discriminate between both class of neuroleptics and NT *in vivo* by monitoring extracellular DOPAC in the nucleus accumbens and the striatum using differential pulse voltammetry with carbon fibre micro-electrodes. Amfonelic acid potentiated the increase of extracellular level of DOPAC induced by typical neuroleptics in both the nucleus accumbens and the striatum but the effect was much greater in the striatum. However, while clozapine, thioridazine and NT also significantly increased the extracellular level of DOPAC in both regions, the administration of amfonelic acid 5 min before totally prevented the rise in DOPAC induced by the atypical neuroleptics and NT. In conclusion, the *in vivo* data confirm that amfonelic acid is a valuable tool to neurochemically differentiate potential typical from atypical neuroleptics. Using this model, it appears that the atypical neuroleptics and NT produced similar *in vivo* neurochemical effects.

We thank the Wellcome Trust, MRC and FCAR for financial support.

354.11

EFFECTS OF DOPAMINE AGONISTS ON EXCITATORY INPUTS TO NUCLEUS ACCUMBENS FROM THE AMYGDALA: MODULATORY ACTIONS OF CHOLECYSTOKININ. R.Z. Liang*, M. Wu*, C.Y. Yim & G.J. Mogenson. Dept. of Physiology, Univ. of Western Ontario, London, Ontario, Canada N6A 5C1.

It has been demonstrated that dopamine (DA) reduces the excitatory responses of neurons of the nucleus accumbens to single-pulse stimulation of the basolateral amygdala (Yim & Mogenson, 1982). It was also reported that cholesytokinin (CCK) co-exists (Kalivas & Nemeroff, 1988) and interacts (Yim & Mogenson, 1990) with DA in accumbens neurons. In the present study the interaction of CCK and DA was investigated by using CCK and DA agonists.

The effects of iontophoretically application of CCK, DA, dopamine D_1 and/or D_2 receptor agonists (SKF-38393 and LY-171555 respectively) were compared. The application of DA, SKF-38393, LY-171555 and LY-171555 + SKF-38393 significantly attenuated the excitatory responses of accumbens neurons to electrical stimulation of basolateral amygdala whereas saline had little or no effect. The application of CCK reversed these attenuating effects of DA, LY-171555 and LY-171555 + SKF-38393.

These observations confirm and extend the results of recent experiments which demonstrated that CCK reduces the modulatory action of DA on excitatory inputs to accumbens neurons. (Supported by MRC of Canada)

354.12

GABAERGIC INNERVATION OF PEPTIDERGIC NEURONS IN THE ARCUATE NUCLEUS OF THE RAT BRAIN: A LIGHT-AND-ELECTRON MICROSCOPIC DUAL LABELING IMMUNOCYTOCHEMICAL STUDY. Z. Katarova*, T.J. Gorcs†, T. Lantos‡, T.F. Freund†, and G. Szabo. *Institute of Biochemistry, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary 6701, †Department of Functional Neuroanatomy, Institute of Experimental Medicine, H-1450 Budapest, Hungary; ‡Neuromorphology Laboratory, Semmelweis Medical University, Budapest, H-1094, Hungary.

Since the arcuate nucleus plays an important role in several neuroendocrine functions, our present aim was to elucidate the GABAergic afferentation of peptidergic (somatostatin, growth hormone releasing hormone, beta endorphin, neuropeptide Y and galanin) neurones using a new antiserum raised against a chemically modified 62 kd form of glutamic acid decarboxylase (GAD). Light and electron microscopic dual labeling immunocytochemical methods were used in this study. At the light microscopic level close apposition of GAD immunoreactive axons with the above mentioned peptide-containing neurons were observed. At the ultrastructural level GAD-immunoreactive axon terminals formed symmetric synaptic junctions with the peptidergic neurons. Based upon the present results a general GABAergic inhibition of peptidergic neurons of the arcuate nucleus may be assumed.

354.13

NEUROPEPTIDES MODULATE THE RELEASE OF ENDOGENOUS AMINO ACIDS FROM THE RAT SPINAL DORSAL HORN. I. Kangrga, G. Pavlaković and M. Randic. Dept. of Vet. Physiol. and Pharmacol., Iowa State University, Ames, IA 50011.

Although the co-existence of substance P (SP) and glutamate (Glut) in some small dorsal root ganglion (DRG) neurons has been reported, and also of SP, neurokinin A (NKA) and calcitonin gene-related peptide (CGRP) in a proportion of DRG cells, the significance of this phenomenon for primary afferent neurotransmission is not well understood. The effects of SP, NKA, CGRP and opioid peptide (Tyr-D-Ala-Gly-MePhe-Gly-ol-enkephalin, DAGO) on the dorsal root (DR) stimulation-evoked release of ten endogenous amino acids have been investigated using the rat spinal cord slice-DRG preparation and HPLC with fluorimetric detection. Activation of the low-threshold primary afferent fibers (PAF) resulted in a 2-fold increase in the basal efflux of aspartate (Asp) and in a smaller increase in Glut and serine. Activation of low- and high-threshold PAF resulted in a 2 to 5-fold increase in the basal efflux of Asp, Glut and serine and a smaller increase of glycine, GABA, alanine and threonine. SP (0.1-1.0 μM) and CGRP (0.1 μM) increased the electrically-elicited release of Glut and Asp. Unlike the response to DR-electrical stimulation that was usually limited to the 5-min period, the SP-response was prolonged lasting 10-15 min. The CGRP-response was oscillatory in character and lasted up to 30 min. DAGO, reduced or abolished the electrically-evoked release of Glut and Asp. The modulation of the release of Glut and Asp by tachykinins, GCRP and opioid peptides acting at the μ receptor subtype may have important physiological implications for regulating the strength of synaptic connnections in the spinal dorsal horn. (Supported by the NIH and NSF).

354.14

MODULATION OF EXCITATORY AMINO ACID-INDUCED CURRENTS BY TACHYKININS AND OPIOID PEPTIDES IN RAT SPINAL CORD NEURONS. H. Hećimović, K. I. Rusin*, P. D. Ryu and M. Randić. Dept. of Vet. Physiol. Pharmacol., Iowa State University, Ames, IA 50011.

The whole-cell patch-clamp technique was used to examine the effects of substance P (SP), neurokinin A (NKA) and opioid peptides (D-Ala^2, D-Leu^5-enkephalinamide, DALEA; Tyr-D-Ala-Gly-MePhe-Gly-ol-enkephalin, DAGO) on excitatory amino acid-induced currents in acutely dissociated (enzymatically and/or mechanically) rat spinal dorsal horn neurons (L.I-IV). In about half of tested cells, SP (10^{-10} - 10^{-6}M) by itself induced an inward current frequently accompanied by an increase in membrane current noise. When applied simultaneously with, or prior to L-glutamate (Glut), SP and NKA enhanced Glut-induced current (184 ± 37%, n=23) in 65% of the cells. The inward currents induced by N-methyl-D-aspartate (NMDA, 30-300 μM) and quisqualate (QA, 0.3-10 μM) were also potentiated by tachykinins. Both transient and sustained components of the NMDA and QA responses were augmented and the effect lasted up to 1 hour. NMDA-induced current, however, was frequently depressed during application of SP (0.2-20 nM). Furthermore the fast, but not the sustained component of the response to QA was enhanced by DALEA (2-200 nM) in 11 cells and reduced in 3 cells. The transient components of the inward currents induced by NMDA and Glut were decreased by DAGO (10-100 nM) in 6 cells and increased in 2 cells. These results suggest that post-synaptic mechanisms of action of tachykinins and opioid peptides may contribute to the regulation of the glutamate-mediated transmission in the rat spinal dorsal horn. (Support by the NIH and NSF).

354.15

CHRONIC D1-DOPAMINE RECEPTOR BLOCKADE INCREASES D1 MEDIATED ACETYLCHOLINE (ACh) RELEASE FROM RAT STRIATAL (S) SLICES. P. Butkerait, H.Y. Wang and E. Friedman, Depts. of Pharmacology and Psychiatry, Med. Coll. of Pennsylvania, Philadelphia, PA, 19129.

The effects of D1-dopamine receptor agonists on S cholinergic neurons were studied by measuring K^+-evoked [^{3}H]ACh efflux from superfused rat striatal slices pre-loaded with [^{3}H]choline. Three D1 receptor agonists including SKF38393 (1-phenyl-7,8-dihydroxy-2,3,4,5-tetrahydro-1H-3-benzazepine HCl), all produced dose dependent (0.1-10μM) increases (24-59%) in K^+-evoked [^{3}H]ACh release from rat S slices. The increase in evoked ACh release by SKF38393 was blocked by 1μM of the D1 antagonist SCH23390 (8-chloro-2,3,4,5-tetrahydro-3-methyl-5-phenyl-1H-3-benzazepin-7-ol hemimaleate). In contrast, quinpirole and carbachol produced dose dependent (0.1-10μM) decreases (31-60% and 15-50%, respectively) in evoked ACh release. To study the regulation of D1 receptors mediating S K^+-evoked ACh release, rats were treated for 5 months with 0.1mg/kg/day SCH23390. This treatment selectively increased SKF38393 mediated S ACh release without altering quinpirole or carbachol mediated S ACh release. These results provide evidence that chronic SCH23390 treatment produces functional sensitization of D1 receptors mediating S K^+-evoked ACh release.

354.16

EFFECTS OF ACETYLCHOLINE ON CORTICAL AND HYPOTHALAMIC RELEASE OF CORTICOTROPIN RELEASING HORMONE (CRH) IN-VITRO. Y. Tizabi and A.E. Calogero*. Howard Univ. Dept. of Pharm., Wash., D.C 20059 and DEB, NICHD, NIH, Bethesda, MD 20892.

Alzheimer's disease (AD) is associated with a reduction in cholinergic markers and CRH levels, but an up-regulation of CRH receptors in the cerebral cortex. There are suggestions that central CRH and cholinergic systems interact. To gain an insight into this interaction, we measured the in-vitro release of immunoreactive CRH (iCRH) from frontal, parietal, temporal and occipital cortex and from the hypothalamus in response to a challenge with varying acetylcholine (ACh) concentrations or KCL. KCL-stimulated iCRH release from the hypothalamus was approxiately 3-fold higher than in cortical tissues. Both 1 or 10 nM concentrations of ACh significantly inhibited iCRH secretion from the frontal cortex (20-26%). 1nM ACh also significantly inhibited iCRH secretion from the parietal cortex (32%). Other cortical areas were not affected. In contrast, hypothalamic release of iCRH was significantly increased by both concentrations of ACh (26-36%). These data indicate differential regulation of CRH release from hypothalamic and cortical tissues under ACh stimulation. The inhibitory effect of ACh on cortical CRH release could have important implication in neurodegenerative disorders.

354.17

DRUG-INDUCED MODIFICATION OF ETHANOL'S HYPOTHERMIC EFFECTS IN TWO LINES OF RATS DIFFERING IN CHOLINERGIC SENSITIVITY. Amir H. Rezvani, David H. Overstreet, and David S. Janowsky. Center for Alcohol Studies and the Dept. of Psychiatry Univ. of North Carolina School of Medicine, Chapel Hill, NC.

In an attempt to understand the mechanisms underlying the differential hypothermic effects of ethanol in the Flinders Sensitive (FSL) and Flinders Resistant (FRL) Lines of rats, animals were pretreated with drugs having relatively specific actions on particular neurotransmitters. Neither scopolamine (1 m/kg), bicuculline (2 mg/kg), haloperidol (1 mg/kg, pindolol (1 mg/kg), nor idazoxan (3 mg/kg), altered ethanol-induced hypothermia in either line. Calcium channel antagonists verapamil (10 mg/kg) potentiated hypothermia to a similar degree in both lines, while nicardipine (10 mg/kg) attenuated it. Mecamylamine (5 mg/kg), dramatically potentiated ethanol-induced hypothermia in FSL rats. However, this potentiation was not seen with hexamethonium (5 mg/kg). Consequently, central nicotinic mechanisms might be involved in the differential ethanol-induced hypothermia.

In conclusion, although several drugs were able to modify the hypothermic effects of ethanol, no drug tested was able to reduce the differential hypothermic effects of ethanol in the FSL and FRL rats. Therefore, the mechanisms underlying the significantly greater hypothermia induced by ethanol in the FSL rats remain to be determined. Supported in part by Grant No. 8802 from NCARA to A.H.R.

354.18

MODULATION OF TRANSMITTER RESPONSES BY VERY LOW CONCENTRATIONS OF ACETYLCHOLINE AND GABA. S. N. Ayrapetyan* and D. O. Carpenter, Wadsworth Labs, NYS Dept. of Health and School of Public Health, Albany, NY 12201.

We have found that extremely low concentrations of both acetylcholine and GABA alter the sensitivity of Aplysia neurons to these same substances applied at the much higher concentrations necessary to elicit voltage and conductance changes. We used acetylcholine at 10^{-5}M and GABA at 10^{-4}M to elicit Cl-dependent conductance increase responses. When acetylcholine was bath perfused at concentrations between 10^{-14} and 10^{-8}M (concentrations at which there were no direct effects on electrical characteristics of the neurons), the responses to GABA were reduced in all of 53 neurons studied. The average reduction was about 30% but in 7 neurons the responses were abolished. A similar effect of low concentrations of acetylcholine was observed on responses to 10^{-5}M acetylcholine. The effect required about 10 min of acetylcholine perfusion to reach maximum, and was abolished by cooling. The threshold concentration varied in different neurons. Low concentrations of GABA (10^{-12} to 10^{-8}M) caused an increase to an average of 130% of control in the response to both acetylcholine and GABA in approximately 65% of neurons studied. This effect had a similar time course. These observations suggest that these conventional neurotransmitters have modulatory as well as direct transmitter actions.

354.19

DIHYDROPYRIDINES ALTER THE INHIBITORY ACTION OF ADENOSINE IN THE RAT HIPPOCAMPAL SLICE.
J.T.Bartrup* and T.W.Stone. Department of Pharmacology, University of Glasgow. G12 8QQ. U.K.
An electrophysiological approach has been used to determine the effect of dihydropyridines (DHP) on adenosine inhibition of synapatic transmission.
Orthodromically evoked population potentials were recorded from the CA1 of rat hippocampal slices. The percentage change in potential size was determined from chart records recorded via a digital storage oscilloscope.
The inhibitory action of adenosine and its analogues was determined in the presence of a number of DHPs. Nifedipine (1-10μM) significantly potentiated the inhibition by adenosine (50μM), increasing it from 58.4±4.87% to 93.1±2.89% at 10μM nifedipine (mean ± sem n=10). In contrast the action of 2-chloroadenosine and other analogues was attenuated in a dose dependent manner, the percentage inhibition by 2-chloroadenosine (0.5μM) being reduced from 57.5±3.63% to 21.9±3.36% at 10μM nifedipine (n=16). Similar effects were shown by the DHP agonist Bay K8644 but not by nimodipine or nitrendipine. The DHPs on their own had no effect on the population potential.
Dipyridamole (10μM) reversed the potentiating effect of nifedipine on adenosine to an antagonism. The results are consistent with uptake and binding studies showing the ability of DHPs to displace ligands from the transporter and receptor sites.

354.20

IN VIVO ELECTROCHEMICAL INVESTIGATION OF ASCORBIC ACID DYNAMICS IN RAT BRAIN. Jon Cammack, Behnam Ghasemzadeh, and R.N. Adams. Dept. of Pharmacology, University of Kansas, Lawrence KS, 66045.
The function(s) of Ascorbic Acid (AA) in the CNS has been an enigma for years, though a variety of roles have been ascribed for this essential vitamin. Our goal was to investigate specific release of AA in brain as a result of various pharmacological manipulations. In vivo electrochemistry, using carbon fiber electrodes with discrete parameters that allows selective detection of AA without any interferences from other oxidizable compounds, was used.
Ascorbic acid oxidase (AAO), was injected into different brain nuclei after attaining stable baselines, the difference between pre- and post-AAO representing basal values. These results, in different regions, are in contrast to reported microdialysis figures. Because of the facile auto-oxidation of AA in solution, we believe our values more accurately describe resting, basal concentrations. Also, various stimulations, such as glutamate infusions and amphetamine injections were performed. These gave rise to AA signals in established areas such as caudate nucleus, as well as regions not previously studied, such as thalamus. The amphetamine induced AA signal in the thalamus may point to a novel function of this agent. Further work in this area should help to elucidate ascorbate's role in the CNS.

TRANSMITTERS IN INVERTEBRATES IV

355.1

PROCTOLINERGIC AND DOPAMINERGIC MODULATION OF A CRUSTACEAN SEXUAL BEHAVIOR: IMMUNOCYTOCHEMICAL AND PHYSIOLOGICAL EVIDENCE Debbie Wood, Charles D. Derby, Georgia State University (SPON: M.-N. Girardot), M.-N. Girardot, BMD
The male blue crab produces a sterotyped posture and rhythmic behavior in response to a sex pheromone produced by the female. Bioassays of neuromodulators have shown the monoamine dopamine and the neuropeptide proctolin are capable of eliciting the postural and rhythmic behavioral components respectively. Proctolin-like and dopamine-like immunoreactivity has been shown in mature male and female crabs. Comparisons across gender, sexual maturity, and hormonal status groups reveal differential behavioral responses to these modulators. We are currently investigating these differences anatomically and physiologically to localize elements of the motor system important to the production of the mature male behavior.

Partially Supported by Whitehall Foundation

355.2

PROCTOLIN IMMUNOREACTIVITY IN CRAYFISH CNS OCCURS IN REGIONS THAT CONTAIN SWIMMERET COMMAND NEURONS. L.D. Acevedo, W.M. Hall* and B. Mulloney. Zoology Dept, Univ. of California, Davis, CA 95616.
Five command axons triggering swimmeret motor pattern generation in crayfish occur in specific locations in the crayfish abdominal connectives, and project to each abdominal ganglion (Wiersma, C.A.G., Ikeda, K., *Comp. Biochem. Physiol.*, 12:509, 1964). The neuropeptide proctolin mimics stimulation of these command neurons (Mulloney, B. et al., *J Neurophysiol.* 58:584, 1987; Acevedo, L., UCD Thesis, 1990). To test the hypothesis that these neurons use proctolin as a transmitter, we used a new affinity-purified proctolin antiserum to trace the projections of proctolinergic neurons in abdominal connectives, and in the core of abdominal ganglia. After visualizing the antibody with an HRP-conjugated secondary antibody and DAB, abdominal ganglia were osmicated, embedded in plastic, and sectioned.
Transverse sections of connectives revealed proctolinerigic axons in regions where the command neurons are found. Horizontal sections of ganglia revealed proctolinergic axons in all longitudinal tracts. Because command neurons project in specific tracts, we traced the proctolinergic axons in these tracts to see whether they branched into the LN, the paired neuropil containing the swimmeret motor centers. We found proctolinergic axons with trajectories that would be predicted for four of the five excitatory command neurons: E_A, E_B, E_C and E_E. These axons are found in the LDT, DLT, LVT, and MVT, respectively; they are intersegmental and project into the LN.
This work was supported by NSF grants BNS 84-06931 and BNS 87-19397, and by a UCD Dissertation Year Fellowship.

355.3

BEHAVIORAL AROUSAL IN THE CRAYFISH (*PROCAMBARUS CLARKII*) IS MODULATED BY SEROTONIN AND OCTOPAMINE. Nicholas C. Hunt* and Richard F. Olivo. Dept. Biol. Sciences, Smith College, Northampton, MA 01063.
Serotonin and octopamine modulate two behaviors that normally increase when a crayfish is aroused: the optokinetic response, and spontaneous walking (Arnesen & Olivo, 1988, Comp. Biochem. Physiol. 91C: 259-263). We show here that the amines alter the firing rates of optomotor units, indicating that their effects on eye movements are not through their known actions on muscles. We also confirm that serotonin suppresses episodes of walking and enhanced optomotor activity, and show that its threshold for action is at doses about a thousand-fold lower than those used in the earlier experiments.
Crayfish were clamped at the center of an oscillating striped drum that elicited continual optomotor responses. Their legs rested on a floating foam-rubber ball, on which they walked during episodes of spontaneous arousal; we monitored walking with a video-based motion detector. A bipolar electrode implanted in a lateral eye muscle recorded the firing of multiple optomotor units, and a cannula in the pericardial space let us inject amines without handling the crayfish. In each experiment, after a 40-minute control period, we injected serotonin-HCl or DL-octopamine in 0.1 ml saline, and monitored the effects on arousal for the next 60 minutes. For serotonin, 4 to 6 experiments at each of three doses showed that 10 ug suppressed arousal for 40 to 60 minutes, after which normal alternating episodes of arousal and quiesence returned. The optokinetic response also was depressed during non-aroused periods. Lower doses of serotonin had smaller (1 ug) or undetectable (0.1 ug) effects. Octopamine in doses from 20 to 500 ug was variable in its actions, but generally increased walking and optomotor activity; this is the opposite of serotonin's effects.

355.4

GLUTAMATE AND ACETYLCHOLINE RESPONSES FROM STOMATOGASTRIC NEURONS IN DISSOCIATED CELL CULTURE. Andrew A. Sharp, Jorge Golowasch and Eve Marder. Biology Department, Brandeis Univ., Waltham MA 02254.
The crustacean stomatogastric ganglion (STG) is a model system for the study of rhythmic pattern generation in neural networks. However, due to the complexity of the interactions between the neurons within the ganglion it is difficult to characterize their biophysical properties. To eliminate these difficulties, several laboratories have developed techniques to grow STG neurons in dissociated, primary tissue culture (Krenz and Fischer,1988,Neurosci. Abstr.14:295; Graf and Cooke,1990,J. Exp. Biol.149:521; Golowasch et al.,Crustacean Systems in Neurobiology,in press). We are characterizing the responses to the primary intraganglionic transmitters, glutamate and acetylcholine, in cultured STG neurons from Cancer borealis and Panulirus interruptus. Iontophoretic or bath applications of glutamate produces inhibitory responses that are blocked by picrotoxin. Acetylcholine produces a strong excitatory response. Most neurons respond to both transmitters after 3 to 7 days in culture and do not require new outgrowth. The responses seen to date represent only a subset of those seen in the intact ganglion, either due to sampling problems or because cultured cells produce only some of the responses seen in the intact ganglion. Supported by NS17813.

355.5

LOCALIZING NEUROPEPTIDE-IMMUNOREACTIVE NEURONS THAT PROJECT TO THE CRAB STOMATOGASTRIC GANGLION. M.J. Coleman and M.P. Nusbaum, Neurobiology Research Center/Dept. of Physiol. & Biophysics, Univ. of Alabama-Birmingham, B'ham, AL 35294.

Different exogenously-applied neuropeptides elicit different motor patterns from the pyloric network, in the stomatogastric ganglion (STG) of the crab, *Cancer borealis* (Marder & Nusbaum, Persp. Neural Sys. & Behav., pp.73-91, 1989, eds. TJ Carew & DB Kelley, AR Liss). Thus far, however, only one of the neurons responsible for these peptide-mediated effects has been identified (Nusbaum & Marder, J. Neurosci. 9:1600-1607, 1989). No STG somata contain these peptides, but many of them appear to originate in somata in the commissural ganglia (CGs). We have now localized several peptide-immunoreactive somata in the CGs that do indeed extend processes to the STG, using the following double-labeling technique.

In *in vitro* preparations, the STG input nerve is cut and backfilled towards the CGs (approx. 15mm) with 5% biocytin (in 50mM $NaHCO_3$) for 48 hours. The wholemount is then fixed and processed for biocytin visualization and immunocytochemistry, using different fluorophores to differentiate the two labels. Biocytin backfills routinely label 5-10 somata/CG. In contrast, Lucifer yellow backfills label 0-4 somata/CG. We have found one biocytin-labeled neuron (approx. 30μm), located anteromedially in the CG, that shows proctolin-like immunolabeling (n=9CGs). Three others (approx. 30μm each), located posteromedially in the CG, show FMRFamide-like immunolabeling (n=5CGs). We are working to identify: (1) the transmitter(s) used by the other biocytin-labeled cells, and (2) these double-labeled neurons with dye-filled microelectrodes, to determine how they influence the pyloric motor pattern.

Supported by NSF Grant BNS-8909613.

355.6

DOPAMINERGIC NEURONS IN LOBSTER CENTRAL NERVOUS SYSTEM: COMPARATIVE IMMUNOCYTOCHEMICAL DISTRIBUTION OF TYROSINE HYDROXYLASE AND DOPAMINE. I. Cournil[1]*, M. Moulins[1] and M.P. Nusbaum[2], [1]Lab. Neurobiol. Physiol. Comp., Univ. Bordeaux/CNRS, Place Peyneau, 33120 Arcachon, France; [2]Neurobiology Research Center/Dept. Physiol. & Biophysics, Univ. Alabama-B'ham, B'ham, AL 35294.

Amines are believed to play crucial roles in controlling and regulating aspects of the behavioral repertoire of animals, and their hormonal action is directed both centrally and at the periphery. In this work, we have explored for dopaminergic neurons in the brain, the ventral nerve cord and the stomatogastric system of the lobster, *Homarus gammarus*. We apply immunocytochemical procedures with specific antibodies to detect, first, tyrosine hydroxylase (the dopamine synthetic enzyme) using immunofluorescence detection on whole mount preparations; second, dopamine transmitter using the peroxidase-antiperoxidase technique on paraffin serial sections. These two antibodies provide consistent mapping of dopaminergic neuronal cell bodies: about 100 neuronal somata are detected in all, of which 30 are found in the brain. A bilaterally symmetrical distribution is evident within each thoracic ganglion (4 somata) and abdominal ganglion (4-5 somata). No labelled somata were found in the stomatogastric and oesophageal ganglia. Stained fibre tracts have been followed through the whole nervous system and into some peripheral nerve roots. In these cases, it is possible to follow a labelled neuron from its central soma until its terminal projections, thus indicating, like other amines, a double action centrally and at the periphery.

355.7

CHARACTERIZATION OF THE PEPTIDE-REGULATED MEMBRANE GUANYLATE CYCLASE OF THE AMERICAN LOBSTER M.F. Goy and C.A. Geary, Department of Physiology, University of North Carolina, Chapel Hill, NC 27599.

In lobsters, and other crustaceans, a family of related peptides (two isoforms of Crustacean Hyperglycemic Hormone and a more-distantly related Molt Inhibiting Hormone) regulate cyclic GMP metabolism in multiple target tissues. These peptides (also called peptides G_1, G_2, and G_3, for their cyclic GMP-promoting effects) are produced abundantly by the sinus gland and, to a lesser degree, by two other neurosecretory structures, the pericardial organs and the proximal secretory regions of thoracic second nerve roots. The peptides have recently been shown to selectively activate the membrane form of guanylate cyclase (M.F. Goy, J. Biol. Chem, in press), a reaction that can be studied in homogenized preparations.

We have used the advantageous properties of homogenates to characterize the peptide/cyclase interaction. (1) The peptide alters the V_{max} of the enzyme without changing the K_m for GTP. (2) Calcium ions may play an inhibitory role in the regulation of cyclase activity, but do not serve to couple or enhance the peptide response. (3) Cytoplasm contains a factor that, when added back to purified membranes, enhances peptide-induced cyclic GMP synthesis. Biochemical techniques have been used to characterize this factor. (4) Molecular cloning experiments, using probes against the membrane cyclases of other species, have been used to define the primary structure of the lobster membrane cyclase, and to compare it to the peptide-responsive cyclases of other species. Supported by NIH grants NS21290 and NS25915.

355.8

IDENTIFICATION OF OCTOPAMINE-CONTAINING NEURONS IN THE LOBSTER NERVOUS SYSTEM. D.E. Valentine, B.A. Trimmer, and E.A. Kravitz, Dept.Neurobiology, Harvard Medical School, Boston, MA 02115.

Octopamine (OA) injection triggers a low-to-the-substrate posture in lobsters which resembles the stance assumed by subordinate animals, and which opposes the dominant-looking posture produced by serotonin. The subordinate-looking posture results from the readout from the ventral nerve cord of a motor program for extension. The action of OA on exoskeletal muscles is to prime them to respond more vigorously, and thus enhance the central effects of OA. We have begun the search for OA neurons involved in postural regulatory mechanisms in order to compare and contrast their actions with those of serotonergic neurons in lobsters.

Octopamine is found in low levels throughout the lobster nervous system and in much higher amounts in peripheral neurosecretory structures found along thoracic 2nd roots. Using antibodies to OA generated in our lab and by Geffard *et al.*, we have constructed partial maps of OA-immunostaining neurons. Stained cells were seen on the dorsal midline of the subesophageal ganglion (SEG), and in anterior and posterior pairs in each of the thoracic ganglia. Processes from the posterior pairs of cells could be traced to the 2nd root neurosecretory regions. Using a modification of the radioenzymatic assay developed by Molinoff *et al.* (1969), we have measured OA levels in single cells identified by location or by neutral red staining. The results show levels of OA in the range of 150 - 250 fmol (0.4 - 0.9 mM) in the anterior pairs of cells from thoracic ganglia and the dorsal midline cells of the SEG, while control neurons showed no measurable amine (limit of detection = 10 fmol). (Supported by NIH).

355.9

ELEVATED TEMPERATURE ALTERS THE IONIC DEPENDENCE OF AMINE-INDUCED OSCILLATIONS IN A CONDITIONAL BURSTER NEURON. B.R. Johnson, J.H. Peck and R.M. Harris-Warrick, Neurobiology and Behavior, Cornell Univ. and Dept. of Psychology, Ithaca College, Ithaca, NY 14853.

The AB neuron in the lobster stomatogastric ganglion is a conditional burster. The amines dopamine (DA, 10^{-4}M), serotonin (5HT, 10^{-5}M) and octopamine (Oct, 10^{-4}M) induce rhythmic oscillations in membrane potential in synaptically isolated AB cells (Harris-Warrick and Flamm. J. Neurosci. 7: 2113, 1987). At 15°, 5HT and Oct-induced oscillation depends upon Na^+ entry (blocked by TTX) while DA-induced oscillation depends upon Ca^{2+} entry (not blocked by TTX but blocked by low Ca^{2+}). At 22°, we found that all three amines can induce oscillatory behavior in the presence of TTX. DA-induced oscillations were strong and rhythmic, while 5HT- and Oct-induced oscillations were often weak and irregular. At 22°, DA-induced oscillations were still blocked by reduced Ca^{2+} (25% of normal). In contrast, 5HT-induced oscillations were not blocked by either low Na^+ (50% of normal) or low Ca^{2+}. Like 5HT, Oct-induced oscillations were not blocked by low Ca^{2+}; however, they were sometimes blocked by low Na^+. Low Ca^{2+} and low Na^+ reduced the undershoot and broadened the slow wave of both 5HT- and Oct -induced oscillations. We suggest that elevated temperature shifts the ionic mechanisms of 5HT- and Oct-induced AB oscillations such that at 22° both Ca^{2+} and Na^+ currents play important roles in burst production. (Supported by NIH grant #NS17323.)

355.10

CARDIAC INHIBITION REVISITED: THE EFFECTS OF GABA AND 5-HT ON THE ELECTRICAL ACTIVITY OF ISOLATED CARDIAC GANGLION IN LIMULUS POLYPHEMUS. B. Washington[1], H. Rucker[2], and J. Townsel[2]. [1]Biomedical Research Center, Xavier University of Louisiana, New Orleans, LA. 70125 [2]Department of Physiology, Meharry Medical College Nashville, TN.

Cardiac inhibition in Limulus polyphemus has been studied, electrophysiologically, during stimulation of the inhibitor nerve from the isolated cardiac ganglion. Computer assisted spike train analysis provided histograms of extra cellular recordings which indicated that burst rate, burst duration and burst content (spike per burst) decreased significantly. This decrease prove to be voltage and frequency dependent. Application of exogenous 5HT or GABA on the isolated ganglion mimic a similar response as stimulating the inhibitor nerve. While concentrations of GABA less than $5x10^{-6}$M showed no significant change in burst content, concentrations greater than $5x10^{-6}$M decrease both burst rate and spike-content significantly. This decrease in burst rate and spike content was also evident with concentrations of 5HT greater than $2x10^{-7}$M.

355.11

DEPLETION OF SEROTONIN IN THE CENTRAL NERVOUS SYSTEM OF LIMULUS POLYPHEMUS. B.S. McAdory* and R.F. Newkirk. Tennessee State University, Nashville, TN 37209 and B. Washington, Xavier University, New Orleans, LA 70125.

It has long been established that serotonin (5-HT) is present in the central nervous system of Limulus polyphemus. Previous studies using techniques of immunocytochemistry demonstrated the presence of serotonin-like immunoreactivity. To further support the contention that these structures are serotonergic, 5,7-dihydroxytryptamine (5,7-DHT) was used to deplete cells and fibers. The chain of paraformaldehyde-fixed abdominal ganglia was pretreated with pargyline before incubating for 24 hours in 5,7-DHT in L-15 (Leibovitz) medium. The tissue was exposed to a rabbit anti-5HT antiserum followed by treatment with a goat anti-rabbit antiserum labeled with fluorescein isothiocyanate (FITC). The results of this study revealed that the neurotoxin depleted 5-HT from cells and fibers of the ventral nerve cord of Limulus, depletion being more complete in cells than in fibers and processes. These findings are supported by HPLC analysis which revealed that the levels of endogenous 5-HT were greatly reduced in experimental ganglia when compared to levels in control ganglia. These results support the contention that the ventral nerve cord of Limulus polyphemus contains 5-HT which serves as a central neurotransmitter.

(Supported by NIH Grant #S06RR08092)

355.12

CRUSTACEAN CARDIOACTIVE PEPTIDE: IMMUNOREACTIVE NEURONS AND PHYSIOLOGICAL ACTIONS IN *MANDUCA SEXTA*. N.T. Davis, T.A. Miller*, H. Lehman, and J.G. Hildebrand. ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721.

We have studied physiological actions of Crustacean Cardioactive Peptide (CCAP) and the localization of CCAP-like immunoreactivity (CCAPLI) in the nervous system of the sphinx moth *Manduca sexta*.

A specific anti-CCAP antibody stains neurons containing CCAPLI in the brain, subesophageal ganglion, and abdominal ganglia of larvae and adults. In addition, the abdominal ganglia have CCAP-immunoreactive neurons that project into the transverse nerve (perivisceral organ), which is known to have a neurohemal function. The number of neurohemal CCAP-immunoreactive neurons increases during larval and pupal development, and in the transverse nerve, a peripheral neuron (L1) and its projections also exhibit CCAPLI.

A comparable system of CCAP-immunoreactive neurons is found in representatives of 5 other orders of insects. Included in this group is *Periplaneta americana*, in which the Lateral White Cells in the abdominal ganglia are CCAP-immunoreactive.

Bioassays using the heart of *M. sexta* show that synthetic CCAP at physiological levels accelerates the heartbeat and stops the reversals of the heart. These results suggest that the heart of *M. sexta* is controlled, in part, by a CCAP-like neurohormone and that this peptidergic control system may occur widely in insects. [We thank Dr. Rainer Keller for generously providing the anti-CCAP antibody. This research was supported by a grant from the Monsanto Company to JGH.]

355.13

A NEUROPEPTIDE RELATED TO CRUSTACEAN CARDIOACTIVE PEPTIDE IN *MANDUCA SEXTA*: CHARACTERIZATION AND DISTRIBUTION. J.G. Hildebrand, H.K. Lehman, T.A. Miller*, and N.T. Davis. ARL Div. of Neurobiology, Univ. of Arizona, Tucson, AZ 85721

Crustacean cardioactive peptide (CCAP) has been identified from the pericardial organs of a crustacean, *Carcinus maenas*, and from the nervous system of an insect, *Locusta migratoria*. Although the cardioactive properties of the peptide have been demonstrated in *Carcinus*, its role in insects remains unexplored. To study the physiological functions of CCAP-like neuropeptide(s) in the sphinx moth *Manduca sexta*, we have used ELISA techniques to monitor the chemical purification of CCAP-like immunoreactivity and determined its distribution in the nervous system, heart, and blood. We have also used bioassays to test the physiological activity of the synthetic and isolated peptides.

A series of three gradient and isocratic HPLC steps were necessary to purify the CCAP-like material from extracts of *M. sexta* ventral nerve cords. In the final purification step, the *M. sexta* peptide eluted with 19.0% acetonitrile as a single UV-absorbing peak, coincident with synthetic CCAP. Preliminary amino acid analysis has indicated, however, that the *M. sexta* peptide is not identical to CCAP. We are currently working to ascertain the primary structure of the *M. sexta* peptide by mass spectroscopy and amino acid sequence analysis.

The distribution of the *M. sexta* CCAP-like peptide corresponds well with that of CCAP-like immunoreactivity visualized with immunocytochemical methods [see Davis et al. in these *Abstracts*]. The greatest concentrations were found in the ventral nerve cord and brain. Little activity was found in the heart, but CCAP-like activity was detected in the hemolymph. Moreover, the purified *M. sexta* peptide mimicked the effects of synthetic CCAP on the semi-isolated heart. Our findings suggest that CCAP-like peptide(s) may function as cardioregulatory agent(s) in insects. [Supported by a grant from Monsanto Co. to JGH.]

355.14

PEPTIDERGIC MODULATION DURING METAMORPHOSIS IN Manduca sexta: CAP_2 CONTROLS GUT EMPTYING IN WANDERING LARVAE. K. Edwards*, C.C. Cheung*, D.P Kimble, A.W. Sylwester*, and N.J. Tublitz. Inst. of Neuroscience, U. Oregon, Eugene, OR 97403.

In preparation for metamorphosis Manduca larvae stop feeding, crawl off their food, and burrow underground in a complex behavior known as wandering. One component of this stereotypic behavior is 'gut emptying', a relatively rapid purging of gut contents. We report here on studies designed to test the hypothesis that the neuropeptide CAP_2 (Cardioacceleratory $Peptide_2$) modulates gut emptying in wandering caterpillers.

Biochemical studies using HPLC and immunoprecipitation experiments using an anti-CAP antibody independently confirmed the presence of CAP_2 in the CNS of wandering larvae. Our first indication that CAP_2 was involved during wandering came from measuring CAP_2 levels in the larval CNS, the results of which showed that CAP_2 declined precipitously at wandering. Although the notion that the larval heart was the primary target of CAP_2 was empirically refuted, additional data did implicate the larval hindgut as a possible target tissue. CAP_2 applied to isolated larval hindguts caused a dose-dependent increase in contraction frequency, amplitude and basal tension of the hindgut. Quantitative analyses indicated that hindgut sensitivity to CAP_2 peaked on the day of wandering. In vivo measurements of hindgut activity in larvae revealed a dramatic increase in contraction frequency associated with gut emptying, which was partially blocked by the anti-CAP antibody. Immunocytochemical staining with the anti-CAP antibody uncovered several terminal ganglion cells projecting to the hindgut. This evidence taken together strongly suggests that CAP_2 is involved in modulating gut activity during wandering behavior.

Supported by grants from the NIH and the Sloan Foundation.

355.15

DISTRIBUTION OF PBAN-LIKE IMMUNOREACTIVITY IN THE CNS OF THE CORN EARWORM, *Heliothis zea*. T.G. Kingan[1], A.K. Raina[1*], M. Blackburn[2*], M. Ma[2*]. 1, Insect Chemical Ecology Laboratory, BARC-East, Bldg. 402, Beltsville, MD, 20705; 2, Dept. of Entomology, U. of MD, College Park, MD 20742

Production of female sex pheromones in several species of moths is under the control of a neuropeptide that is synthesized in the suboesophageal ganglion (SOG). The mechanism of action of this neuropeptide, termed pheromone biosynthesis activating neurohormone (PBAN), is of significant current interest. Early work indicated that PBAN-like bioactivity could be detected in the blood of scotophase females (Raina and Klun, *Science* **225**, p. 531, 1984) while a more recent investigation suggested axonal transport to the terminal abdominal ganglion (TAG) and release of a second messenger from the TAG to induce pheromone biosynthesis. (Teal et al., *PNAS* **86**, 2488, 1989). We have begun to address these possibilities with the production of antisera against PBAN and their use in immunochemical studies. In immunocytochemical studies, antisera reveal two sets of immunoreactive soma in the SOG. Processes from one set are apparently confined to the SOG and the adjacent tritocerebrum, while processes from the other set project to and apparently terminate in the corpora cardiaca, putative release sites for peptide neurohormones. Immunoreactive PBAN can be detected in the thoracic ganglia and the segmental ganglia of the abdomen; a single pair of immunoreactive fibers can be detected in the connectives of the ventral nerve cord in scotophase females. Using these antisera, we have developed a competitive ELISA for PBAN; we are now using this assay to quantify immunoreactive PBAN in hemolymph and in extracts of CNS ganglia of scotophase and photophase moths.

355.16

EFFECTS OF 5,7-DIHYDROXYTRYPTAMINE UPON THE *IN VITRO* UPTAKE, RELEASE AND SYNTHESIS OF SEROTONIN IN *Rhodnius prolixus*. H. Cook and I. Orchard. Dept. of Zoology, Univ. of Toronto, Toronto, Canada M5S 1A1

5,7-dihydroxytryptamine (5,7-DHT) is a neurotoxic analogue of serotonin which lesions serotonergic neurons. In *Rhodnius prolixus*, 5,7-DHT treatment (1 μL of 10^{-3} M 24h injected previously) led to a severe depletion of serotonin immunoreactivity of the neurohaemal areas of the abdominal nerves. This toxin-induced depletion of serotonin was confirmed using HPLC. There was no significant change in the serotonin content of the brain, subesophageal ganglion, prothoracic ganglion or the mesothoracic ganglionic mass (MTGM).

In order to elucidate the mode of action of this neurotoxin in *Rhodnius*, we have examined the effects of 5,7-DHT upon the *in vitro* uptake, release and synthesis of serotonin by the neurohaemal areas of the abdominal nerves and the associated MTGM. We have found that 5,7-DHT elicits a significant release of previously accumulated ^{3}H-serotonin in the presence or absence of calcium in the bathing medium. 5,7-DHT also reduces the specific uptake of ^{3}H-serotonin but does not affect the uptake of ^{3}H-tryptophan nor the subsequent synthesis of ^{3}H-serotonin. The effects of this dosage 5,7-DHT appear to be transient as the neurohaemal areas appear to be fully recovered 4 weeks after injection, as witnessed by immunohistochemistry.

355.17

GABA RECEPTORS OF INSECTS SUSCEPTIBLE AND RESISTANT TO CYCLODIENES. N. M. Anthony, E. A. Benner*, C. D. Lamison*, J. G. Vassallo*, D. B. Sattelle and J. J. Rauh. Agricultural Products Dept., DuPont Experimental Station, Wilmington, DE 19880 and AFRC Lab of Molecular Signalling, University of Cambridge, Cambridge CB2 3EJ U.K.

The interaction of insecticidal cyclodienes at the convulsant binding site of insect GABA receptors is believed to play a role in the toxic properties of these compounds. Previous studies suggested that cyclodiene resistance in roaches is due to insensitivity at the convulsant site. We examined GABA receptors from cyclodiene resistant (CYW) and susceptible (CSMA) housefly strains to determine if alterations in the convulsant binding site exist. In toxicity tests, the CYW flies exhibited a high degree of resistance to the following compounds: ketoendrin, dieldrin, heptachlor epoxide and lindane. Using [35S]-TBPS as a probe for the convulsant site, we demonstrated specific binding to thorax/abdomen membranes prepared from resistant and wild type strains. Multiple membrane preparations from each strain failed to provide evidence for differences in either density or affinity of convulsant sites. Although cyclodienes appear to competitively displace TBPS, a mutation in the convulsant site might alter the binding of cyclodienes without altering TBPS binding. Therefore, we are currently assessing the relative efficacy of a variety of cyclodienes at displacing [35S]-TBPS from membranes of each housefly strain.

355.18

MULTIPLE SEROTONIN-ACTIVATED CURRENTS IN ISOLATED, NEURONAL SOMATA FROM LOCUST THORACIC GANGLIA. I. Bermudez*†‡, D.J. Beadle*† and J.A. Benson‡. †School of Biological and Molecular Sciences, Oxford Polytechnic, Oxford OX3 0BP, England and ‡R & D Plant Protection, Agricultural Division, CIBA-GEIGY Ltd., CH-4002 Basel, Switzerland.

Serotonin (5-hydroxytryptamine, 5-HT) evokes changes in membrane potential and membrane conductance when applied to the somata of insect neurones *in vitro* (Usherwood, P.N.R. *et al.*, In: *Insect Neurobiology and Pesticide Action*, pp. 115-128, 1980; Neumann, R. *et al.*, In: *Sites of Action for Neurotoxic Pesticides*, pp. 25-43, 1987). To investigate the voltage-dependence, ionic basis and pharmacology of these serotonin responses, we voltage-clamped mechanically isolated neurones from the thoracic ganglia of *Locusta migratoria*. These neurones remain viable *in vitro* for many hours and exhibit characteristic responses to GABA (Lees, G. *et al.*, *Brain Res.* 401:267, 1987), nicotine and muscarine (Benson, J.A., *Soc. Neurosci. Abs.* 15:365, 1989). Pressure micro-application of serotonin (500 ms, 1 mM) evoked three different inward currents, not all of which were recorded in every neurone. I(5HT)Na was Na-dependent, increased with membrane hyperpolarisation and was insensitive to ketanserin, a vertebrate 5HT2 receptor antagonist. ICS 205 930, a 5HT3 antagonist, showed agonism at the receptor mediating the I(5HT)Na response, while MDL 72222, another 5HT3 antagonist, blocked this response. I(5HT)K was K-dependent and maximally activated at a membrane potential of -60 to -70 mV. It was blocked by ketanserin, in a dose-dependent manner, and by 10 mM Cs and Rb but not by Mn. A third current, I(5HT)X, with faster kinetics than the other two, was evoked at membrane potentials more positive than -50 mV and increased with depolarisation. This current was blocked by 5 mM 4-aminopyridine. Numerous immunocytochemical studies have revealed serotonergic neurones in insects, receptor binding and re-uptake of radio-labelled serotonin by insect neurones has been demonstrated and serotonin is known to affect many physiological functions in insects as a neurohormone and possibly as a neurotransmitter. Here we identify individual serotonin-activated currents that are candidates for rôles in mediating neuronal responses to serotonin in insects.

355.19

ACTIONS OF GLUTAMATE, GABA AND OTHER PUTATIVE AMINOACID NEUROTRANSMITTERS ON THE NEUROPILE ARBORIZATIONS OF LOCUST MOTONEURONS. F. Dubas, Zoologisches Institut der Universität, 4051 Basel, Switzerland.

In locusts, there is evidence that certain amino-acids (aa) are centrally released neurotransmitters. To establish the pharmacological profile of aa receptors involved in synaptic transmission on locust flight motoneurons, the effect of pressure application of putative aa neurotransmitters and certain of their agonists was recorded from neuropile impalement sites, in a preparation where neuropile recordings can be made during expression of the flight motor output.

The prevalent effect triggered by glutamate, GABA, aspartate and taurine was an inhibition of spontaneous or evoked activity, accompanied by a chloride conductance increase. Responses to aspartate and glutamate had identical reversal potentials and cross-desensitized. Responses to GABA and taurine had more negative reversal potentials and neither cross-desensitized with those elicited by glutamate. Responses to GABA were blocked by picrotoxin but those to glutamate were not.

NMDA and glycine were without effect while kainate and quisqualate caused large depolarizations and spiking even in cells inhibited by glutamate. Ibotenate triggered hyperpolarizations with reversal potentials similar to the responses to glutamate. Characterization of the receptors for GABA and glutamate is in progress.

EXCITATORY AMINO ACIDS: NMDA RECEPTOR ANTAGONISTS

356.1

ANTAGONISM OF EXCITATORY AMINO ACID NEUROTRANSMISSION IN VIVO: COMPARISON OF SEVERAL ARTHROPOD TOXINS. M.G. Jones* and D.Lodge. The Royal Veterinary College, London, NW1 0TU, U.K.

Examining the specificity of arthropod toxins for the N-methyl-D-aspartate (NMDA), quisqualate (QUIS) and kainate (KAIN) subtypes of glutamate receptor, we have combined the techniques of microiontophoresis and extracellular recording to study the effects of several toxins, including philanthotoxin (PhTx), argiotoxin (ATX) and Nephila Spider toxin (NSTX) on responses of rat central neurones to excitatory amino acids in vivo.

Under pentobarbitone anaesthesia a lumbar laminectomy was performed and 7-barreled glass microelectrodes inserted into the spinal cord.

PhTx markedly reduced excitatory responses to QUIS and KAIN whilst those to NMDA showed a small overall increase. ATX was more potent but markedly less selective, reducing NMDA as well as Quis responses. Selectivity of NSTX resembled that of PhTx but recovery was more variable.

Whilst existing data from in vitro studies favour selectivity of such toxins for NMDA rather than non-NMDA mediated responses, factors perhaps operating only in vivo appear to reverse this selectivity.

356.2

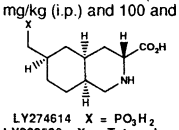
INHIBITION OF ^{3}H-PROLINE BINDING BY AP-7 Cordero, M.L.*, Negrón, A.*, Blanco, C.*, Santiago, G.*, Ortiz, J.G. Department of Pharmacology, School of Medicine, University of Puerto Rico, San Juan, Puerto Rico 00936

Proline has been described as a possible neuromodulator in mammalian CNS (Giacobini, 1983, Nadler et al., 1989). Several binding sites for proline have been identified (Greene et al., 1986, Ortiz et al., 1989). Glutamate neurotransmission antagonists partially block proline actions in CNS (Ault et al., 1987). L-glutamate and AP-7 (10^{-9}-10^{-6} M), an NMDA antagonist, inhibit proline binding by 60% in midbrain. In hippocampus, AP-7 inhibits proline binding by 80%. However, AP-7 does not affect proline binding in cerebellum. These results are consistent with the possible neuromodulatory role of proline in the CNS, possibly through the NMDA receptor(s). Further studies with more specific agents, such as MK-801, could clarify the possible interaction(s) between proline and glutamate. (Supported by NIH/MBRS, and in part by RCMI)

356.3

THE DISCOVERY OF LY274614 AND LY233536 AND THEIR CHARACTERIZATION AS SELECTIVE AND COMPETITIVE NMDA ANTAGONISTS. Paul L. Ornstein, M. Brian Arnold*, Nancy K. Augenstein*, Darryle D. Schoepp, J. David Leander and David Lodge, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285 and Royal Veterinary College, London, NW10TU, UK.

We sought to develop an array of potent, selective competitive NMDA antagonists that have good systemic activity. Our efforts led to the discovery of LY274614 and LY233536. We will discuss the SAR for this novel series of amino acids. LY274614 and LY233536, the most potent compounds in this series, selectively displaced ^{3}H-CGS19755 binding (IC_{50}'s = 55 ± 14 and 627 ± 188 nM, respectively) and selectively inhibited NMDA-induced depolarizations in a cortical wedge preparation (IC_{50}'s = 0.15 ± 0.01 and 1.4 ± 0.3 μM, respectively). These new antagonists also showed potent systemic activity in animals, e.g., against NMDA-induced lethality in mice; the MED's for LY274614 and LY233536 were 1.25 and 2.5 mg/kg (i.p.), respectively. In blocking NMDA-induced convulsions in neonatal rats, these amino acids were active following both parenteral and oral administration (MED's for LY274614 and LY233536 were 1 and 20 mg/kg (i.p.) and 100 and 200 mg/kg (p.o.)).



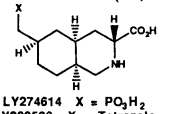
LY274614 X = PO_3H_2
LY233536 X = Tetrazole
(Racemic)

Thus, LY274614 and LY233536 are potent antagonists of neurotransmission at NMDA receptors *in vitro* and *in vivo*. These compounds may provide novel therapeutic intervention in a number of neurological disorders where excitotoxicity may have a role in neuronal damage, including cerebral ischemia and Alzheimer's disease.

356.4

THE BEHAVIORAL PHARMACOLOGY OF LY233536 AND LY274614: TWO NOVEL, SELECTIVE, COMPETITIVE NMDA ANTAGONISTS. J. David Leander and Paul L. Ornstein, Lilly Research Laboratories, Eli Lilly and Company, Indianapolis, IN 46285.

LY233536 and LY274614 are two 6-substituted decahydroisoquinoline-3-carboxylic acid competitive NMDA antagonists (Ornstein, et al., this meeting). After i.p. administration (+30 min), the minimally effective doses (MED) of LY233536 and LY274614 to protect against maximal electroshock (MES) seizures were 10 and 5 mg/kg, respectively. At 5 min after i.v. administration, the ED_{50}'s were 6.6 and 2.9 mg/kg, respectively. LY233536 was most potent at the earliest time point of testing (5 min after i.v. administration), and the protection from LY233536 was gone by 40 min. In contrast, LY274614 was most potent (ED_{50} = 1.25 mg/kg) at 60 min after i.v. administration and protection was sustained. The MED's (i.m.) for antagonizing the behavioral suppressant effects of 10 mg/kg (i.m.) of NMDA on schedule-controlled responding in pigeons were 2.5 and 0.16 mg/kg for LY233536 and LY274614, respectively. The MED for producing the phencyclidine-like cataleptic response in pigeons was 5 mg/kg (i.m.) for LY274614, whereas LY233536 was ineffective (>160 mg/kg). The cataleptic effect of LY274614 had a delayed onset and long duration irrespective of the route of administration (i.v., i.m. or p.o.).



LY274614 X = PO_3H_2
LY233536 X = Tetrazole
(Racemic)

Thus, both compounds are competitive NMDA antagonists; they differ in that LY233536 has a fast onset and short duration of effect, whereas LY274614 has a slow onset and long duration of effect.

356.5

CHARACTERIZATION OF MDL 100,453, A NEW COMPETITIVE ANTAGONIST OF GLUTAMATE AT THE NMDA RECEPTOR COMPLEX. J.P. Whitten[1], B.M. Baron[1], D.M.Muench*[1], A.L. Slone*[1], B.W. Siegel*[1], C.J. Schmidt*[1], H.S. White[2] and I.A. McDonald*[1] [1]Merrell Dow Research Institute, 2110 E. Galbraith Road, Cincinnati, OH 45215, [2]Department of Pharmacology, University of Utah, Salt Lake City, UT 84112.

MDL 100,453 ((R)-4-oxo-5-phosphononorvaline) binds to the glutamate recognition site on the NMDA receptor complex (IC_{50} = 109 nM vs. [^{3}H]CPP) with IC_{50} values > 100 μM at other glutamate binding sites, such as those labeled by [^{3}H]kainate and [^{3}H]AMPA and those mediating [^{3}H]glutamate uptake into synaptosomes. This compound inhibits [^{3}H]TCP binding in a manner reversible by NMDA but not by glycine. MDL 100,453 is a competitive inhibitor of several NMDA-mediated physiological responses including NMDA-stimulated cGMP accumulation in rat cerebellar slices (K_D = 7.9 μM by Schild analysis), NMDA-evoked release of [^{3}H]NE from hippocampal slices (IC_{50} = 17 μM), and NMDA-induced elevation of cytosolic calcium concentration measured in single cultured hippocampal pyramidal and cerebellar granule cell neurons (IC_{50} = 5 - 10 μM).

In vivo, i.p administered MDL 100,453 antagonized the harmaline-induced elevation of cerebellar cGMP content of mice (ED_{50} = 11.5 mg/kg) and displayed anticonvulsant activity vs. quinolinic acid-induced seizures in mice (ED_{50} = 16.3 mg/kg), maximal electroshock seizures in rats (ED_{50} = 2.3 mg/kg), and sound-induced convulsions in the audiogenic seizure-prone DBA/2J mouse (ED_{50} = 3.5 mg/kg). Upon oral administration to rats, MDL 100,453 (50 mg/kg) markedly suppressed the expression of kindled seizures for > 24h.

356.6

COMPARISON OF THE BEHAVIORAL EFFECTS OF COMPETITIVE AND NON-COMPETITIVE NMDA ANTAGONISTS IN MICE. D. Luttinger and D. Koonz*. Department of Neuroscience, Sterling Research Group, Rensselaer, NY 12144.

Many of the effects of PCP and other similar non-competitive NMDA antagonists are believed to be mediated by inhibition of NMDA coupled ion channels. The present studies compared the behavioral effects of competitive and non-competitive NMDA antagonists.

The effects of NMDA antagonists were assessed in male, Swiss-Webster mice following bilateral ICV administration. The mice were observed once every ten minutes for ninety minutes post-drug administration by a "blind" observer using a modification of the rating scale of Sturgeon et al., EJP., 59:169, 1979.

Non-competitive NMDA antagonists (e.g. PCP, MK-801, (-)alpha- and beta-cyclazocine) increased locomotion, stereotypic head-weaving and ataxia. In contrast, the competitive antagonists; CPP, AP-5, and AP-7 in doses sufficient to antagonize NMDA-induced lethality, did not increase locomotion or stereotypies but did increase ataxia.

The results indicate differences in the behavioral effects of non-competitive and competitive NMDA antagonists in mice. These differences might be due to differences in distribution, or to the involvement of non-NMDA coupled PCP sites underlying the mechanism for some of the behavioral effects in mice.

356.7

EVIDENCE FOR NMDA ANTAGONIST PROPERTIES OF SIGMA AGENTS. S.Borosky, J. Ferkany, M. Pontecorvo and W. Karbon. Nova Pharmaceutical Corporation, Baltimore, MD 21224.

Ifenprodil is a potent sigma agent (Karbon et al., *Eur. J. Pharmacol.*, 176:247, 1990) that also has cerebroprotective and NMDA antagonist properties (Carter et al., *JPET.*, 247:1222, 1988; Reynolds and Miller, *Mol. Pharmacol.*, 36:758, 1989), suggesting that other sigma agents might interact with the NMDA receptor/ionophore complex. In the present study sigma agents including caramiphen, haloperidol and BMY 14802, as well as ifenprodil, were tested for their ability to interact with [^{3}H] TCP binding sites in well-washed rat cortical membranes *in vitro*, and to antagonize NMDA-induced seizures in mice. In the absence of glutamate, ifenprodil inhibited [^{3}H] TCP binding in a biphasic manner with IC_{50}'s of 5 nM and 14 μM, whereas in the presence of glutamate (10 μM) the IC_{50}'s were 16 nM and 19 μM. Similarly, haloperidol inhibited glutamate-stimulated [^{3}H] TCP binding in a biphasic manner exhibiting IC_{50}'s of 8 nM and 39 μM, whereas caramiphen inhibition of [^{3}H] TCP binding was monophasic in the absence and presence of glutamate, exhibiting IC_{50}'s of 14 μM and 22 μM, respectively. In contrast to the results obtained in well-washed cortical membranes, neither caramiphen nor haloperidol inhibited [^{3}H] TCP binding to crude rat forebrain membranes. *In vivo*, ifenprodil (10 mg/kg, i.p.), haloperidol (0.75 mg/kg, i.p.) and BMY 14802 (30 mg/kg, i.p.) potentiated the ability of MK-801 to protect against NMDA (250 mg/kg, i.p.)-induced seizures at doses that provided little or no protection alone, whereas caramiphen itself was effective. These findings suggest a possible functional interaction between sigma binding sites and the NMDA receptor/ionophore complex.

356.8

TETRAHYDROAMINOACRIDINE (THA) IS AN OPEN CHANNEL BLOCKER OF *N*-METHYL-D-ASPARTATE (NMDA)-GATED CATION CHANNELS. N. Hershkowitz and M. A. Rogawski. Medical Neurology Branch, NINDS, NIH, Bethesda, MD, 20892.

Among the proposed mechanisms of action of THA, an agent which may provide symptomatic benefit in Alzheimer's dementia, are inhibition of acetylcholinesterase and blockade of voltage-dependent K^+ channels. Radioligand binding studies have indicated that THA may interact with the NMDA receptor-channel complex. In the present study, we examined the effects of THA on excitatory amino acid responses in cultured hippocampal neurons. In whole cell voltage-clamp recordings (V_H, -60 mV), THA produced a dose-dependent block of NMDA-evoked inward current (IC_{50}, 190 μM), without effect on quisqualate or kainate responses. The block was nearly completely relieved at positive holding potentials, indicating that THA may act as a steric blocker of the channel pore. Single channel recordings in excised outside-out membrane patches demonstrated that THA produces a decrease in the frequency of channel opening and in the mean channel open time without affecting amplitude, supporting the concept that THA is an open channel blocker.

(N.H. was a NIH Resident Research Associate of the National Research Council.)

356.9

KINETIC ANALYSIS OF NMDA ANTAGONISM BY NOVEL ANTICONVULSANT PHENCYCLIDINE ANALOGS: COMPARISON TO PCP AND MK-801. Susan M. Jones and Michael A. Rogawski. Neuronal Excitability Section, Medical Neurology Branch, NINDS, NIH, Bethesda, MD 20892.

Non-competitive antagonists of the *N*-methyl-D-aspartate (NMDA) receptor channel complex including phencyclidine (PCP) and MK-801 are anticonvulsant in several animal seizure models, but cause motor toxicity at similar doses that protect against seizures. We have identified several PCP analogs that are comparable in potency to PCP in the maximal electroshock (MES) seizure test but produce less motor toxicity. In the present study, we examined the ability of one of these analogs phenylcyclohexlamine (PCA) to block NMDA mediated responses in cultured hippocampal neurons using whole-cell voltage-clamp recording techniques. The characteristics of the inhibition were compared to those of PCP and MK-801. The peak currents elicited by repetitive applications of NMDA or NMDA plus antagonist were plotted as a function of cumulative time of exposure to the antagonist. Kinetic parameters were estimated by fitting exponential curves to the successive peak current values. As reported by several groups, the inhibition of NMDA-induced current by PCP (3 μM) was use-dependent (τ_{on}, 112 s; τ_{off}, 222 s at -60 mV) and also highly voltage-dependent. MK-801 (1 μM) also showed use-dependency (τ_{on}, 52 s); however, the inhibition by MK-801 did not exhibit the marked voltage-dependence seen with PCP. Full recovery from MK-801 could only be obtained with agonist application at depolarized potentials. PCA produced a voltage-dependent blockade of NMDA responses (66% block by 5 μM at -60 mV). However, in contrast to PCP and MK-801, the onset and recovery of block by PCA was rapid (τ_{on}, 4 s; τ_{off}, 1 s). The ability of PCA to block seizures without causing motor toxicity may relate to its rapid block of central NMDA mediated neurotransmission during the excessive stimulation that occurs at the onset of a seizure discharge.

356.10

INHIBITION OF [^{3}H]TCP BINDING TO RAT BRAIN PCP RECEPTORS BY ALIPHATIC ALCOHOLS. **F.R. DePietro and J.C. Byrd. Developmental Neurobiology Program, Center for Neuroscience, University of Pittsburgh, W.P.I.C., Rm. E-1226, Pittsburgh, PA 15213.**

Ethanol is now known to inhibit the function of NMDA/PCP receptors at pharmacologically relevant concentrations. Since the binding site for PCP is thought to reside in the NMDA/PCP receptor-linked ion channel, it seemed probable that ethanol might also inhibit TCP binding to this site. Accordingly, we examined the effect of aliphatic alcohols on TCP binding to the PCP receptor in well-washed rat brain membranes. Ethanol did indeed inhibit TCP binding, but only at high concentrations (IC_{50}=1275 mM); the pseudo-Hill coefficient was -1.5. This inhibition was fully reversible after washing ethanol-treated membranes. At the IC_{50} for ethanol, Scatchard analysis revealed an increase in the K_d of TCP for the PCP site from 9.5 nM to 25 nM. Methanol was a less potent inhibitor (IC_{50}=2200 mM), while propanol was more potent (IC_{50}= 0.82 mM). This pattern of inhibition correlates with the membrane fluidizing potencies of these compounds. We have recently shown (J. Mol. Neurosci., in press) that membrane fluidizing agents are also capable of inhibiting TCP binding to the PCP site, suggesting that this parameter is important for NMDA/PCP receptor functioning. The high concentrations of ethanol required to inhibit TCP binding may indicate that the protein:lipid ratio is a critical factor in binding studies, as we have found in our previous work.

356.11

EXTRACELLULAR MAGNESIUM CONCENTRATION DETERMINES THE N-METHYL-D-ASPARTATE (NMDA) EVOKED FIRING PATTERN OF LATERAL HABENULA (LHB) NEURONS, *IN VIVO*. C. L. Meier* and P. L. Herrling, Sandoz Research Institute, P.O.Box, CH-3001 Berne, Switzerland

Iontophoretic application of NMDA to LHb cells elicited a regular firing pattern in contrast to previous observations in other brain regions, e.g. caudate, where it caused bursty firing (Herrling et al., *J.Phys.*, 339:207, 1983). The present study was designed to determine if NMDA induced regular excitations in the LHb could be converted to bursts by increasing extracellular [Mg^{2+}]. Extracellular single cell recordings were made from neurons in halothane anaesthetized cats.

Iontophoretically applied quisqualate (QUIS, N=77), α-amino-3-hydroxy-5-methyl-5-isoxazolepropionate (AMPA, N=4) and kainate (KA, N=12) elicited predominantly regular firing patterns in all cells tested. Surprisingly, in 106 (88%) out of 121 neurons tested, NMDA also elicited regular firing up to inactivation. In 5 cells (4%), NMDA application elicited bursts, and a mixed firing pattern in 10 (8%) cells. NMDA, but not AMPA, QUIS or KA excitations were antagonized by AP7 or CPP.

When Mg^{2+} (0.3-1M, $MgCl_2$ or $MgSO_4$, retaining current -5 to -50nA) was added to one barrel of the iontophoretic assemblies, NMDA now evoked irregular bursty firing in 9 (39%) out of 23 cells tested. In 4 (17%) additional cells, NMDA initially induced regular firing that could be changed to bursty firing by actively ejecting Mg^{2+}. AMPA evoked regular firing could not be changed to bursts by Mg^{2+}.

These results suggest that in the lateral habenula of the cat *in vivo* the Mg^{2+} concentration in the extracellular microenvironment determines the type of firing pattern evoked by activation of the NMDA receptor.

356.12

ANTAGONISTS AT NMDA AND GLYCINE RECEPTORS MIMIC ANXIOLYTICS IN REDUCING RAT PUP SEPARATION RESPONSES. J.T. Winslow, T.R. Insel, R. Trullas, P. Skolnick. Lab. Clin. Sci., NIMH, Poolesville, MD 20837 and Lab. of Neurosci., NIDDK, NIH, Bethesda, MD 20892

Several recent studies have suggested that drugs affecting the NMDA receptor-glycine receptor-cation channel complex might have anxiolytic properties as assessed in rodent conflict models. To determine whether these agents might mimic anxiolytics in a non-conflict model, we studied rat pup ultrasonic vocalization (USV) emitted during social isolation. Previous studies have shown that this "distress call" is reduced by anxiolytics (benzodiazepines and 5-HT1a agonists) and increased by anxiogenics (pentylenetetrazol and 5-HT1b agonists). In the current studies, 10 day old pups were isolated for 2 min. as a pre-test, injected SC with drug or vehicle, returned to their litters for 30 min., then retested for 2 min. to assess change from baseline. The functional glycine antagonist 1-aminocyclopropanecarboxylic acid (ACPC) reduced the rate of USV in a dose-dependent fashion (12.5-200 mg/kg). Calling was reduced 40-90% at doses that did not affect locomotor or thermoregulatory measures and the effect on USV was still evident 4 hrs. after injection. Although glycine (50-200 mg/kg) did not show consistent intrinsic effects on USV, a glycine dose of 200 mg/kg doubled hippocampal glycine conc. and reversed the effects of 50 mg/kg ACPC. The glycine antagonist 7-chlorokynurenic acid (25 mg/kg) also decreased the rate of USV. In addition, rat pup separation behavior was sensitive to compounds binding to the NMDA receptor. NMDA increased the rates of USV as much as 50% at doses that were not convulsant (2.5-5 mg/kg). The NMDA competitive antagonist AP-5 (30 mg/kg) decreased the rate of USV. Two non-competitive antagonists: MK-801 (0.05 mg/kg), which appears to bind to a site within the cation channel, and the polyamine site ligand ifenprodil (12.5 mg/kg) also decreased the rate of USV, but both compounds had a number of non-specific motor effects. Taken together, these results support previous behavioral studies with adult animals suggesting that agents which reduce activity at NMDA receptor-coupled cation channels may constitute a new class of anxiolytic agents.

356.13

ANXIOLYTIC POTENTIAL OF DRUGS WORKING AT THE NMDA RECEPTOR COMPLEX. J.H. Kehne, T.C. McCloskey*, B.M. Baron, B.L. Harrison*, J.P. Whitten and M.G. Palfreyman. Merrell Dow Research Institute, 2110 E. Galbraith Rd., Cincinnati OH 45215.

Compounds with known or putative anxiolytic activity were evaluated for their potencies in suppressing "separation induced ultrasonic vocalizations" (SIV) in 10 day old rat pups isolated from the litter. Muscle relaxant/sedative potential was assessed using "time on an inclined plane" (TIP), the amount of time a pup was able to retain its position on a 70 degree incline. TIP was preferentially suppressed by mephenesin. The benzodiazepine agonist diazepam and the 5-HT_{1a} partial agonists buspirone or MDL 73,005EF all suppressed SIV, but the latter two compounds were inactive against TIP, reflecting their reported lack of muscle relaxant/sedative effects. The 5-HT_2 antagonist MDL 11,939 and the 5-HT_3 antagonist MDL 73,147EF were inactive against either SIV or TIP. The NMDA antagonists AP5 or MDL 100,453 and the ion channel blocker MK-801 decreased both SIV and TIP. In contrast, 5,7-dichlorokynurenic acid, an antagonist at the strychnine insensitive glycine receptor, preferentially suppressed SIV, with little effect on TIP. Thus, antagonists acting at different sites of the NMDA/glycine complex all exhibited anxiolytic activity, but the glycine antagonist was unusual in its lack of prominent muscle relaxant/sedative side effects. These data generally support the utility of the SIV and TIP measures in separating anxiolytic activity from muscle relaxation/sedation.

356.14

EFFECTS OF N-METHYL-D-ASPARTATE (NMDA) AND NMDA RECEPTOR COMPLEX SITE-SELECTIVE AGENTS ON FIXED-RATIO RESPONDING IN SQUIRREL MONKEYS. J. Willetts and W.H. Morse*. NERPRC, Harvard Medical School, Southborough, MA 01772.

The effects of NMDA and its enantiomer NMLA, and of agents which antagonize NMDA-induced convulsions, including the competitive NMDA antagonists NPC 12626 and CGS 19755, the ion channel blocker phencyclidine (PCP), the glycine-site ligand HA-966, and dextromethorphan (DXM), were determined in squirrel monkeys trained under a fixed-ratio 20 schedule of food presentation. NMDA (3-17 mg/kg i.m.) dose-dependently reduced response rates. NMLA was less potent, having only limited rate-decreasing effects at 100 mg/kg. No effects on response rates were observed with NPC 12626 (1-10 mg/kg) or CGS 19755 (0.01-1 mg/kg) alone, but both antagonists shifted dose-effect curves for NMDA to the right. Effects of lower doses (3-10 mg/kg) but not of 17 mg/kg of NMDA were antagonized by PCP (0.01-0.1 mg/kg). PCP (0.1 and 0.3 mg/kg) alone decreased response rates and responding was abolished by 0.3 mg/kg PCP and 5.6 mg/kg NMDA. Neither HA-966 (0.1-3 mg/kg) nor DXM (0.01-1 mg/kg) antagonized NMDA; rather rate-decreasing effects of NMDA were enhanced by 3 mg/kg HA-966 and by 0.3-1 mg/kg DXM. Thus, PCP, HA 966 and DXM, unlike the competitive antagonists, NPC 12626 and CGS 19755, have limited ability to antagonize effects of subconvulsant doses of NMDA in squirrel monkeys.

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356.15

BLOCKADE OF NMDA RECEPTORS POTENTIATES DOPAMINE MEDIATED TURNING BEHAVIOR IN THE 6-OHDA MODEL OF PARKINSON: DIFFERENT ROLE OF D1 AND D2 RECEPTORS
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Increase in neuronal activity of some basal ganglia structures and motor thalamus has been described in Parkinson and after administration of MPTP, suggesting the presence of an unbalance between the glutamatergic and GABAergic systems after dopaminergic (DA) neuron lesion. In the 6-OHDA model of Parkinson, we studied the effect of the antagonist of the N-metyl-D-aspartate (NMDA) receptor (+)MK 801 on the contralateral turning induced in rats, by various DA agonists, after unilateral lesion. Administration of 0.1 mg/kg of (+)MK 801 which do not induce any change in the behavior per se, potentiated the turning induced by the D1/D2 agonist apomorphine and L-dopa. This potentiation seemed to be due to an action on D1 receptors since (+)MK 801 strongly potentiates the turning induced by SKF 38393 but not that induced by the D2 agonist LY 17555. In addition (+)MK 801 completely blocked the sensitization (priming) to the subsequent administration of DA agonists. Blockade of NMDA receptors therefore, acts synergistically with D1 agonists in inducing turning and prevents the development of adaptive mechanisms such as priming.

356.16

STRUCTURE AND BIOLOGICAL ACTIVITY RELATIONSHIPS OF RIGID FLUORENAMINE-BASED PHENCYCLIDINE ANALOGUES.
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We reported previously the design, synthesis, and testing (binding affinity) of a class of rigid fluorenamine-based phencyclidine analogues and deduced the structural determinants of high binding affinity (A. P. Kozikowski and Y.-P. Pang, *Mol. Pharmacol.*, **37**:352, 1990). Recently, we tested the ability of this class of analogues to inhibit NMDA-induced calcium influx using an established protocol (J. T. Wroblewski *et al.*, *Proc. Natl. Acad. Sci., USA*, **84**:5068, 1987). For all -NH_2 bearing analogues, stereoselectivity of inhibition of Ca^{2+} influx was observed; the relative potency of inhibition of Ca^{2+} influx by these analogues was consistent with their binding affinities. Analogue **3** with the highest binding affinity (IC_{50}=19 nM) showed the most potent inhibition (IC_{50}=59 nM) while PCP with a binding affinity of 35 nM showed an inhibition of Ca^{2+} influx of IC_{50}=100 nM. For all -NHEt bearing analogues, no stereoselectivity of action was observed; all compounds showed weak inhibition of Ca^{2+} influx, and the relative potencies of such inhibition was inconsistent with binding affinities. Thus analogue **7** with a binding affinity of 68 nM had an IC_{50} for blockade of Ca^{2+} influx of 450 nM. These results indicate that: (a) analogue **3** is a potent NMDA receptor antagonist; (b) the presence of an ethyl group on the nitrogen atom reduces the ability to block Ca^{2+} influx, but only slightly reduces binding affinity. The anomalous effect of the ethyl group may provide some insight into the design of NMDA receptor ligands showing partial antagonism.

REGULATION OF AUTONOMIC FUNCTION: PERIPHERAL AUTONOMIC ORGANIZATION AND FUNCTIONS

357.1

COMPARISON OF THE DISTRIBUTION OF NEUROPEPTIDE Y (NPY) AND VASOACTIVE INTESTINAL PEPTIDE (VIP) IN RENAL POSTGANGLIONIC NEURONES OF THE RAT.
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Noradrenergic nerves innervating the blood vessels and calyx of the kidney in rats have been shown to have NPY-like immunoreactivity. Moreover, some noradrenergic nerves on renal vessels show VIP-like immunoreactivity. The extent of this peptidergic innervation cannot be readily quantified by identifying nerve terminals within the target organ. Therefore, the proportion of renal sympathetic cell bodies containing these peptides was determined. Renal postganglionic neurones were labelled in anaesthetised rats using retrograde transport of fluorescent dyes and serial sections of ganglia were then processed immunocytochemically for the presence of NPY or VIP. The following table illustrates the distribution of peptides in 2144 renal neurons of five rats.

	T12-T13 chain ganglia	splanchnic ganglion
NPY	6-26%	19-35%
VIP	1%	0%

The majority of remaining neurones in the sympathetic ganglia examined showed NPY-like immunoreactivity, whereas, only few reacted positively for VIP. In conclusion, (a) VIP appears to make a minor contribution to the control of the kidney by postganglionic neurones, and; (b) the small proportion of NPY-immunoreactive cell bodies of renal neurones conflicts with the reported high density of NPY-immunoreactive sympathetic postganglionic terminals in the kidney.(Research support: Medical Research Council,Canada)

357.2

INCREASED CYTOCHROME OXIDASE ACTIVITY IN SUPERIOR CERVICAL GANGLION CELLS OF HYPERTENSIVE, BUT NOT HYPERACTIVE RATS.
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A number of neural changes have been described in spontaneously hypertensive rats (SHR) as compared to their normotensive control, Wistar-Kyoto (WKY) rats. Since the SHR is both hypertensive and hyperactive, the extent to which these changes reflect hypertension-associated changes *per se* is unknown. To obviate this problem, two inbred strains of rats derived from the SHR have been developed; these are the WKHA, which is hyperactive, but not hypertensive, and the WKHT, which is hypertensive, but not hyperactive (Hendley et al., *Hypertension*, 5:211, 1983). We have used these strains to determine the level of metabolic activity in sympathetic ganglion cells as reflected by cytochrome oxidase levels.

Cryostat sections (20 μm) of superior cervical ganglia from WKY, SHR, WKHT and WKHA rats were processed for cytochrome oxidase histochemistry utilizing diaminobenzidine (DAB) as a chromogen. The relative level of cytochrome oxidase activity was determined by video densitometry of the DAB reaction product. Strain differences in the level of cytochrome oxidase in the ganglia were assessed by ANOVA.

Significantly greater levels of cytochrome oxidase activity were present in the superior cervical ganglia of SHR and WKHT rats as compared with WKY and WKHA. WKY and WKHA superior cervical ganglia exhibited similar levels of cytochrome oxidase activity. These results suggest that ganglion cells are more active in hypertensive animals than in either normal or hyperactive animals. Whether this increase in metabolic activity reflects electrophysiologically more active cells is currently under investigation.
Supported by the AHA and PHS K0401344 & RO126390.

357.3

MORPHOLOGICAL ORGANIZATION OF GANGLION CELLS AND SMALL INTENSELY FLUORESCENT (SIF) CELLS OF THE CANINE INTRACARDIAC GANGLIA.
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Canine intracardiac atrial ganglia are innervated by vagal preganglionic and interganglionic nerves. Forty ganglion cells were intracellularly recorded (resting membrane potential > 45 mV) and labeled using horseradish peroxidase technique. In whole tissue mounts, cell morphology was traced using camera lucida technique. Somata were elongated (mean 40 x 63 μm) and had 2-12 primary dendrites (mean 5.5) which ranged in length from 16 - 276 μm (mean 80 μm) with shorter secondary and tertiary branches and numerous spines. On 34 cells no dendrites were observed to extend beyond the ganglion. Axons could be distinguished by their length (110-2389 μm), small diameter and sparcity of spines. All cells had one axon which arose from either the soma (n=28) or primary dendrite (n=6). Almost half of the axons (n=19) can be traced only within the ganglion, sometimes terminating on other neurons. These may be axons of interneurons. Another type of axon (n=21) coursed to the muscle or fat tissue or to interganglionic nerves. These axons were long (1043 vs 214 μm, P<0.05), usually parallel to the long axis of the ganglion. These neurons could be parasympathetic postganglionic projection cells or interneurons that synapse on neurons in another ganglion. These two types of cells had similar somas and dendrites. Glyoxylic acid fluorescence staining for catecholamines in these ganglia revealed SIF cells which were near to other neuron soma but only 1/3 the diameter. Catecholamine containing nerve fibers ran along interganglionic nerves and around neurons, which were most likely sympathetic postganglionic axons. No large ganglion cell bodies were labeled. Conclusions: 1). Canine intracardiac ganglion is composed of vagal postganglionic projection neurons, interneurons and SIF cells. 2). Interganglionic nerves contained vagal pre and postganglionic axons, axons from interneurons, and sympathetic postganglionic axons. (Supported by HL 27595)

357.4

LIGHT AND ELECTRON MICROSCOPIC STUDY OF NEURONS IN CARDIAC GANGLIA OF THE RAT AND DOG.
M.H. Mostafa, X. Xi, R.D. Wurster, F. LaVelle, and E.J Neafsey. Neuroscience Graduate Program and Departments of Physiology and Anatomy, Loyola Univ. Sch. of Med., Maywood IL 60153.

Cardiac ganglia were processed for electron microscopy where 70 profiles of cell bodies and their surrounding neuropil from each species were examined. In both rat and dog the somas were 25-30 um in diameter and were completely ensheathed by satellite cell processes, thus preventing synapses on them. On dendritic branches two types of synaptic boutons were found: boutons with homogeneous, round agranular vesicles (40 nm) and boutons with similar vesicles that also contained a few larger (70 nm) dense-cored vesicles. The predominant type of synaptic contact in the rat was asymmetric and in the dog was symmetric. The large, wide based dendrites commonly seen in the dog were missing in 20 cell bodies of rat cardiac ganglia cut serially (2 um thick) and studied under light microscopy, leaving the rat with rather simple ovoid or round cell bodies. In agreement, neurons of rat cardiac ganglia injected intracellularly with horseradish peroxidase showed only few (1-3) long spiny dendrites compared to the many dendrites seen on dog ganglionic neurons. There are significant differences in the morphology of cardiac ganglia neurons in rats and dogs that may explain some of the differences in cardiac control between these species. (Support: NIH HL 27595)

357.5

VAGAL PROJECTIONS TO THE CELIAC PLEXUS OF THE RAT.

T.L. Powley and H.-R. Berthoud. Lab. of Regulatory Psychobiology, Purdue University, West Lafayette, IN 47907.

Distal branches of the abdominal vagus are known to course into or through the region of the celiac plexus, but the exact trajectories and targets of these fibers have not been traced, in part due to difficulties both in discriminating vagal axons from the nonvagal elements with which they mix and in following them over long distances. In the present experiment, we used a protocol that combines anterograde DiI labeling, Fluoro-gold counterstaining, wholemount preparations of tissues, and confocal as well as conventional fluorescence microscopy to map these projections.

Three weeks before sacrifice, male SD rats received multiple small DiI injections (3 to 4 per side; 40 nL per injection; 5% solution) into the dorsal motor nucleus and adjacent vagal complex; three days before sacrifice, they also received an i.p. injection of 5 mg Fluoro-gold. Whole mounts of the celiac plexus region were prepared, processed in glycerin, and examined.

DiI-labeled vagal axons could be followed through connecting branches and nerves into both the celiac ganglia and the smaller associated ganglia in the plexus. Vagal fibers evidenced a selective projection pattern insofar as they distributed into--as well as terminated in--some ganglia but not others. Further, within those ganglia innervated, vagal terminals were observed on some cells and not others. Both large varicosities and finer terminal endings were observed. Additionally, a small population of neuronal somata in the celiac and accessory ganglia were retrogradely labeled with DiI. Apparently these cells (as well as similar neurons we have observed in the myenteric plexus of the proximal small intestine) must project centrally to the NTS or other regions within the dorsal vagal complex. NIH grants DK27627 and NS26632.

357.6

LOCATION, CHARACTERIZATION, AND PROJECTIONS OF MECHANOSENSITIVE ENTERIC NEURONS IDENTIFIED BY ACTIVITY INDUCED EXPRESSION OF C-FOS IMMUNOREACTIVITY.

A. L. Kirchgessner and M. D. Gershon, Department of Anatomy and Cell Biology, Columbia Univ. P & S, New York, NY.

The bowel is the only peripheral organ that is able to manifest reflex activity in the absence of input from the CNS. This activity depends on intrinsic primary afferent neurons, which have not previously been identified. Peristaltic reflexes are initiated by mechanical distension of the mucosal surface of the gut. We studied nuclear immunoreactivity (ir) of the protein encoded by the proto-oncogene, *c-fos*, in order to visualize and characterize intrinsic mechanosensitive enteric neurons. Initial experiments utilized mucosal applications of cholera toxin (CTx), which activates enteric neurons, in order to determine whether *c-fos* is a marker for stimulation of these cells. No nuclear *c-fos*-ir was found in the neurons of non-stimulated gut; however, following CTx, nuclear *c-fos* -ir appeared in neurons of each plexus. This effect of CTx was blocked by tetrodotoxin (TTx). In order to distend the mucosal surface of the bowel *in vitro*, segments of gut were opened and puffs of N_2 were delivered to the intestinal lining with a Picospritzer™. After 1 hr, *c-fos*-ir was found in neuronal nuclei; this effect was blocked by TTx and 5-HT antagonists. A microinjection of the retrograde tracer, FluoroGold (FG), was placed in a single myenteric ganglion in order to label those submucosal neurons which project to the myenteric plexus. When this technique was combined with mucosal stimulation and demonstration of *c-fos*-ir, a set of submucosal neurons was found that was doubly labeled by retrograde transport of FG and antibodies to *c-fos*. Some of these neurons were found to contain calbindin-ir and/or substance P-ir. It is concluded that the gut contains submucosal mechanosensitive neurons that project to the myenteric plexus. 5-HT is involved in stimulating these cells, a proportion of which contain calbindin and/or substance P. Supported by NIH grants 27645 and NS 12969.

357.7

DiI - TRACING OF DIRECT VAGAL INNERVATION OF RAT PANCREAS.

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Activation of abdominal vagal motor fibers can powerfully stimulate pancreatic endocrine and exocrine secretions, and it is thought to play a major role in metabolic regulation during the cephalic phase of ingestion. Anatomically, the vagal fibers and terminals in the pancreas have not been well characterized, because silver staining for degenerating axons was the only method applied and yielded inconclusive results. Having recently identified the vagal innervation of the GI-tract with the carbocyanine dye DiI in combination with Fluorogold labeling of the enteric nervous system (Berthoud et.al., Neurosci. Abstr. 15 : 264, 1989), we used the same strategy to visualize the vagal innervation of the pancreas.

DiI was injected bilaterally (3X40 nL per side, 5% in Eth) into the dmnX of male SD rats. After 3-6 weeks, animals were perfused with formalin, the pancreas was postfixed in 10% formalin, transferred to PBS, and systematically dissected into approx. 60 pieces, which were dehydrated in a series of glycerin, whole mounted, and examined by means of epifluorescence or laser scanning confocal microscopy. Fluorogold labeled pancreatic islets as well as ganglia and connecting nerve bundles in the interlobular septa could be clearly visualized using the appropriate filter cube, and then checked for vagal innervation by switching the filter.

Of the mean number of 232 $\pm$ 28 (n=5) pancreatic ganglia per rat, an average of 19 $\pm$ 5 (8%) of mainly the larger ones (10-30 neurons), located in the head of the pancreas, were vagally innervated. Highly varicose DiI labeled profiles, indicative of vagal motor terminals, characteristically encircled either only select, or most of the neurons of a given ganglion. No DiI labeled terminals could be found in islets and acinar tissue. These findings support the view that vagal preganglionics control the diverse pancreatic functions by affecting relatively few postganglionic neurons in the larger relay stations of the pancreas. (NIH grants DK27627 and NS26632)

357.8

ULTRASTRUCTURE OF HRP LABELED NEURONS IN THE GANGLIONATED PLEXUS OF THE GUINEA PIG GALLBLADDER.

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Little is known regarding the extent to which gallbladder function is regulated by neurons that are intrinsic to the organ. The wall of the gallbladder contains a prominent neural plexus comprised of small, irregularly shaped ganglia of 2-10 neurons. In this study, individual, physiologically identified neurons within these ganglia were injected intracellularly with horseradish peroxidase (HRP). The labeled cells were examined with light and electron microscopy to determine the morphology of galbladder neurons and their association with the surrounding ganglion.

HRP labeled cells were large (avg. size 54 X 30 μm) with irregular contours. Most cells gave rise to a single unbranched axonal processes that exited the ganglion through a connective; some cells had a second process that passed directly across muscle fibers adjacent to the ganglion. Ultrastructurally, labeled cells revealed numerous short somal extensions that closely apposed adjacent neurons or enclosed terminals containing dense core and clear spherical vesicles or clear spherical vesicles only. Abundant glial processes surrounded most somal extensions within the ganglia and ensheathed individual axons in the ganglia and connectives. At the periphery of the ganglia, labeled cells had frequent direct contacts with the basal lamina. Overall, ganglia were compact, lacked internal basal lamina and collagen, and were surrounded by a basal lamina and distinct connective tissue capsule.

These studies reveal that the intrinsic ganglia of the gallbladder more closely resemble those of the enteric nervous system than traditional autonomic ganglia, and may similarly play an extensive role in the local regulation of the function of this organ. However, the simpler neuronal geometry, the greater extent of glial investment and a lower level of innervation indicate differences from enteric ganglia that may be functionally significant. Supported by NS26995 and UVM College of Medicine Postdoctoral Fellowship (to EBC).

357.9

DIFFERENTIAL 5-HT RECEPTOR-MEDIATED RESPONSES IN AORTIC RINGS FROM THYROPATHOLOGIC RATS.

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The present study examined the effects of thyropathology upon 5-HT receptor-mediated contraction/dilation in 2mm aortic ring sections excised from Sprague-Dawley rats (175-200g). Following 28 days of pathology induced by administration of propylthiouracil (PTU: hypothyroid), thyroxine (TRX:hyperthyroid) or saline (control), Rats were sacrificed and aortic sections were assayed for isometric contraction in response to increasing concentrations of 5-HT (0.1nM-10uM). 5-HT increased contraction in TRX rings, and reduced contraction in tissue from PTU-treated rats. Mechanical removal of the endothelium (denudation) reduced effects in PTU, but not TRX rings. The observed increased contraction in TRX rings was blocked by the 5-HT_2-antagonist, ketanserin (KT). The contractile response elicited by high concentrations of 5-HT was also attenuated by the putative 5-HT_3-antagonist ICS 205-930 (10uM). Distinct effects in PTU, TRX treated tissue following denudation suggests the possible involvement of 5-HT-induced endothelial relaxing/contractile factors. The efficacy of KT in antagonizing the differential contractile response in thyropathologic rings implies that a role for the 5-HT_2 receptor in these effects. The action of ICS 205-930, in light of the apparent lack of 5-HT_3 sites in this tissue, suggests that the contractile response may be subserved, in part, by a 5-HT site with properties similar to the newly identified 5-HT_4 receptor.

357.10

RESTING SYMPATHETIC ACTIVITY IN DIABETIC NERVE.

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Resting sympathetic activity(SA) was examined in saphenous nerve of 32 BB rats with diabetic durations of 3-15 mo., maintained on either a good (G) or poor (P) glycemic control regimen. Twenty two nondiabetic littermates and cousins served as controls (C). Unilateral whole nerve conduction velocities CV were obtained, then the nerve was cut near the knee and 30 microfilaments were dissected and examined for SA.

Results: CV of 3-6 mo. G were not different than C, but 3-6 mo. P CV were significantly slower than C ($p<0.001$) or G ($p<0.05$). CV were also significantly slower than C in both 9-12 mo. G ($p<0.029$) and P ($p<0.04$), but did not differ between treatment regimens. SA was found in significantly fewer microfilaments than controls in 3-6 mo. G ($p<0.022$) and P ($p<0.001$), but was not affected by treatment regimen. SA in 9-12 mo. diabetics did not differ from matched control level, nor did treatment groups differ. However, although CV did not differ among age groups, there was significantly less SA in the older C than in the younger($p<0.028$).

Conclusions: (1) CV was slower in diabetic BB rat nerve than in controls. (2) Good glycemic control maintained normal CV in young adults, but the effect diminished with age. (3) SA levels in young adult BB rats were lower than in controls. (4) SA levels in old BB rats were low whether or not diabetes was present.

357.11

PEAK SYMPATHETIC NERVE ACTIVITY DURING FATIGUING ISOMETRIC EXERCISE IN HUMANS. D. R. Seals, M.J. Reiling*, and D. G. Johnson*. Depts. of Exercise and Sport Sciences, Physiology and Internal Medicine, Univ. of Arizona, Tucson, AZ 85721.

We tested the hypothesis that peak sympathetic activity is independent of the level of exercise during voluntary, constant-force muscle contractions sustained to the point of fatigue. In 6 healthy subjects, muscle sympathetic nerve activity (MSNA; peroneal microneurography), venous plasma norepinephrine levels (NE), arterial blood pressure (AP) and heart rate (HR) were determined before (control) and at the end (last 20% of contraction) of isometric handgrip exercise performed to fatigue at 20% (431±68 [SE] s), 40% (115±23 s) and 60% (64±9 s) of maximal voluntary force (random order). MSNA averaged 14 bursts/min and 175 units (total min activity) during the control periods and increased during all 3 levels of exercise ($p<0.05$). Peak MSNA was greater ($p<0.05$) during exercise at 40% (47±9 bursts/min, 710±257 units) and 60% (49±7 bursts/min, 720±186 units) of maximum than at 20% (31±3 bursts/min, 397±119 units). The increases in NE (136±11, 134±6, 135±12% of control at 20, 40 & 60%) and mean AP (28±6, 33±6, 32±2 mmHg) were not different among the 3 exercise levels, but the increase in HR (bt/min) was greater ($p<0.05$) at 60% (39±6) than at 40% (29±7) and 20% (22±5) of maximum. We conclude that during fatiguing isometric exercise in humans, peak sympathetic nervous system activity to non-active skeletal muscle depends on the force of contraction. This influence is not reflected in plasma neurotransmitter levels, and there is no obvious effect on arterial blood pressure. (Supported by NIH HL 39966)

357.12

POSTNATAL MATURATION OF CARDIAC RESPONSES TO STELLATE GANGLIA (SG) STIMULATION. C.V. Coren*, N.A. Kaplan*, F.M. Pisana*, N. Gootman*, P.M. Gootman and B.J. Buckley.* Dept. Cardiology, Schneider Children's Hospital, New Hyde Park, NY 11042 and Dept. Physiology, SUNY-Health Science Center, Brooklyn, NY 11203.

SG were exposed via bilateral thoracotomy in pentobarbital-anesthetized, paralyzed, artificially ventilated and ganglionic blocked piglets (1 wk, n=10; 2 wks, n=19; 1 mo, n=19). SG were subdivided into 30 sites for standardization and each site stimulated with bipolar tungsten electrodes for 20 sec (0.3-10.0 mA, pulse width 1.1-3.0 msec, 1-20 Hz). EKG, heart rate (HR), left ventricular (LV) and aortic pressures, and LV dP/dt max were continuously recorded. Cardiac responses were:

TOTAL # SITES ELICITING RESPONSES	# SITES ELICITING RESPONSES IN: HR	LV dP/dt max
RIGHT STELLATE (n=18)		
143/485 (30%)	118 (83%)	118 (83%)
LEFT STELLATE (n=30)		
149/822 (18%)	4 (3%)	131 (88%)

There were no responses to stimulation of the left SG in 53% of animals ≤ 2 wks. Cardiac responses differed depending upon site of stimulation within the left or right SG. The results indicate: 1) age-related maturation of responses between the two ganglia, 2) laterality of function with respect to chronotropy and 3) positional specificity of responses within each ganglion. (Supported by HL-20864 and Faculty Research Awards Program of LIJMC.)

357.13

POSTNATAL DEVELOPMENT OF CARDIOVASCULAR AND SYMPATHETIC RESPONSES TO FICTIVE VALSALVA MANEUVERS. B.W. Hundley, P.M. Gootman, H.L. Cohen and G. Condemi*, Department of Physiology, State University of New York, Science Health Center, Brooklyn, N.Y. 11203.

Studies of postnatal neural regulation of the circulation indicate variability of maturation of cardiovascular reflexes (Gootman, "Developmental Neurobiology of the Autonomic Nervous System," Humana Press, 1986, pp. 279-325). We have now examined responses to more complex stimuli, the Valsalva Maneuver, using maintained lung inflations (MLI) as the test in Saffan-anesthetized, paralyzed piglets. Both efferent phrenic (PHR) and preganglionic sympathetic (SYMP) activity were recorded. Prior to 1 month of age, no overshoot of aortic pressure (AoP) or heart rate (HR) change could be elicited at the cessation of the MLI although PHR activity was inhibited. In 1 month old piglets, changes (@5%) in AoP and HR were obtained. It was not until 48 days of age that classical Valsalva Maneuver responses were obtained in the developing swine. In the >1 month old swine, SYMP responses included decreased activity followed by an increase and, upon removal of the MLI, inhibition followed by recovery. Thus, while the various component reflex pathways involved in complex cardiovascular response patterns are present in younger animals, the complete integration of responses to more complex stimuli was delayed until the piglets were at least 6 weeks of age. (Supported by NIH Grant HL-20864.)

357.14

ALTERED EXPRESSION OF PEPTIDYLGLYCINE α-AMIDATING MONOOXYGENASE (PAM) IN HYPERTENSIVE AND HYPERACTIVE RATS. K.M. Braas, E.D. Hendley, and V. May. Departments of Anatomy & Neurobiology and Physiology & Biophysics, University of Vermont, Burlington, VT 05405

Many bioactive peptides influence cardiovascular function, and altered amidated peptide levels have been associated with hypertension. Peptidylglycine α-amidating monooxygenase (PAM) catalyzes the formation of bioactive α-amidated peptides from their inactive glycine-extended precursor molecules. Highest levels of PAM expression were found in the cardiovascular system. The expression of PAM in neuroendocrine tissues is coordinately regulated with amidated peptide biosynthesis. We have examined the tissue specific expression of soluble and membrane-associated PAM in tissues involved in cardiovascular regulation in the normotensive Wistar-Kyoto (WKY) and spontaneously hypertensive (SHR) rat strains. To determine whether altered PAM expression is associated with either the hypertensive or hyperactivity trait of the SHR, tissues from two inbred strains were also examined. The WK-HT is hypertensive but not hyperactive, while the WK-HA is hyperactive but normotensive (Hendley, et al., Hypertension 5:211, 1983). Tissue PAM activities in the SHR, WK-HT and WK-HA strains were compared to activities in the WKY strain. Membrane-associated PAM activity levels were elevated 1.5 - 2.0 fold in heart atrium and ventricle of the SHR and WK-HT strains, suggesting that peptidergic levels in these tissues are altered in the hypertensive animals. Ventricular soluble PAM activity was elevated over 4 fold in the WK-HT strain. Atrial PAM activity was lower in the WK-HA strain. The anterior pituitary gland, which is composed of many distinct endocrine cell types, expressed 2 - 3 fold increases in PAM activity in the SHR, WK-HT, and WK-HA rats, which may reflect changes in different cell populations in hypertension and hyperactivity. PAM activity in the neurointermediate pituitary lobe increased 2 - 4.5 fold only in the SHR and WK-HA rats, suggesting a specific association with hyperactivity. Tissues from the CNS are heterogeneous in cell populations, and changes in PAM activities were expectedly smaller. Current studies using Northern blot analysis, immunocytochemistry and in situ hybridization will help define the sites of altered bioactive amidated peptide production in hypertension and hyperactivity. Supported by PHS F07RR05429 and NS26390.

357.15

ADRENOMEDULLARY CONTRIBUTION TO STRESS-INDUCED VASODILATION MEDIATED BY β_2 ADRENOCEPTORS IN RAT. R. Davisson, R. Kirby & A.K. Johnson. Dept. of Psychology and Pharmacology, Univ. of Iowa, Iowa City, IA 52242.

We have previously found that blockade of β_2 adrenoceptors increases the pressor response to acute stressors in rats. This response is most likely due to antagonism of epinephrine's action on β_2 adrenoceptors mediating vasodilation in skeletal muscle vasculature. The present study examined the relative contributions of the sympathetic nerves and the adrenal medulla to the pressor response evoked by acute stress in Sprague Dawley rats.

The stressors, transfer to a novel chamber and footshock, led to immediate pressor responses in vehicle-treated animals. Blockade of sympathetic terminal release of norepinephrine with bretylium abolished this immediate pressor response to the stressors, with no effect on the delayed (7-9 sec) blood pressure response. The β_2 adrenoceptor antagonist ICI 118,551 led to an increased blood pressure response to transfer and a delayed pressor response to footshock as compared to vehicle-treated controls. Blockade of sympathetic terminal release with bretylium did not affect the increased pressor responses to the stressors produced by antagonism of β2 adrenoceptors. These results indicate that acute stressors produce skeletal muscle vasodilation that is mediated by adrenomedullary epinephrine release on β2 adrenoceptors.

357.16

CALCIUM, BLOOD PRESSURE & SODIUM FLUX IN SHR & WKY RATS. A. Derick Dalhouse , Dawn Erickson*, and Patrick Bellgowan* Dept. of Psy., Moorhead State University, Moorhead, MN.

There is evidence that calcium (Ca) physiology is altered in essential hypertension. Intracellular Ca ions have direct effect on peripheral vascular tone, and hypertensives have increased intracellular free Ca that decreases to normal levels with anti hypertensive treatment. Serum Ca levels have been negatively associated with 24-hour urine sodium (Na) and positively associated with potassium (K) excretion. If abnormal cations transport is in fact a marker and precursor of hypertension it is important to identify potentially modifiable environmental or life style variables that might be useful for preventive purposes. In this experiement SHR and WKY rats were placed on normal, high, or low calcium diets for 7 weeks to investigate the effects of dietary calcium on blood pressure and red blood cell (RBC) Na flux. SHR pressures were significantly higher than the WKY in all groups ($p<.009$), but not different from each other. The low calcium WKY rats showed a significant elevation in blood pressure ($p<.003$) compared to the normal and high calcium WKY rats. Thus high dietary calcium seems to have no effect on the blood pressure of genetically hypertensive or normotensive rats while low dietary calcium seems to have an elevating effect on that of normal rats. The Ca diets produced no significant differences in RBC Na flux, however the SHR rats showed higher Na flux than the WKY rats.

357.17

DIETARY CALCIUM ALTERS BP REACTIVITY TO NOREPINEPHRINE BUT NOT TO THE ALPHA-1 AGONIST METHOXAMINE

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Variations in dietary calcium cause blood pressure (BP) alterations in the spontaneously hypertensive rat (SHR). Animals fed supplemental dietary calcium have lower blood pressure than those fed restricted calcium diets. Along with the change in basal blood pressure, blood pressure reactivity to infusions of norepinephrine (NE) is altered with animals on low calcium diets having larger and more prolonged pressor respones to infused NE. To determine whether the alteration in blood pressure reactivity is due exclusively to activation of alpha-1 adrenergic receptors, the alpha-1 adrenergic agonist methoxamine (100, 200, 400 ug/kg) was given to 5-week-old SHRs maintained on either high (2.0%) or low (0.1%) calcium diets for two weeks. There was no difference in pressor response to methoxamine between dietary groups. However, as in previous studies, there was a significant difference in basal blood pressure between dietary groups (118 vs 105 mmHg, $p<.01$) as well as a significant difference ($p<.001$)between dietary groups in their response to doses of NE. The results suggest that diet-induced alterations in blood pressure reactivity to NE are not a consequence of changes in alpha-1 receptor activity.

357.18

MULTIPLE EFFECTS OF SPANTIDE IN THE ISOLATED PERFUSED GUINEA PIG HEART. D.B. Hoover. Dept. Pharmacol., Col. of Med., East Tennessee State Univ., Johnson City, TN 37614.

The present study was designed to determine the effect of spantide on perfusion pressure in isolated guinea pig heart and the ability of this drug to block vasodilator responses to substance P (SP). Hearts from male, Hartley guinea pigs were perfused at a constant flow rate with isotonic buffer containing 20 or 40 mM KCl. High KCl was used to increase perfusion pressure and eliminate cardiac contractions. Spantide (1 μM) increased the ED_{50} for vasodilation by SP (1.3 vs 26 pmol; n=6 each; 40 mM KCl). Bolus injections of $\leq$ 0.25 nmol spantide had no effect on perfusion pressure. However, 2.5 and 25 nmol spantide produced a biphasic perfusion pressure response. With the 25 nmol dose, an initial 12 $\pm$ 2% decrease in perfusion pressure was followed by a prolonged increase with a maximum 58 $\pm$ 5% above baseline (20 mM KCl, n=5). Spantide also caused a brief resumption of cardiac contractions. Histamine produced a similar response pattern to that of spantide, and a combination of H_1 and H_2 receptor blockers antagonized vascular and cardiac responses to spantide. Therefore, spantide is a weak antagonist at SP receptors in the guinea pig coronary vasculature and at high doses elicits vascular and myocardial responses mediated by histamine released from cardiac mast cells. Supported by NIH Grant HL38705 and a Grant-in-Aid from the American Heart Association, Tennessee Affiliate.

357.19

AMPLIFICATION OF ANP-RELATED GENE FRAGMENTS FROM FISH BRAIN cDNA BY PCR. D.A. Price and B.M. Byrne*. Whitney Laboratory, 9505 Ocean Shore Blvd., St. Augustine, FL 32086.

We previously isolated an ANP-related peptide from fish (Fundulus heteroclitus) brain by use of an antibody to rat ANP. The sequence of this peptide:

G W N R G C F G L K L D R I G S M S G L G C

is similar to the known mammalian peptides within the disulfide ring but differs from them in its N-terminal sequence and most remarkably by ending with the second half-cystine of the disulfide-linked ring. The lack of a C-terminal tail is a unique feature among the known ANP-related peptides, so we wished to determine its biosynthetic basis, and first of all the structure of its precursor. A mixed oligonucleotide, encoding the N-terminal eight amino acids of the peptide, was synthesized. To reduce the degeneracy, inosine was used for all positions where any of the four bases might occur. The downstream primer consisted of an oligo(dT_{15}) extended at the 5' end by ten bases containing an EcoRI recognition site. Total RNA from fish brain was reverse transcribed using the downstream primer, and subsequently subjected to 30 cycles of amplification (48°C annealing temperature). The products were fractionated on a low melting point agarose (NuSieve) gel and selected regions of the expected size (250-400 bp, based on homology to mammalian sequences) were re-amplified for a further 25 cycles. The products of these second amplifications were made blunt-ended using T4 DNA polymerase and, after digestion with EcoRI, they were directionally cloned into SmaI x EcoRI-digested pGEM-3Z. We have sequenced three recombinants; each contained both primers but the remaining sequence showed no similarity to ANP. Additional recombinants are currently being characterized by sequencing. (Supported by NIH grant HL28440).

357.20

ALTERED NEUROPEPTIDE-Y IMMUNOREACTIVITY (NPY-IR) IN LEFT VENTRICULAR HYPERTROPY RESULTING FROM AORTIC STENOSIS. S. Love, C. Nyquist-Battie, and B.M. Chronwall, Schools of Pharmacy and Basic Life Sciences, University of Missouri, Kansas City, MO 64108.

Myocardial stores of norepinephrine(NE) are depleted in most models of hypertrophied and failing heart. The effect of cardiac hypertrophy on NPY, a cardiovascular regulatory peptide released from cardiac adrenergic nerve endings, is the focus of this study. Left ventricular hypertrophy was induced in female guinea pigs by subdiaphramatic aortic stenosis. Animals were sacrificed five weeks after surgery. The left ventricular/body weight ratio was elevated 49% by aortic stenosis. Cryostat sections of 4% paraformaldehyde fixed tissue were stained for NPY-IR using an indirect fluorescence method. A dense innervation by NPY-IR fibers was seen in the atria of control animals with less innervation present in the ventricles. NPY-IR fibers were present in all layers of the heart wall. Hearts from animals with aortic stenosis showed a similar NPY-IR distribution in all chambers except the left ventricle which was nearly devoid of NPY-IR. These results indicate that NPY-IR is reduced in the pressure-overloaded ventricle in addition to NE depletion. Given the direct and indirect roles of NPY in cardiovascular functions, a reduction in NPY levels may contribute to the congestive heart failure seen in cardiac hypertrophy.

357.21

DIFFERENTIAL DISTRIBUTION OF CATECHOLAMINES IN WHITE ADIPOSE TISSUE REVEALED BY VIDEO-ENHANCED AND CONFOCAL SCANNING LASER MICRSOSCOPY. M. Rebuffe-Scrive*, R. J. Wyman, and C. L. Keenan, Depts. of Psychology and Biology, Yale University, P.O. Box 11A, Yale Station, New Haven, CT 06520-7447.

Abdominal distribution of fat is associated with increased risk factors for cardiovascular diseases and diabetes. It has been suggested that the elevated catecholamine (CAT) stimulated lipolysis found in abdominal, particularly intraabdominal fat depots might be a potential generator for the pathology of abdominal obesity. However, whether the sympathetic nervous system directly and differentially regulates the different depots of white adipose tissue (WAT) is not known. We used a modified de la Torre and Surgeon (1976) method for fluorescence histochemical detection of CATs in combination with video-enhanced and confocal scanning laser microscopy to localize CATs in four different WAT depots (mesenteric, retroperitoneal, epidydimal, and inguinal) of rats. We also studied the sensitivity of isolated adipocytes to norepinephrine (NE) stimulation. Three-dimensional and quantitative localization of the CATs in whole mounts of WAT depots allowed us to show that CATs are differentially distributed among WAT depots. Visceral depots contain the highest CAT innervation and non-neural CATs, particularly in the mesenteric region. Inguinal WAT had the lowest CAT content. CATs not specific to nerve terminals were distributed around the adipocytes primarily at the nexuses formed by the junctures of the adipocytes and the nerve terminals. Sensitivity of isolated adipocytes to bath applied NE (10^{-5}M) also provided direct evidence that fat cell size is regulated by CATs. These preliminary results suggest that the higher content of CATs in intraabdominal fat tissues might explain the regional differences observed in lipolysis. Furthermore, it was shown that the methods utilized provide a direct in vitro means to study further the role and interactions of CATs with environmental and hormonal factors that might affect regional fat distribution.

357.22

ACETYLCHOLINESTERASE (AChE) EXPRESSION IN CULTURED PERINATAL CARDIAC MYOCYTES. K. Hagler* and C. Nyquist-Battie. School of Basic Life Sciences, University of Missouri, Kansas City, MO 64108.

Globular(G) and asymmetric(A) molecular forms of AChE are present in the ventricles of rat heart. However, the relative contribution of A-forms is higher in the fetal period compared to the perinatal and adult periods. To determine if this change is the result of altered expression of AChE forms by cardiac muscle cells, ventricles from 17-19 day old embryos and 2-4 day old rats were dissociated by 0.1% collagenase. Dissociated cells were preplated in M199 and 0.5% fetal calf serum to remove non-muscle cells. Subsequently, myocytes were plated at 1.5×10^5 cells per 35mm dish, coated with fibronectin and laminin, in a defined medium. Myocytes were confluent and beating for at least 48-h before harvest. The specific activity of AChE in the cellular and secreted pools did not differ as a function of age of hearts from which myocytes were isolated. The tetrameric G_4 form was the predominate secreted form in both groups. G_1 (monomeric) and G_4 forms were the predominate forms in the myocyte-associated pool of both groups and exhibited a similar activities ratio. A-forms were barely detectable (< 5%) in the cell-associated and secreted pools in cultures from both pre- and post-natal hearts. In conclusion, there was no increased AChE synthesis or G_4 expression in cultured myocytes as a function of maturation. Secondly, A-form production is low in perinatal myocyte cultures compared to rates in vivo, suggesting that trophic factors are necessary in developing heart for A-AChE expression.

357.23

SINGLE JUNCTIONAL CHANNEL EVENTS IN HEART CELL PAIRS AND cAMP. W. C. De Mello. Department of Pharmacology, School of Medicine, Univ. of Puerto Rico, PR 00936.

Evidence is available that cAMP increases the junctional conductance (gj) in isolated heart cell pairs. (De Mello, 1988). The mechanism by which cAMP increases gj might be related to: 1) increase in unitary channel conductance; 2) an increase in nº of channels or 3) a change in open-closed time kinetics. To investigate these possibilities heart cell pairs were isolated from adult rat ventricle. Only pairs with low gj (2-6 nS) were used. With a transjunctional voltage of -50 mV single junctional channel events were recognized. The unitary channel conductance was found to be 101 pS (± 33). No change in single channel conductance was seen with isoproterenol (10^{-6}M) despite the increase in gj (75%). The open-time distribution was not changed by the drug. An increase in open probability is assumed to explain the increase in gj caused by cAMP. (Supported in part from grant #HL-34148 from NIH and M0786E87 from MBRS).

REGULATION OF AUTONOMIC FUNCTION: GASTROINTESTINAL CONTROL

358.1

PARTICIPATION OF THE PERIPHERAL AUTONOMIC NERVOUS SYSTEM IN EMESIS. M.T. Makale* and G.L. King, Dept. Physiology, AFRRI, Bethesda, MD 20814.

It is well established that the vagi play a key part in emesis. However, participation of the sympathetic n.s. is less well examined, and our study explored this topic. Ferrets were anesthetized with urethane i.p. and the right jugular and carotid cannulated. For bilateral vagotomy (vgx) or splanchnisectomy (spx), the animals were laparotomized and the nerves transected. The ferrets were then given 10 ml of $CuSO_4$ (5mg/ml) gastrically, or irradiated using a ^{60}Co source (γ) or reactor (γ/n=20:1) for 6 Gy at 1 Gy/min. Another group, anesthetized with isofluorane, cannulated, and allowed to recover, received cyclophosphamide (200 mg/kg). Ferrets not subjected to nerve transection received: atropine-CH_3Br (1 mg/kg), propanolol (3 mg/kg), α-blocker (DCMB 10 mg/kg), or $5HT_3$ antagonists zacopride (0.3 mg/kg) and granisetron (0.5 mg/kg), i.v. before radiation, $CuSO_4$, or cyclophosphamide. All controls responded by retching or vomiting. After vgx 0/8 animals responded to radiation, and 0/2 to $CuSO_4$. With spx 2/8 ferrets responded to radiation. After atropine, 3/4 animals responded to $CuSO_4$. With propanolol 12/15 responded to $CuSO_4$, and 3/3 to cyclophosphamide. After DCMB 4/6 animals responded to $CuSO_4$. With zacopride 0/4 ferrets responded to $CuSO_4$, and with granisetron 4/4. These early results suggest that both autonomic branches contribute to emesis, but afferent ACh and adrenergic contributions are minor.

358.2

SOURCES OF GASTRIC VAGAL EFFERENT INNERVATION IN RATS. J.Y. Wei, Y. Taché and L. Kruger. CURE/VA Wadsworth Medical Center, Dept. of Medicine, Dept of Anatomy & Brain Research Institute, UCLA, CA 90073.

Gastric vagal efferent sources were studied by recording ventral gastric vagal efferent discharges (VED) in urethane-chloralose anesthetized rats. After a single or multi-unit basal VED was recorded, the right and left cervical vagus and then the distal end and proximal end of the ventral gastric vagus were transected successively at about 30 min intervals, and comparisions of VED before and after each transection were made. Results: 1) the VED were increased in 15/21 cases by 4-53% (median 18%) and were reduced in 2 cases by 4 and 22% after the right cervical vagus was cut, suggesting that there is a numerous crossed inhibitory afferent influence and few, if any, crossed gastric vagal efferents. 2) After both cervical vagi were cut, the VED were decreased by 10-99% (median 90%) in 14/17 of experiments. 3) A segment (4-5 mm) of the gastric vagus was isolated by additional cuts at the distal and proximal end of the nerve, abolition of VED were obtained in only 4/14 of successful cuts, 1 to 3 residual units of VED were recorded in 10 cases. Morphological evidence suggests that the presence of paraganglionic cells in the isolated specimen may account for the residual VED. These findings reveal that in rats, gastric vagal efferents do not all originate from the brain stem. Alternative descending sources may be located between the lower cervical to upper and/or lower abdominal esophageal levels. (Supported by NIH grants, DK33061, DK30110 and NS5685)

358.3

TOPOGRAPHIC REPRESENTATION OF THE PROXIMAL AND DISTAL STOMACH IN THE DORSAL MOTOR NUCLEUS IN RAT. S.M. Altschuler, R.R. Miselis, T.H. Moran, P.R. McHugh, & G.J. Schwartz. Dept. Ped., Anim. Biol. & Inst. Neurol. Sci., Univ. of Penn., Phila., PA, 19104, & Dept. Psychiatry, Johns Hopkins Univ. Sch. Med., Balto., MD 21205.

Small quantities (5-10 µl) of cholera-toxin conjugated to horseradish peroxidase (CT-HRP) were injected into the ventral pyloric/antral region (N=8) or the ventral forestomach (N=9) of rats to determine the topographic representation and dendritic projections of motoneurons within the dorsal motor nucleus (DMN). Although both injections labelled cells throughout the rostro-caudal extent of the DMN unilaterally, the majority of labelled cells in both cases were located along the rostral-caudal extent of the area postrema. Second, there was a prominent topographic representation of the two gastric regions injected. Motoneurons innervating the pylorus/antrum were found in the medial 1/3 -1/2 of the DMN. In contrast, motoneurons innervating the ventral forestomach were located in the lateral 1/3 of the DMN. Finally, dendritic projections from medially labelled DMN sites tended to extend into the lateral aspect of the subnucleus gelatinosus of the nucleus tractus solitarius, while those from lateral DMN sites tended to extend into more medial portions of the subnucleus gelatinosus. The results extend the notion of a topographic representation of visceral structures within the DMN to include intra-visceral topography. (Supported by NIH grants GM27739, DK01747, & DK19302).

358.4

ABDOMINALLY PROJECTING NON-CHOLINERGIC NEURONES IN THE DORSAL MOTOR NUCLEUS OF THE VAGUS (DMN) IN THE RAT. E.K. Tayo and C.J. Helke. Dept. of Pharmacol., Uniformed Services Univ., Bethesda, MD 20814.

Efferent fibres arising from perikarya within the dorsal motor nucleus of the vagus (DMN), form a major autonomic, motor pathway involved in the regulation of abdominal visceral function. These neurones are classically regarded as being cholinergic and have been previously identified by a number of investigators using immunocytochemistry (icc) to choline acetyltransferase (ChAT) or acetylcholinesterase (AChE) histochemistry.

The presence of AChE-negative abdominally projecting neurones has been previously reported (Hoover & Barron 1982, Tayo & Williams 1988), however, the fact that the same had not been shown using ChAT icc precluded the classification of these cells as non-cholinergic.

The present study combines retrograde tracing with True Blue, immunocytochemistry to ChAT, AChE histochemistry and Nissl staining techniques on individual 20µm rat medullary sections and also on adjacent serial sections.

The results show the distributions of ChAT-immunoreactive and AChE-containing cells to be identical throughout the rostrocaudal extent of the DMN. In addition, a comparison of the cholinergic cell localization with those cells identified subsequent to Nissl staining and/or retrograde transport of True Blue, reveals a population of cells which do not appear to contain AChE or ChAT.

358.5

DIRECT HUMORAL ACCESS TO DORSAL MOTOR NUCLEUS OF THE VAGUS PREGANGLIONIC NEURONS? F.B. Wang and T.L. Powley. Lab. of Regulatory Psychobiology, Purdue U., West Lafayette, IN 47907.

The autonomic outflow of the dorsal motor nucleus of the vagus (dmnX) is influenced by the concentrations of peptides and other active compounds in the ventricular and systemic circulations. These factors have traditionally been assumed to affect preganglionics indirectly by acting through afferents. In experiments on dmnX dendroarchitecture, we have observed some preganglionics which send dendrites to the ependymal wall as well as to blood vessels, raising the possibility of additional, direct humoral effects on dmnX motorneurons.

In male SD rats, the preganglionics of the dmnX were labeled by retrograde transport of Fast Blue. The brains were lightly fixed by perfusion, sections of the medulla oblongata were vibratomed, and the dendrites of individual neurons were stained by intracellular iontophoresis of lucifer yellow. Dendritic profiles were reconstructed with both laser scanning confocal and conventional microscopy.

Although dendrites of dmnX preganglionics tended overall to remain within the nucleus and even within the longitudinal columnar subnucleus of origin (see Fox and Powley, Soc. for Neurosci. Abst. 15(1): 662, 1989), a number of medial gastric column neurons sent one or more dendritic process to the subependymal region of the fourth ventricle or central canal. Other preganglionics had one or more dendrite(s) within the dmnX running to and along blood vessels. Although EM analyses will be required to specify what if any contact occurs between dmnX neurons and these ventricular and vascular zones, the present observations--particularly when taken in conjunction with previous observations suggesting a reduced blood-brain barrier in the region--raise the possiblity that dmnX neurons could be directly influenced by humoral factors. (DK27627 and NS26632)

358.6

DIFFERENTIAL GASTRIC EFFECTS OF MICROINJECTION OF L-GLUTAMATE, TRH, AND A SEROTONIN$_{1A}$ RECEPTOR AGONIST INTO THE ROSTRAL AND CAUDAL DMV OF CATS. C.D. Rossiter, R.A. Gillis*, and P.J. Hornby. Dept. of Pharmacology, Georgetown University, Washington, D.C. 20007.

Recently we reported that increases and decreases in lower esophageal sphincter pressure occur after activation of neurons in the DMV rostral and caudal to the obex, respectively (Rossiter et al., Gastroenterology Abst.,1990). The purpose of the present study was first to determine the effects on intragastric pressure and gastric motility of activation of neurons in the DMV rostral to obex (0-3.0mm) or caudal to the obex (0 to -1.5mm) in chloralose anesthetized cats. A second purpose was to determine the gastric effects of TRH or a serotonin1A receptor agonist, [3H]8-hydroxy-N,N-dipropylaminotetraline (8-OH-DPAT), microinjected into the rostral and caudal DMV. DMV rostral to obex. Microinjection of L-glutamate (5-10nl of 500mM; N=6) resulted in significant increases in intragastric pressure (+6.9±2.5mmHg, p<0.05) and pyloric minute motility (MMI; +8.9±2.3MMI, p<0.05). Microinjection of TRH (25-200ng in 5-20nl) also significantly increased intragastric pressure (+6.8±1.5mmHg, N=6, p<0.05) and pyloric MMI (+6.7±2.2, N=6, p<0.05). However, no significant alteration in gastric function occurred after microinjection of 8-OH-DPAT (20ng in 20nl, N=6). DMV caudal to obex. Microinjection of L-glutamate (-0.0 to-1.5mm) significantly decreased intragastric pressure (-7.5±2.2, p<0.05, N=4) without changing pyloric motility (+1.3±1.0, N=10). TRH microinjection (N=7) had no significant effect on either intragastric pressure or pyloric MMI. A decrease in intragastric pressure was noted in each of the three cases where 8-OH-DPAT was microinjected into the caudal DMV (mean decrease was -5.0±2.0mmHg), although no change in pyloric MMI occured in these cases (+0.2±0.2). No significant change in heart rate or blood pressure occurred upon microinjection of any of the above drugs into either the rostral or caudal DMV. Conclusions: Activation of neurons in the rostral DMV stimulates gastric function whereas activation of neurons caudal to the obex results in relaxation of the stomach. TRH appears to be excitatory to gastric function only in the rostral DMV, and serotonin1A receptors may be involved in gastric relaxation in the DMV caudal to the obex. Supported by NIH grant AM29975 and an AHA Grant-in-Aid.

358.7

EVIDENCE FOR GLUTAMATE AS A NEUROTRANSMITTER IN THE GASTRIC MECHANORECEPTOR AFFERENTS PROJECTING TO THE NUCLEUS OF THE SOLITARY TRACT (NTS). Richard Rogers, Monica McCann, and Robert Stephens. Dept. of Physiology, Ohio State University, Columbus, OH 43210.

It is now well known that gastric distention can activate a subset of neurons in the medial NTS. These NTS neurons are probably involved in gastric vago-vagal reflexes and in the transmission of information regarding gastric fill to the central nervous system. Our electrophysiological studies demonstrate that the activation of NTS neurons by gastric inflation may depend upon the release of glutamate by the first order afferents. NTS neurons, identified by their increased firing rate upon inflation of the stomach, were excited by iontophoretically-applied glutamate and NMDA. As predicted, the glutamate antagonist, kynurenic acid, inhibited the excitation produced by glutamate. Furthermore, kynurenic acid blocked the increased firing rate of NTS neurons elicited by gastric inflation. These findings suggest that glutamate is released upon inflation from the gastric mechanoreceptor afferents projecting to the NTS. Supported by NIH grants NS24530 to RCR and NS08690 to MJM.

358.8

ATRIAL NATRIURETIC FACTOR (ANF) INJECTIONS INTO THE DORSAL MOTOR NUCLEUS (DMN) INCREASE GASTRIC MOTILITY AND ACTIVATE DMN NEURONS. Monica J. McCann and Richard C. Rogers. Dept. of Physiology, Ohio State University, Columbus, OH 43210.

ANF has been associated with the central and peripheral regulation of cardiovascular (CV) homeostasis. In the brain, ANF-containing terminals and ANF receptors are located in sites known to influence CV function such as the DMN, which contains the vagal preganglionic neurons. However, ANF applied to the DMN has no effect on CV parameters. Given the role of DMN in the regulation of the digestive tract, we hypothesized that ANF may be a regulator of vagal outflow to the stomach. By applying picomolar amounts of ANF to the DMN, we established that this peptide significantly reduced gastric motility and tone; vagotomy eliminated this reduction. In electrophysiological studies, we observed that femtomolar quantities of ANF excited antidromically-identified DMN neurons. We conclude that ANF reduces gastric motility by activating inhibitory vagal projections to gastric smooth muscle. Supported by NIH grants NS24530 to RCR and NS08690 to MJM.

358.9

TRH-IMMUNOREACTIVE (IR) PATHWAYS PROJECTING TO THE DORSAL VAGAL COMPLEX (DVC) IN RATS. R.B. Lynn[1], M. Kreider[2], R.R. Miselis[3]. Depts. of Medicine[1], Psychiatry[2], Animal Biology[3] and Institute of Neurological Sciences, University of Pennsylvania, Philadelphia, PA 19104

TRH microinjections into the DVC stimulate marked gastric motor and sensory responses. TRH receptors and TRH-IR nerve terminals are present in the DVC and these terminals synapse on gastric motor neurons (Rinaman & Miselis JCN '90). However, the origins of these neurons are not mapped in detail. Fluoro-gold and a Texas-red fluorescent secondary antibody were used in a double labeling protocol. RESULTS: Double labeled (DL) neurons were found exclusively in the medulla and were located in the nucleus raphe obscurus (nRO), nucleus raphe pallidus (nRP) and along the ventral edge of the medulla. A cluster of DL'd neurons was located medial and ventral to the lateral reticular nucleus. Representative quantitation of DL'd neurons from every fourth section of a rat brain that received multiple injections bilaterally at the level of the area postrema found: 31 in the nRO, 37 in the nRP and 38 in the ventral medulla. Many FG and TRH-IR neurons were in the paraventricular nucleus of the hypothalamus but these were separate populations of neurons with no double labeling. SUMMARY: We have identified specific sites in the ventrocaudal medulla that contain TRH-IR neurons that project to the DVC, and presumably are the neurons providing the endogenous TRH which stimulates gastric function. This work was supported by NIH Grant GM27739.

358.10

COMPARISON OF MICROINJECTION OF TRH ANALOGUE RX 77368 AND ELECTRICAL STIMULATION OF THE NUCLEUS AMBIGUUS (NA) ON GASTRIC MOTOR AND SECRETORY RESPONSES IN CATS. H-S. Feng*, J. Han* and F.P. Brooks. Depts. of Medicine and Physiology. Sch. Med., Univ. of PA, Philadelphia, PA 19104.

We wished to compare the force of gastric contraction and gastric acid output during electrical stimulation with the response to microinjections of TRH analogue into the NA of anesthetized cats. Cats were equipped with force transducers on the antrum and a gastric cannula. A semi micro-electrode (16 cats) or a micropipette (22 cats) was inserted into the NA on the left and right side under stereotaxic guidance. Current was held constant at 100μ Amp and frequency and pulse duration varied independently. Microinjections of TRH analogue varied from 50-1000 ng in 100 nl. The force of gastric contractions was determined over 15 min. periods. Acid output was determined by measuring volume and acid conc titrimetrically. Force of antral contractions reached 26.2± 1.34g with 2 msec pulse durations. Peak force of 28.7± 2.1g was obtained with 50 Hz. There was no increase in acid output. The peak force of contractions occurred after microinjection of 500 ng of TRH analogue (33.2 ± 1.7g). Acid output also peaked at 0.33 ± 0.04 mM/15 min with 500 ng of TRH analogue. Taché and her colleagues reported an increase in force of contraction and acid output in different rats microinjected with TRH analogue. The force of antral contractions in our experiments continued to increase after acid output had returned to baseline. We think TRH analogue acts on two different pathways in the NA to excite force of contraction and acid output. Electrical stimulation excites only the motor response.

358.11

BOMBESIN MICROINJECTED INTO THE NUCLEUS AMBIGUUS (NA) INHIBITS GASTRIC ACID SECRETION THROUGH PARASYMPATHETIC PATHWAY. H. Yang, T. Ishikawa* and Y. Taché. CURE, V A Wadsworth Medical Center, Dept of Medicine and Brain Research Institute, UCLA, Los Angeles, CA 90073.

Microinjection of bombesin (0.6-6.2 pmol/100 nl)into the NA and the surrounding areas including C1 in the rat brain stem inhibited pentagastrin stimulated gastric acid secretion (FASEB J. 2:A498,1988). This work was designed to identify whether 1) the NA itself is an effective site for bombesin to inhibit gastric acid secretion and 2) the effect of bombesin in the NA is mediated through the parasympathetic pathway. Male SD rats (250-300g, n=5-7/group) fasted for 24 h were anesthetized with urethane. A PE-90 cannula was inserted into the cervical artery to measure blood pressure and heart rate. Gastric acid secretion was measured by titration of the flushed perfusate. The basal parameters were monitored after the obex region of the dorsal medulla was exposed, then a glass micropipette was positioned into the NA or the dorsal motor nucleus (DMN) according to the atlas of Paxinos and Watson. The location of microinjection sites was identified after experiment. Bombesin (6.2 pmol, 5 - 50 nl) microinjected into the NA inhibited the gastric acid secretion induced by : 1) pentagastrin (16 μg/kg/h iv) by 41% (5 nl); 2) RX77368 into the DMN (30 ng) by 63.3% or into the NA (3 ng, together with bombesin) by 55.4% (50 nl) and inhibited heart rate (5 nl). The inhibitory effect of bombesin on pentagastrin-stimulated gastric acid secretion and on heart rate was compeletly abolished by vagotomy. The antisecretory effect of bombesin co-injected into the NA with RX77368 was still observed after spinal cord transection. Conclusions: 1) The NA is a site of action for bombesin to inhibit gastric acid secretion; 2) the inhibitory effect is mediated at least in part through parasympathetic pathway.

358.12

EFFECT OF CHOLECYSTOKININ (CCK) AND LiCl ON GASTRIC MOTILITY IN RATS WITH LESIONS OF THE HYPOTHALAMIC PARAVENTRICULAR NUCLEUS (PVN). L.M. Flanagan, J. Dohanics, J.G. Verbalis and E.M. Stricker. Departments of Behavioral Neuroscience and Medicine, University of Pittsburgh, Pittsburgh, PA 15260.

Parvocellular neurons in the PVN projecting to the dorsal motor nucleus of the vagus may participate in the inhibition of gastric motility seen after administration of CCK (1.0 ug/kg, ip) or LiCl (1.5 mEq/kg, ip) in rats. To test this hypothesis, rats were given bilateral lesions of the PVN made by rotating a triangular knife positioned in the third ventricle 2 mm behind bregma. When rats were allowed to recover from this procedure for 2-3 weeks, systemic CCK and LiCl each produced inhibitory effects on gastric motility similar to that seen in intact or sham lesioned rats (n=6 per group). In addition, the inhibitory effect of these treatments on deprivation-induced food intake was not discernibly different 2-3 weeks after knifecut lesion of the PVN. In contrast, rats studied 12 h after PVN lesions showed almost no inhibition of gastric motility in response to either CCK or LiCl (n=3), and analogous results were found 20 min after injection of lidocaine bilaterally into the PVN (n=3). These data therefore suggest that the PVN normally modulates gastric motility inhibitory responses to CCK and LiCl. The return of normal responses 2-3 weeks after PVN lesions, however, suggests functional recovery of inhibitory vagal efferent activity by this time.

358.13

CCK ACTIVATES C-FOS IN PARAVENTRICULAR NEURONS PROJECTING TO THE DORSAL VAGAL COMPLEX. B.R. Olson, J.G. Verbalis, A.F. Sved, E.M. Stricker and G.E. Hoffman. Departments of Medicine and Behavioral Neuroscience, University of Pittsburgh, Pittsburgh, PA 15261.

Our previous results suggest that systemic administration of cholecystokinin-8 (CCK) decreases food intake and gastric motility through oxytocinergic (OT) projections from the paraventricular nucleus (PVN) of the hypothalamus. To study the functional anatomy of this phenomenon, we measured CCK-stimulated c-fos expression in PVN cells of male rats (n = 2) 2 weeks after injecting 4% fluorogold (100 nl) into the dorsal vagal complex (DVC). One hour after CCK administration (100 μg/kg ip), rats were perfused for immunohistochemical staining with antiserum directed against the N-terminus of c-fos antigen (Cambridge Research Biochemicals) as described previously (Hoffman et al., Endocrinol, 1990). Previous studies with this antiserum have demonstrated absent expression of c-fos in the PVN under basal conditions, but marked stimulation of OT and CRF neurons in response to CCK. Brains were cut into 25 μm sections, and retrogradely-labelled cells in every twelfth section were counted. In the PVN sections counted, a mean total of 563 cells were retrogradely labelled; of these, 310 (54%) also expressed c-fos. These double-labelled cells distributed predominantly in the medial parvocellular subdivision, with fewer occurring in periventricular, anterior and dorsal parvocellular PVN. We also observed many cells in the PVN expressing c-fos that were not retrogradely labelled, as well as numerous c-fos negative neurons labelled intensely with fluorogold in the perifornical area, lateral to PVN, and in the dorsal hypothalamus, caudal to PVN. The overall distribution of c-fos activation closely resembled that found previously in CCK treated animals. These results suggest that CCK activates parvocellular neurons in several subdivisions of the PVN that project to the DVC of the brainstem, and support the hypothesis that the PVN modulates autonomic activity in response to systemically-administered CCK.

358.14

VAGAL STIMULATION INDUCED GASTRIC DAMAGE IS DEPENDENT UPON AN INTACT PVN. L.E. Hierlihy and A.V. Ferguson. Dept. of Physiology, Queen's University, Kingston, ONT K7L 3N6.

Recent work from our laboratory has implicated the paraventricular nucleus (PVN) and the vagus nerve in the development of gastric mucosal erosions. The present studies were therefore undertaken to investigate the influential role of the PVN in vagal stimulation induced gastric damage.

Sprague Dawley rats were anesthetised with sodium pentobarbital and placed in a stereotaxic frame. A monopolar electrode was advanced toward the region of the PVN and lesions were made by passing direct current (0.5mA, 30s). One week later following 24 hours of fasting, rats were anesthetised with urethane. Cervical vagus nerves were isolated and laid over hook electrodes which were used to deliver supramaximal stimulation (5Hz) for 1 hour. Following stimulation, the stomach was removed, examined macroscopically and assigned a damage score ranging from 0-3 based on an arbitrary scale in which 0 indicates no damage and 3 indicates extensive gastric damage. The brain was perfused, removed and prepared for histological determination of lesion sites.

Electrical stimulation of intact vagus nerves following bilateral lesion of PVN resulted in significantly reduced gastric damage scores (0.42±0.20, mean±SEM) as compared to damage elicited in rats with an intact PVN (2.00±0.33). Similar vagal stimulation was found to produce significant gastric damage (1.50±0.22) in animals lesioned in non-PVN regions. No visible gastric damage was observed in lesioned animals in which no vagal stimulation was applied. In conclusion, these experiments demonstrate the dependence of vagal stimulation induced gastric damage upon an intact PVN and further emphasize a role for vagal afferents as well as efferents in the development of gastric mucosal erosions.

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358.15

EFFECT OF HYPOTHALAMIC STIMULATION ON ILEAL WATER ABSORPTION IN RATS. X. Zhang*, T. Kong*, W.E. Renehan, R. Fogel. Depts of Medicine and Anatomical Sciences and Neurobiology, University of Louisville, Louisville, KY

Although a cephalic phase of intestinal water and ion absorption has been proposed, the specific central nervous system regions that serve this function are not clear. The aim of this study was to determine the effects of lateral (LH) and paraventricular (PVH) hypothalamic stimulation on intestinal water absorption, and to examine possible pathways for these effects. Intestinal water absorption was measured by the single pass perfusion technique with ^{14}C-PEG, M.W. 4,000, as the nonabsorbable marker. Electrical stimulation of the LH (4Hz, 4V, 0.2 msec) increased basal water absorption. This effect was prevented by systemic reserpine, yohimbine or prazosin but not by propranolol. In contrast, electrical stimulation of PVH and intracerebroventricular injection of vasopressin or oxytocin did not alter water absorption. These results suggest that the LH increases water absorption by a pathway involving alpha adrenergic receptors. The specific location of these receptors is not yet known.

358.16

PHARMACOLOGIC INVESTIGATION OF THE DIGESTIVE TRACT RESPONSES TO MOTION SICKNESS IN THE CAT. I. M. Lang and M. Steensrud*. Depts of Surgery & Physiology, Med. Coll. Wisc. & Zablocki VAMC, Milwaukee, WI 53295.

We found previously that motion sickness is associated with three distinct gastrointestinal (GI) events: 1) decreased motor activity, 2) bradygastria, and 3) the retrograde giant contraction. The aim of this study was to determine the role of dopaminergic, serotonergic, opioid, and adrenergic receptors in mediating these responses. Five cats, preselected for their susceptibility to motion sickness, were chronically instrumented with strain gauge transducers and bipolar electrodes on their GI tract. Motion sickness was induced by vertical oscillation (VO) at 0.5 HZ. The effects of the following pharmacologic agents, given 5 min before VO, were tested: haloperidol, Sch 23390, domperidone, 1-1-naphthyl piperazine (1-NP), fentanyl, guanethidine, and phentolamine. We found that all three responses were blocked by haloperidol or Sch 23390 but not domperidone, 1-NP, or fentanyl. The decrease in motor activity was inhibited by guanethidine or phentolamine. These results suggest that 1) motion sickness may be mediated by 5HT-1 or D-1 but not 5HT-2 or D-2 receptors, 2) the decrease in motor activity may be mediated by the sympathetic nervous system through alpha receptors, and 3) cats respond to antiemetic agents differently than dogs.

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358.17

5-HYDROXYTYPTAMINE$_{1A}$ (5-HT$_{1A}$) RECEPTORS LOCALIZED TO NON-CHOLINERGIC MOTORNEURONS IN GUINEA-PIG ILEUM (GPI). J. Galligan* and R. Rech. Dept. Pharmacol., Michigan State Univ. E. Lansing, MI 48824

Previous studies have shown that 5-HT$_{1A}$ agonists inhibit synaptic transmission but not cholinergic neuromuscular transmission in the GPI. In the present study, the actions of 5-HT and the 5-HT$_{1A}$ agonist, 5-carboxamidotryptamine (5-CT) on non-cholinergic (1 μM scopolamine present) longitudinal muscle contractions of the GPI were studied in vitro. Contractions were evoked by trains of transmural electrical stimuli (10 Hz for 1 s); 5-HT (EC_{50}, 0.1 μM) and 5-CT (EC_{50}, 6 nM) inhibited these contractions. The actions of 5-CT were blocked by spiperone (pA_2, 7.25, K_B, 56 nM), a 5-HT$_{1A}$ antagonist. The contractions were reduced by 80% by the substance P (SP) antagonist D-arg^2, D-phe^5, D-trp^{7,9}, leu^{11} SP (1 μM), indicating that SP is a mediator of non-cholinergic contractions. 5-CT (0.1 μM) did not affect contractile responses to exogenous SP (1-30 nM) suggesting that the actions of 5-CT on nerve-mediated contractions are prejunctional. Conclusions: 5-HT$_{1A}$ receptors are localized to motor nerve terminals releasing SP. As 5-HT$_{1A}$ receptors are not localized to cholinergic motor nerves, it is likely that SP and acetylcholine are released from different motor neurons in GPI. (Supported by PMAF and DK40210)

NEUROENDOCRINE REGULATION: OTHER II

359.1

ANGIOTENSIN II (AII) ENHANCED ACTIVITY OF SUPRAOPTIC VASOPRESSIN (VP) NEURONS IS POTENTIATED IN RATS WITH IBOTENATE LESIONS OF EITHER DIAGONAL BAND OF BROCA (DBB) OR THE PERINUCLEAR ZONE (PNZ). **L.P. Renaud, R. Nissen and J.T. Cunningham. Neuroscience Unit, Ottawa Civic Hospital, Ottawa, Ontario, K1Y 4E9.**

Circulating AII increases the activity of supraoptic VP neurons. Due to the potent pressor actions of AII, this facilitation is often masked by simultaneous baroreceptor activation. The latter transiently suppresses the spontaneous activity of VP-neurons, presumably through involvement of relay neurons in the DBB and inhibitory neurons in PNZ. In order to separate these opposing mechanisms, the excitotoxin ibotenic acid (1.25 ug/250 nl) was injected into the vertical limb of the DBB or into the PNZ surrounding the supraoptic nucleus (SON) in pentobarbital anesthetized male Long-Evans rats. After a minimum recovery period of 3 days, rats were reanesthetized with pentobarbital and prepared for extracelluar recording of identified SON neurons using a transpharyngeal approach. In control rats (N=5), intracarotid infusions of AII (500 pmol/kg) increased the activity of VP neurons by 33.2 ± 11.7%. By contrast, DBB lesioned rats (N=5) displayed a 101.8 ± 21.1% increase in activity following identical AII infusions, significantly elevated from the control group ($F(1,8)= 8.06$, $p<0.05$). Similarly, in PNZ lesioned rats (N=4), VP-neurons displayed a 131.8 ± 34.6% increase in their activity in response to AII ($F(1,7)= 8.85$, $p<0.05$). The blood pressure elevations coincident with AII administration tended to be higher in both lesioned groups but were not significantly different from the control group. Therefore, lesions of forebrain pathways for baroreceptor mediated inhibition of VP neurons potentiate the facilitatory actions of blood borne AII. **(Supported by FRSQ, NRSA MH0977 and MRC).**

359.2

IBOTENATE LESIONS IN THE PERINUCLEAR REGION OF THE SUPRAOPTIC NUCLEUS (SON) ATTENUATES BARORECEPTOR BUT NOT MEDIAN PREOPTIC NUCLEUS - INDUCED INHIBITION OF VASOPRESSIN (VP) NEURONS IN RAT. **R. Nissen, J.T. Cunningham and L.P. Renaud. Neuroscience Unit, Ottawa Civic Hospital, Ottawa, Ontario, K1Y 4E9.**

Brief elevations in arterial pressure, sufficient to activate peripheral baroreceptors, transiently suppress spontaneously active VP-synthesizing neurons in rat SON. Recent evidence suggests that this inhibition is GABA mediated, presumably through inhibitory interneurons located in the perinuclear zone (PNZ) adjacent to the SON. Therefore, in pentobarbital anesthetized male Long-Evans rats, neurons in PNZ were lesioned by stereotaxic injections of the excitotoxin ibotenic acid (1.25ug/250nl). After a minimum of 3 days recovery, extracellular recordings were obtained from identified SON neurons using a transpharyngeal approach in rats reanesthetized with pentobarbital. In vehicle injected control rats (N=2), 18/23 phasic VP-synthesizing neurons were inhibited by pressor responses of at least 50mmHg produced by administration of metaraminol (10ug/10ul iv). The number of phasically active neurons that were inhibited by similar metaraminol induced pressor responses (16/31) was significantly decreased in PNZ lesioned rats (N=6, $X^2(df=1)=4.02$, $p < 0.05$). In contrast, electrical activation of an alternate (presumably direct) inhibitory pathway to SON from the median preoptic nucleus remains unaffected. Thus, destruction of cell bodies in the PNZ selectively attenuates baroreceptor mediated inhibition of VP-synthesizing neurons and supports the hypothesis that neurons in PNZ mediate this response. **(Supported by FRSQ, NRSA MH0977 and MRC).**

359.3

REGIONAL BRAIN AND PITUITARY ENKEPHALINS CHANGES WITH AGE AND METAMORPHOSIS Ambystoma tigrinum. A. Cano-Martínez*, A. Vargas-González*, G. Matamoros-Trejo*, F. Antón-Tay (1) and M. Asai*. Neurosci. Div. Instituto Mexicano de Psiquiatría. Calz. Méx-Xochimilco 101, San Lorenzo Huipulco, Tlalpan, México 14370, D.F. (1) UAM Iztapalapa, México.

The concentrations of Metionine and Leucine-Enkephalin in the telencephalon, diencephalon-mesencephalon, rombencephalon and hypophysis of juvenile and neotenic adults A. tigrinum individuals were determined before and after metamorphosis. A radioimmunoassay method was used. Whit exception of unchanged Leu-enkephalin concentration in the diencephalon-mesencephalon, all other levels of enkephalins were found to be 2 to 10 fold higher in neotenic adults compared to juvenils. The concentration of both enkephalins increased between 35 to 70% in the diencephalon-mesencephalon and the rombencephalon of juvenile and adults after metamorphosis. In metamorphosed organisms the Metionine-Enkephalin levels showed a 30% increase in the telencephalon of the juveniles. In contrast a 30% decrease was found in the hypophysis of adults. These results suggest that either the enkephalins play a role in the maturation, neotenia and metamorphosis of A. tigrinum, or the changes are derived from aging of the individuals.

Work supported in part by Grant P228CCX880172 from CONACYT.

359.4

EFFECTS OF SYMPATHECTOMY AND CAPSAICIN DENERVATION ON SKELETAL AND CALCITONIN CHANGES IN RESPONSE TO TAIL- SUSPENSION. E.L. Hill[1], C. M. Cone*[1], R.B. Martin*[2], S.B. Arnaud*[1], P. Fung*[1], and E. Morey-Holton*[1]. [1]NASA-Ames Research Center, Moffett Field CA 94035 and [2]Orthopaedic Research Laboratory, U.C. Davis, Davis, CA 95616.

Sympathetic and partial sensory (capsaicin) denervation were both previously shown to affect bone resorption (Hill et al, 1990 submitted). To evaluate the effect of such denervations on bone formation, tail-suspension (SUSP) which results in reduced bone formation and maturation in the unloaded hindlimbs of growing rats was used. The skeletal and hormonal response in 49-day old male rats which had either neonatal guanethidine-induced sympathectomy (GUAN) or neonatal capsaicin-induced denervation (CAP) was investigated in both SUSP and weight-bearing (WTB) controls. At the end of a 7 day experimental period, chemical (bone Ca, ^{45}Ca and ^{3}H-proline uptake), histological (cortical bone formation rates and area), calcitonin (CT), and mechanical (three-point breaking strength) parameters were measured.

Vehicle-treated (VEH) SUSP animals had decreased ^{45}Ca and ^{3}H-proline uptake in the femur, decreased bone formation and apposition rates at the tibio-fibular junction (TFJ), decreased plasma CT levels and reduced mechanical strength in the femoral shaft as compared to VEH WTB, while mechanical and chemical parameters were largely unaffected in the humerus which was weight-bearing. CAP rats responded to SUSP in the same manner as VEH. GUAN SUSP rats showed similar chemical changes in the femur and histological changes at the TFJ as other SUSP animals, but did not have lower CT levels than GUAN WTB. Mechanical and chemical parameters were altered in the humerus of GUAN SUSP rats as compared to GUAN WTB.

The results of these studies indicate that the skeletal and CT responses to tail-suspension are not altered by the selective destruction of unmyelinated and fine myelinated sensory nerves by capsaicin. However, sympathetic innervation may be important in a systemic and/or hormonal response to unloading.

359.5

Angiotensin II applied to the subfornical organ increases glucose utilization in the hypothalamo-neurohypophysial system. M.L. Terrell, M.Kadekaro and S. Freeman. Division of Neurosurgery, University of Texas Medical Branch, Galveston, Texas 77550.

Using quantitative [^{14}C]deoxyglucose (DG) autoradiography, we studied the neural pathways activated by topical stimulation of the subfornical organ with angiotensin II (AII). Under Equithesin anesthesia a 23 ga. stainless steel guide cannula was stereotaxically implanted 1 mm above the subfornical organ in male, adult Sprague-Dawley rats. Seven to ten days later, an internal cannula connected to a Hamilton microsyringe containing AII or normal saline was inserted into the guide cannula to deliver 2 pulses of 10^{-10} moles of AII in 0.5 ul of saline (n=8) or same volume of saline in control animals (n=7). AII increased arterial blood pressure and stimulated water intake (4.9±0.5ml) with a short-latency (58.5±13.6s); means±SEM. Glucose utilization increased ($p<0.05$) in the subfornical organ (+27%), paraventricular (+24%) and supraoptic nuclei (+24%) and pituitary neural lobe (+73%). Glucose utilization tended to increase in the nucleus medianus ($p=0.065$) and in the organum vasculosum laminae terminalis ($p=0.08$). These results indicate that specific chemical stimulation of the subfornical organ increases functional activity of the hypothalamo-neurohypophysial system and demonstrate that the DG method is useful for quantifying transient metabolic responses in the neuroendocrine circuit.

359.6

LESIONS OF THE CIRCUMVENTRICULAR ORGANS DISRUPT THE PRESSOR RESPONSE TO RELAXIN IN ANAESTHETISED RATS. L.J.Parry & A.J.S. Summerlee. Biomedical Sciences, Ontario Veterinary College, Guelph, Ontario, N1G 2W1, Canada.

The role of the subfornical organ (SFO) and the antero-ventral region of the third ventricle (**AV3V**) in mediating the pressor effects of **iv** relaxin (**RXN**) was tested in urethane-anaesthetised female rats. Blood pressure was recorded directly from a cannulated common carotid artery. The animals were placed in a stereotaxic frame, and radiofrequency lesions were created in either the **SFO** or the **AV3V** region. In each case, the pressor response to **iv** porcine **RXN** (5μg in 0.1ml saline) was determined, and compared with the response in animals with control lesions in the wall of the left lateral ventricle. In **SFO** lesioned animals, the pressor response to **RXN** (n=9) was significantly ($P<0.05$: ANOVA) attenuated, compared with control lesioned **RXN**-treated animals (n=12), but was still present. Blood pressure started to rise 1-3 minutes after **RXN** treatment in the control lesioned animals, with increases of 10.0 ± 1.84 systolic and 14.0 ± 2.07 diastolic (mean ± s.e.m. percentage increases over baseline values), compared with 4.4 ± 0.59 systolic and 7.0 ± 0.99 diastolic in **SFO** lesioned animals. Lesion of the AV3V (n=14) further reduced this pressor response significantly ($P<0.05$), 3 and 6 minutes after **RXN** injection. These data imply that both the **AV3V and SFO** have a role in mediating the pressor response to **RXN**. Supported by NSERC Canada.

359.7

GROWTH HORMONE SECRETION DURING THE PERIPUBERTAL PERIOD IN MALE AND FEMALE RATS. S.M. Gabriel, Department of Psychiatry, Mount Sinai School of Medicine, New York, NY 10029.

A sex-specific pattern of **growth hormone (GH)** secretion is recognized in adult rats. Males exhibit high amplitude GH pulses at 3-4 hour intervals, while females have lower amplitude GH pulses at 1-2 hour intervals. This sexually dimorphic pattern is thought to contribute to the differential growth rates observed in rats. Episodic GH release has not been studied in the young rat, however. In the present study, *25-50 d.o.* rats received jugular catheters and were housed in chambers designed for stress-free blood sampling. Four to 7 days after surgery, *300µl* blood was collected every 10 minutes between *1600h* and *2000h*. Red cells were returned in artificial plasma. Rats were grouped by age and endocrine state as: **immature** *(<30 d.o., immature testes; anestrus)*; **early puberty** *(30-37 d.o., increased testes weight, first proestrus)*; and **late puberty** *(>37 d.o., mature testes, estrous cycles)*. GH secretion was similar in **immature** male and female rats, being characterized by baseline GH secretion with few GH pulses. During **early puberty**, male rats weighed more than female rats. However, both male and female rats showed low amplitude, high frequency GH pulses. In **late puberty**, the sex difference in body weight increased and a sexual dimorphism in GH secretion became apparent. Male rats had increased GH pulse amplitudes and decreased pulse frequency, while females continued to have low amplitude, high frequency GH pulses. These studies suggest that the sexually dimorphic pattern of GH release emerges during puberty in the rat. Because a sex difference in body weight develops before this pattern is established, other factors may contribute to the sex difference in growth rates.

359.8

LOCALIZATION AND REGULATION OF PREPROGRF mRNA IN ARCUATE NUCLEUS AND IN NEURONS IN PERIVENTROMEDIAL HYPOTHALAMUS, J.D. White, J. Bruno*, D. Olchovsky*, M. Kershaw* and M. Berelowitz, Div. Endocrinology, SUNY, Stony Brook, NY 11794

Growth hormone releasing factor (GRF) is the principal stimulatory hypothalamic neuropeptide controlling growth hormone (GH) secretion from pituitary somatotrophs. Recently, we demonstrated that hypothalamic preproGRF mRNA levels are decreased approximately 5-fold in animals subjected to 72 hr food deprivation and in streptozotocin diabetic rats. In this study we examined the hypothalamic and extra-hypothalamic sites of preproGRF mRNA expression using *in situ* hybridization and determined which brain loci modulate preproGRF mRNA levels in response to food deprivation or experimental diabetes mellitus. Control and experimental male Sprague-Dawley rats were anesthetized with ketamine / xylazine and perfused with 4% paraformaldehyde. The brains were removed, post-fixed in paraformaldehyde and free-floating 30 μm coronal sections were processed for preproGRF mRNA localization using a ^{35}S-cRNA probe. The distribution of hybridization was analysed using both film and emulsion autoradiographs. These studies confirmed that the principal site of GRF expression in CNS is within the hypothalamic arcuate nucleus with an addtional population of neurons lying in the periventromedial region of the hypothalamus. In agreement with data from nuclease protection studies, preproGRF mRNA levels in both of these populations of hypothalamic neurons declined following food deprivation or streptozotocin-induced diabetes mellitus. Hybridization signal was also detected in paraventricular nucleus of the hypothalamus, in hippocampus and in piriform cortex; however, hybridization signal in these regions was not markedly affected by either experimental protocol. These data implicate two neuronal populations in modulating GH secretion and suggest specific modulation of hypothalamic preproGRF mRNA levels by food deprivation and diabetes. NIMH 42074, 00801 (JDW); NIH AM 36831 (MB)

359.9

SOMNOGENIC DOSES OF GHRH AND IL1 INDUCE GH BUT NOT PRL RELEASE IN RATS. L.C. Payne*, F. Obal Jr.*, L. Kapas*, and J.M. Krueger. Dept. of Physiology Univ. of TN. Memphis TN. 38163.

Growth hormone-releasing hormone (GHRH) promotes non-rapid eye movement sleep (NREMS) and rapid-eye movement sleep (REMS) (1). Interleukin-1 (IL1) elicits growth hormone (GH) secretion via a hypothalamic mechanism (2), probably GHRH, and it strongly enhances NREMS (3). IL1 stimulates PRL release via the hypothalamus in vivo (2) but inhibits PRL secretion from the pituitary in vitro (4). The effects of hypnogenic doses of GHRH (0.16 ug) and IL1 (2.5 ng) plasma concentrations of GH and PRL in free moving rats provided with intracerebroventricular cannulae were determined. GH and PRL were determined by RIA in plasma samples taken every 20 min. GHRH and IL1 caused a significant increase in GH secretion 20 and 40 min postinjection. PRL levels were not affected by any of the treatments. These data provide evidence for the hypothesis that the NREMS promoting effect of IL1 may be mediated via GHRH, and provide an additional link between NREMS and GH.

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359.10

ACUTE EFFECTS OF STRESS ON SOMATOSTATIN (SS)-CONTAINING CELL BODIES AND FIBERS IN THE PREOPTIC-ANTERIOR HYPOTHALAMUS (PO-AH) AND MEDIAN EMINENCE (ME) OF FEMALE RATS. M. Bhatnagar, G. Nillaver and V. Critchlow. Oregon Regional Primate Research Center, Beaverton, OR 97006.

Stress in rats causes acute hypothalamic release of SS that induces a marked, prolonged suppression of growth hormone (GH) secretion. The objective of this study was to determine whether stress causes acute changes in neuronal content of SS that can be detected with immunocytochemistry (ICC). Groups (N= 5) of adult female rats were decapitated under nonstress conditions or 30, 60, 120 or 180 min after 15-min leg restraint stress. Treatments were assigned and performed with a randomized block design. Brains were rapidly removed, blocked and fixed by immersion for ICC; 50 μm serial sections were cut on a vibratome and stained with an Avidin biotin-peroxidase complex method. The rabbit antiSS used detects SS-14 and -28. SS-containing cell bodies were most numerous, largest and filled with SS granules in brains from nonstressed rats. Most cell bodies were in the periventricular (PeV) zone of the PO-AH; some were found in sites not heretofore described. SS was largely in the internal zone of the ME in nonstress brains. At 30, 60 and 120 min after initiation of 15 min stress, there was a marked decrease in the number and size of SS cell bodies, a reduction in cytoplasmic SS granules and a striking increase in SS in the external zone of the ME. The most conspicuous and reproducible changes in cell bodies involved subsets of PeV neurons; some clusters of SS neurons were not visibly affected by stress. The stress-induced changes were largely reversed by 180 min. These results demonstrate that stress causes acute changes in the PO-AH SS system as assessed by ICC. It appears that stress acutely affects both synthesis and secretion of SS. Supported by NIH grants DK32442 and RR00163.

359.11

COLOCALIZATION OF THE ANDROGEN RECEPTOR AND SOMATOSTATIN MESSENGER RNA IN NEURONS IN THE PERIVENTRICULAR NUCLEUS.

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Growth hormone secretory patterns in the adult male rat are influenced by sex steroids. Somatostatin (SS) is involved in the regulation of growth hormone secretion, and we have previously demonstrated that SS gene expression in neurons of the periventricular nucleus (PeN) is dependent upon the activation of androgen receptors. It is uncertain, however, whether androgens act directly on SS neurons or, alternatively, act through an intermediate neuronal pathway to effect their action. We tested the hypothesis that androgen receptors are localized in neurons in the PeN expressing the SS gene. Coronal sections were taken through the anterior hypothalamus of the adult male rat brain and double-labeled by immunocytochemistry with an antibody against the rat androgen receptor (kindly provided by Dr. S. Liao) and *in situ* hybridization with an ^{35}S-labeled RNA probe complimentary to pre-pro SS mRNA. Androgen receptor immunostaining was found in approximately 50% of the SS mRNA-containing neurons in the PeN. Fewer than 30% of the SS mRNA-containing neurons in the amygdala and less than 5% of SS neurons in the cortex were immunopositive for the androgen receptor.

Conclusion: Androgens, such as testosterone, are capable of acting directly on a subpopulation of SS neurons in the PeN to regulate SS gene expression. Moreover, this effect may subserve sexually dimorphic GH secretory patterns and underlie the developmental changes in GH secretion in the male rat.

359.12

DIHYDROPYRIDINE-SENSITIVE CALCIUM CHANNEL ACTIVITY IN PERINATAL SOMATOTROPHS.

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The factors that regulate somatotroph function in immature animals are not well understood. We have previously found that, compared with mature animals, perinatal somatotrophs are highly sensitive to the GH-secretory effects of GH-releasing factor (GRF), phorbol myristate acetate (PMA), and forskolin (FSK). To assess the role of dihydropyridine-sensitive Ca^{2+} channels in mediating these actions, we tested the effects of the antagonist, nisoldipine (NIS; 1 μM) and the agonist, BAY K8644 (BAY; 1 μM) on GRF-, PMA-, and FSK-induced GH release from cultured pituitary cells of 2-day-old and adult male rats (n=3-5 experiments/group; also see adjacent abstract). GRF stimulated GH release in an age-dependent manner; in response to 1 nM GRF, GH release was 509 ± 51 and 279 ± 32% of controls from pituitaries of 2-day and adult rats, respectively ($P<0.01$). Similar results were obtained with 0.15 μM PMA and 1 μM FSK. NIS markedly reduced 1 nM GRF-induced GH release in both age groups ($P<0.02$); GH release was only 168 ± 17 and 114 ± 23% of controls in 2-day-olds and adults, respectively. NIS also reduced PMA-and FSK-induced GH release in both age groups. BAY augmented GRF-, TPA-, and FSK-induced GH release from pituitaries of both neonatal and adult rats. The degree of enhancement by BAY was age-dependent. For example, BAY increased GRF-induced GH release 1.8 fold from pituitary cells of 2-day-olds but only 1.3 fold from adults ($P<0.01$); the effects of BAY on PMA- and FSK-induced GH release were similar to those on GRF. Conclusion: Immature rat somatotrophs possess functional dihydropyridine-sensitive Ca^{2+} channels, which may mediate GH release in response to regulatory agents during the perinatal period.

359.13

DOES FREQUENCY ENCODING OF [Ca2+]i OSCILLATIONS REGULATE GROWTH HORMONE RELEASE IN SOMATOTROPHS?

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Although spontaneous [Ca2+]i oscillations have been described in somatotrophs, their role in mediating somatotroph function is unknown. We studied the effects of known regulators of growth hormone (GH) release on [Ca2+]i in individual cultured adult male rat anterior pituitary cells using fura-2 based digital video microscopy. [Ca2+]i oscillations were characterized with the pulse analysis program ULTRA (Van Cauter, Am. J. Physiol. 254, E786-94). Somatotrophs were identified by their response to the specific and Ca2+-dependent secretagogue GH-releasing factor (GRF; see adjoining abstract). Individual GRF responses were defined as mean [Ca2+]i >mean±2SD prestimulus [Ca2+]i. Cumulative GRF dose-response data gave a sigmoidal curve with an EC50=0.67nM. However, individual cells exhibited primarily all-or-none responses over a dose range of 10pM-100nM. No evidence of desensitization was observed at the near EC90 dose of 1nM. 21% of the total pituitary cells surveyed (n=457) responded to 1nM GRF, with a cumulative mean increase in [Ca2+]i of 186nM. GRF significantly increased [Ca2+]i and pulse amplitude relative to baseline, without significantly increasing frequency. Forskolin (10nM) had similar effects. Somatostatin (1nM), the major physiological inhibitor of GH release, blocked the GRF-mediated increase in [Ca2+]i and pulse amplitude, without affecting basal or GRF-response frequency. The calmodulin antagonist calmidizolium (40μM) produced similar effects. Following chronic phorbol ester treatment (100nM PMA, 20-120 min), both basal and GRF-response frequency were increased, but the increase in [Ca2+]i and pulse amplitude produced by GRF was attenuated. Nimodipine (1μM) reversibly inhibited all three components of both basal and GRF-response [Ca2+]i signalling. These data suggest that, in cultured rat somatotrophs: 1) GRF produces parallel increases in mean GH release and [Ca2+]i that are dependent on Ca2+ influx. These responses may be mediated via recruitment of cells rather than by augmented responses in individual cells; 2) Major regulators of GH release alter [Ca2+]i and pulse amplitude in a parallel manner; 3) Oscillation frequency does not appear to be coupled to the other components of [Ca2+]i signalling. Therefore, frequency encoding may not regulate GH release in somatotrophs.

359.14

CHARACTERIZATION OF A NA^{++}, CL^{-},AND CA^{++} -INDEPENDENT [^{3}H] GLUTAMATE BINDING SITE IN RAT PINEAL.

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Neuronal control of melatonin production in the pineal gland appears to be primarily mediated by alpha and beta adrenoceptors. The pineal, however, possesses a number of other receptor types whose functions are at present unknown. Using quantitative receptor autoradiography, we have detected a Na^{++},Cl^{-} and Ca^{++} independent binding site for [^{3}H]glutamate in the rat pineal. The binding site is saturable, stereospecific, temperature dependent, and displays an apparent dissociation equalibrium constant (Kd) of .556 + .136 X $X10^{-6}$M. Competition studies showed that radioinert L-glutamate was the most potent displacer of [^{3}H] glutamate binding with a Ki value of .536 + .037 X $X10^{-6}$M. Quisqualate displayed moderate affinity with a Ki value of 34 + .06 $X10^{-6}$M. All other compounds tested were ineffective competitors. Peripheral dennervation of the pineal gland by removal of the superior cervical ganglion increased the number of [^{3}H]glutamate binding sites by approximately 50% suggesting the possibility of receptor upregulation. A day/night difference in the number of pineal [^{3}H]glutamate binding sites was not observed . The presence of a binding site for [^{3}H]glutamate in this gland suggests an important role for glutamate as a modulator of pineal function. (Supported in part by Sigma Xi, Grants-in-Aid of Research and NSF #BNS-88-1726)

359.15

DISTRIBUTION OF MELATONIN RECEPTORS IN NEUROENDOCRINE TISSUES OF THE EWE.

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Photoperiod controls reproduction in ewes by regulating the duration of nightly melatonin secretion by the pineal gland. Melatonin acts on brain targets which have not been identified in this species. We examined specific high affinity binding of 2-[^{125}I]-melatonin in 20 micron sections prepared from intact Suffolk ewes killed during late anestrus or the breeding season. The pars tuberalis contained by far the highest concentration of receptors of all areas studied. Within the telencephalon, intense labeling was found in the mediolateral septum, the ventrolateral septal and septohypothalamic nuclei, entorhinal cortex, subiculum, and the inner and outer molecular layers of CA1 adjacent to the dentate gyrus. Melatonin binding in the bed nucleus of the stria terminalis, medial preoptic nucleus, and medial preoptic area was less striking but still distinct. Among diencephalic regions, melatonin receptors exist in low concentrations in anterior hypothalamus, ventral tuberal region, paraventricular thalamic and supramammillary nuclei. Little binding was evident in the suprachiasmatic or ventromedial nuclei of the hypothalamus. In the midbrain, significant binding was restricted to the ventral raphe complex and the inferior colliculus. Little specific binding was found in the pituitary or pineal glands. Several sites of intense IMEL binding are areas in which immunocytochemical studies indicate high concentrations of estrogen receptors. These data suggest sites at which melatonin might act to regulate reproduction and other photoperiod-dependent functions. Supported by NSF BNS86-16935, NIH MH44132, and NIH NRSA HD-06976.

359.16

ELECTROPHYSIOLOGICAL INTERACTION BETWEEN MELATONIN AND MONO AMINES IN THE ANTERIOR HYPOTHALAMUS.

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Local application of MELATONIN (MEL) on the anterior hypothalamus (AH) induces antigonadotropic effects as a result of an interaction with monoaminergic neurons. When MEL is microiontophoresed, a decrease on the spontaneous and evoked activity is observed. If such electrophysiological effect of MEL provokes the antigonodotropic action, then this would be related also to monoaminergic neurons. This study analyses the electrophysiological effect of MEL and 5-Metoxytriptophol (5-MTP), applied by micropressure, on neurons from AH of rats pretreated with reserpine (RES), 6-hydroxidopamine (6-OHDA) or 5-7,dihydroxytryptamine (5,7-DHTP). Both, 6-OHDA (200 µg) and 5,7-DHTP (200 µg) were applied intracerebroventricularly and RES (1 mg/kg) IP. In the recording sesion, a four micropipette multibarrel containing: MEL (0.1 M), 5-MTP (0.1 M), glutamate (0.05 M), and NaCl 3M with fast green, was lowered in AH. In control rats, MEL and 5-MTP elicited a consistent decrease in the spontaneous and glutamate-provoked activity on AH units. However, in RES, 6-OHDA and 5,7 DHTP pretreated rats, both, the intensity and number of affected units, were significant reduced as compared to untreated rats. These results indicate an interaction between these metoxyindols and mono amines in the AH.
Supported by Grant DGAPA IN-02-50-89.

359.17

ONTOGENY OF TYROSINE HYDROXYLASE (TH) mRNA LEVELS AND CATALYTIC ACTIVITY IN THE RAT HYPOTHALAMUS. L. A. Arbogast and J. L. Voogt, Physiology Dept., Univ. Kansas Med. Ctr., Kansas City, KS 66103.

Tuberoinfundibular dopaminergic (TIDA) neurons with cell bodies in the arcuate nucleus (AN) and nerve terminals in the stalk-median eminence (SME) release dopamine, a PRL inhibiting hormone. The objectives of this study were: 1) to determine the catalytic activity of TH in the SME during neonatal, peripubertal and adult life, 2) to examine the TH mRNA signal levels in the AN, and 3) to relate changes in TIDA neuronal function to circulating PRL levels. Male and female rats were used at 5 day intervals between days 5 and 40 of age and diestrous females and intact males were used as adults (60-80 days). *In vitro* TH activity was assessed by incubating hypothalamic explants with brocresine, a decarboxylase inhibitor. Dihydroxyphenylalanine (DOPA) accumulation in the SME was measured by HPLC. TH activity (ng DOPA/mg protein/30 min) rose between days 10 and 15 from 4.04 ± 0.8 to 13.23 ± 1.86 and from 5.09 ± 1.14 to 9.95 ± 1.75 in females and males, respectively, and remained unchanged in both sexes between days 15 and 35. TH activity increased in females, but not males, between days 35 and adulthood and was 2.5-fold higher in diestrous females than in males. In a second group, frozen brain sections (15 μM) through the AN were hybridized at 45 C with a ^{35}S-labeled riboprobe for TH. After RNAase treatment and stringent post-hybridization washes, slides were dipped in emulsion and exposed for 4 weeks. TH mRNA signal levels in the AN were low on days 5 and 10 of age and increased by day 20 in both sexes and between days 30 and 40 in females, but not males. The TH mRNA levels in the AN were higher in diestrous females than males during adulthood. Serum PRL levels were low (2-3 ng/ml) until day 15, rose 4-6 fold by day 20 and an additional 2-4 fold by day 40 in both sexes. Conclusions: 1) The early (days 10-15) increase in TH mRNA levels and activity in the TIDA neurons occurs before the rise in circulating PRL and may be controlled by a mechanism common to both sexes. 2) The later (day 40) increase in TH is sex-specific and may be related to the hormonal events surrounding puberty in females. Supported by HD 24190.

359.18

THE STIMULATORY AND INHIBITORY MODULATION OF AMPHETAMINE (AMPH) STIMULATED DOPAMINE (DA) RELEASE FROM THE CORPUS STRIATUM (CS) OF FEMALE RATS BY PROGESTERONE (P) IS STEREOSPECIFIC. D. Dluzen and V. Ramirez. Department of Physiology and Biophysics, University of Illinois, Urbana Illinois 61801.

Progesterone linked to bovine serum albumin at position 3 of the steroid ring (P-3-BSA) increased AMPH stimulated DA release *in vitro* from superfused corpus striatal (CS) tissue fragments of ovariectomized-estrogen treated female rats when administered in a pulsatile mode directly into the superfusion chambers, but inhibited AMPH stimulated DA release when administered in a continuous mode (Dluzen and Ramirez, Brain Res. 476:388, 1989). To examine the structure-activity of these effects we used P linked with BSA at the 11 position (P-11-BSA) and deoxycorticosterone-21-hemisuccinate (DOC-BSA). The areas under the AMPH stimulated DA release curves (χ±SEM-pg/50 min) in response to DOC-BSA (5ng/ml) were not significantly altered by either pulsatile (613±72, N=4) or continuous (565±137, N=4) infusion modes compared to control superfusions (473±93, N=4). In contrast, both the pulsatile (937±182, N=4) and continuous (1025±111, N=4) infusion of P-11-BSA (5 ng/ml) significantly increased AMPH stimulated DA release over control levels. The results demonstrate that P-11-BSA was capable of exerting a stimulatory but no inhibitory effect upon AMPH stimulated DA release while DOC-BSA was without effect.

359.19

HYPOTHALAMIC PARAVENTRICULAR NUCLEUS ALPHA$_2$ ADRENERGIC RECEPTORS, BUT NOT ALPHA$_1$ OR BETA RECEPTORS, MAY BE INVOLVED IN THE REGULATION OF RAT BLOOD CORTICOSTERONE LEVELS. M.W. Gunion, M.J. Rosenthal*, S. Miller*, and B. Zib*. GRECC, Sepulveda VAMC, Sepulveda, CA 91343.

Neurons in the paraventricular nucleus of the rat hypothalamus synthesize and release corticotropin releasing factor to regulate blood corticosterone levels, and have also been implicated in metabolic fuel regulation. This nucleus receives ascending adrenergic and noradrenergic projections from lower brainstem sites which may be involved in corticosterone and metabolic fuel regulation. Unilateral microinfusions of the agonists methoxamine (α_1), clonidine (α_2), and isoproterenol (β) (0,10,30,100 nmol/500 nl 0.9% NaCl+0.1% BSA) were made into the hypothalamic paraventricular nucleus or caudate nucleus of adult male albino rats through chronic guide cannulae. Blood samples (120 ul) taken from the tail tip 0, 15, 30, 60, 90, and 120 min postinfusion were assayed for serum corticosterone, glucose, and free fatty acids. Although both the α_2 and the β agonists cause dose-related increases in serum glucose and free fatty acid levels, there was no detectable difference between paraventricular nucleus and caudate nucleus infusions, suggesting that the effects seen on these measures were due to leakage of agonists into the periphery. Corticosterone was strongly affected by paraventricular nucleus infusions of α_2 agonist and only weakly changed by caudate infusions, but was affected equally by β agonist infusions into the paraventricular and caudate nuclei. The α_1 agonist had no effect on any measure at any site. These data suggest a possible role for α_2 receptors in the regulation of corticosterone by paraventricular nucleus neurons. [Supported by NS20660 (MWG), AG04793 (MJR), and Veterans Administration funds (MWG, MJR).]

SENSORY SYSTEMS—AUDITORY SYSTEM: MODELS

360.1

A NEURAL NETWORK MODEL OF SOUND LOCALIZATION BASED ON SPECTRAL CUES Chalapathy Neti and Eric Young, Dept. of Biomedical Engineering and Ctr. for Hearing Sciences, The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

Psychophysical evidence suggests that localization of sound depends upon directional filtering of the pinna in mammals in addition to interaural disparity cues. We are interested in studying the kinds of response properties of neurons that are useful in extracting sound location information from spectral cues provided by the pinna. Using a systematic map of pinna transfer functions of a cat for different points in space (Rice et. al, *JASA* 85: S67) We have constructed a layered neural network that transforms pinna filtered stimuli to a two dimensional representation of spatial location (modelled according to the spatial code in superior colliculus). The resulting network solutions are characterized by generating response maps of network units to tones. Network inputs to tones are rate-profiles of auditory nerve (AN) fibers for single tones. Rate-profiles at different stimulus levels are generated by a computational model of AN fiber rate level functions (Sachs. et. al *Hear.Res.* 41) and AN fiber tuning curves (from B.Delgutte). We will describe computational properties of the network solutions and response properties of network units. Particularly interesting are first, the existence of level invariant network solutions; Second, response properties of network units resemble cochlear nucleus types III and IV units (Young and Brownell) and their composites (including non-monotonic rate responses); and third, particular frequency regions of the spectra are relatively more important for the computation. Supported by NIH grant DC00115.

360.2

APPLICATION OF NEW ELECTROTONIC MODELING METHODS: RESULTS FROM TYPE I CELLS IN GUINEA PIG VENTRAL COCHLEAR NUCLEUS. J.A. White*, E.D. Young, and P.B. Manis, Center for Hearing Sciences, Johns Hopkins Univ. Sch. of Med., Baltimore, MD 21205.

Stellate cells of the mammalian ventral cochlear nucleus (VCN) modify the neural signal conveyed to them by the auditory nerve (AN) into spike trains that are more regular than those of AN fibers and less strongly phase-locked. We have proposed that an important mechanism underlying these transformations is low-pass filtering by the passive electrotonic structure of the dendritic tree. In this study we quantitatively assess this hypothesis by determining the parameters of the somatic shunt model (Durand, D., *Biophys. J.*, 46:645-53, 1984) which best describe Type I (stellate) cells in the guinea pig VCN.

We use Monte Carlo analysis to show that the parameter identification problem for the somatic shunt model is unstable, in that low-level electrophysiological noise can lead to grossly inaccurate parameter estimates and predicted synaptic responses. However, morphologic information can be used to add stability to the problem. Intracellular electrophysiological data required for these analytical techniques were collected from Type I cells in a guinea pig VCN brain slice preparation. Data include I-V curves for cell response type characterization, responses to AN electrical stimulation, and averaged responses to injected steps of hyperpolarizing current. Some neurons were stained with Lucifer Yellow to gather morphologic data. For Type I cells we obtain a mean estimated electrotonic length of 1.6 length constants, a dendritic membrane time constant of 6.6 ms, and a dendritic dominance factor of 1.8 (5.9 in shunt-corrected cells). These values support the hypothesis that the passive electrotonic architecture of stellate cells contributes strongly to their response properties. Supported by NIH grants DC00115 (EDY) and DC00425 (PBM).

360.3

RESOLUTION OF μSEC TIMING IN A NEURAL COMPARTMENTAL MODEL. W.E. Sullivan, Dept. of Biology, Princeton Univ., Princeton, N.J. 08544.

Behavioral discrimination of μsec. time differences has been described in owls, bats and weakly electric fish. The neural basis of this acuity has thus been subject to speculation since physiological events are thought to operate in msec. rather than μsec. ranges. Either these groups have membrane and synaptic processes which are many times faster than normal or μsec. resolution is in fact possible with conventional cells. Simulations using a neural model with both passive and active compartments (i.e. the Hodgkin-Huxley model) show that μsec. accuracy can be achieved without rapid membrane processes. Synaptic input is modelled by a rising then falling membrane conductance in one of the passive compartments. Synaptic duration was 1.0 msec. To measure time resolution, twin inputs of 52.5, 55, 60, 75 and 90 % of single pulse threshold were presented at varying time intervals. The largest interval evoking a spike was then found for each input level.

In single active compartment models, time resolution was minimized by increasing active-to-passive conductance, raising voltage sensitive K+ channel density or by adding steady inhibition. These produce onset responses to sustained excitation. In these models, time resolutions of only about 100 μsec could be achieved before the size difference between subthreshold and suprathreshold responses became so gradual as to be arbitrary. Improvements in resolution were accompanied by higher threshold so that the "best" models appeared to be attenuating input pairs with large delays rather than selecting for short delays. By adding active compartments, both temporal tuning and the all-or-none nature of the response were improved. In these models, time resolutions of <10 μsec for inputs each 75% of single pulse threshold were achieved, but only in the most distal compartment. If spikes in proximal segments fire too slowly, they become asynchronous and attenuate each other. A key feature of models with fine time resolution is the rate of voltage change rather than peak voltage. Slight differences in the rising phase of spikes in proximal segments are magnified by intracellular temporal filtering so that only the most rapidly activated spikes are propagated. Distal compartments fired larger spikes because they were isolated from the passive compartments and were subject to less loading.

360.4

MODELING AMPLITUDE-MODULATED (AM) TONE ENCODING BEHAVIOR OF COCHLEAR NUCLEUS NEURONS.
S. Ghoshal, D.O. Kim and R.B. Northrop, Div. Otolaryn., Surg. Res. Ctr, Ctr. Neurol. Sci., Biol. Engin. Prog., Univ. Connecticut Health Ctr., Farmington, CT 06032

A study of AM tone envelope encoding behaviors of dorsal and posteroventral cochlear nucleus (DCN & PVCN) neurons by Kim et al. (Hearing Res., 1990) reported that DCN pause build-type-III neurons and chop-S neurons exhibited band-pass modulation transfer functions (MTFs) and intrinsic oscillations (IOs) whereas chop-T neurons exhibited low-pass MTFs and no IOs. The goal of this study is to develop models for band- and low-pass MTFs of these neurons. We used the models of stellate and fusiform cells developed by Arle and Kim (Assoc. Res. Otolar., 1990) based on a modified MacGregor-type neuron model. We represented a high carrier-frequency AM tone by applying at the soma of a model neuron a current consisting of DC, AC and zero-mean Gaussian noise. We found that, when a relatively strong noise was present, the stellate-cell model exhibited a low-pass MTF and no IO, thus reproducing a chop-T neuron's behavior. We also found that, with proper DC, AC and noise amplitudes, both the fusiform-cell model and the stellate-cell model exhibited band-pass MTFs and prominent IOs, thus reproducing the behaviors of a pause-build neuron and a chop-S neuron. The fusiform-cell model exhibited a more robust band-pass MTF and IO than the stellate-cell model in the sense that a strong noise tended to abolish the IO and band-pass MTF more readily in the latter model. These results support a hypothesis that intrinsic cellular mechanisms of fusiform and stellate cells similar to those postulated in the present models may underlie the observed MTFs and IOs of CN neurons.

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360.5

PARAMETERS AFFECTING GAP DETECTION IN THE RAT. D. S. Leitner, G. Hammond*, C. Springer*, K. Ingham*, A. Mekilo*, P. Bodison*, M. Aranda* and M Shawaryn*. Dept. of Psychology, St. Joseph's University, Philadelphia, PA 19131.

An important aspect of the functioning of the auditory system is temporal acuity, the ability to track rapidly changing acoustic transients. It is particularly important in human speech comprehension; if the auditory system continues to respond as if a recently terminated stimulus is still present, then it will interfere with the perception of a new stimulus, blurring the transition. In theory, a failure by the nervous system to resolve temporal detail will occur to the extent that the activity engendered by an auditory stimulus outlasts the actual physical event.

The present research used a startle amplitude reduction paradigm to investigate the ability of the mammalian auditory system to track acoustic transients. Specifically, the ability of brief gaps in otherwise continuous acoustic stimulation to reduce the amplitude of a subsequently elicited acoustic startle reflex was examined. In the first experiment, 12 rats were exposed to a gap, embedded in otherwise continuous 60 dB (SPL; A-weighted) white noise, whose duration varied among 5, 10, 15, 20, 25, 30, 40, and 50 msec. Also included was a "0" gap condition, consisting only of the 1 msec rise-fall time used in the other gap conditions, and a control condition in which the 50 msec, 125 dB (SPL; A-weighted) white noise startle-eliciting stimulus (SES) was presented without a gap preceding it. These 10 conditions were presented to each rat in a block-random fashion, with a total of 30 blocks being presented. The gaps were presented either 30, 50, 100, 200, 300, or 500 msec before the SES. A second experiment used gaps with durations varied among 0, 1, 2, 3, 4, 5, 6, 8, and 10 msec, and the interstimulus intervals were varied among 25, 50, 75, 100, 150, and 200 msec. A third experiment used gaps varied among 0, 1, 2, 3, 4, 5, 6, 8, and 10 msec, presented at a fixed interstimulus interval of 50 msec, and with rise/fall times varied among 0, 1, 2, and 4 msec.

The results of these experiments indicated that the most sensitive test for assessing the detectability of brief gaps by rats was achieved by positioning the gap so that its offset occurred 50 msec before the presentation of the SES. The maximum effect produced by a gap was a reduction in startle amplitude to approximately 40% of mean control amplitude. The subjects could reliably detect gaps that were less than 5 msec in duration. The longer the rise/fall time, the more readily detectable was a gap of a specific duration. These data are consistent with measures taken using other techniques, and are comparable to gap detection thresholds in a variety of other species, including birds and human beings. They suggest that the ability of the nervous system to identify and track gaps is limited only by the response characteristics of the neurons involved.

360.6

SOUND LOCALIZATION IN THE BARN OWL: A QUANTITATIVE MODEL OF BINAURAL INTERACTION IN THE NUCLEUS LAMINARIS. S. Grün*[1], A. Aertsen[1], H. Wagner[1], C. Carr[2]. [1]*Max-Planck-Inst. für biol. Kybernetik, Spemannstr. 38, 7400 Tübingen, FRG;* [2]*Dept. of Neurobiol. and Anatomy, Univ. of Rochester, NY 14642, USA.*

The barn owl is a nocturnal hunter that uses interaural time difference (ITD) to determine the azimuth of a sound source. The first two stages in the neural pathway dealing with ITD are the nucleus magnocellularis (NM) and the nucleus laminaris (NL). Neurons in these two nuclei exhibit phase-locking, as demonstrated in the period histogram (PH). Magnocellular neurons are spontaneously active, respond only to ipsilateral stimulation, and project bilaterally to NL. Laminaris neurons are binaural: their response varies in a cyclic manner with ITD. The response to monaural stimulation is generally between the minimum and the maximum of the ITD-curve.

We found that the underlying neural computations can be described by a simple two-stage model: linear summation followed by an algebraic nonlinearity. The model is formulated in terms of firing probabilities, as measured by the PHs. Phase-locking of the NM-neurons is described by modulation of firing rate around the spontaneous level. In the NL, inputs from both sides, together with an unspecific, hyperpolarizing inhibition, are linearly summated. The result is subsequently transformed by a sigmoid-shaped nonlinearity, modeling the probability of spike generation in the NL-neuron. The inhibition effectively serves as a control parameter, shifting the operating point on the nonlinearity. The model parameters are: spontaneous activity and strength of phase-locking in NM, strength of inhibition, and gain and slope of the nonlinearity in NL. For physiologically realistic values of these various parameters, this simple, general model correctly describes the monaural and binaural response properties of laminaris neurons, without having to invoke any problem-specific mechanisms. The model predicts that weakening of the inhibition would reduce the sensitivity for ITD.

SENSORY SYSTEMS—AUDITORY SYSTEM: HAIR CELLS AND COCHLEA I

361.1

TWO-TONE DISTORTION PRODUCTS IN THE BASILAR MEMBRANE OF THE CHINCHILLA COCHLEA. L. Robles*#, M. A. Ruggero and N. C. Rich*
Dept. of Otolaryngology, Univ. of Minnesota, Minneapolis, MN 55414.

Motion of the basilar membrane (BM) in response to two-tone stimuli was measured in the chinchilla cochlea using a new application of laser velocimetry. BM velocity was determined from the Doppler frequency shift of laser light reflected from glass microbeads (10-30 um) placed on the BM in the basal turn of the cochlea. Two locked primary tones (f_1 and f_2, 10-ms duration) were digitally generated and delivered to the ear canal via two acoustically-coupled earphones. BM responses were averaged over 1024 stimulus repetitions and Fourier transformed to obtain frequency spectra. Distortion products (DPs; i.e., spectral components with frequencies such as $2f_1$-f_2, $2f_2$-f_1, $3f_1$-$2f_2$, $3f_2$-$2f_1$, etc.) were observed in responses of 6 cochleae to primary tones with intensities as low as 30 dB SPL. Some pairs of primaries evoked multiple DPs at frequencies both lower and higher than those of the primaries. Under optimal conditions, the effective SPLs of the $2f_1$-f_2 and $2f_2$-f_1 DPs were only 11 dB and 22 dB, respectively, below the levels of the primaries. [Effective SPL is the SPL of a single tone at the DP frequency required to elicit the same response magnitude as that evoked at the DP frequency by the two-tone stimulus.] Such prominent DPs undoubtedly originated in the cochlea, since controls showed that artifactual DPs in the acoustical and measuring systems were at least 50 dB below the primary levels. These results are the first to demonstrate that mechanical DPs are present in the BM at low and moderate stimulus levels and that they travel along the cochlea in both directions.

#On leave from Depto. de Fisiologia y Biofisica, Fac. de Medicina, Univ. de Chile.

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361.2

DEVELOPMENT OF THE ORGAN OF CORTI IN THE RAT: A SCANNING ELECTRON MICROSCOPIC STUDY. L.P. Rybak, C. Whitworth,* A. Weberg*and V. Scott* Dept. of Surgery, SIU School of Medicine, Springfield, IL 62794-9230.

The rat is an altricial animal which has proven to be a useful model for the study of auditory development. The purpose of the present study was to study the surface development of the organ of Corti using scanning electron microscopy (SEM). Rat pups from 1-30 postnatal days of age were anesthetized with pentobarbital and the cochleas were removed and processed for SEM using a modification of the TOTO procedure (Davis and Forge, J Microsc 1987; 147: 89-101). The specimens were viewed with a Hitachi S500 Scanning Electron Microscope. The stereocilia of the apical region were very immature but underwent sequential maturation. By contrast, the outer hair cells of middle and basal turns were more mature in appearance. Kinocilia were present on both inner and outer hair cells in very young pups, but disappeared by 10 days of age. The inner hair cell stereocilia of the middle and lower turns of the cochlea had a V-shaped configuration similar to that of the outer hair cells of early postnatal ages, but became arranged into a linear row with maturation. The effects of maturational changes in stereocilia may have important effects on cochlear micromechanics in development.

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361.3

THE MAGNITUDE OF THE NEGATIVE SUMMATING POTENTIAL VARIES DIRECTLY WITH PERILYMPH CALCIUM LEVELS. R.P. BOBBIN and M. FALLON*. Kresge Hearing Research Lab., LSU Medical Center, New Orleans, LA 70112.

Previous results (Soc. Neurosci. Abstr., 15: 210, 1989) demonstrated that nimodipine, an L-type of Ca^{2+} channel antagonist, abolished the negative summating potential (-SP), suggesting that Ca^{2+} is involved in generation of the -SP. This study examined the effect of Ca^{2+} on cochlear potentials. Perilymph spaces of guinea pig cochleae were perfused with Ringer solutions containing zero Ca^{2+}, zero Ca^{2+} with 2 mM EGTA, and increasing levels of Ca^{2+} (2, 4, 8, 16 mM) at a rate of 2.5 µl/min for 10 min. Immediately after each period of perfusion the compound action potential of the auditory nerve (CAP), cochlear microphonics (CM) and the -SP evoked by 10 kHz tone bursts of varying intensities were recorded from a wire inserted in the basal turn scala vestibuli. Decreasing the level of Ca^{2+} decreased the magnitude of the -SP whereas increasing the levels of Ca^{2+} progressively increased the magnitude of the -SP. The results are in harmony with the hypothesis that the -SP is Ca^{2+} dependent. We speculate that the -SP represents a Ca^{2+} current and/or a Ca^{2+} dependent change in the length of the outer hair cells. (Supported by NIH grant NS-22024, Kresge Foundation and the Louisiana Lions Eye Foundation.)

361.4

EFFECT OF INTERMITTENT NOISE ON COCHLEAR POTENTIALS IN THE CHINCHILLA. F.A. Boettcher and R.J. Salvi. Hearing Research Lab, State University of New York at Buffalo, Buffalo, NY 14214.

The effect of intermittent noise exposure on sensitivity of the whole-nerve action potential (AP) and amplitude of the AP and cochlear microphonic (CM) were examined in the chinchilla. The subjects were exposed to an octave band of noise (95 dB SPL, 0.5 kHz center frequency) for 3 hours twice daily. The threshold of the AP was elevated by approximately 46 dB on the third day of exposure, then the shift decreased to approximately 10 dB by the 10th day of exposure. The amplitude of the AP decreased across intensity on the first day of exposure and gradually recovered across the duration of the exposure. The slope of the AP input-output functions showed little change. The amplitude of the CM response decreased on the first day of exposure and remained depressed until the termination of the exposure. The mechanism of recovery of sensitivity during exposure will be discussed in terms of outer hair cell function and data will be compared to other investigations of modification of susceptibility to noise-induced hearing loss by previous acoustic stimulation. (Work supported by NIH 5-RO1-16761-09).

361.5

ADENYLATE CYCLASE SYSTEM AS A RESERVE BATTERY IN THE GUINEA PIG COCHLEA. K. Doi*, N. Mori*, K. Wada and R. J. Wenthold. Dept. of Otolaryngology, Osaka Univ. Med. Sch., Osaka 553, Japan and Lab. of Molecular Otology, NIDCD, Bethesda, Maryland 20892.

We reported (Doi et al., Hear. Res., 45: 157, 1990) that the perilymphatic perfusion with forskolin, known as an adenylate cyclase (AC) stimulant, produced a reversible 10-20 mV elevation of the endocochlear potential (EP). To examine whether or not this EP elevation is mediated by the activation of the AC-dependent protein kinase (A-kinase), we studied the effect of H-8, an A-kinase inhibitor, on the EP and the EP elevation produced by forskolin. During the recording of the EP, the scala vestibuli was perfused from the basal turn to the apex at a rate of 8µl/min with forskolin (10^{-4}M, $2x10^{-4}$M), H-8 (10^{-4}M) or the mixture of forskolin and H-8. We found that 10^{-4}M H-8 eliminated the effect of 10^{-4}M forskolin (n=5) but did not significantly affect that of $2x10^{-4}$M forskolin (n=5). We also found that 10^{-4}M H-8 did not significantly affect the EP itself (n=3).

The results suggest that the cochlear AC system may be inactive in the normal state and that it may be activated and play a role as a reserve battery in some pathological conditions.

361.6

SUSCEPTIBILITY TO TONAL OVER-EXPOSURE IN AWAKE AND ANESTHETIZED RABBITS. M.C. Patterson,*[1] B.D. Mensh,[2,3] M.L. Whitehead,*[1] B.L. Lonsbury-Martin[1,2] and G.K. Martin[1]. [1]Dept. of Otolaryngol. Commun. Sci., [2]Div. of Neurosci., [3]Med. Sci. Tr. Prog., Baylor Col. of Med., Houston, TX 77030.

Exposure to moderate pure tones causes cochlear damage, as measured by a reduction in the amplitude of 2f1-f2 distortion-product otoacoustic emissions (DPOAEs). Previous work in our laboratory has shown that the susceptibility to damage is greater in anesthetized rabbits during a second exposure, 40 min after the first. The present study examined the role of the acoustic reflex in the increased susceptibility and extended the results to awake animals. The paradigm consisted of: 1) establishing baseline DPOAEs to 50-dB SPL primaries at 2.3 kHz (geometric-mean), 2) exposing the ear to multiple 10-s, 100-dB SPL stimuli at 1.768 kHz, and 3) monitoring the reduction in DPOAEs during a first and second exposure separated by 40 min. The contralateral acoustic reflex was monitored throughout the protocol. Each animal was exposed to the paradigm three times at 2-3 day intervals while awake, and three times while anesthetized.

The increase in susceptibility to overstimulation 40 min after the initial exposure in anesthetized rabbits was not accompanied by a diminished acoustic reflex, implying a cochlear origin of the altered susceptibility. However, anesthetized rabbits were found to be more susceptible to overstimulation than awake rabbits. Because the acoustic reflex was considerably smaller in anesthetized animals, it may be a significant contributor to the difference in susceptibility to overstimulation between the awake and anesthetized states. [DC00313, ES003500].

361.7

TEMPORAL REGULARITY MEASURES OF THE FIRING PATTERNS OF AUDITORY NERVE FIBERS TO COMPLEX SOUNDS. D. Lim and R.R. Capranica. School of Electrical Engineering and Section of Neurobiology and Behavior, Cornell University, Ithaca, NY 14853.

Spike trains in response to 300-msec duration simple and complex tones and to natural and synthetic calls were studied in detail for over 50 single auditory fibers in the eighth nerve of the green treefrog (Hyla cinerea). The acoustic stimuli were generated on-line by means of a Macintosh-based auditory workstation in our laboratory (J. Vrieslander and R. Capranica, J. Acoust. Soc .Am. Suppl.1,87: S25,1990). Each stimulus was presented once every 1.5 seconds for five repetitions at each of several different sound intensities, and resultant spike times were determined with an accuracy of 1.0 µsec. For each stimulus and each intensity, cross-correlation functions were computed within the set of spike trains for the same fiber (iso-fiber correlation functions) and also between spike trains from different fibers (cross-fiber correlation functions). For each correlation function, it s peak value was divided by its area to yield a normalized temporal regularity index between 0 and 1. This index provides a more meaningful measure of the ability of an auditory neuron to encode dynamic temporal patterns, compared to other techniques such as coefficient of synchronization or PST histograms which are based on averaged activity . Our results indicate that there is a variety of spike train regularities within the population of fibers in the peripheral auditory system. [Supported by NINCDS.]

361.8

HISTOPATHOLOGICAL CHANGES FOLLOWING IMPLANTATION OF INTRACOCHLEAR ELECTRODES VARY WITH THE LENGTH OF DEAFNESS PRECEDING THE PLACEMENT OF THE ELECTRODE. S.A.Larsen, Ph.D., and T.M. Kirchhoff, M.D., Depts. of Anat. Sci. & Neurobiol. and Surgery, Div. of Otolaryngol., Univ. of Louisville, Ky. 40292.

Previous studies have shown that insertion, presence and stimulation of cochlear prostheses result in damage to the auditory system; such changes are of concern for long-term use of these devices. We report results from two studies following implantation of intracochlear electrodes. We used Macaca Nemestrina that had been deaf five months prior to the insertion of the electrodes in one study while in the second investigation, normal-hearing and white-deaf cats that had been deaf for as long as five years were implanted. Cochleas and auditory nerves of implanted and control animals were studied with electron microscopy. Differences were not observed when comparing cochleas or auditory nerves of implanted or control animals in either study, however, degeneration found in the normal-hearing animals could be distinguished from that found in deaf animals. In addition, auditory nerves of the primates appeared to have a normal density of fibers compared to that found in the deaf cats with the remaining auditory fibers failing to show the degree of degeneration observed in deafened primates. Nerves of normal-hearing animals showed patches of fibers that appeared normal intermingled with patches of degenerating fibers that were similar in appearance to the degenerating fibers observed in the deaf primates. We conclude that degeneration seen in both deafened primates and white-deaf cats is due to the deafness itself not to the insertion or the presence of the electrodes while degeneration of cochleas and auditory nerves seen in normal-hearing animals is due to the insertion or presence of the electrodes. In our opinion, the period of the deafness prior to electrode insertion is an important factor determining the degree of degeneration. (Supported by grants from Deafness Research Foundation and University of Louisville Graduate School grant)

361.9

[^{125}I] Sar^{1}, Ile^{8}-Angiotensin II Receptor Binding in the Cochlea of SHR, WKY and SD Rat Pups. J.K.M. Coleman, V.I. Cook, K.L. Grove*, H.A. Dengerink, J.W. Wright and R.C. Speth. Departments of Psychology and VCAPP, Washington State University, Pullman, WA 99164.

Our laboratory has been investigating the effects of arginine vasopressin (AVP), angiotensin II (AII), and substance P on cochlear blood flow (CBF) because these vasoactive hormones may be implicated in otopathologies such as Meniere's disease, noise induced hearing loss, tinnitus and sudden deafness syndrome. The present investigation utilized the radioligand [^{125}I]Sar^{1},Ile^{8}-AII to determine the distribution of AII receptor populations in the cochlea of 3 day old spontaneously hypertensive rats, and Sprague-Dawley and Wistar-Kyoto normotensive controls. The results indicate substantial levels of AII binding in the cochlear vasculatures of each strain and represent the first autoradiographic analyses of the cochlea for AII binding. Our previous research has shown dose-related decreases in CBF with the infusion of AII into the supplying vessels of the cochlear vasculature. Taken together these results support an important role for AII in the control of CBF.

361.10

PHOSPHOINOSITIDE METABOLISM IN THE GUINEA PIG COCHLEA: DIFFERENCES BETWEEN BASE AND APEX. A. Niedzielski and J. Schacht. Kresge Hearing Research Institute, University of Michigan, Ann Arbor, MI, 48109-0506.

Functional differences between the base and apex of the cochlea are believed to have an important role in auditory processing. It has been suggested that these differences are the result of longitudinal gradients in cochlear anatomy, biochemistry, and physiology. Gradients in efferent innervation and in Na^{+}-K^{+} ATPase are two examples with functional significance. Previously, we have reported that in whole tissue preparations *in vitro*, ^{32}P-labelling of phosphoinositides is significantly higher in the apex of the guinea pig cochlea. The phosphoinositide second messenger system has been hypothesized to mediate cell signalling in the cochlea, including outer hair cell contractility. We have now determined that the differential distribution of phosphoinositide labelling is not due to differences in ATP pools or in substrate availability between the base and apex of the cochlea. In contrast, direct assay of diacylglycerol kinase revealed 1.6-fold higher activity in the apical cochlea. Similar differences may exist in other enzymes of phosphoinositide metabolism. The differential distribution of the phosphoinositide second messenger system may have important implications for cochlear function.

(Supported by NIH grant DC-00078)

361.11

ARGIOTOXIN-636 BLOCKS EFFECTS OF N-METHYL-D-ASPARTATE ON LATERAL LINE OF *XENOPUS LAEVIS* AT CONCENTRATIONS WHICH DO NOT ALTER SPONTANEOUS OR EVOKED NEURAL ACTIVITY. S.L. Guth*, D.A. Scapini*, M.J. Drescher* and D.G. Drescher, Laboratory of Bio-otology, Wayne State University School of Medicine, Detroit, Michigan, 48201

Excitatory amino acids (EAA), their analogs and antagonists can modulate the activity of afferent neurons in acousticolateralis organs, and EAAs are among the better candidates for afferent transmitter between hair cells and afferent neurons. Argiotoxin-636 (ATX) is a spider venom toxin that is a selective antagonist of the N-methyl-D-aspartate (NMDA) subtype of excitatory amino acid receptors in vertebrates (Priestley et al., Brit. J. Pharmacol. 97: 1315-1323, 1989). We compared the effects of NMDA alone and in combination with ATX on resting firing rate (spontaneous activity) and mechanically stimulated activity of lateral line afferent neurons of early post-metamorphic *Xenopus laevis*. Perfusion of NMDA (100 or 200 μM) typically produced a biphasic effect on spontaneous activity, which consisted of a transient increase followed by a decrease in firing rate. After spontaneous activity had stabilized in the presence of NMDA, mechanical stimulation evoked an increase in firing rate which was not significantly different from the increase in firing rate evoked by mechanical stimulation in the absence of NMDA. The biphasic effect of NMDA on spontaneous activity was blocked in a reversible manner by concentrations of ATX (1-2 μM) which, when applied alone, did not alter either spontaneous or mechanically evoked activity. While these results suggest that NMDA receptors are present in the lateral line, it appears that the generation of action potentials by the afferent transmitter in this organ is not dependent on activation of postsynaptic NMDA receptor-ion channels.

SENSORY SYSTEMS—AUDITORY SYSTEM: CENTRAL PHYSIOLOGY II

362.1

REGULARITY ANALYSIS OF UNITS IN THE ANESTHETIZED GERBIL DORSAL COCHLEAR NUCLEUS (DCN). J.C. Maghirang*, R. Wallace*, S. Xue*, T.E. Benson and H.F. Voigt. Dept. of Biomedical Engineering, Boston University, Boston, MA 02215.

Responses of units in the DCN of barbiturate-anesthetized gerbils to 50-ms tone-bursts were recorded in order to establish a normative data-base for peri-stimulus-time (PST) histograms and regularity analysis in this species. Responses to 100 stimulus presentations were recorded at best frequency (BF) and up to 30 adjacent frequencies (0.2 octave steps) for several sound pressure levels (10 dB steps). This report is based on the analysis of BF responses at 20 dB re threshold in 61 units. A decision tree (after Blackburn and Sachs, J. Neurophysiol., 62:1303, 1989 and Young et. al., J. Neurophysiol., 60: 1, 1988) was developed for unit classification based on PST-type, spikes/peak test, regularity analysis (mean interval and standard deviation (SD) vs. time following stimulus onset), coefficient of variation (CV = SD/mean interval). Of 61 units classified, 7 are Primary or Pri/Notch; 24 are Chopper-S; 5 are Chopper-T, 1 is a Chopper-I; 11 are Pausers; 5 are Buildups; 7 are Onset or Onset-sustained, and 1 is an Off-responder. To our knowledge, this is the first use of regularity analysis applied to unit classification in gerbil cochlear nucleus. [Work supported by NIH]

362.2

RESPONSE MAP MEASUREMENTS OF UNITS IN THE ANESTHETIZED GERBIL DORSAL COCHLEAR NUCLEUS (DCN). G.T. Gdowski*, K. Kreitner*, T.E. Benson, H.F. Voigt. Dept. of Biomedical Engineering, Boston University, Boston, MA 02215.

Responses of units in the DCN of barbiturate-anesthetized gerbils to 32-, 50-, and 200-ms tone- and 200-ms noise-bursts were recorded in order to establish normative response-map (RM) data in this species. Three-dimensional RMs (rate vs sound level vs frequency) and two-dimensional RMs were generated from responses to single presentations of the 32-ms tones and compared to those based on responses to 100 50-ms tones. Units were typed according to the presence and location of excitatory and inhibitory RM regions, rates of spontaneous activity (SPAC), and relative noise index [p= (maximum noise response -SPAC)/(maximum best frequency (BF) tone response -SPAC)]. Of 126 units classified, 20 are type I (no inhibition, SPAC $\geq$2.5 Spk/s); 18 are type II (no inhibition, SPAC <2.5 Spk/s, p$\leq$0.3); 56 are type I/III (no inhibition detected, SPAC <2.5 Spk/s, p>0.3); 30 are type III (side-band inhibition, SPAC >2.5 Spk/s); and 2 are type IV (inhibition above excitation at BF; SPAC >2.5 Spk/s). These are consistent with data from the DCN of other anesthetized species. [Work supported by NIH]

362.3

EVALUATION OF NEURONAL ACTIVITY IN THE MEDIAL GENICULATE BODY OF THE RAT IN RESPONSE TO NOVEL STIMULI. D. S. Weiss and S. Reinis, *Department of Psychology, University of Waterloo, Waterloo, ONT, Canada.*

Extra-cellular activity was recorded using tungsten microelectrodes. Subjects were Long-Evans hooded rats anaesthetized with Rompun and Ketaset. Data were digitized at 10 kHz. Spikes in the multi-unit record were separated and discriminated according to voltage into leading cell and mass activity categories. Frequency histograms of interspike intervals (auto-correlograms) were calculated in order to determine significant interactions between neurons. Stimulus conditions were No Stimulus (NS); Tone Continuous (TC); Tone Burst (TB), 100 msec at 500 msec intervals; and novel stimulus (CL). Tone stimuli were presented at characteristic frequencies. The novel stimulus was a sharp clanging sound produced by a mechanical device at a rate of 2 Hz.

Neuronal systems recorded under the NS and TC conditions reacted in a similar manner. Both mass and leading cell correlograms were characterized by either predominantly short-term interspike intervals of less than 100 msec or evenly distributed patterns of short, medium, and long-term intervals over the full 1000 msec correlogram. A completely different configuration was evident in the TB and CL conditions where both leading cell and mass activity correlograms were characterized by a W-like shape with peaks congregated at interspike intervals of 5-100, 450-550, and 900-1000 msec.

362.4

LESION OF THE ACOUSTIC NERVES OR VENTRAL COCHLEAR NUCLEI ABOLISH MOTOR BUT NOT CARDIOVASCULAR RESPONSES TO AIRPUFF STARTLE STIMULI. B.K. Taylor, R. Casto and M.P. Printz. Pharmacology M-036, Univ. of California, San Diego, La Jolla, CA 92093.

Air-puff stimuli (12.5 psi, 100-ms duration) elicit behavioral (motor) startle reactions accompanied by a complex cardiovascular (**CV**) constellation which includes pressor, bradycardic and tachycardic components. We previously found an acoustic component of the airpuff. After tympanic membrane rupture (**TMR**) the motor response to air-puff (and acoustic) stimuli was abolished but not the **CV** responses. The present study extends this observation by transecting the VIII cranial nerve (acoustic) and lesioning the ventral cochlear nucleus (VCN).

Under halothane anesthesia, the acoustic nerve of Sprague-Dawley rats was severed bilaterally by aspiration and animals tested after 7-10 days of recovery. Successful transection (**TRANS**) was verified. Shams had surgery without transection. For trial 1, motor responses to air-puff stimuli were abolished by acoustic nerve transection (SHAM: 50.3±12.5, TRANS: 4.2±2.1) but **CV** responses were intact (pressor SHAM: 31.3±2.5, TRANS: 31.8±4.9 mmHg; bradycardia SHAM: -21.2±7.8, TRANS: -26.1±4.9 bpm; tachycardia trial 15: SHAM: 20.4±6.4, TRANS: 24.9±3.8 bpm).

Under anesthesia, the VCN was bilaterally RF-lesioned. Shams received no current. Testing was 9-12 days after surgery. Again, after VCN destruction motor but not **CV** responses to air-puff stimuli were abolished. Results were similar between the VCN lesioned, VIII-transected, and TMR groups providing further evidence that the acoustic component of the airpuff stimulus is essential to the behavioral motor response.

362.5

RECOVERY OF AUDITORY NEURONS FROM PRIOR STIMULATION VARIES WITH SPONTANEOUS ACTIVITY. E.M. Relkin and J.R. Doucet*. Institute for Sensory Research and Dept. of Bioengineering, Syracuse Univ., Syracuse, NY 13244.

Studies of forward masking in primary auditory neurons have revealed that probe-tone thresholds recover more slowly from the effects of a 100 ms masker for low and medium spontaneous-rate (SR) neurons compared to high SR neurons. Complete recovery takes 2.0 s for the former groups and 200 ms for the latter. These results suggest the hypothesis that recovery from adaptation is slower for low and medium SR fibers. PST histograms were recorded for the responses to 100 ms toneburts at a neurons's characteristic frequency with interstimulus intervals (ISI) of 100 ms, 300 ms, and 2.0 s. For all SR groups, both onset and average responses are reduced for the 100 ms ISI compared to the 2.0 s ISI. However, only for low and medium SR neurons are these measures also reduced for the 300 ms ISI. These results are consistent with the hypothesis, and explain why onset responses are often small or absent when low and medium SR neurons are studied with typical ISI's that are chosen to avoid the effects of adaptation in high SR neurons.

362.6

VOICE ONSET TIME ENCODING FOR SYLLABLES IN PRIMATE AUDITORY CORTEX. M. Steinschneider, J. Arezzo, C. Schroeder and H.G. Vaughan, Jr.. Albert Einstein College of Medicine, Bronx, NY 10461.

Voice onset time (VOT) is a speech parameter which denotes the interval between stimulus onset and the onset of periodic portions of speech sounds. The differential perception of consonants such as /d/ and /t/ is partially determined by the VOT. We investigated the cortical encoding of VOT by examining laminar profiles of auditory evoked potentials, current source density and multiple unit activity (MUA) elicited by the syllables /da/ (VOT=0 msec) and /ta/ (VOT=80 msec) in auditory cortex of an awake macaque. Stimuli were delivered to the contralateral ear at 85 dB SPL.

In thalamocortical (TC) fibers, responses to /da/ predominantly reflect the 0 msec VOT by phase-locked activity to the syllable periodicity that begins at stimulus onset and persists throughout its duration. In contrast, the response to /ta/ consists of an initial burst time-locked to stimulus onset followed by phase-locked responses delayed by the increased 80 msec VOT. Two cortical patterns reflecting VOT are observed. In the first pattern, the temporal sequence of current sinks mirrors that seen in TC fibers, with prominent phase-locked responses extending throughout the duration of /da/ and delayed by the increased VOT for /ta/. In the second pattern, the onsets of the periodic and aperiodic segments of the syllables are accentuated in the initial laminae 4 and lower 3 current sinks and MUA while the phase-locked responses are diminished in amplitude. The presence of MUA inhibition suggests that inhibitory processes help shape this response transformation.

We conclude that auditory cortex encodes VOT by: 1) the faithful transmission of TC fiber input patterns, and 2) a transformation of thalamic input that accentuates important acoustic transients used in the VOT distinction. (supported by MH06723)

362.7

STEREODYNAMIC STUDIES OF AUDITORY POTENTIAL (AER) OF THE LIMBIC AND NON-AUDITORY NEOCORTEX. C.C. Turbes and G.T. Schneider*. Creighton University, School of Medicine, Omaha, NE 68178.

These studies use 24 cats under non-anesthetic states. Electrodes are implanted, under appropriate anesthetic and stereotoxic procedures in non-auditory neocortical areas, nucleus accumbens, and certain nuclei of the amygdala complex.

Auditory stimulation was done using the free field method with periodic tones at 2.0 KHz to 3.0 KHz at 30db. The auditory stimulation and recording was performed with hard wire and telemetry methods.

The analog data is collected on FM tape and processed with minicomputer. Digital filtering, cross, coherence, phase, spectral and cycle time analyses are used on the analog data.

The (AER) potentials and spontaneous electro potentials in these brain areas are investigated noting simularities and differences relating to physiological interactions.

362.8

SPATIAL EXTENT OF COHERENT SENSORY-EVOKED CORTICAL ACTIVITY. Z. Lü* and S.J. Williamson, Neuromagnetism Lab., Dept. of Physics and Center for Neural Science, N.Y.U., New York, NY 10003.

To estimate the extent of cortical involvement that could account for observed human sensory-evoked magnetic fields or electric potentials, we have analyzed data on current source-density analyses in the somatosensory cortex of macaque monkey (Cauller, L.J., Kulics, A.T., submitted) and visual cortex of cat (Mitzdorf, U., J. Neurosci. 33: 33, 1987). By integrating through cortex the deduced *intracellular* current density flowing perpendicular to the cortical laminae, we deduced the net current dipole moment per unit area of cortical surface for several evoked components of long latency. Similar values were obtained across species and modalities, suggesting that suprathreshold response levels for natural stimuli are similar when characterized this way. The overall average current-dipole moment density is about 50 μA/m (S.D. = 30 μA/m). We suggest because of the similarities of mammalian cortical structure that this may also be taken as a reasonable estimate for human cortex as well. Since the total current dipole moments obtained from neuromagnetic studies on human subjects fall in the range 2-20 nA·m, the corresponding coherent neuronal activity extends over a cortical area that is typically 40-400 mm^2. At least for the auditory system, this is incompatible with a columnar organization where the response is limited to an area corresponding to linear dimensions that determine the just-noticeable differences for pitch (about 10 μm) and loudness (about 100 μm). One implication of this comparison is that neuronal activity in human auditory cortex evoked by the onset of a tone burst is sufficiently extensive to be largely overlapping for the close-lying but distinguishable attributes of pitch and loudness.

362.9

RESPONSES OF INFERIOR COLLICULUS NEURONS IN YOUNG ADULT C57BL/6J MICE AS A FUNCTION OF STIMULUS AZIMUTH. S.L. McFadden* and J.F. Willott. Psychology Dept., Northern Illinois University, DeKalb, IL 60115.

To examine neuronal responses as a function of sound source location in the horizontal azimuthal plane, recordings were made from inferior colliculus neurons in anesthetized 2-month-old C57BL/6J mice. Pure tone stimuli were presented at 7 angles in the free field. Many neurons (approximately half of the sample) behaved as though they were binaurally excited, with discharge rates varying little as a function of stimulus azimuth. Other units had discharge rates that varied markedly with speaker location. Maximal excitation nearly always occurred when the speaker was positioned in the hemifield contralateral to the recording site. Some units responded as though they were monaurally excited; in these cases, response variations could be attributed to the head shadow effect (attenuation of the signal at the distal ear by the presence of the animal's head in the sound field). In other cases, response variations were greater than could be accounted for by the head shadow effect alone, suggesting binaural interactions. Similar types of units (e.g., omnidirectional, hemifield) appear to be found in the mouse as in other animals (e.g., cat), although proportions may differ between species. (Supported by NIH grant 5R01-AG06241 to J.F.W.)

362.10

RESPONSE PROPERTIES OF NEURONS IN THE COCHLEAR NUCLEUS OF BEHAVING CATS. B. May *, M.B. Sachs and C. M. Aleszczyk*. Ctr. for Hearing Sciences and Depts. of Biomedical Engineering and Otolaryngology-Head and Neck Surgery, The Johns Hopkins Univ. School of Medicine, Baltimore, MD 21205.

We have recorded single unit responses from over 100 neurons in the cochlear nucleus (CN) of awake and behaving cats. Microelectrodes are directed to the CN by a chronically implanted recording chamber. When an auditory neuron is encountered, the unit's best frequency (BF) and threshold are determined. Each unit is classified by the shape and regularity of peristimulus time (PST) histograms describing responses to 50-ms tone bursts. Dynamic range properties of the neuron are measured in behavioral tests. Our subjects must press a lever as 200-ms BF tone bursts covering a 100-dB range are presented. When the testing sequence is interrupted at random by an increase in the rate of stimulus presentation (i.e., an abrupt decrease in stimulus duration and interstimulus interval), subjects momentarily release the lever to obtain a food reward. A function relating the unit's discharge rate to stimulus level is computed for the 200-ms tones in the testing sequence. Rate-level functions are also obtained using noise bursts as stimuli and BF tone bursts in continuous background noise. A computational model (Sachs et al., Hearing Res., 41:61-70, 1989) is applied to these data to quantify the effects of noise on dynamic range. This report will compare sensitivity, PST types and dynamic range properties of units obtained in behaving cats to responses observed in anesthetized and other nonbehaving preparations, and to psychophysical measures of performance.

(This research was supported by NS08333 and DC00023.)

362.11

PHASE-SENSITIVE BINAURAL INHIBITION IN LATERAL SUPERIOR OLIVARY NEURONS. P.G. Finlayson and D.M. Caspary, Dept. of Pharmacology, Southern Illinois Univ. School of Medicine, Springfield, IL 62794-9230.

The lateral superior olive (LSO) is involved in the localization of sound. LSO neurons in the lateral limb receive low frequency (<1.2 KHz) inputs. Tone-evoked responses from 96 chinchilla LSO neurons and 10 rat LSO neurons with CFs less than 1.2 KHz were characterized, and most displayed phase-locked tone-evoked temporal discharge patterns to ipsilateral CF stimuli. Low-CF LSO neurons comprised between 5% and 10% of all LSO neurons encountered. Discharge rate of low-CF LSO neurons increased as ipsilateral intensity increased and decreased as contralateral intensity increased. In over 60% of neurons, binaural inhibition, inhibition of ipsilaterally-evoked activity by contralateral stimuli, was dependent on interaural phase differences (IPD). Maximal binaural inhibition does not appear to occur more frequently with in-phase or out-of-phase stimuli for neurons tested with like binaural stimuli. Maximal binaural inhibition was observed at intermediate IPDs (i.e. between 0 and 180°) in cells tested with smaller phase increments. Interaural phase differences had profound effects on binaural inhibition in many low-CF LSO neurons. Responses of phase-sensitive neurons to binaural stimuli often varied with 90° or 180° changes in IPD from total inhibition to a facilitated response (compared to responses to control ipsilateral stimuli alone). The present study finds that most or all low-CF LSO neurons do receive a functional inhibitory contralateral input and that these neurons are sensitive to interaural phase differences as well as interaural intensity differences. The role of these low-CF LSO neurons in shaping the activity of inferior colliculus neurons should be considered and contrasted with low-CF inputs originating from the medial superior olive.

Supported by NIH Grant # DC 00151-10, SIU CRC funds and Canadian MRC Fellowship to P. Finlayson.

362.12

DYNAMIC EFFICACY OF THE TYPE II/TYPE IV SYNAPSE IN THE CAT DORSAL COCHLEAR NUCLEUS. M.R. Sydorenko and E.D. Young. Department of Biomedical Engineering & Hearing Science Center, Johns Hopkins University, Baltimore, MD 21205.

The dynamic efficacy of the synapse between type II and type IV cells in the cat dorsal cochlear nucleus (DCN) was investigated *in vivo*. Type II cells provide a mono-synaptic inhibitory input to type IV cells. Data for estimating the synaptic impulse response were collected by simultaneously recording the relative occurrence times of action potentials (APs) from a type II and a type IV neuron with similar best frequencies. Synaptic efficacy is expressed as the expected number of post-synaptic (type IV) APs eliminated per pre-synaptic (type II) AP. The efficacy of the synapse was estimated over a series of brief time windows spanning the stimulus duration. Also, various other parameters such as pre- and post-synaptic rate were computed for each window. Results reveal a consistent inverse relationship between average pre-synaptic rate and synaptic efficacy. No consistent relationship between synaptic efficacy and post-synaptic rate or post-stimulus onset time was observed. An analysis of synaptic efficacy conditional on instantaneous pre-synaptic interspike interval (ISI) indicated that the efficacy of the synapse is not dependent on the previous ISI. Rather, this study suggests that type II/type IV synaptic efficacy is a long memory, pre-synaptic history predictable process; and, that type II/type IV synaptic efficacy is inversely and roughly linearly related to type II average rate. Supported by NIH grant 5 R01 DC00119.

362.13

DIRECTIONAL SENSITIVITY OF AUDITORY NEURONS IN THE OWL. T.T. Takahashi and H. Wagner. Institute of Neuroscience, University of Oregon, Eugene, OR 97403; Max-Planck Institut für Biologische Kybernetik, D-7400 Tübingen, FRG.

The barn owl captures moving prey using only its sense of hearing. To study the neural basis of sensitivity to moving sound sources, we recorded the responses of 68 neurons in the owl's superior and inferior colliculi to the motion of a target simulated by sequentially activating 7 speakers placed at 30° intervals in the horizonal plane. Stimuli were typically 100-msec bursts of broadband noise, presented every 95 msec, simulating a speed of 102°/sec. The response of the neurons were plotted as post-stimulus time histograms to determine whether spike discharge was greater in one direction than in the other. As a control, we disconnected all speakers except the most effective speaker and repeated our stimulus presentation. The single speaker condition, which contains no motion cues, represents a reference with which to judge the directional preference of a neuron statistically (chi-squared). Of the total, 37% displayed a significant directional preference. Inhibition in the null direction (89% of cells) facilitation in the preferred direction (11%), and both (11%) accounted for directional sensitivity.

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362.14

AUDITORY-NERVE RATE REPRESENTATION OF SOUND LOCALIZATION INFORMATION PRESENT IN SPECTRA GENERATED BY DIRECTION-DEPENDENT PINNA FILTERING E.D. Young, J.J. Rice*, and G.A. Spirou*. Dept. of Biomedical Engineering & Hearing Science Center, Johns Hopkins Univ., Baltimore, MD 21205

The cat pinna modifies the spectra of stimuli in a fashion that depends on the direction of sound origin; a prominent component of the resulting spectra are notches with center frequencies above 8 kHz which move in an orderly fashion as sound source direction changes. Because these features occur at frequencies above the cutoff for phase-locking, they must be represented at the level of the auditory nerve (AN) by profiles of discharge rate versus best frequency (BF). In this paper we report on AN rate profiles in decerebrate cats for responses to stimuli generated by filtering broadband noise with pinna transfer functions. Rate profiles reveal a rather poor representation of the spectra of pinna-filtered stimuli when plotted as rate, as driven rate, or as normalized rate versus BF. However, when plotted as the difference in response rate between two stimuli, a clear representation of the ratio of the two magnitude spectra is seen. This result suggests that precise information about stimulus spectra is present in discharge rate, and that the poor quality of rate profiles is due to scatter that is not controlled by normalization. If stimuli are presented simultaneously to the left and right ears with spectra appropriate to the same position in the free field, a weak inhibitory effect of the contralateral stimulus is seen. The inhibition may be due to activation of the olivocochlear bundle. Sharp spectral features of the contralateral stimulus such as notches are not observed in a frequency-specific fashion in the contralateral inhibition. Supported by NIH grant 5 R01 DC00119.

362.15

ATTENUATION OF SALICYLATE-INDUCED TINNITUS BY EXOGENOUS CALCIUM IN RATS. J.F. Brennan[1] and P.J. Jastreboff[2]. [1]Dept Psychology, Univ of Massachusetts/Boston, MA 02125 and [2]Dept Surgery, Yale Sch Med., New Haven, CT 06510.

The influence of 0.05 M calcium chloride solution substituted for tap water on salicylate-induced tinnitus was evaluated in 72 pigmented rats through a procedure described earlier for our animal model of tinnitus (Beh.Neurosc., 1989, 188, 811-822). Combinations of 2 doses of salicylate (200- and 300-mg/kg) and 2 levels of continuous background noise (42- and 62-dBC) were used with groups of 18 water deprived rats trained to lick for the calcium solution and then given an acclimation session of exposure to the to-be-conditioned stimulus (CS), consisting of 5 offsets of the background for 1 min durations. 2 training sessions involved the 5 CSs' terminating with a 0.5 s, 1 mA footshock, and 7 extinction sessions followed. 6 subjects began daily salicylate injections before the 1st training session, 6 rats after training and before the 1st extinction test, and the remaining 6 rats served as saline injected controls. Under calcium supplement, the salicylate effects were attenuated compared to water alone, consistent with reversal of other salicylate effects by exogenous calcium. (NIH DC00299).

362.16

AGE-RELATED TEMPORARY THRESHOLD SHIFTS AND COCHLEAR MICROPHONIC ALTERATIONS AFTER CALCITONIN INFUSION IN A MACAQUE MONKEY MODEL. A.M. SHAPIRO*, R.N. STROMINGER*, H. ARBABZADEH*, A.T. CACACE*, S.M. PARNES* and N.L. STROMINGER. Departments of Surgery (Otolaryngology) and Anatomy, Albany Medical College, Albany, N.Y. 12208

Recent studies have demonstrated a relationship between hypocalcemia and temporary auditory threshold shifts (TTS) using surface recorded brainstem auditory evoked potentials (BAEPs); promontory recorded cochlear microphonics (CMs) also are affected. These phenomena have been shown in rabbits and monkeys after calcitonin infusion, and in rabbits after thyroparathyroidectomy. There is evidence suggesting that calcium is involved with age-related alterations in active neural processing. Thus, we studied the response of calcitonin infusion and consequent transient hypocalcemia on BAEPs and CMs in four young (2-4 years) and four aged (>22 years) macaque monkeys. Hypocalcemia was correlated with increased auditory thresholds and CM alterations in all animals. The older monkeys displayed larger and longer lasting TTS and CM changes than younger animals. These findings support the premise that age-related calcium regulation plays a role in sensorineural auditory functions.

362.17

EVALUATION OF BRAINSTEM EVOKED RESPONSE AIMED AT DETECTION OF TINNITUS IN HUMANS. [1]P.J. Jastreboff, [2]C.L. Ikner, and [3]A. Hassen. [1]Dept Surgery, Yale Univ Sch of Med, New Haven, CT 06510, [2]Dept Clinical Sci and [3]Physiol, W.V. Sch of Osteopathic Med., Lewisburg, WV, 24901.

This study attempted to create an objective method for tinnitus detection in humans based on mathematical analysis of brainstem evoked response (BSER). In a blind study, (PJJ) was provided with BSERs from 3 groups of 7 patients with normal hearing. Extending a previously developed method (Biol.Cybernetics 33:113-120, 1979), an approach was made to detect tinnitus related changes in BSERs, employing both frequency and time domains. The method enabled the correct identification of each group of patients (1 normal and 2 tinnitus groups). Highly significant differences ($p<0.001$) were obtained between the normal group and either of the two tinnitus groups. There were no differences between tinnitus groups, which were statistically indistinguishable. The time domain analysis indicated differences occurring for the potentials of latencies 4-7 msec. The frequency analysis showed differences for 600 Hz and below. At present, work is in progress in an effort to expand this method for evaluation of a single individual with tinnitus. Support: NIH DC00299.

362.18

VISUAL INFORMATION FROM ARTICULATION MODIFIES ACTIVITY OF THE HUMAN AUDITORY CORTEX. *Mikko Sams, *Reijo Aulanko, Matti Hämäläinen, Riitta Hari, Olli V. Lounasmaa, Sing-Teh Lu, and Juha Simola.* Low Temperature Laboratory, Helsinki University of Technology, 02150 Espoo, Finland, and *Department of Phonetics, University of Helsinki, 00170 Helsinki, Finland.

Neuromagnetic measurements were used to find out in which cortical area the heard and seen speech are integrated. The auditory stimuli were Finnish consonant-vowel syllables /pa/ presented together with a videotaped face articulating either the concordant syllable /pa/ (84% of the stimuli, V=A) or the discordant syllable /ka/ (16% of the stimuli, V≠A). Magnetic field was recorded over the left hemisphere with a 24-channel SQUID-magnetometer.

The subjects heard the V≠A stimulus as /ta/ or /ka/ or their mixture. The magnetic responses to these stimuli had similar initial deflections as those to the V=A stimuli. However, at the latency of about 150 ms the responses started to differ. This long-lasting difference peaked at 200 - 500 ms depending on the subject, and could be explained by activity at the supratemporal auditory cortex. This indicates that seeing the articulatory movements of the face has an effect on signal processing occurring at the auditory cortex.

The present results show that visual information from the articulatory movements has an entry into the auditory cortex. Interestingly, the neural activity observed correlates with auditory, especially phonetic, perception rather than with the acoustical energy of the stimuli.

362.19

EFFECTS OF TEMPORAL LOBE LESIONS ON THE PRECEDENCE EFFECT IN SOUND LOCALIZATION. J. L. Cranford, E.T. Goldstein, S. Preskorn, C. Moore*. Dept. Communicative Disorders, Wichita State Univ., & Psychiatric Res. Unit, St. Francis Regional Med. Center, Wichita, KS 67208.

The precedence effect involves presentation of pairs of clicks from spatially separated speakers in the sound field, with one speaker leading the other in time (.2 to 8 msec.). Normal subjects perceive a fused auditory image which appears to originate from the side of the leading speaker. Cats with unilateral auditory cortex lesions (Cranford, J.L., Oberholtzer, M., Brain Res., 111:225, 1976) correctly identify the leading source only when it is located opposite the intact hemisphere; when the leading speaker is located opposite the lesion side, cats approach the side of the trailing source. Eight humans with unilateral neocortical lesions were tested with this procedure. An effect similar to that seen with cats was found in 4 subjects in which the lesions involved areas 41 and 42 of the temporal lobe. Lesions located in non-auditory areas had no effects. The severity of the deficit appeared to be related to both the size of the lesion as well as the length of time since the occurrence of the lesion. Additional tentative evidence suggests the deficits may be more severe with left hemisphere lesions than with right lesions.

362.20

THE ROLE OF MYELINATION IN THE DEVELOPMENT OF NUCLEUS LAMINARIS IN THE BARN OWL. C. E. Carr and G. A. Higgins. Dept. of Zoology, University of Maryland, College Park, MD 20742 and Gerontology Res. Ctr., NIA/NIH, Baltimore MD 21224.

The barn owl uses interaural time differences to localize sound in azimuth. Sensitivity to these interaural time differences arises in the brainstem nucleus laminaris (NL). Maps of interaural phase difference are formed in the dorso-ventral dimension of NL by interdigitating axons from the ipsi- and contralateral magnocellular cochlear nuclei (NM). The amount of delay mapped in NL depends on the length and conduction velocity of the axons. Since myelination is important in determining conduction velocity, we have used immunohistochemical and in situ hybridization techniques to describe development of myelination in the month posthatch.

At hatching, the auditory nerve fibers are myelinated, and the NM axons that project to NL are myelinated up to the border of NL. In the first week post-hatch, oligodendrocytes line the borders of NL, and a wave of myelination of the NM axons in NL begins towards the end of the first week posthatch. Myelination proceeds slowly and is complete at 1 month posthatch. At this time the circuit in NL is almost adult in appearance.

Thus the delay lines acquire a functional appearance towards the end of the first month of life. The maturation of this circuit coincides with the time that the head stops growing, and the bird is no longer subject to changing interaural time differences.(Supported by R29 NS25507 to C.E.C)

363.1

CYCLIC GMP RELEASE AND CAROTID CHEMORECEPTOR INHIBITION PRODUCED BY ATRIAL NATRIURETIC PEPTIDE. L. He*, J. Chen, W.-J. Wang, B. Dinger and S. Fidone. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108.

Recent studies in our laboratory demonstrated the existence of ANP in preneural type I cells in the carotid body and showed that brief exposures to nanomolar concentrations of the ANP fragment atriopeptin III (APIII) induced large increases in the content of cyclic GMP (cGMP) in these cells. Furthermore, 100 nM APIII was shown to inhibit by more than 80% the carotid sinus nerve (CSN) activity evoked by a moderate hypoxic stimulus in vitro (media equilibrated with 20% O_2); but APIII did not significantly alter the stimulus evoked release of catecholamine from the tissue. We have now examined the hypothesis that the CSN inhibition produced by APIII is mediated by cGMP.

Rabbit carotid bodies were superfused in vitro in studies of cGMP release evoked by APIII. RIA measurements of superfusion media collected during sequential 10 min control, stimulus and poststimulus periods showed that basal levels of cGMP released from the tissue (7.62 ± 0.60 fmol/mg tissue; $\bar{X}$± SEM) were elevated by 64.9-fold ± 21.9 in the presence of 100 nM APIII. Also, CSN activity was recorded in vitro to examine the effects of cGMP and the cell-permeant analogue dibutyryl-cGMP (db-cGMP). In six experiments the CSN response evoked by 20% O_2-media was inhibited by 66% ± 6% in the presence of db-cGMP (2 mM). In contrast, cGMP did not alter the response evoked by hypoxia, even when the phosphodiesterase inhibitor isobutylmethyl-xanthine was present in the incubation media. Although APIII evokes a large increase in the generation and release of cGMP, the results suggest an intracellular site of action for the inhibition produced by this cyclic nucleotide second messenger.
Supported by USPHS Grants NS12636 and NS07938.

363.2

EFFECT OF BRIEF HYPOXIC EXPOSURE ON CYCLIC AMP LEVELS IN THE RABBIT SUPERIOR CERVICAL GANGLION IN VITRO. J. Chen, W.-J. Wang, B. Dinger and S. Fidone. Dept. of Physiol., Univ. Utah Sch. of Med., Salt Lake City, UT 84108

Earlier studies by Libet and Owman (J. Physiol. 237: 635, 1974) established that preganglionic nerve stimulation evokes the release of dopamine (DA) from small intensely fluorescent (SIF) cells of the rabbit superior cervical ganglion (SCG). In addition, DA release from these cells is believed to activate adenylate cyclase via D-1 dopaminergic receptors on post-ganglionic neurons. Previous studies in our laboratory (Soc. Neurosci. Abstr. 13: 1674, 1987) demonstrated that incubation of SCG in low-O_2 media increases the content of DA and its metabolite, dihydroxyphenyacetic acid (DOPAC); findings which are consistent with the hypothesis that hypoxia, like preganglionic stimulation, evokes DA release from SIF cells. To further test this hypothesis, we have examined the possibility that hypoxia elevates the content of cAMP in the SCG.

Desheathed SCG were preincubated for 30 to 40 min in modified Tyrode's media equilibrated with 100% O_2 and then exposed for 10 min to 100% O_2-, 10% O_2- or 5% O_2-media. The tissues were then processed by RIA for determination of cAMP. In 100% O_2- media, the cAMP content of SCG was 2.80 ± 0.18 pmole/mg tissue ($\bar{X}$± SEM). Incubation in 10% O_2- and 5% O_2-media elevated the cAMP content to 4.00 ± 0.38 ($p<0.01$) and 4.74 ± 0.19 pmole/mg tissue ($p<0.0005$), respectively. Because the cAMP generated by the action of DA has been demonstrated to modulate slow synaptic potentials in rabbit post-ganglionic neurons, our data suggest that local tissue PO_2 can influence impulse traffic through sympathetic ganglia.
Supported by USPHS Grants NS12636 and NS07938.

363.3

AN IMMUNOCYTOCHEMICAL STUDY OF BIOGENIC AMINES AND NEUROPEPTIDES IN THE HYPOXIC CAT CAROTID BODY. Z.-Z. Wang, B. Dinger, S.J. Fidone and L.J. Stensaas. Dept. of Physiology, Univ. of Utah Sch. of Med., Salt Lake City, Utah 84108

Long-term hypoxia induced by high altitude has been shown to elicit a number of structural and functional changes in chemosensory elements of the carotid body. Current concepts of chemoreception implicate multiple neuroactive agents in chemotransmission. The present immunocytochemical study investigated the effects of chronic hypoxia on biogenic amines and neuropeptides in intact, sinus nerve denervated, and sympathectomized cat carotid bodies. Adult cats were exposed to a hypoxic gas mixture (10% O_2 and 90% N_2) for 2 weeks, and fixed by perfusion. Semithin sections (1 μm thick) of carotid body embedded in Epon were immunostained with antisera to tyrosine hydroxylase (TH), substance P (SP), met-enkephalin (M-ENK) and serotonin (5-HT) using the avidin-biotin-peroxidase method. M-ENK and 5-HT immunoreactivity in type I cells was unchanged following chronic hypoxia, while the high SP level found in 70% of type I cells of normoxic cats was reduced with hypoxia to less than 10%. A similar effect of hypoxia was also consistently seen in chronically CSN-denervated and sympathectomized carotid bodies. Chronic hypoxia significantly increased both the number and intensity of TH immunostained type I cells, an effect slightly attenuated by sympathectomy. However, sympathectomy increased the number of SP and TH positive nerve profiles nearby to type I cells in the hypoxic carotid body. CSN denervation eliminated these profiles. In conclusion, chronic hypoxia had profound effects on catecholamine and neuropeptide stores in the carotid body, findings which suggest their involvement in chemoreceptor adjustments to long-term hypoxia.
Supported by USPHS Grants NS12636 and NS07938.

363.4

EFFECTS OF HYPOXIA ON TYROSINE HYDROXYLASE AND CYCLIC GMP IMMUNOREACTIVITY IN SIF CELLS OF THE RAT SUPERIOR CERVICAL GANGLION. L.J. Stensaas, Z.-Z. Wang, B. Dinger and S.J. Fidone. Dept. Physiology, Univ. Utah Sch. of Med., Salt Lake City, UT 84108

Previous neurochemical studies in our laboratory demonstrated that hypoxic stimulation of superior cervical ganglia (SCG) *in vivo* or *in vitro* reduced the content of substance P, increased the release of catecholamines, and triggered the induction of tyrosine hydroxylase (TH) activity. Parallel studies with the arterial chemosensitive tissue of the carotid body (CB) revealed similar changes which suggested that the SCG may, therefore, contain intrinsic chemosensory mechanisms. The present immunocytochemical study attempted to localize hypoxia-induced changes to specific cellular elements of the rat SCG.

SCGs and CBs were removed from rats either immediately, or 48 hr following a 3 hr exposure to a hypoxic gas mixture (10% O_2 and 90% N_2). TH immunoreactivity in frozen sections stained with the avidin-biotin peroxidase method was found in ganglion cells and in small intensely fluorescent (SIF) cells. Forty-eight hours after hypoxia, the number and intensity of stained SCG SIF cells and chemosensitive CB glomus cells was increased. A less remarkable effect was apparent immediately following hypoxia. For *in vitro* studies of cGMP, rat SCG and CB were incubated in Locke's solution equilibrated with either 100% O_2 or 10% O_2 for 10 min and then fixed. cGMP immunoreactivity was found in SIF cells and glomus cells exposed to 100% O_2 media, but was visibly reduced following hypoxic exposure; postganglionic sympathetic neuron TH and cGMP levels were not altered by hypoxia. The findings that a similar TH and cGMP response to hypoxia occurred both in SIF cells and in glomus cells suggest the possible involvement of SIF cells as chemosensory elements in the SCG.
Supported by USPHS Grants NS12636 and NS07938.

363.5

IMPLANTS OF RAT PERIPHERAL GANGLIA AND CAROTID BODY ONTO THE CHICK EMBRYO CHORIOALLANTOIS. A. Gual, J. Eugenin, J. Alcayaga, V. Gotzems*, L.J. Stensaas and C. Eyzaguirre. Dept. of Physiol., Univ. of Utah Sch. of Med., Salt Lake City, UT 84108.

To study reciprocal influences between arterial chemoreceptors and sensory or sympathetic neurons, we implanted rat carotid bodies, nodose- and superior cervical ganglia, either alone or together, on the chicken chorioallantoic membrane (CHAM). Grafts obtained from 100 g anesthetized rats were placed over the CHAM of 6-12 days chick embryos. After 5-6 days, successful grafts (50-90%) were surrounded by the CHAM and were well vascularized. Light microscopy revealed survival of glomus cells and neurons, hyperplasia of glial cells and proliferation of capillaries. Electron microscopy showed that regenerating axons from nodose ganglia penetrated the carotid body stroma, entered the lobules, and contacted the surface of glomus cells. Immature junctional complexes (membrane densities and synaptic vesicles) between glomus cells and the regenerated unmyelinated axons were identified. Intracellular recordings of nodose ganglion neurons are in progress. Preliminary observations show properties similar to those of normal ganglia. Thus, chorioallantoic grafting may permit the study of interrelations between chemoreceptors and neurons.
Supported by granst NS 05666 and NS 07938. J.E. is a Fogarty International Fellow; J.A. is an O.A.S. Fellow.

363.6

CHEMOSENSORY RESPONSES OF NODOSE GANGLION NEURONS CO-CULTURED WITH CAROTID BODY CELLS. J. Alcayaga and C. Eyzaguirre. Dept. Physiol., Univ. Utah., Sch. Med., Salt Lake City, UT 84108.

Nodose ganglion (NG) neurons and carotid body (CB) cells, enzymatically dissociated and plated on Plys- or FN-coated dishes, were cultured with Ham's F-12 supplemented with 10% FBS, 10% HS, 20mM HEPES and NGF (15 ng/ml). NG neurons, cultured either alone or with CB cells for up to 78 days were recorded intracellularly during superfusion with Earle's solution equilibrated with 95% O_2 and 5% CO_2 (pH 7.35-7.47). Acid solutions, added to the bath or pressure-ejected near the neurons, were used as "natural" chemoreceptor stimuli.

NG neurons had similar passive and active properties in both cultures, but co-cultured neurons had smaller and shorter after-hyperpolarizations. Neuronal spontaneous activity was higher in co-cultures (16%) than in pure nodose cultures (≈3%). Acid applied to pure nodose cultures produced small changes in membrane potential and blocked the spikes evoked by supra-threshold pulses; increased pulse strength restored the response. Similar results were found in 80% of co-cultured neurons, but the rest showed clear acid-related responses. Acid added to the bath induced slow depolarizations up to 45 mV, that returned to the baseline within 3 min with no associated action potentials. Pressure-ejected acid elicited rapid depolarizations, up to 25 mV, increased discharge frequency with either small changes in membrane potential, or a combined response. Depolarizations resembled synaptic potentials, suggesting synapses (morphologically confirmed) near the recordings. Thus, nodose neurons acquire or resume chemosensory properties in the presence of glomus cells probably mediated by synaptic interactions.
Supported by grants NS 05666 and NS 07938.

363.7

ACTIVATION OF SENSORY NEURONS BY HISTAMINE, SEROTONIN AND ATP INCREASES INTRACELLULAR CALCIUM CONCENTRATION. J.F. Fiekers, S.B. Bevan, J.C. Yeats* and H.P. Rang. Dept. of Anat. and Neurobiol., Univ. of Vermont Coll. Med., Burlington, VT and The Sandoz Inst. Med. Res., 5 Gower Place, London, England.

The mechanisms of nociceptor activation and sensitization are believed to originate from receptor activation by endogenous mediators. Calcium ions and intracellular mediators have been implicated in this response. The present study examined the action of histamine (H) serotonin (S) and ATP on fura-2-loaded adult DRG neurons. Calcium measurements were obtained by analyzing the ratio of the fura-2 fluorescence. H (10uM), S (1-10 uM) and ATP (1uM) each caused a marked rise in calcium levels which reached a peak and decayed exponentially to the resting level. When examined in the same neuron responses were obtained to both H and S. Increases in calcium were also obtained in the absence of external calcium. When tested, neurons responding to H and S were responsive to capsaicin. These results demonstrate that increases in intracellular calcium may be involved in the activation and/or sensitization of sensory neurons. Supported by IBRO/UNESCO and NIH (NS27319) grants to JFF.

363.8

PHYSIOLOGICAL STUDIES ON CULTURED RAT SENSORY NEURONS FROM THE PETROSAL AND JUGULAR GANGLIA. A. Stea* and C.A. Nurse. Department of Biology, McMaster University, Hamilton, Ontario L8S 4K1.

As part of our investigations on chemosensory mechanisms in the rat carotid body we are interested in the physiological properties of the chemoreceptor neurons in the petrosal ganglion which supplies the afferent innervation to the carotid body. Whole-cell recordings from dissociated cell cultures of the petrosal and (juxtaposed) jugular ganglia from 6-9 day old pups revealed distinct populations of neurons. With depolarizing voltage steps all neurons produced a transient inward current followed by a prolonged outward current that often contained an inactivating (presumably I_A) component. At least 3 types of inward currents, present in variable proportions, were recorded from 30 cells: a TTX-sensitive Na^+ current; a TTX-insensitive Na^+ current; and a Co^{2+}-sensitive Ca^{2+} current. The mean input resistance was 492 ± 243 Mohms (n = 30). Under current clamp, a few neurons showed multiple spiking whereas the majority responded with only a single or at most a few spikes with depolarizing currents. The fact that several of these neurons survive in long-term co-culture (>40 days) with chemosensory glomus cells of the carotid body provides the opportunity for studying chemosensory mechanisms in vitro. Supported by NIH #1 R01 HL 43412-01.

363.9

PRESENCE OF D-1 RECEPTORS IN THE RABBIT CAROTID BODY. C. Gonzalez, M.T. Pérez-Garcia* and L. Almaraz*. Dept.Fisiol.Fac.Med.Valladolid,(Spain).

The carotid body (CB) contains dopamine (DA) that is released in a Ca^{++}-dependent manner during natural stimulation. Exogenously applied DA behaves as an inhibitory, an excitatory or a neutral agent depending on the experimental conditions. These observations may reflect a preferential action of DA on different sets of receptors. Only D-2 receptors, located in the sensory nerve endings and in the chemoreceptor cells, have been recognized in the C.B.

Working with an in vitro preparation of rabbit CB we found that: 1) DA (10^{-7} to 10^{-4} M) increases C.B. cAMP levels in a dose-dependent manner; 2) + -sulpiride and haloperidol (10^{-6} M) reduced to 50% the cAMP accumulation induced by 10^{-5} M DA; 3) the specific D-1 blocker SCH-23390 (10^{-6} M), inhibited completely the effect of DA, 4) SKF-38393, a D-1 agonists, increases also cAMP and its efect is fully blocked by SCH-23390, and; 5) SCH-23390 does not modify the ongoing release of DA. These findings suggest that, in addition to the well recognized D-2 receptors, the CB contains D-1 receptors that do not appear to be located in chemoreceptor cells. Supported by grant PB86/0325 DGICYT (Spain).

363.10

CHARACTERIZATION OF A Ca PUMP FROM *PARAMECIUM*: PUTATIVE COMPONENT OF CHEMOSENSORY TRANSDUCTION. J.L. Van Houten, M. V. Wright*, and N. Elvess*, Dept. of Zoology, University of Vermont, Burlington, VT, USA 05405

Previously, we presented evidence that implicated a calcium ATPase pump in the hyperpolarizing 0.2 nA current that is an integral part of *Paramecium* chemoresponse (Wright et al., Chem. Senses 14 762-3, 1989). Here we report a surface associated Ca^{2+}-ATPase activity and a corresponding phosphoprotein intermediate characteristic of a pump. The activity requires 3 mM Mg for optimal Ca^{2+} stimulation (K_{Ca}= 90 nM) and is specific for ATP as substrate (K_m=75 uM). Vanadate and calmidazolium inhibit Ca^{2+} stimulated activity with EC_{50}s of ~2 uM and 0.5 uM respectively. 10 uM trifluoperazine or 2 uM melittin inhibit ~80% of activity, but bovine calmodulin fails to stimulate. The Ca^{2+}-ATPase is not inhibited by Na Azide (10 mM), oligomycin (10 ug/ml) or ouabain (0.2 mM). [δ-^{32}]-ATP specifically labels a 133 kDa protein from the surface membranes in a Ca^{2+} dependent, hydroxylamine sensitive manner, and the level of phosphorylation is increased by 100 uM La^{3+}. Similar phosphorylation of an endoplasmic reticulum enriched fraction results in labeling a protein of lower molecular mass, unaffectd by La^{3+}. Ca^{2+} uptake by the alveolar sacs, integral components of the surface membrane complex, is poorly coupled to Ca^{2+} stimulated ATP hydrolysis and is much less sensitive to vanadate inhibition (EC_{50} ~20 uM) compared to the total Ca^{2+}-ATPase activity. Supported by NSF, Whitehall, VRCC.

CHEMICAL SENSES: PERIPHERAL MECHANISMS III

364.1

DETERMINATION OF CELL TYPE IN ACUTELY ISOLATED TASTE CELLS USING QUINACRINE FLUORESCENCE. R.J. Delay, C.J. Ruiz, S.C. Kinnamon, & S.D. Roper. Dept. of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523 and Rocky Mountain Taste & Smell Center, Denver, CO 80262.

Studies indicate that *Necturus* taste buds contain four different cell types:basal (stem), dark, light and Merkel-like cells. Using the patch-clamp recording technique, we have shown that cells isolated from *Necturus* have a variety of voltage-dependent currents and show differential responses to chemical stimuli. In order to correlate physiology with taste cell type, we have developed a fluorescent technique to distinguish taste cell type in the light microscope prior to recording. Stripped lingual epithelium was soaked in 5 X 10^{-6} M quinacrine for 15 min and washed for 20 min prior to our standard cell dissociation procedure (Chem. Senses **13**: 355-66, 1988). Isolated cells were plated onto Cell Tak-coated plastic petri dishes and observed for fluorescence. Two types of staining were observed. Most elongated receptor cells and a subpopulation of small round basal cells showed bright, punctate fluorescence in the cytoplasm. In contrast, a small number of receptor cells and basal cells showed little or no cytoplasmic fluorescence. Following fixation and examination in the electron microscope, we have correlated the presence of punctate fluorescence with membrane-bound granules characteristic of dark cells and membrane-bound vesicles of Merkel-like cells. Elongated receptor cells lacking fluorescence were found to be light cells, and the non-fluorescent small round cells were found to be an undifferentiated cell type, presumably the basal (stem) cell. Preliminary whole-cell patch-clamp recordings show no deleterious effects of the quinacrine fluorescence with short exposures to fluorescence illumination. This technique should prove useful for correlation of structure and function in isolated taste cells. (Supported by N.I.H. grants DC00378, DC00244, & AG06557).

364.2

CHEMICAL SENSITIVITY IN TASTE CELLS OF *NECTURUS MACULOSUS* IS VOLTAGE-GATED. A. R. Bigiani* and S. D. Roper. Dept. of Anatomy and Neurobiology, Colorado State University, Ft. Collins, CO 80523 and Rocky Mountain Taste & Smell Center, Denver, CO 80262.

Studies have shown that *Necturus* respond to a wide variety of chemical stimuli other than sweet compounds as glucose (Chem. Senses **10**: 341, 1985). To investigate transduction mechanisms, we impaled taste cells in thin slices (200 μ) of lingual epithelium and recorded responses elicited by brief pulses of focally applied solutions of KCl (100 mM), citric acid (400 mM), $CaCl_2$ (200 mM) and glucose (400 mM). These chemicals were delivered to the pore of the taste bud from multi-barrelled micropipettes (overall tip dia., 15-20 μ). Only taste cells with stable resting potentials (-40 to -70 mV) and that responded with action potentials to brief depolarizing current pulses (Science **220**: 1311, 1983) were studied. At resting membrane potentials, KCl, $CaCl_2$, and citric acid elicited depolarizing responses that could trigger action potentials in taste cells. These responses did not desensitize with repeated application of the stimulus if a sufficient interval elapsed between chemostimuli. The amplitude of these responses proved to be highly voltage-dependent. The more negative was the membrane potential, the smaller were the receptor potentials. Hyperpolarization below -100 mV abolished receptor potentials. In contrast, glucose did not evoke responses at any membrane potential. A single taste cell typically responded to all chemostimuli (other than glucose). These data indicate that the first step of the chemosensory transduction pathway for KCl, $CaCl_2$ and citric acid perception is a depolarizing response in taste cells. The marked voltage-sensitiviy of chemoresponses is consistent with the interpretation that voltage-gated ion channels, especially voltage-gated K channels, play an integral role in taste transduction. (Supported by N.I.H. grants DC00374, PO NS20486 & AG06557).

364.3

LOCALIZATION OF RECEPTORS FOR EPIDERMAL GROWTH FACTOR (EGF) IN TASTE BUDS OF NEONATAL RATS: EFFECTS OF SODIUM RESTRICTION DURING DEVELOPMENT. R.E. Stewart and D.L. Hill, Departments of Psychology and Neuroscience, Univ. of VA, Charlottesville, VA 22903.

Epidermal growth factor is an important regulator of growth and differentiation in most epithelia. In several tissues, initial biological activity of EGF is noted post-partum, initial receptor expression and ligand availability. Because the majority of taste bud and taste response development occurs postnatally in rats, we sought to establish taste cells as a target for EGF. In addition, we hypothesized that alterations in the expression EGF receptors in Na^+-restricted rats might be correlated with altered peripheral taste function. Frozen sections of tongue from control and Na^+-restricted rats were incubated with EGF-biotin and then with either streptavidin-Texas Red or extravidin-peroxidase for visualization. Analysis of labeled sections revealed the presence of apparently specific EGF binding sites in lingual epithelium and in taste buds. In 2 day old rats of either group, binding of EGF-biotin was rarely detected in taste buds. In contrast, the percentage of taste buds showing EGF-biotin binding was markedly increased in both groups at 6 days of age. Ten day old rats showed profiles of EGF-biotin binding similar to those observed in tongue from 45 day old rats. This time course parallels the appearance of amiloride-sensitive taste responses in rat. At no age examined were any group differences in the percentage of taste buds showing EGF-biotin binding noted. We conclude that EGF could be involved in postnatal taste development, and that differences in the time of EGF receptor expression cannot account for altered peripheral sodium taste function in Na^+-restricted rats. We are currently assessing receptor kinetics and tissue responsiveness to EGF in normal and Na^+-restricted rats. Supported by NIH grants DC00025, DC00407 and HD07323.

364.4

TASTE AVERSION TO INTENSE NATURAL SWEETENERS BY THE GERBIL. W. Jakinovich Jr. and E. Vasquez*, Dept. of Biological Sciences, Herbert H. Lehman College and the Graduate School, City University of New York, Bronx, NY 10468; N.P.D. Nanayakkara*, R.A. Hussain*, and A. Douglas Kinghorn*, Program for Collaborative Research in the Pharmaceutical Sciences, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612.

Last year (Neurosci Abs #302.9, 1989), we reported that the INTENSE NATURAL SWEETENERS stimulated the gerbil's chorda tympani nerve. Since that time, we have conducted conditioned taste aversion studies to determine how the gerbil responds behaviorally to these compounds, such as hernandulcin (Hern), mogroside V (Mogro), periandrin III (Peri), rebaudioside A (Reb-A), and stevioside (Stev). It was observed that gerbils trained to avoid these compounds generalized an avoidance to sucrose but not to HCl. Also, animals trained to avoid Hern, Mogro, Peri, and Reb-A generalized an avoidance to quinine HCl. In addition, animals trained to avoid Hern, Peri, and Reb-A generalized an avoidance to NaCl. Except for the salty and bitter tastes, these results indicate that the INTENSE NATURAL SWEETENERS taste like sucrose to the gerbil as they do in the human. (Supported by grants RO3-DE-07560-01 of NIDRS,NIH, RO1-NS2538-01 of NINCDS,NIH and S06 RR08225 of MBRS/NIH).

364.5

ODOR AND VOLTAGE-DEPENDENT CURRENTS IN CULTURED LOBSTER OLFACTORY NEURONS. D.A. Fadool, W.C. Michel, and B.W. Ache. Whitney Laboratory and Depts. of Zoology and Neuroscience, University of Florida, St. Augustine, FL 32086.

Previously, both excitatory and inhibitory conductances have been identified in the olfactory receptor cells of the Caribbean spiny lobster, *Panulirus argus*. To facilitate voltage clamping and to provide access to the long and thin dendritic processes for single channel recordings of odor activated currents, the olfactory organ cells were placed in sustained primary culture. Following L-cysteine-activated papain treatment (0.25 mg/ml; 50 min) and mechanical disruption, individual cells were plated on poly-D-lysine coated coverslips in L15 media supplemented with salts, serum, and nutrient factors. Neurons rapidly expressed processes and were viable for 3-4 weeks. Four neuronal cell types, with an average soma diameter of 7 to 12 microns, were defined by their number of processes: (1) no visible processes, (2) unipolar with thin process over 30 μ in length, (3) bipolar, and (4) multipolar. The current-voltage relationships of cell types 1-3 were identical to those of cells recorded *in situ*. Both the average maximum outward and inward current of multiprocess cells were only *ca.* 1/3 the amplitude of the other cell types. In whole cell voltage clamp experiments (day 3 - day 10), application of various odors resulted in the generation of rapid (latency < 20msec) inward or outward current with a percentage of all neuronal cell types responding to the applied odorant: TetraMarin fish food extract (100% responded, avg pA; 58.7 inward/22.0 outward), a five component mixture (34% responded, avg pA; 33.4 inward/ 10.3 outward), and proline (88% responded, avg pA; 35.2 inward/ 16.2 outward). These results indicate that cultured lobster olfactory receptor neurons retain their voltage dependent conductances and express odor-activated conductances identical to those seen *in situ*. Supported by ONR N00014-90-J-1566 and NSF 88-10261.

364.6

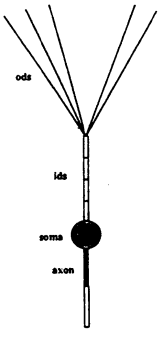
OLFACTORY RECEPTOR CELL OUTPUT IS SHAPED BY SUMMATION OF OPPOSING, ODOR-ACTIVATED CONDUCTANCES. W.C. Michel and B.W. Ache, Whitney Lab. & Depts. Zool. & Neurosci., Univ. Florida, St. Augustine, FL 32086.

Whole cell current clamp recording from lobster olfactory receptor cells shows that odors activate excitatory sodium, calcium or non-selective cation conductances, as well as an inhibitory potassium conductance. Excitatory and inhibitory conductances occur on the same receptor cell. To determine if opposing conductances shape the output of the cell, we examined the effect of increasing concentrations of inhibitory odorants on the magnitude, rate, and duration of excitation. Inhibitory odorants attenuated the magnitude of odor-evoked depolarizations in a dose dependent manner with a threshold below 1 μM. Inhibitory odorants slowed the rate of depolarization, which delayed the peak of the response as long as 1000 ms (average *ca.* 100 ms). Inhibitory odorants truncated odor-evoked depolarization; cells with opposing conductances depolarized in a characteristic phasic/tonic manner, which changed to a strictly tonic response in the absence of inhibitory odorants. Corresponding effects were observed in extracellular recordings. Olfactory receptor cells in the lobster, at least, are not just selectivity filters but must be viewed as the first level of olfactory integration. This research was supported by awards from the ONR and the NSF.

364.7

THE ELECTROTONIC STRUCTURE OF LOBSTER OLFACTORY RECEPTOR CELLS: A COMPARTMENTAL MODEL. F.Pongracz*, T.S. McClintock, B.W. Ache and G.M. Shepherd. Sec. Neuroanatomy and Sec. Molecular Neurobiology, Yale Univ. Sch. Med., New Haven, CT 06510 and Whitney Lab., Univ. Florida, St. Augustine, FL 32086.

The electrotonic structure of lobster olfactory (antennular) receptor cells was evaluated in a compartmental model derived from EM reconstruction (Cell Tiss Res, 251:95, 1988) using SABER simulation software. Values for R_N (1.6 GΩ) and T_m (100 msec), but not T_i (11 msec), determined from whole-cell recordings, could be represented in models with a uniform R_m only by assuming $C_m \geq 1.5$ μF/cm². Selectively increasing R_m in the outer dendritic (ods) compartments, however, had little influence on R_N while markedly decreasing the electrotonic length (L). In vivo determinations were best represented with R_m in the ods compartments between 120-150K ohm.cm² and R_m in the inner dendritic (ids), soma and axon compartments between 40-50K ohm.cm², holding C_m = 1μF/cm². The total value for L was 1.5. Calculations of current spread in the model cell indicate that 1.6 nS total input (e.g., 160-10 pS channels) applied equally to all ods compartments depolarizes the soma compartment 32 mV, a value consistent with whole-cell odor evoked depolarizations (J. Neurophysiol, 61:994, 1989). The model suggests that receptor potentials arising in the ods could passively invade a spike generator in the initial segment, in spite of the extreme ods-soma distance (1 mm) of these cells.



Supported by awards from the ONR (to BWA and GMS) and the NIH (to TSM).

364.8

SPATIAL INFORMATION CONTAINED IN THE THREE-DIMENSIONAL FINE STRUCTURE OF AN AQUATIC ODOR PLUME. Paul A. Moore, Nat Scholz, Jelle Atema, and Greg A. Gerhardt (B.U.M.P., Marine Biological Laboratory, Woods Hole, MA 02543 Departments of Psychiatry and Pharmacology, University of Colorado Health Science Center, Denver CO 80262)

Odor plumes serve as sources of information for many animals during chemically mediated orientation. However, the information contained within the odor plume is poorly understood due to inadequate stimulus detection techniques. Using high speed electrochemical recording techniques, we have quantified the three dimensional structure of an aquatic odor plume within a laboratory flume. The plume was created in a uni-directional seawater flume (90 x 250 x 20 cm). Odor profiles were recorded for three minutes at 10 Hz from 63 different sample sites. Analysis showed that spatial gradients of odor pulse parameters point to the source. Specifically, the pulse height and slope values increase towards the source. We conclude that the type of information available to the orienting animal depends upon the chemoreceptor filter properties.

Supported by USPHS (AG06434 and AG00441) to GAG and NSF (BNS 90-12952).

364.9

MEMBRANE PROPERTIES OF FROG OLFACTORY RECEPTOR NEURONS.
S. Kleene*, R. Pun, & R. Gesteland, Anatomy & Cell Biology, Univ. of Cincinnati Medical Center, OH 45267.

In isolated frog olfactory receptor neurons it is difficult to elicit an action potential except after hyperpolarizing the cell. We are studying the cellular mechanisms underlying this lack of excitability. With whole-cell recording under voltage clamp, the threshold for eliciting a fast inward current is about -40 mV. The current peaks near -20 mV. The voltage for half-inactivation is -81.8 ± 3.4 mV (range -72 to -98 mV; $n=8$). This is about 30 mV more negative than the resting membrane potential. The voltage dependence of decay for the fast inward current was studied using Cs^+ and TEA^+ in the recording pipette to block outward currents. Under these conditions, a slower inward current occasionally follows the fast inward current. The threshold for the slow current is about -20 mV; it peaks near 0 mV. This current runs down, disappearing about 10 min after establishing whole-cell recording. The fast inward current is unaffected by rundown. The decay of the fast inward current has a time constant of 1.63 ± 0.19 msec at -20 mV and 1.10 ± 0.14 msec at 0 mV ($n=5$). The fast inward current is strongly blocked by tetrodotoxin (0.5-1.0 μM), indicating that this current is mediated by voltage-dependent Na^+ channels.

Currents of single cilia in isolated neurons are measured by drawing one of the cilia into a pipette and forming a seal near the base of the cilium. All experiments are done under voltage clamp. At rest, few cells produce spontaneous action potentials, but injecting current through the cilium often elicits spikes. About 15% of the cells respond to an odorant mixture. The pipette containing the cilium can be excised from the cell and immersed in a pseudointracellular solution. Addition of 10 μM cAMP to the bath causes a large increase in the ciliary membrane conductance, indicating that the cytoplasmic face of the membrane is exposed to the bath. This conductance increase is reversible. Adding Ca^{2+} to the bath increases the ciliary conductance and inhibits the response to cAMP.

Supported by NIH NS23523 and DC00347.

364.10

ANTIBODY IDENTIFICATION OF CELL TYPES IN THE AMPHIBIAN OLFACTORY EPITHELIUM: DIFFERENCES ACROSS SPECIES.
M.J. Crowe and S.K. Pixley. Dept. of Anatomy & Cell Biology Univ. of Cincinnati, Cincinnati, OH 45267.

To facilitate studies of the amphibian olfactory epithelium (OE), in particular the electrophysiological properties of olfactory receptor neurons (ORNs), it would be useful to have cell-type-specific antibody markers. However, immunostaining specificity differs drastically across amphibian species. We report here on immunostaining with a monoclonal antibody (Mab) directed against carnosine synthetase (CS) (from Dr. F. Margolis) and a novel Mab (1D9) developed by M. Crowe. Staining was done on cryostat sections of paraformaldehyde-fixed OE from bullfrog (Rana catesbiana), grass frog (Rana pipiens) and salamander (Ambystoma tigrinum). Anti-CS labelled ORNs in bullfrog and grass frog OE, but showed no specific staining in salamander OE. This distribution of synthetic enzyme follows the biochemical localization of carnosine in OE from these species as determined by Dr. F. Margolis. Mab 1D9, in frog tissues, appears to label all cells in the OE, nerve bundles and Bowman's glands, but did not stain other lamina propria cells. In contrast, in the salamander, Mab 1D9 identified a subset of basally located epithelial cells resembling ORNs. These data show the usefulness of these antibodies and support a concept of biochemical differences between species.

Supported by NIH NS23523 and DC00347.

364.11

PERIPHERAL OLFACTORY GLIA IN DISSOCIATED CELL CULTURE ARE IMMUNOPOSITIVE FOR S100, GFA, 217C AND NSE.
S.K. Pixley. Dept. Anat. & Cell Biol., Univ. of Cincinnati Coll. Med., Cincinnati, OH 45267.

Peripheral olfactory glial cells ensheath olfactory receptor neuron (ORN) axons between the olfactory epithelium and the olfactory bulb, and they may participate in regeneration of ORN axons. We confirm glial immunostaining for S100, a Schwann cell-specific protein, and glial fibrillary acidic protein (GFA) in adult Spraque-Dawley rat nasal tissue sections. We further report that, in dissociated cell cultures of newborn rat nasal tissues, glial cells were immunopositive for S100 or GFA. They were also immunostained by the monoclonal antibody 217C, which binds to NGF receptors (Kumar and de Vellis, Neurochem. Abs., p.158, 1990). Glial cells in serum-free medium, compared to those in serum-containing medium, were more frequently bipolar, had longer and thinner processes and more often had beaded processes. Immunostaining with an antiserum specific for neuron-specific-enolase (NSE) showed some staining of cultured olfactory Schwann cells although cultured ORNs were more densely stained. Cultures of olfactory glia will be useful in studying ORN and glial cell physiology. This study also illustrates some problems in ORN identification in culture. {Supported by Amer. Paralysis Assoc. Grant PB1-8803-1}.

364.12

2-MERCAPTOETHANOL PROMOTES THE SURVIVAL OF RETROGRADELY LABELLED OLFACTORY RECEPTOR NEURONS IN VITRO.
R. J. Grill and S. K. Pixley. Dept. of Anat. and Cell Biol., Univ. of Cincinnati, Cincinnati, OH 45267.

A primary culture system of olfactory receptor neurons (ORNs) has been developed in which ORNs were characterized by immunocytochemistry and the whole cell 'tight-seal' recording technique (Pixley, S.K. and Pun, R., Dev. Brain Res., 53:125, 1990). Cultured ORNs responded to odors. Here we describe further identification of ORNs and a culture medium which enhances survival of ORNs. Rhodamine-labelled latex microspheres were injected into newborn Sprague Dawley rat olfactory bulbs. Two days were allowed for retrograde transport of microspheres to the ORNs, after which the nasal tissues were taken for culture. ORNs containing microspheres were seen in culture and were electrically active. The culture medium which supported survival of these ORNs varied from other neuronal growth media in that it contained 2-mercaptoethanol (2ME). We have determined that 2ME is a significant survival enhancing factor for cultured ORNs. These ORN cultures should prove useful for studies of neuronal regeneration and the mechanisms of olfactory transduction. (Supported by American Paralysis Association Grant # PBI-8803-1).

364.13

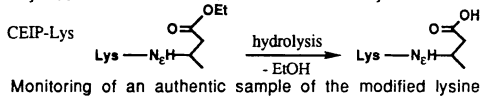
ISOLATION AND MOLECULAR CLONING OF A TISSUE SPECIFIC MEMBRANE PROTEIN WHICH IS A MAJOR COMPONENT OF OLFACTORY DENDRITES AND WHICH POSSESSES ODORANT BINDING PROPERTIES.
R.G. Vogt and M.R. Lerner†. Molecular Neurobiology, Yale Univ. Sch. of Med., P.O. Box 3333, 333 Cedar Street, New Haven, CT 06512.

Odorant detection is thought to be mediated through receptor proteins associating with the membrane of olfactory cilia/dendrites, though the isolation of such proteins has been elusive. A 69 kDa protein of the silk moth *Antheraea polyphemus* was previously implicated as an olfactory receptor protein: it was a component of the olfactory dendrite membrane; it could be labeled with a photoaffinity analog of a sex-pheromone odorant, and labeling could be blocked by sex-pheromone; it was specific to olfactory tissue (Vogt, R.G. et. al., *J. Biol. Chem.*, 263:3952, 1988). *In vivo* labeling with ^{35}S-methionine has subsequently shown that a 69 kDa protein is a major component of olfactory dendrite membranes in two moth species, that it is absent from other neuronal tissue, and that it is developmentally expressed at a time coincident with other olfactory proteins. We isolated olfactory dendrite membranes from 800 male *A. polyphemus* antennae, and electrophoretically purified the 69 kDa protein in sufficient quantity to obtain a partial N-terminal amino acid sequence. We designed PCR primers to the N-terminus, and synthesized, purified, cloned and sequenced a 90 base PCR product corresponding to this region. We then synthesized a 65 base oligonucleotide which was used as a probe for Northern blot analysis of mRNA. Specificity to olfactory tissue was confirmed by both PCR and Northern blot analysis. We then successfully used this probe to screen oligo-dT and random primed cDNA libraries generated against antennal mRNA. The properties of the 69 kDa protein will be presented, with respect to tissue distribution and deduced amino acid sequence.

364.14

IRREVERSIBLE RADIOLABELING OF SCHIFF BASE-FORMING MEMBRANE PROTEINS IN THE OLFACTORY EPITHELIUM OF TIGER SALAMANDERS(*Ambystoma tigrinum*).
L.E. Grishow,* M.G. Kelcher,* and T. H. Morton. University of California, Riverside, CA 92521.

Specific odor blindness results from irreversible covalent modification of the olfactory epithelium. Replication of this procedure in vivo with tritiated ligand shows significant incorporation of non-dialyzable radiolabel. Lavage of salamander olfactory sacs with [1,2-^{3}H]-ethyl acetoacetate followed by $NaBH_3CN$ yields greater ($p<0.0002$) incorporation of radioactivity versus a control without $NaBH_3CN$. In vitro labeling of isolated membrane fractions and in vivo labeling of intact tissue are both specific for olfactory epithelium, as compared to buccal epithelium, brain, and tongue membranes, which show no significant radioactivity in the experimental (w/$NaBH_3CN$) versus control (w/o$NaBH_3CN$) fractions ($p>0.15$). Partial purification of detergent-solubilized membrane protein yields a fraction that can still be irreversibly labeled.



Monitoring of an authentic sample of the modified lysine unit (CEIP-Lys) by NMR shows that hydrolysis of the ethyl ester is pH-dependent and can be fit to the rate expression $k=k_0k_1[OH^-]/(k_0+k_1[OH^-])$, where $k_0=.005min^{-1}$ and $k_1=10^3M^{-1}\ min^{-1}$. Modified protein appears to lose radiolabel by hydrolysis when denatured, but has greater stability when undenatured.

364.15

AUTOLOGOUS OLFACTORY EPITHELIAL TRANSPLANTS IN MICE: A SYSTEM FOR EXAMINING EFFECTS OF LOCAL FACTORS ON THE PROCESS OF NEURON RENEWAL. M.A. Schwartz, C.B. Heilman and J.S. Kauer. Neurosci. Program, Tufts/NEMC, Boston, MA 02111.

Little is known about the regulation of olfactory neuron renewal which occurs by differentiation of the epithelial basal cells. To determine whether local, diffusible factors are involved, autologous olfactory epithelial transplants were made into contralateral mucosae of C57BL/6J mice. Transplants consisted of epithelium taken 4 days after unilateral bulbectomy (4-Bx), unoperated control epithelium (0-Bx), and frontalis muscle (M). Transplants were placed between the cartilaginous septum and lamina propria on the undisturbed side and examined at 4 and 11 days. BrDU labelling (BrDU-I), using injections 1 hour prior to sacrifice, was used to examine cell proliferation. In the 4-Bx condition, there was no change in BrDU-I in the recipient epithelium, but the transplant showed the normal heavy BrDU-I of bulbectomized tissue, suggesting continued ability to proliferate. In the 0-Bx condition, there was increased BrDU-I in the recipient epithelium and normal BrDU-I was seen in the transplant. These data suggest that the 0-Bx transplant may have proliferative signals not present in the 4-Bx condition. Sham operations and M transplants indicated these effects were not due to surgical trauma. Examination for the expression of cytokeratin (CK) showed both 4-Bx and 0-Bx transplants and recipient epithelia were CK+, indicating the persistence of horizontal basal cell populations. In the 4-Bx transplant, GAP43+ fibers were seen penetrating into the recipient epithelium, with no change in GAP43 staining in the 0-Bx condition. Taken together, these findings suggest that different local signals may expressed during the degeneration-regeneration process observed in the olfactory system.

Supported by grants from NIH, Pew Freedom Trust and Dept. of Neurosurgery.

SOMATIC AND VISCERAL AFFERENTS III

365.1

DISTRIBUTION AND ULTRASTRUCTURE OF SPLANCHNIC VISCERAL AFFERENTS AND EFFERENTS IN THE RAT THORACIC SPINAL CORD. S. E. Kapadia , C. M. Kocol, and C. C. LaMotte. Section of Neurol. Surgery, Yale Univ. Sch. of Med., New Haven, CT. 06510.

The greater splanchnic nerve of rats anesthetized with 40mg/kg pentobarbitol was injected with a combination of 0.5 mg of WGA-HRP and .05 mg bCT-HRP. Spinal sections from T4-T11 were cut on a vibratome and reacted using a TMB method for LM and an AHM (ammonium heptamolybdate) method for EM; EM sections were also stabilized with DAB. Visceral afferents (VA's) were found in a distinct bundle in Lissauer's tract, and terminals were located in lamina I, where they occupied distinct, superficial outcroppings of the medial and lateral margin of the dorsal horn. VA's also terminated in lamina X dorsal to the central canal or adjacent to the ependyma, and ocassionally in the contralateral lamina X or intermediate grey. Terminals in lamina I were of several types: 1) dark, sinuous glomerular terminals, with both large dense core vesicles and large clear vesicles, which contacted many dendrites; 2) simple terminals with clear and dense core vesicles; and 3) simple terminals with mainly clear round vesicles. The last two types were also found in lamina X. Labelled preganglionic splanchnic motoneurons were seen in the intermediolateral (IML) and intercalatus (IC) nuclei. Often their dendrites extended to lamina X; these dendrites received few synapses before reaching lamina X, where they became finely branched and were contacted by many terminals. (NIH grant NS13335)

365.2

INNERVATION OF THE PYLORIC REGION OF THE STOMACH IN THE BRAZILIAN OPOSSUM. J. K. Elmquist, C. A. Fox, and C. D. Jacobson. Dept. of Vet. Anatomy, Iowa State Univ., Ames, Iowa 50011.

Vagal afferents from the pyloric region of the stomach (PRST) are thought to be involved in the regulation of food intake. In this study, we have used DiI to trace the innervation of the PRST in the Brazilian opossum, Monodelphis domestica. DiI was injected intramurally into the PRST. After 24 hours, animals were killed and perfused with 4% paraformaldahyde. Sections of the brain, stomach and nodose ganglion (NDG) were viewed with a fluorescence microscope. DiI labeled cell bodies were found in the NDG, and the motor nucleus of vagus. Other neuronal elements (fibers, and terminals) containing DiI were found in the nucleus of the solitary tract (Sol) and lateral reticular nucleus (LRt). In addition to labeling in the brainstem, DiI containing structures were located in the paraventricular (Pa) and ventromedial (VMH) nuclei of the hypothalamus. In order to verify specific DiI labeling in the vagal afferents to the stomach a group of animals were exposed to DiI as above. However, prior to injection of the dye the gastric branches of the vagus nerve were severed as they ran along the terminal part of the esophagus. Fluorescence microscopy of tissue obtained from these animals did not yield any labeled structures in the regions stated above. Consequently, these results indicate that vagal afferents from the stomach communicate with neurons in the Sol, LRt, Pa and VMH. Two possible mechanisms can explain the hypothalamic staining patterns observed: 1- There are direct neuronal projections from the stomach to the Pa and VMH of the hypothalamus. 2- The DiI can label neurons trancellularly. Further studies are underway to explain these findings. These results support the use of this animal model in studies aimed at describing the ontogeny of the neuronal circuitry involved in control of food intake. Supported by Iowa State University, NSF grant BNS 8909751 and USDA Formula Funds Section 1433.

365.3

EFFECTS OF URINARY BLADDER DISTENSION (UBD) AND SOMATIC INPUT ON VENTROPOSTEROLATERAL (VPL) THALAMIC CELLS IN PRIMATES. M.J. Chandler, S.F. Hobbs, Q.-G. Fu*, D.R. Kenshalo, Jr., R.W. Blair, R.D. Foreman. Univ. of Okla. Hlth. Sci. Ctr., Okla. City, OK 73190. NIH-NIDR, Bethesda, MD 20892.

Urinary bladder distension (UBD) increases activity of some lumbosacral spinothalamic tract (STT) neurons antidromically activated from the VPL nucleus of the thalamus (Milne et al., 1981). These same cells receive convergent excitatory somatic input from lower-body regions. The purpose of this study was to determine effects of visceral input from UBD on activity of neurons located in VPL thalamus. We hypothesized that UBD would excite VPL neurons that were excited by stimulation of skin and muscle of the lower body. Extracellular action potentials of 27 cells were recorded from the right VPL thalamus in 11 monkeys (Macaca fascicularis) anesthetized with alpha-chloralose. UBD excited 9 VPL cells, inhibited 4 cells and did not affect activity of 14 cells. Eight of 9 VPL cells excited by UBD also were excited by stimulation of lower-body somatic fields. Five VPL neurons were activated antidromically from the SI cortex. Activity of 3 of these cells was affected by visceral input from UBD. These results showed that (1) stimulation of visceral afferent input by UBD affected activity of neurons located in VPL thalamus, (2) VPL cells affected by UBD also were excited by input from skin and muscle, and (3) visceral input stimulated by UBD reached the SI cortex. (Supported by NIH grant HL22732)

365.4

IMMUNOCYTOCHEMICAL CHARACTERIZATION OF NEUROPEPTIDES WITHIN THE AFFERENT RENAL NERVE OF THE RAT. M. Burg*, D.S. Zahm, M.M. Knuepfer, Dept. of Pharmacol., Anat. & Neurobiol. St. Louis Univ., St. Louis, MO 63104.

We have shown that afferent renal nerves (ARN) in the rat contain only unmyelinated and small myelinated axons. Although the function of different types of ARN fibers is not clear, small caliber fibers in other nerves often mediate nociception. Nerve fibers within the renal pelvis contain substance P and calcitonin-gene-related peptide (CGRP) and are suggested to be sensory. This study was designed to determine directly whether substance P, CGRP and/or vasoactive intestinal polypeptide (VIP) exist within ARN. Fluoro-Gold was placed on the central cut end of the renal nerve 3-4 days before intrathecal application of colchicine (25 μg at T10). 24 hours later, rats were perfused for immunocytochemical localization of substance P, CGRP and VIP in dorsal root ganglionic soma (T9-L1). Fluoro-Gold labelling was observed only in soma of the ipsilateral ganglia. Neurons were located primarily at T12 and T13, were generally small in diameter (20-40 μm) and were not numerous (less than 10% of all neurons). Substance P, VIP or CGRP immunofluorescence was observed in a number of Fluoro-Gold labelled neurons. These results indicate ARN fibers project predominatly to ipsilateral dorsal roots at spinal levels of T12 and T13 and that specific neuropeptides are present in many of these fibers. The presence of substance P in small diameter fibers suggests the possibility that these axons mediate nociception. (Supported by HL38299 and NS23805).

365.5

NON-PAINFUL SENSATIONS EVOKED BY THERMAL STIMULATION OF TEETH IN MAN. B.Matthews, W.Kollmann*, J.E.Crapper* and A.S.Walters*. Dept. of Physiology, University of Bristol, England BS8 1TD.

Much of the available evidence indicates that pain is the only sensation which is produced in man by natural stimuli which excite nerves in teeth, although electrical stimulation close to threshold is known to produce non-painful sensations. The present experiments were carried out to investigate the sensations evoked by thermal stimuli close to sensory threshold.

Stimuli in the range 5 to 60°C and 10s in duration were applied by switching the flow of saline from thermostatically-controlled reservoirs through a small chamber (o.d. 6mm, i.d. 4mm) which was cemented to the enamel over the centre of the labial surface of the crown of a maxillary central incisor. Saline at 30°C was used as control. The temperature in the chamber and that of the adjacent gingival margin were recorded with thermocouples. Six different stimuli were each applied twice in random order. The subjects were told that stimuli might be a change in temperature, pressure or chemical composition of the solution in the chamber. Apart from effects due to changes in tooth temperature, they had no cue as to when stimuli were applied. The subject indicated the onset and time-course of any sensation perceived with a push-button and visual-analogue scale. After each stimulus, the subjects identified the sensations from a list of descriptors.

In 10 subjects, stimuli of 5-10°C or 50-60°C evoked responses in up to 100% of applications, but no more than 50% were described as uncomfortable or painful. The other sensations were difficult to describe and most frequently attributed to cold stimuli, even when they were hot. Stimuli of 20 and 40°C were detected in up to 40% of applications and were rarely uncomfortable. Such stimuli are within the range likely to occur during eating and drinking and indicate that intradental nerves are often activated under physiological conditions. The results demonstrate that sensations other than pain may be produced by thermal stimulation of teeth.

365.6

SEGMENTAL DISTRIBUTION AND CENTRAL PROJECTIONS OF CLITORAL AFFERENT PATHWAYS OF THE CAT. M. Kawatani and W.C. de Groat. Depts. Pharmacol. and Behavioral Neuroscience, Univ. of Pittsburgh, Pgh., PA 15261

Axonal tracing techniques were used to label the afferent pathways to the clitoris of the cat. Under halothane anesthesia 5 µl of 1%, WGA-HRP was injected into the clitoris. After 5 days for transport of tracer anesthetized animals were perfused with fixative and tissues were removed and processed for HRP using the TMB method. An average of 650 neurons were identified in the lumbosacral dorsal root ganglia (DRG) with the majority of cells in the S1-S2 DRG. The average cell size was 2280 μm^2. In the spinal cord HRP reaction product was present in fibers passing through lamina I on the medial edge of the dorsal horn into the dorsal gray commissure (DGC). Very little HRP reaction product was present in Lissauer's tract or on the lateral edge of the dorsal horn. Labeled axons in medial lamina I and the DGC occurred in bundles, averaging 70 µm in width and occurring at approximately 200 µm intervals in the rostrocaudal axis. No labeled neurons were identified in the spinal cord. In conclusion, the clitoris is innervated by a large number of afferent neurons which project to a discrete region of the medial spinal cord. This central projection contrasts with that from the uterine cervix which terminates in the lateral dorsal horn.

365.7

CENTRAL REPRESENTATION OF PERIPHERAL INNERVATION TO THE LOWER EXTREMITY: TRANSGANGLIONIC HRP STUDY IN DORSAL COLUMN AND ADJACENT PRECEREBELLAR NUCLEI OF CAT AND RAT. T. Ueyama, T. Houtani*, M. Ikeda* and T. Sugimoto. Dept. of Anatomy Kansai Medical Univ., Osaka 570 Japan.

Primary afferents from the skin of lower extremity were traced in the dorsal column nuclei and in a novel precerebellar cell group of the cat and rat. HRP was applied to the central cut end of the lateral femoral cutaneous, posterior femoral cutaneous, saphenous, genitofemoral superficial peroneal, plantar, sural and pudendal nerves. In the cat, afferent fibers containing large-sized terminal knobs were densely distributed to the cell nest region of the ipsilateral gracile nucleus. Each nerve terminal was arranged in this sequence with a lateromedial direction. In the gracile reticular region such a topographic distribution could not be detected. In contrast, the middle region of rat gracile nucleus showed an overlapping, lateromedially-directed arrangement of plantar, superficial peroneal, saphenous and pudendal nerve terminals. In the cat we identified a novel cell group with HRP-labelled afferents from the lower extremity. This compact neuronal collection was located medial to the external cuneate nucleus and lateral to the descending vestibular nucleus, and was here shown to project to the cerebellar cortex. In the rat a homologous cell group, though organized by a fewer neurons, was also identified at the medial border of the external cuneate nucleus. The results suggest different primary afferent organization in feline and rodent gracile nuclei, and emphasize the presence of a medullary station to convey inputs from the lower extremity to the cerebellum.

365.8

EXPRESSION OF "CLASSICAL NEUROTRANSMITTERS" BY PRIMARY SENSORY NEURONS OF THE CHICK DORSAL ROOT GANGLIA. **E. PHILIPPE, C. ZHOU*, M. GEFFARD and M. DESCHENES**. Neurobiology Res. Center and Laval University, 1401, 18e Rue, Quebec - CANADA and IBCN-CNRS -Bordeaux - FRANCE.

Neurons of the dorsal root ganglia (DRG) subserve a great variety of sensory modalities and subpopulations of these cells appear to contain different neurotransmitters including neuropeptides, amino acids and catecholamines.

Among the amino acids which could be considered as putative neurotransmitters, gamma aminobutyric acid (GABA) was immunocytochemically detected in ganglion cell bodies belonging to 2 subclasses of sensory neurons. These 2 subclasses were related, at least in part, to the peripheral sensory innervation of the skin and the striated skeletal muscle. Moreover, by using polyclonal antibodies raised against dopamine, a small percentage of sensory neurons expressed a light immunoreactivity. These immunostained cell bodies were mainly related to a subclasse of large A sensory neurons. In contrast, after immunostaining performed with antibodies raised to serotonin, the few immunostained sensory neurons were mainly related to a subpopulation of small B neurons.

Thus, the characterization of "classical neurotransmitters" in the sensory neurons and their related peripheral target tissues allowed a better understanding of the various sensory modalities. The fact that these classical neurotransmitters constitute only a small proportion of neuronal cell bodies suggest that there may be considerable heterogeneity among the sensory neurons with regard to both neurotransmitters and peptide content. (Supported by grants of C.R.M. and F.R.S.Q.).

365.9

A LIGHT MICROSCOPIC STUDY OF INNERVATION OF THE LUMBAR FACET JOINT CAPSULE. AC Ozaktay*, T Yamashita*, JM Gavanaugh, AI King*, Bioengineering Center, Wayne State University, Detroit, MI 48202.

The innervation of the facet joint capsule of the human lumbar spine was investigated using high-power light microscopy. The description and localization of nerve fibers are presented. **MATERIALS and METHODS**: Four lumbar facet joint capsules were obtained from three fresh adult cadavers. Two capsules were from L4/L5, one from L3/L4, and one from L5/S1. The specimens were treated by using a silver impregnation method for whole mount (Winkelman, Staff Meet., Mayo Clin., 32; 217-222, 1957) and 70 µm thick frozen sections were made and mounted on slides. They were examined at magnifications of 40 to 1000. **RESULTS**: Many nerve fibers with small diameters were found in the facet joint capsules. 76.9 % of the nerve fibers had diameters of 0.5-2 µm, 17.6 % had diameters of 2-5 µm, and 5.5 % had diameters of 5-10 µm. A majority of the fibers were found in the latero-inferior area of the capsule close to the inferior articular recess. 68.5 % of the nerve fibers were in the deeper capsular layers which consist mainly of elastic connective tissue. Also, free nerve endings and encapsulated nerve endings were seen, primarily in the elastic fiber layers of the capsules.

Supported by a Whitaker Foundation Grant (JMC), NIH Grant NS28994 (AIK, JMC), and WSU Research Stimulation Fund (TY).

365.10

INNERVATION PATTERNS OF COLLATERAL KNEE LIGAMENTS AS REVEALED BY SILVER STAINING AND IMMUNOHISTOCHEMISTRY. K.A. Sharkey and R.C. Bray. Departments of Medical Physiology and Surgery, University of Calgary, Calgary, Alberta, Canada.

Articular ligaments are known to contain neural elements responsible for proprioception and nociception. However, little is known about the pattern and distribution of nerve terminals in discrete ligaments. Collateral ligament from adult rats, rabbits and human cadavers were fixed and frozen sections were stained with a modified Bielshowsky-Gros technique or for substance P- and CGRP-immunoreactivity (IR). In all species nerve bundles entered the ligaments obliquely and became longitudinally oriented in the deep tissue. The commonest nerve termini identified by silver staining were free endings arising from myelinated and unmyelinated intraligamentous nerves. Encapsulated endings (e.g. Golgiform, and Ruffiniform) were less frequently observed, but when present were usually found superficially, near ligament attachments to bone. Nerve bundles entered the tissue with blood vessels and often form multiple "tangles" of unmyelinated fibres which become intimately associated with branching vessels deep in the ligament. Substance P- and CGRP-IR together mirrored the pattern of unmyelinated fibres identified by silver staining and were predominately found associated with blood vessels. The structures described above could provide proprioceptive and nociceptive input from ligament. The predominant association between nerves and blood vessels suggests important vasomotor functions previously unrecognized in this tissue.

365.11

INTRACELLULAR NEUROBIOTIN INJECTION: A NEW METHOD FOR RAPID STAINING OF THE LONG-RANGE PROJECTIONS OF FUNCTIONALLY DIVERSE TRIGEMINAL GANGLION CELLS. J.W. Hu*, B.J. Sessle, & M.F. Jacquin. Fac. of Dentistry, Univ. of Toronto, Toronto, Ontairo M5G1G6 & Dept. of Anat. & Neurobiol., St. Louis Univ. Sch. of Med., St. Louis, MO 63104.

Currently available methods for study of the morphology of physiologically characterized primary afferents are limited by 2 factors: it is very difficult to impale and stain thin fibers, and staining is restricted to, at best, 0.5 cm from the region of the injection site. Sugiura et al., (Science 234:358, '86) have shown that intracellular PHA-L injections are effective in long-range staining of the projections of unmyelinated spinal primary afferents; however, long survival times are required. We have encountered an alternative method that overcomes the above limitations. Intracellular injection of Neurobiotin (Vector Labs) into rat trigeminal ganglion cells provided long-range staining with 10-55 min. of depolarizing injection currents of 6-10 nAmp (2 Hz, 250ms pulses) through Microstar capillaries beveled to 50-80 mOhms. Pipettes contained 2% Neurobiotin in 1.0M KCl. Rats were perfused with 4% paraformaldehyde within 4.5-10 hr. of tracer injection and brainstems were processed by avidin-biotin immunohistochemistry. Reliable axonal labeling in the medulla occurred in 5 cases attempted to date. Three were skin-or-hair-responsive A-delta fibers with relatively thin axons, and 2 were whisker-sensitive A-beta fibers. In all cases the parent axon was stained in the V spinal tract to the level of the obex. Collaterals were visible at the level of nucleus principalis and each of the spinal V subnuclei. Support: DE07662, DE07734 and DE04786.

365.12

HERPES SIMPLEX VIRUS (HSV) INFECTION OF MOUSE DORSAL ROOT GANGLIA (DRG): EFFECTS OF INOCULATION ROUTE AND VIRUS STRAIN. D.B. Henken & J.R. Martin, Laboratory of Experimental Neuropathology, NIH, NINDS, Bethesda, MD, 20892.

DRG neurons can be divided into two populations based on morphology, connections and peptide content: smaller diameter neurons contain peptides (e.g., calcitonin-gene related peptide (CGRP)) and larger diameter neurons do not. We examined whether HSV-2 strains differing in virulence preferentially infect one or the other of these populations following unilateral peripheral (footpad) or intraneural (sciatic nerve) inoculation. Anaesthetized female BALB/c mice were infected with one of three HSV-2 strains: 186, MS or HG52 (descending order of virulence); five days later they were perfusion-fixed. Vertebral columns, with associated spinal cords and DRG, were decalcified and paraffin sections immunoreacted to detect HSV-2 antigen. Adjacent sections were processed for CGRP-immunocytochemistry. Somal areas for all HSV- and CGRP-labelled and unlabelled neurons in the infected DRG were compared with the somal areas of the contralateral uninfected DRG. With footpad inoculation, 9.5% of DRG neurons were HSV-antigen positive with 186, 7.7% were positive with MS strain and thus far labelling has not been detected with HG52. Labelled HSV-positive cells were in the category of smaller DRG neurons. With the sciatic nerve inoculation model, 80% of DRG neurons were antigen-positive with MS. Labelled HSV-positive cells were not confined to the small cell range. In all cases, equal numbers of CGRP-positive neurons were seen in infected and uninfected ganglia. These results suggest that the footpad inoculation model targets the small population of DRG neurons. With sciatic nerve inoculation, more neurons, with a broader range of cell sizes are infected. The proportion of neurons infected appears to be strain related. Modification of host peptide production is not detectable five days following infection.

365.13

THE VGF8a PROTEIN IS EXPRESSED BY PRIMARY SENSORY AND OTHER PERIPHERAL NEURONS AND BY CERTAIN ENDOCRINE CELLS. G.-L. Ferri*, P. Possenti, L. Abelli*, A. Levi, W. Neuhuber*. Dept. Cytomorphology, Univ. Cagliari, 09100 Cagliari; CNR Inst. Neurobiology, Rome; Dept. Pharmacology, Menarini Ricerche Sud, Pomezia, Italy. Dept. Anatomy, University Zürich, Switzerland

The 712 aminoacid protein VGF8a was recognized in PC12 cells on the basis of its rapid induction by NGF. A variety of mammalian peripheral tissues were studied by immunocytochemistry, using two antisera raised against beta-galactosidase fusion proteins, corresponding to non-overlapping regions of the VGF8a sequence. Controls included cross-absorption of antisera with both fusion proteins. Striking immunoreactive fibres were seen in the dorsal spinal cord, which were almost absent in rats treated neonatally with capsaicin, and greatly decreased after dorsal zhizotomy. Neuronal perikarya were stained in spinal ganglia, and intrinsic gut neurons were seen after colchicine. Nerve fibres were detected in para- and pre-vertebral ganglia, which decreased after capsaicin. Endocrine cells in adenohypophysis and adrenal medulla showed immunoreactivity, and Western blot of bovine adrenal extracts revealed high MW material reactive for both antisera, comigrating with PC12 cells-VGF8a protein.

In conclusion, the VGF8a protein was demonstrated in a variety of peripheral sites, including primary sensory and visceral neurons and certain endocrine populations.

365.14

ELECTRICAL CHARACTERISTICS OF ACUTELY ISOLATED NEURONS FROM RABBIT NODOSE GANGLIA. J.H. Leal-Cardoso*, G. Koschorke*, G. Talyor* and D. Weinreich. University of Maryland, Sch. of Med., Baltimore, MD 21201.

Passive and active membrane properties were examined to determine whether isolated neurons possessed membrane properties similar to those found in intact ganglia. Neurons were dissociated by the method of Wonderlin & Weinreich (J. Neurosci. Methods, 22:53, 1987) and allowed to settle onto round glass coverslips for 2-3 hr before recording in a HEPES-containing Locke solution at 22°C. Fifty-two neurons were studied. The following values were obtained (mean ± sem): cell dia., 59.3 ± 2.2 μm; E_m, -50.1 ± 1.5 mV; tau, 6.2 ± 0.9 msec; R_i, 86.8 ± 9.9 MΩ; C_m, 0.78 ± 0.11 μF/cm^2. Action potential amplitude, overshoot, dV/dt and duration were 84.0 ± 2.4 mV, 19.7 ± 1.4 mV, 198.1 ± 12.6 V/sec and 8.9 ± 0.4 msec, respectively. Spike threshold was -40.0 ± 2.0 mV. All cells revealed a fast spike after-hyperpolarization immediately following the spike (11.5 ± 0.7 mV, 60.8 ± 8.8 msec). Nine neurons manifested an additional spike after-hyperpolarization that was long-lasting (15.0 ± 2.1 sec) and 14.3 ± 2.0 mV in amplitude. These values are comparable to those obtained with similar recording micropipettes in neurons from intact ganglia. We conclude that isolated nodose neurons retain the properties of intact neurons. Supported by NIH grant NS22069.

365.15

TEMPERATURE SENSITIVITY OF EFFECTS OF STATIC MAGNETIC FIELDS ON ACTION POTENTIALS OF SENSORY NEURONS IN CELL CULTURE. R.R. Holcomb, A.W. Wamil*, J.D. Pickett* and M.J. McLean. Depts. of Neurology and Neurosurgery, Vanderbilt Univ. Med.Ctr.,Nashville, TN 37212.

We investigated effects of static magnetic fields on adult mouse dorsal root ganglion neurons in dissociated cell culture using conventional intracellular electrophysiological recording techniques. In some neurons, 0.5 msec current pulses elicited action potentials (APs) of 0.5-0.5 msec duration and 500 msec pulses elicited one or a few APs. Other neurons fired 2-4 msec APs in response to brief stimuli and fired throughout 500 msec depolarizations. Placing quadripolar arrays of permanent magnets beneath the recording chamber reversibly abolished APs elicited by brief pulses and limited firing during long pulses at 37°C. Reduction of the temperature to 33°C led to recovery of APs in the continuing presence of magnets. Rewarming to 37°C once again limited firing and removal of the magnets led to recovery of APs. Magnets had no significant effect on chronaxie or input resistance of the neurons. These findings suggest that specific configurations of static magnetic fields limit firing by a temperature-sensitive effect on AP generating mechanisms. These effects may be important in controlling different components of pain.

366.1

INCREASED RECIPROCAL I-A INHIBITION FOLLOWING SPINAL CORD INJURY IN HUMANS. G.Boorman*, R.G. Lee, W.J. Becker, and R. Tanaka*. Dept. of Clinical Neurosciences, University of Calgary, Calgary, Alberta, Canada T2N 4N1.

Damage to descending motor pathways due to spinal cord injury (SCI) might interfere with mechanisms which normally produce reciprocal I-a inhibition between agonist-antagnoist muscle groups. We have used the method of Mizuno et al (1971) to investigate this question.

H-reflexes were recorded from the soleus muscle while subjects maintained a relaxed sitting position. Tibial nerve stimuli to elicit the H-reflex were preceded by stimulation of the peroneal nerve at varying intervals. The effect of the conditioning peroneal nerve stimulus on the soleus H-reflex was analyzed.

When the peroneal nerve stimulus preceded the tibial nerve stimulus by 1-2 ms an inhibition of the H-reflex was seen. In normal subjects this early inhibition reduced the H-reflex by about 10%. A later inhibition was also detected when the interval between stimuli was 15-20 ms. The extent of the later inhibition was more variable and reduced the soleus H-reflex in normal subjects by 10-20%.

In SCI patients a significant increase in the early inhibition was seen, with the H-reflex being reduced by 30-50%. The later inhibition was variable and did not differ significantly from the later inhibition seen in normals. We are now investigating the effect that voluntary activity of ankle muscles has on the above results.

366.2

DISSOCIATION OF SOLEUS SINGLE MOTOR UNIT RESPONSES WITH PERONEAL NERVE STIMULATION. J.S. Halle* and C.G. Kukulka. Physical Therapy Laboratories, University of Iowa Medical College, Iowa City, IA 52242.

Consistency of human soleus motor unit (MU) response to peroneal nerve stimulation was investigated in adults with no known pathology. Single 0.5 msec electrical pulses were delivered 3/sec at fixed intensities of 0.9 or 1.25 motor threshold. In 11 cases, two MU's (a MU pair) were detected and isolated for the same supraspinal set (voluntary activation) and reflex input (peroneal stimulation). Peristimulus time histogram analysis demonstrated qualitative dissociation of the MU's response in 8 of the 11 pairs (73%). Five of the 8 MU's demonstrated a robust dissociation, with one MU exhibiting a strong, short latency (mean 37.5 msec), short duration, enhanced firing probability, that was not observed in the second MU. Longer latency responses were usually qualitatively similar in the MU pair. These results indicate that soleus MU's recruited at low force levels do not always respond homogeneously to external stimuli. The dissociation of reflex effects may represent a means of selective activation of low threshold soleus motoneurons, and may provide a mechanism for reflex reversal of these motoneurons. Supported by Grant #5-R29-NS24991, National Institute of Neurology and Communicative Disorders and Stroke, Department of Health and Human Services.

366.3

VOLTAGE THRESHOLDS OF HUMAN MOTONEURONES. K.S. Türker and T.S. Miles. Department of Physiology, The University of Adelaide, Adelaide, S.A. 5000, Australia.

In reduced animal preparations, the amplitude of the initial-segment voltage threshold of motoneurones of different functional types is said to be similar. However, it is not clear whether this is also true in the absence of anaesthesia, when the motoneurones are subject to a constant barrage of inhibitory and excitatory inputs. Here we describe a novel technique for indirect estimation of voltage threshold of motoneurones in conscious humans. The essence of the technique is to establish the minimal stimulus required to activate a resting soleus motor unit via the Ia reflex pathway, and subsequently to determine the size of the EPSP evoked in the motoneurone by this stimulus: this then equals the voltage threshold of the resting motoneurone. The method used to measure the amplitude of the EPSP has been described in detail elsewhere (Miles, T.S., Türker, K.S. & Le, T.H. *Exp. Brain Res.* 77, 628-636, 1989). Based on an AHP of 10 mV, the voltage thresholds of 4 soleus motoneurones ranged from 4.1 to 5.9 mV. Further experiments are in progress to determine the relationship between the motor unit type and its voltage threshold.

Supported by the NH & MRC of Australia.

366.4

SPATIAL SUMMATION OF MECHANICALLY EVOKED PERIORAL REFLEXES IN HUMAN. S.M. Barlow. Orofacial Physiology Lab., Indiana University, Bloomington, IN 47405.

An issue of special importance to studies of facial motor control concerns how systematic variations in the spatial properties of external mechanical inputs influence the excitability of trigemino-facial reflexes during voluntary motor activity. In the present report, the skin contactor was systematically varied in size to quantify the effects of spatial summation on the amplitude and time course of the early component (R_1) of the human perioral reflex sampled bilaterally from tonically active muscles of the lower face.

Results indicate that the amplitude of R_1 responses from orbicularis oris and mentalis muscle recording sites are local in sign and positively related to the size of the contactor array using constant amplitude mechanical inputs delivered to the lip vermilion. Varying skin contactor size from 2 to 16 points was also effective in shortening the latency of R_1 by 3 to 5 milliseconds. It appears that spatial summation is effective in modulating the excitability of trigemino-facial pathways in humans. Supported by NIH grant DC 00365-04.

366.5

THE GAIN OF THE HUMAN STRETCH REFLEX. M. Gorassini*, Q. Mao*, and A. Prochazka. Division of Neuroscience, Univ. of Alberta, Edmonton, Alberta, T6G 2S2, CANADA.

The gain of the human stretch reflex is difficult to measure since, anatomically, it is impossible to open this negative feedback pathway. We have, however, developed a two part strategy, the result of which was equivalent to opening the stretch reflex loop. First, sinusoidal movements of varying frequencies were imposed on a subject's arm. Rectified EMG from the triceps muscle was recorded and averaged, along with the displacement produced by the perturbation. In the second part of the experiment, the subject was instructed to produce a volitional, unloaded, sinusoidal movement, whose EMG matched in amplitude and frequency that of the EMG produced in the imposed trial. The ratio of the displacement produced in the volitional trial to that of the imposed trial gave us a measure of the open loop gain of the stretch reflex. Net phase around the loop was obtained by summing the phase advance of EMG on movement in imposed trials and the phase lag of movement on EMG in volitional trials. Similar to the stretch reflex in decerebrate cats (Rosenthal et al., J. of Neurophys.33:713, 1970), the gain decreased with increasing frequency of modulation, with a concomitant increase in phase lag. Gains of 3 or more were inferred at frequencies of 1 Hz, dropping below 1 at 4 Hz and beyond. The phase lag exceeded 180° at 4.5 Hz. From these results, a more accurate and quantitative prediction can be made as to the contribution of the stretch reflex in reducing the effects of an external perturbation. For example, a gain of 2 @ 3 Hz will reduce an external perturbation by a factor of 1/3rd (1/1+GAIN). Supported by Medical Research Council of Canada.

366.6

REFLEX CHANGES ACCOMPANYING ISOMETRIC STRENGTH TRAINING OF THE CONTRALATERAL LIMB A.J. Butler, W.G. Darling. Department of Exercise Science, University of Iowa, Iowa City, IA 52242.

It has been shown by numerous investigators that exercising one limb is usually associated with an increase in strength in the homonymous muscles of the contralateral limb. The mechanisms of this cross-education of strength have not been completely investigated. It has been suggested, however, that the improvement in strength in the contralateral untrained limb may be attributed to neural adaptations at various levels of the nervous system (i.e. increased facilitation or disinhibition of motor neurons). The purpose of this project was to determine whether training of the left triceps surae muscle would produce changes in the excitability of spinal motor neurons and interneurons controlling the right, untrained triceps surae.

The following measurements were made prior to and immediately following unilateral, isometric strength training three times a week, for 4 weeks: (1) Electromyography's (EMG) of triceps surae, H-reflexes and conditioned H-reflexes, and plantarflexion torque during maximal plantarflexion contraction, (2) maximal twitch and M-responses of triceps surae. The subjects ankle and knee joint angles along with EMG and stimulating electrode positions were held the same for pre and post testing sessions. After training, increases in plantarflexion torques of the trained and untrained leg, triceps surae EMG's, and reflexes were observed. Although inter-subject variability was high, these observations support the theory that cross-education effects result from neural adaptations.

366.7

A PILOT STUDY INVESTIGATING A REFLEX EXCITABILITY MEASUREMENT TECHNIQUE TO ASSESS CEREBRAL SPASTICITY IN HUMANS. *K. HARBURN, A. VANDERVOORT*, A. HELEWA*, C. GOLDSMITH*, A. KERTESZ*, R. TEASELL*, (K. HILL*). Departments of Occupational and Physical Therapy, The University of Western Ontario, London, Ontario, N6G 5C1.*

The purpose of this study was to evaluate the feasibility of a reflex excitability measurement technique (REMT) to assess spasticity related to cerebral stroke. The H-reflex was evoked at 4 degrees of plantarflexion during a stationary condition and during slow passive movement of the ankle. Pilot testing demonstrated less inhibition of the H-reflex during passive movement of the affected ankle in stroke subjects (n=12) than matched control subjects (n=12) ($p<.05$). This finding was felt to be related to faulty presynaptic inhibition. A positive, but weak correlation was found between the REMT index and a clinical measure of spasticity in stroke subjects (Spearman R=.49). Stroke subjects demonstrated greater average stiffness (Nm·deg/deg) of the affected ankle than control subjects ($p<.05$). Size of cerebral lesion was not significantly related to any of the variables examined. Further testing of the reliability and validity of the REMT index is required to establish its usefulness as an outcome measure in a clinical trial of therapy.

Supported by a grant: Health and Welfare Canada (NHRDP)

366.8

EFFECT OF STIMULANT MEDICATION ON MOTONEURONAL EXCITABILITY IN ADDH CHILDREN. **R.T. PIVIK AND F.W. BYLSMA* DEPTS. PSYCHIATRY, PHYSIOLOGY AND SCHOOL OF PSYCHOLOGY, UNIV. OF OTTAWA, ONT.**

Previous reports have indicated unmedicated ADDH children may exhibit disparate levels of spinal motoncuronal excitability as indexed by H-reflex recovery function profiles. The present study provides a comparison of these profiles in ADDH children both while receiving stimulant medication and while unmedicated.

Fifty-two children (26 ADDH: 18 males, 8 females; $\bar{X}$ ages = 9.58 $\pm$ 1.58 years; 26 controls: 22 males, 4 females; $\bar{X}$ ages = 10.12 $\pm$ 1.66 years) participated in the investigation. Clinical diagnoses were based on psychological evaluations, DSM-III criteria and scores of $\geq$ 15 on the hyperactivity scale of Connors' Parent and Teacher Behavioral rating scales. Muscle action potentials were recorded from gastrocnemius-soleus muscles. Above threshold equal intensity stimuli were delivered in pairs at intra-pair intervals ranging from 50-2000 msec. Ratios of test (H_2) to conditioning (H_1) reflexes were averaged (5/interval) and analyzed using repeated measures analysis of variance. Group comparisons of indexes of secondary facilitation (the average of reflex ratios at 100, 200 and 300 msec intervals) revealed that, relative to controls, reflex amplitude was significantly depressed in ADDH children when nonmediated ($p = .013$) and that medication was associated with a reduced difference between control-ADDH data ($p=.094$). Approximately 1/3 of the ADDH subjects actually showed reflex facilitation when unmedicated. In conjunction with medication administration, these subjects showed a 10% decrease in excitability, whereas the remaining subjects showed a 34% increase in excitability. These data, obtained under resting conditions, reinforce the notion that motor-related symptomatology generally noted in this disorder is fundamental and does not require activation of complex behavioral patterns to become evident. Furthermore, the data suggest that stimulant medication has the effect of modifying levels of motoneuronal excitability in the direction of normality, regardless of initial levels of excitability. -- Research assisted by Ontario Mental Health and Hospital for Sick Children's Foundations.

366.9

ELICITATION OF A LARYNGEAL EXTEROCEPTIVE REFLEX FOLLOWING SUPERIOR LARYNGEAL NERVE STIMULATION IN HUMANS. C. L. Ludlow, S. G. Yin*, J. Kohda* and M. Hallett, Speech and Voice Unit, NIDCD and Human Motor Control Section, NINDS, Bldg.10/5N226, Bethesda, MD 20892.

Previously a long latency response between 55 and 80 ms was elicited bilaterally in the thyroarytenoid (TA) and sometimes in the ipsilateral cricothyroid (CT) muscle following electrical stimulation of the recurrent laryngeal nerve on one side containing glottic afferents and motor fibers for several laryngeal muscles including the TA (Ludow C.L., Yin S.G. and Hallett, M., Neurosci Abstracts, 1989). The purpose of this study was to determine if a similar response could be elicited from the TA and CT muscles bilaterally by stimulating the superior laryngeal nerve containing supraglottic afferents and motor fibers for the CT. During nerve stimulation, simultaneous bilateral hooked wire recordings were made from the TA and CT muscles with unilateral surface recordings from the posterior cricoarytenoid and obicularis oculi. The stimulation levels were varied between threshold and supramaximal for direct ipsilateral CT muscle responses. The ipsilateral direct CT response occurred at 3 ms, and the adductor response occurred at low levels of stimulation in the ipsilateral CT and TA, and the contralateral TA at 13, 15 and 22 ms respectively. The long latency response occurred at low levels of stimulation starting at 50 ms and lasting to 80 ms in the laryngeal muscles, but not the obicularis oculi. This was more consistent and appeared at lower stimulation levels in the TA than in the CT muscles. This later response was more consistant, higher in amplitude and longer than the adductor response. The results demonstate that an exteroceptive response appears following stimulation of either glottic or supraglottic afferents.

366.10

EFFECTS OF GRADED JENDRASSIK FACILITATION ON TYPE Ia AND II MEDIATED REFLEXES IN ABLE-BODIED AND SPINAL CORD INJURED HUMANS. P.W. Nance, R. Leckey*. Dept. of Medicine, U. Manitoba, Winnipeg, Man. and Dalhousie U., Halifax, N.S., CANADA

The soleus deep tendon reflex (DTR) is a type Ia mediated spinal relex which can be facilitated by a Jendrassik Maneuver (JM) or inhibited by vibration (v). Since v activates dynamic muscle spindle receptors, only the static stretch receptors, mediated by Type II fibers, respond to a tendon tap during v. Type Ia and Type II mediated reflexes were tested in able-bodied (n=10) and spinal cord injured (SCI) (n=10) men before and during a graded JM:a 3 s Maximum Hand Grip (MHG), 50%MHG and 25%MHG. Both Ia and II reflex facilitation was greatest during 50%MHG. Type Ia and II reflex amplitudes of motor spared SCI subjects were greater ($p<0.001$) than motor complete SCI subjects and able-bodied subjects. Reflexes of the motor complete SCI subjects failed to facilitate at any grade of the JM. Able-bodied subjects (3/10) showed reflex inhibition during MHG which none of the SCI subjects showed. Thus, reflex facilitation by the JM is mediated via a descending motor pathway and requires the normal compliment of innervation for inhibitory gating.

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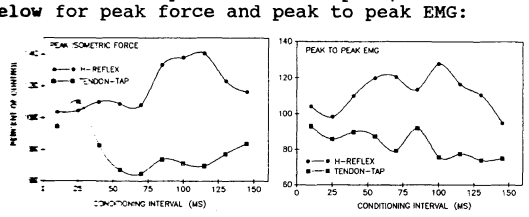
366.11

DIFFERENTIAL EFFECTS OF A CONDITIONING STIMULUS UPON THE CONTRALATERAL ALPHA MOTONEURON POOL. D.M. Koceja & G. Kamen, Motor Control Lab, Indiana University & Neuromuscular Research Center, Boston University.

In this study, the tibial nerve H-reflex (50% max.) was conditioned by either a contralateral tibial nerve H-reflex or a tap to the contralateral Achilles tendon in a group of normal college-age subjects (n=12). Conditioning intervals of 10, 25, 40, 55, 70, 85, 100, 115, 130 and 145 ms were used. The results indicated that the conditioning H-reflex stimulus produced a facilitation while the conditioning tendon-tap stimulus produced an inhibition to the contralateral alpha motoneuron pool, as shown below for peak force and peak to peak EMG:



367.1

Potentiation and diminution of a Pavlovian UR (rabbit eyeblink) as a function of the CS - US interval in training and testing. T. Canli, W.M. Detmer*, N.H. Donegan. Dept. of Psych., Yale Univ., New Haven, CT 06520.

Defensive motor reflexes elicited by a US can be 1) potentiated when the target US is preceded by a CS associated with an aversive US (fear potentiated startle; Davis et al., 1979, *Psychopharm, 65*, 111); or 2) diminished when the target US is preceded by a CS with which it has been paired (the conditioned diminution of the UR; Donegan, 1981, *JEP:ABP, 7*, 295). We propose that these different outcomes result from two separate associative processes that develop when a CS is associated with an aversive US: 1) conditioning of a fear response that is present during much of the CS; 2) conditioning of a brief, discrete motor CR (e.g., eyeblink) timed to occur near the US onset. This is assumed to produce a short lasting inhibition of the US sensory pathway, and thus a diminished UR (Donegan et al., *Psy. of L & M*, 1989, *23*, 109). Consequently, a CS paired with an aversive US should diminish the UR when the *same* US occurs at the training interstimulus interval (ISI), but should potentiate the UR when the same US occurs during the CS, but outside the training ISI.

Seven rabbits received discrimination training in which a 1040 msec CS was paired with a 40 msec periorbital shock US (5 msec pulses, 100 Hz, 4.0 mA) on half of the trials (CS+), thus defining a training ISI of 1.0 sec. On the other half of the trials, a different CS was presented alone for 1040 msec (CS-). Subsequently, probe test-trials in which the US was presented alone, preceded by a 1 sec CS+ or CS-, and a 10 sec CS+ or CS-, were introduced in the course of discrimination training sessions. The findings were consistent with the above hypotheses. When the US was presented 1.0 sec after CS onset (the training ISI), the eyeblink UR amplitudes were diminished on CS+US trials, compared to CS-US and US alone test trials. Importantly, UR amplitudes were diminished on CS+ trials in which there was an eyeblink CR, but not on CS+ trials in which a CR was absent. However, when the US occurred at the end of a 10 sec CS, the UR amplitude was potentiated by CS+ compared to CS- or US alone test trials. The potentiation produced by CS+ was greater on trials on which an eyeblink CR occurred compared to trials on which a CR did not occur.

367.2

DIRECT STIMULATION OF MUSCLE IS SUFFICIENT TO PRODUCE PERSISTENT HINDLIMB FLEXION IN RAT. M.F. Anderson and B.J. Winterson. Depart. of Physiology, Univer. of New England College of Osteopathic Medicine, Biddeford, ME 04005.

Electrical current (2mA, 7ms, 100Hz for 1 hr) delivered through wound clips attached to the medial and lateral thigh skin induces a persistent flexion. Since current also passes through the underlying muscle, we assessed the relative contribution of muscle and skin in this paradigm. Long-Evans rats (200-275g) were anesthetized and assigned to three groups: MUSCLE & SKIN; MUSCLE; SKIN. In the MUSCLE & SKIN group, wound clips were attached to the thigh skin. In the MUSCLE group, skin was removed from the thorax to the hindlimb footpads and exposed muscle was wrapped in sterile gauze soaked in normal saline. Wound clips were applied through the gauze and into the underlying muscle. Body temperature was maintained by wrapping a thermostatically controlled heating pad around the thorax and upper thighs. In the SKIN group, wound clips were applied to the thigh skin and 1cc of Vaseline was injected subcutaneously at each site. Capacitative current was monitored continuously at the muscle via insulated 27g needles and did not exceed 0.3mA. Following stimulation, mean flexion was 9.5g for the MUSCLE & SKIN group, 8.9g for the MUSCLE group and 4.0g for the SKIN group. These results suggest that muscle afferents are more influential than skin afferents in the induction of a persistent hindlimb flexion.

367.3

In Search for a Primary Startle Pathway in the Rat.

Karin Rüttgers, Karl Kandler*, and Horst Herbert; Univ. of Tübingen, Dept. Animal Physiol., Morgenstelle 28, 7400 Tübingen, FRG.

The acoustic startle response is a short latency reflex. Head muscles are the first to react with minimal latencies of 5.5 ms (*Caeser et al., 1989, Behav. Neurosci. 103: 1075-1081*). Therefore we assume a startle pathway with only one synapse between the sensory input (cochlear nucleus, CN) and the motor output (motor nuclei of the trigeminal and facial nerve, Mo5 and Mo7). In the rat brain a structure receiving input from the CN and in turn projecting to Mo5 and Mo7 is not known. In search for such a sensorimotor interface we injected Mo5 and Mo7 with the retrograde tracer Fluoro-Gold and mapped the distribution of labeled neurons. In a second set of experiments we injected Phaseolus vulgaris leuccoagglutinin into CN and looked for labeling in areas that were previously shown to contain premotor neurons.

We found four sites where premotor neurons and auditory terminal fibers coincide: the lateral paragigantocellular nucleus (LPGi), an area including the ventral part of the caudal pontine reticular nucleus (PnC) and the adjoining dorsal periolivary nucleus, the pontine nuclei (Pn), and the ventrolateral tegmental nucleus (VLTg). Retrograde double-labeling experiments revealed that some neurons in these areas project to both, Mo5 and Mo7. This is in accordance with *Caeser et al.*, who demonstrated that different groups of head muscles show a common fluctuation in response latency and amplitude.

Our data implicate, that the above mentioned nuclei are potential sensorimotor interfaces responsible for mediating the acoustic startle response. Currently retrograde/anterograde double tracing experiments are done to clarify whether the auditory efferent fibers from CN synapse on the premotor neurons.

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367.4

THE EFFECT OF LOW TEMPERATURES ON POST-TETANIC POTENTIATION IN THE FROG SPINAL CORD. M.-S. Rioult-Pedotti and H.P. Clamann, Dept. of Physiology, University of Bern, CH-3012 Bern, Switzerland.

Temperature is known to affect neuron excitability, the size of post-synpatic potentials, and the size of reflex responses. Action potential (AP) duration increases with decreasing temperature, increasing the reliability of AP propagation across axonal branch points. It has been suggested that post-tetanic potentiation (PTP) increases the reliability of AP propagation in a similar manner. Thus low temperature should increase the size of a reflex response while reducing the effect of PTP.

Frogs were cooled in ice, decapitated and the spinal cord removed. The spinal cord was hemisected, placed in a plexiglass chamber and superfused with cooled Ringer's solution. Lumbar dorsal (DR) and ventral roots (VR) were drawn into suction electrodes for stimulation and recording of reflex responses. The hemicord was kept at a constant temperature between 5-22°C. Stimuli of 0.1 ms duration were applied to the DR singly or at 1/s before and after 500/s tetanic trains and the reflex response recorded from the VR.

A single supramaximal DR stimulus elicited a reflex response with a large polysynaptic component which was greater the lower the temperature. At 22°C a reflex response was often virtually absent. Monosynaptic or short-latency polysynaptic components were most prominent at 5-15°C. A tetanus always augmented the shorter-latency response at the expense of longer-latency responses. PTP was most effective at the highest and weakest at the lowest temperatures tested. In some cases a hemicord at > 20°C showed no reflex response until after PTP. No PTP could be evoked at 6°C in some experiments, while the same hemicord showed marked potentiation at higher temperatures. These results are consistent with the hypothesis of Lüscher et al. (J. Neurophysiol. 50:1045, 1983) that branch-point failure of presynaptic axonal arborizations and its relief by PTP can modulate spinal reflexes. (Supported by the Sandoz-Stiftung.)

367.5

MOTOR UNIT- MUSCLE SPINDLE INTERACTIONS DURING FATIGUE. R.M. Enoka[1,2], M.A. Nordstrom[1], R.M. Reinking*[1] and D.G. Stuart[1]. Depts. of Physiology[1] and Exercise & Sport Sciences[2], Univ. of Arizona, Tucson AZ 85724.

The effect of motor unit (MU) activation on spindle afferent discharge was studied during a 6-min fatigue test (cf. Burke et al., J. Physiol., 234:723, 1973) in the tibialis posterior muscle of anesthetized cats. Single MUs were activated repetitively by stimulating ventral root filaments, while afferent activity was recorded from dorsal root filaments. The interaction between a MU and a muscle spindle was quantified using a coupling index (CI; Munson et al., J. Neurophysiol., 51:1268, 1984), where CI = (1 - a/p) x 100; "a" is the number of action potentials from the afferent during the last 250 ms of each 330 ms epoch of MU activation (at 1/s), and "p" is the afferent action potential count from a 250 ms epoch during the quiescent period following each tetanus. The CI was plotted against the corresponding MU peak force for the 360 activations of the fatigue test. They usually changed in parallel, provided the MU and spindle were mechanically coupled. However, for selected fatigable units it was possible to compare spindle responses to the same MU peak force after different periods of activity, because an initial force potentiation was followed by fatigue. The ability of a MU to influence spindle discharge was time-dependent; a given peak force was more effective in unloading the muscle spindle during the period of fatigue when force was declining, compared to the same force during the period of potentiation. This finding is consistent with the earlier suggestion using a different paradigm (Christakos & Windhorst, Brain Res. 365:388, 1986), that the gain of the system linking MU force and spindle discharge increases during fatigue. Supported by USPHS grants NS 20544, HL 07249, NS 25077, NS 07309 and RR 05675. M.A.N. is a C.J. Martin Fellow of the NH&MRC of Australia.

367.6

DESCENDING INPUTS AFFECT CROSSED EXTENSION MORE THAN STRETCH REFLEX RECORDED FROM QUADRICEPS FEMORIS IN DECEREBRATE CATS. J.A. McMillan, M.J. Serwacki* and W.A. Yuhas*. Dept. of Biol. and WAMI Program, Montana State University, Bozeman, MT 59717

We examined effects of changing body position, known to affect excitability of the crossed extension reflex (McMillan, J.A. and Koebbe, M.J., Exp'l Neurol. 73:233, 1981), on both crossed extension (CER) and stretch reflex (SR) recorded from right quadriceps femoris in midcollicular decerebrate cats. Reflexes were recorded with the cat on the right and left body sides respectively. Magnitude of response was determined by measuring the area under the force X time curve.

The CER was stronger when the cat was on the right as compared to left side (mean R/L=3.15±0.96, P=0.03) whereas the SR was not changed (R/L=1.00±0.13, P=0.45). Since SR is a monosynaptic reflex, these results suggest that incluences of descending inputs, as proposed by Bruggencate and Lundberg for the lateral vestibulospinal tract (Exp. Brain Res. 19:248, 1974), are exerted predominantly on interneurons in the CER pathway rather than directly on motor neurons. (Supported by NSF BNS 86-19148 and NIH RR08218-06. MJS was supported by Univ. of Wash. School of Med. MSRTP)

367.7

RECURRENT COLLATERALS OF FLEXOR HALLUCIS LONGUS AND FLEXOR DIGITORUM LONGUS MOTONEURONS OF THE CAT. T.M. Hamm and M.L. McCurdy. Div. Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85013.

Recent studies have demonstrated differences in recurrent inhibition to and from flexor hallucis longus (FHL) and flexor digitorum longus (FDL) motoneurons (Hamm, J. Neurophysiol. 63:395, 1990), including an absence of recurrent inhibition from FDL motoneurons in several species of motoneurons (Fleshman et al., Exp. Brain Res. 54:133, 1984; Hamm, 1990). In the present study, the recurrent collaterals of FHL and FDL motoneurons have been examined for a potential morphological basis of differences in patterns of recurrent inhibition. Experiments were performed using cats which had been ischemically decapitated under isoflurane anesthesia. Individual motoneurons were injected intracellularly with horseradish peroxidase. To date, 4 FHL and 1 FDL motoneurons with distinct, well-stained axonal systems have been examined. Three of the FHL motoneurons had one first order collateral; the fourth cell had two. Each collateral tree had no more than 5 swellings and 6 blunt endings located in the ventral part of lamina VII. The one FDL motoneuron studied to date had no recurrent collaterals. This preliminary data indicates that these motoneurons have very simple axon collateral systems in relation to most other species of hindlimb motoneurons, consistent with the small recurrent IPSPs, and the absence of recurrent IPSPs, produced by FHL and FDL motoneurons, respectively (Hamm, 1990). Considering the absence of recurrent collaterals in motoneurons that innervate the short plantar muscles (Cullheim and Kellerth, J. Physiol. Lond. 281:285, 1978), these data provide evidence for a morphological basis for a proximal-distal gradient in the strength of recurrent inhibition in motor nuclei that innervate the cat's hindlimb.

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367.8

PARTICIPATION OF FLEXOR HALLUCIS LONGUS MOTONEURONS IN THE PLANTAR CUSHION REFLEX AND ITS RELATIONSHIP TO DENDRITIC ORIENTATION IN THE CAT. M.L. McCurdy and T.M. Hamm. Div. of Neurobiology, Barrow Neurological Inst., Phoenix, AZ 85015

Motoneurons innervating short plantar muscles of the foot respond with short latency EPSPs to natural stimulation of the plantar footpad (Egger et al., J. Physiol. 306:349-363, 1980). These motoneurons have a bundle of dorsomedially oriented dendrites which project into Rexed's laminae VI of the dorsal horn. Flexor hallucis longus (FHL) and/or flexor digitorum longus (FDL) and plantaris (Pl) motoneurons also respond to plantar cushion stimulation (Engberg, I., Acta Physiolog. Scand. 52: Suppl. 235:1, 1964). We tested the responses of FHL, FDL and Pl motoneurons to electrical stimulation of the plantar cushion (1.5-2T) in ischemically decapitated low-spinal cats and subsequently injected some cells with horseradish peroxidase to investigate dendritic specialization in the dorsal horn.

Our preliminary findings indicate that short latency (< 3 msec) EPSPs vary from < 1 to 3 mV in FHL and Pl motoneurons and the cells with the strongest responses have a substantial dendritic projection in the dorsal horn. These dendrites do not extend as far medially and do not form a discrete bundle as in motoneurons of short plantar muscles but rather fan across the lateral half of laminae VI. Some FHL motoneurons lack prominent dorsomedial dendrites. In FDL cells studied to date, only weak EPSPs from plantar cushion stimulation have been observed. The morphology of one FDL cell has been examined and dorsomedially directed dendrites were confined to the lateral edge of lamina VI. Our results to date are consistent with a correspondence between the dendritic morphology of FHL and Pl motoneurons and their participation in the plantar cushion reflex.

Supported by USPHS Grants NS22454, NS08773 and NS07309.

367.9

HETEROGENIC REFLEX ORGANIZATION WHICH MIRRORS SPECIALIZED ACTIVATION PATTERNS OF FLEXOR DIGITORUM LONGUS AND FLEXOR HALLUCIS LONGUS MUSCLES IN THE CAT. T. R. Nichols and S. J. Bonasera*. Department of Physiology, Emory University Atlanta, GA 30322.

The different activity patterns of flexor hallucis longus and flexor digitorum longus muscles (FHL and FDL) during locomotion and the facultative activation of FDL are discordant with the strong Ia synergism between these two muscles (O'Donovan et al., J. Neurophysiol., 47:1126, 1982). In an attempt to obtain a more global view of the reflex organization of these muscles, we evaluated stretch-evoked reflexes among FHL, FDL and other hindlimb muscles in decerebrate cats. FHL and FDL were indeed linked by powerful excitatory pathways, but the interactions between either FHL and FDL and quadriceps, gastrocnemius, plantaris soleus and pretibial flexors were largely, although not exclusively, inhibitory. Reflex interactions for FDL were weaker and more variable than those of FHL, suggesting a greater flexibility of control for FDL. Additionally, the distribution and strength of inhibitory interactions for FHL and FDL were similar to the pattern of recurrent inhibition for these muscles (Hamm, T. M., J. Neurophysiol. 63: 395, 1990). The strong Ia synergism between FDL and FHL may serve to reinforce the response of these muscles to external, mechanical disturbances rather than mediate patterns of activity during locomotion.

(Supported by NIH grant NS20855)

367.10

COMPUTER SIMULATIONS OF SOLEUS MOTONEURON POOL DYNAMIC RESPONSES TO RAMP STRETCHES STARTING FROM DIFFERENT OPERATING POINTS. Dj. T. Boškov and W. Z. Rymer. Dept. of Physiology, Northwestern University, Chicago, IL, 60611.

Previously we have shown that, during ramp and hold stretches of the soleus muscle in decerebrate cats, the reflex components of EMG and force show a variety of behaviors for nearly stereotyped spindle afferent input. In order to simulate the reflex output under these conditions, we developed a model of neural network of the soleus motoneuron pool using the neuron model by MacGregor and Oliver (Kybernetik16:53, 1974). The neural network consisted of 50 simulated soleus motoneurons innervated by two groups of 50 input fibers, one corresponding to spindle afferent input and another representing the central input. The thresholds of motoneuronal firing were determined from the previously reported rheobase currents and synaptic currents across the pool and the other model parameters were based on experimental data on single neurons. Different initial forces were simulated by changing the background activity of the central input.

We first tested the hypothesis that simple changes in central tonic activity, coupled with stereotyped spindle afferent activity, are sufficient to explain changes in recruitment patterns during stretch. As the simulated tonic activity increased, the number of newly recruited units during stretch decreased and most of these new units were recruited shortly after the simulated onset of stretch. These results were entirely in accord with experimental data, suggesting that intrinsic properties of motoneurons are sufficient to account for the observed recruitment patterns under a wide range of background activities

We have previously shown that the sensitivity of force responses to reflexive recruitment pattern decreases with background force. We performed a sensitivity analysis of simulated recruitment patterns to the step increase and slope of spindle rate during stretch. The results indicated that, as the background activity increased, the normalized sensitivity decreased from 2-5 to «1 shortly after stretch onset, whereas the slope effect was insignificant. These results suggest that the sensitivities of recruitment patterns to variations in step phase of afferent input are well suited to muscle properties.

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367.11

COMPUTER SIMULATION OF THE STEADY-STATE BEHAVIOR OF THE MOTONEURON POOL: COMPARISONS WITH EXPERIMENTAL DATA ON FREQUENCY MODULATION. C.J. Heckman, J.J. Gemperline and M.D. Binder. Dept. of Physiology, Northwestern University, Chicago, IL, 60611.

A realistic computer simulation of the medial gastrocnemius motoneuron pool and muscle was used to investigate the nature of the synaptic input required to generate normal steady-state frequency modulation of single motor units. We recently hypothesized that synaptic inputs which generate larger effective synaptic currents (*In*) in type S than F motoneurons (e.g., the spindle Ia input) are used to produce low forces, while production of higher forces requires the addition of inputs with the opposite distribution of *In* (Heckman and Binder, JNP 60:1946, 1988).

Here we consider the hypothesis that such a "crossover" between very different input systems is also sufficient to account for frequency limiting of low threshold units as high threshold units are recruited. Simulation outputs were compared to plots of single unit discharge rates versus background force in human subjects (Monster and Chan, JNP 40:1432, 1977). Various versions of a "crossover" input scheme were found to simulate frequency limiting. However, accurate simulation of the experimental data required that the synaptic input meet 3 specific criteria: 1) the low level input had to generate greater *In* in S than FF units; 2) conversely, the high level input had to generate much less *In* in S than FF units; and 3) the crossover point had to occur at a low *In* amplitude, at less than 5% of maximum force and well before the recruitment of any FF units.

These results appear to be consistent with some of the known characteristics of the monosynaptic Ia input (a putative "low level" input favoring S units) and the polysynaptic rubrospinal/sural inputs (putative "high level" inputs favoring F units). Further, the simulations predict that a good indicator of the distribution of synaptic input should be the slope of the relationship between the rates of two different motor units as force is increased. Experiments are underway to obtain detailed measurements of rates of pairs of motor units to evaluate this approach.

367.12

COMPUTER SIMULATION OF THE STEADY-STATE BEHAVIOR OF THE MOTONEURON POOL: COMPARISONS WITH EXPERIMENTAL DATA ON RECRUITMENT ORDER. M.D. Binder and C.J. Heckman. Dept. of Physiol. and Biophysics, University of Washington, Seattle, WA 98195.

We have developed a computer simulation of the steady-state behavior of the cat medial gastrocnemius (MG) motoneuron pool and muscle. A neural network was constructed consisting of 100 motor units with neural and mechanical properties that very closely matched the available experimental data. The use of realistic single elements permits quantitative comparisons between simulation outputs and normal motor outputs and creates new approaches for testing hypotheses about the single unit basis of motor behavior.

We investigated the hypothesis that the intrinsic properties of motor units are sufficient to account for the phenomenon of orderly recruitment. All units received precisely equal shares of the effective synaptic current, so that recruitment thresholds were solely determined by current thresholds for rhythmic firing (*Ithres*). We began with a relationship between *Ithres* and maximal unit force output (*Fmax*) that was monotonic, giving perfect recruitment order. We then used Monte Carlo techniques to add varying amounts of random variance to the *Ithres-Fmax* relationship. Pairwise comparisons of recruitment order were performed to relate *Ithres-Fmax* correlation coefficients to the orderliness of recruitment.

Our results showed that reducing the *Ithres-Fmax* covariance to that reported in the experimental data (Fleshman et al., J. Neurophysiol. 46: 1981) generated recruitment orders that were much more variable (28% reversals on average) than those reported for hindlimb muscles of decerebrate cats (0-8%: Zajac and Faden, J. Neurophysiol. 49: 1985; Botterman and Tansey, Soc. Neurosci. Abstr. 15: 1989). Application of steady-state homonymous Ia effective synaptic current to the pool, which is about twice as large in low threshold as in high threshold units, substantially improved the orderliness of recruitment (18% reversals). These results suggest that intrinsic motoneuron properties alone are not sufficient to account for rank-ordered recruitment by motor unit force and that synaptic input must play an important role.

367.13

RELATIVE ENDURANCE AND RECRUITMENT ORDER AMONG PAIRS OF FAST-TWITCH MOTOR UNITS IN THE CAT MEDIAL GASTROCNEMIUS MUSCLE. A.K. Yee, K.E. Tansey and B.R. Botterman. Depts. of Cell Biology & Neuroscience and Physiology, UT Southwestern Medical Center, Dallas, TX 75235.

A muscle's ability to maintain a specified force output is largely determined by the relative forcefulness and fatigability of motor units recruited during a contraction. Generally the earliest recruited and most frequently used units of a muscle produce the smallest tensions and have highest resistance to fatigue. Indeed, a number of laboratories, including our own, have shown that a unit's tetanic tension (P_o) is an excellent predictor of its order within a motor pool. It is less clear whether a similarly strong correlation between recruitment order and fatigability exists among fast-twitch units of medial gastrocnemius (MG). Based on the strong correlation ($r=-.85$) between P_o and endurance time (E_t) in MG units (*J. Neurophysiol. 60:1215,1988)*, such a relationship might be expected. The present experiments were undertaken to test directly this possibility.

In decerebrate, unanesthetized animals spinalized at C_1, pairs of motor axons were isolated from the MG nerve and their recruitment order determined during monosynaptic reflexes elicited by stimulation of the L_7+S_1 dorsal roots. If one or both units of a pair were recruited during the reflex, then their P_o's and E_t's were determined. E_t was the length of time that a unit could maintain 25% of its P_o. Unit tension was clamped at this output level by altering the stimulation rate of a unit's axon through computer feedback control. For 6/7 pairs of units studied in 3 experiments, the lower threshold unit had the smaller P_o and the larger E_t. In the remaining pair, the lower threshold unit had a P_o and E_t of 28.7g and 156.5s, compared to 20.6g and 236.4s for the higher threshold unit.

The present study provides additional evidence that motor units of a pool are generally recruited in order of increasing fatigability. Among fast-twitch units of MG, the correlation between recruitment order and E_t would seem to be as strong as that observed previously between recruitment order and P_o. (NIH NS17863)

367.14

MEMBRANE ELECTRICAL PROPERTIES OF EXTERNAL URETHRAL SPHINCTER AND EXTERNAL ANAL SPHINCTER MOTONEURONS IN THE DECEREBRATE CAT. S. Hochman, B. Fedirchuk, and S.J. Shefchyk. Depts. Med. and Physiol., Univ. of Manitoba, Winnipeg, Canada R3E 0W3.

Intracellular measurements were made of passive and threshold membrane properties of antidromically identified pudendal motoneurons innervating either the external urethral spincter (EUS) or the external anal sphincter (EAS). Mean values for EUS and EAS motoneurons were similar and are combined in the Table below. A strong correlation between motoneuron input resistance and membrane time constant suggests that the variations in input resistance between motoneurons depend primarily on membrane resistivity rather than cell size and geometry. The mean electrotonic length of 1.4 is identical to that of hindlimb motoneurons. Compared to hindlimb motoneurons, however, these cells have higher input resistance, lower rheobase and conduction velocity and a longer after-hyperpolarization duration. Although the electrical properties most closely resemble those of hindlimb motoneurons innervating slow twitch muscle, EAS is a predominantly fast muscle in the cat (Krier et al., Am. J. Physiol., 255). Subthreshold rectification processes (i.e. anomalous rectification and SAG) were found in several motoneurons. A preliminary examination of motoneuron firing properties suggest that these motoneurons can be made to fire rhythmically with the interval between first and second spikes reaching a minimum of 3.6 - 7.0 ms near 4 times rheobase current.

afterhyperpolarization duration (ms)	98	rheobase (nA)	3.4
conduction velocity (m/s)	48	threshold voltage (mV)	8.1
input resistance (MΩ)	2.1	action potential (mV)	73
membrane time constant (ms)	3.5		

Supported by the Rick Hansen Man in Motion Legacy Fund, the Medical Research Council of Canada and the Manitoba Health Research Council.

367.15

STRETCH EVOKED MONOSYNAPTIC EPSP AMPLITUDES IN ANKLE EXTENSOR MOTORNEURONS ARE INCREASED IN THE CHRONIC SPINAL CAT A. Jones, S. Hochman, and D. McCrea. Dept of Physiology, University of Manitoba, Winnipeg, Manitoba Canada, R3E 0W3.

Earlier work in this lab showed that the amplitudes of electrically-evoked homonymous EPSPs in 6 week chronic spinal cats were increased in lateral gastrocnemius but not medial gastrocnemius motoneurons. The present study used muscle stretch to evoke combined homonymous and heteronymous EPSPs in triceps surae and plantaris motoneurons in unlesioned and chronic spinal (L1-L2) barbiturate-anaesthetized cats. Brief (< 2 ms) small (5 to 100 μm)stretches of the Achilles tendon were used to evoke EPSPs in ankle extensors with initial muscle tensions of 100-500 g. Motor units were also mechanically typed as FF, Fint, FR, or S. Amplitudes of EPSPs evoked by 20 μm (200g initial tension) stretches were increased by more than 70% in ankle extensors including MG in the chronic spinal preparation. In Fast-Fatiguable motor units of chronic spinal cats EPSPs increased by 83%. As stretch amplitude increased, however, the difference between the unlesioned and chronic spinal preparations diminished. Thus stretches that produced sub-maximal EPSPs in the unlesioned state produced considerably larger EPSPs in the chronic spinal preparation. The use of stretch to evoked combined homonymous and heteronymous EPSPs is more likely to mimic clinical assessment of monosynaptic reflexes. We conclude that increased Ia EPSPs would contribute to increased reflex recruitment of all ankle extensor motoneurons in the chronic spinal state. Supported by the Medical Research Council of Canada and Rick Hansen Man in Motion Legacy Fund.

367.16

MOTOR UNIT PROPERTIES AND REFLEX RECRUITMENT IN THE MEDIAL GASTROCNEMIUS OF THE DECEREBRATE CAT. B.D. Clark and T.C. Cope. Dept Physiol & Biophys, Hahnemann Univ, Philadelphia, PA 19102.

It has been reported that in decerebrate cats the motor unit of a pair with the lower axonal conduction velocity (CV) was almost always recruited first in muscle stretch reflexes (Bawa et al., J. Neurophysiol., 1984). We measured multiple motor unit properties under these same conditions to determine their relationships with recruitment sequence and to assess the possible dependence of recruitment on the source of synaptic drive. Axons of medial gastrocnemius (MG) motoneurons were penetrated 2 at a time with microelectrodes in ventral rootlets of decerebrate cats. The rank order within unit pairs by CV (42/55), maximum isometric force (40/53) and potentiated twitch contraction time (32/37) were significantly associated (G test; $p<0.005$) with recruitment order elicited by MG stretch. These findings differ from those of Zajac and Faden (J. Neurophysiol., 1985), who found recruitment by high frequency stimulation to be perfectly ordered by tension. Reversal of the recruitment order obtained with MG stretch was observed in 5/19 pairs during electrical stimulation of the sural nerve; reversal also occurred in 2 trials involving combined stretch of MG and synergistic muscles. We conclude that recruitment does not proceed in strict accord with any measured physiological property, and that it may indeed depend on the source of synaptic drive. (Supported by NIH NS21023)

367.17

THE EFFECT OF FATIGUE-INDUCED FEEDBACK ON MOTONEURON LATE RATE ADAPTATION IN THE DECEREBRATE CAT. L. Hayward, U. Wesselmann and W.Z. Rymer, Dept. of Physiology, Northwestern University and The Rehabilitation Institute of Chicago, Chicago, IL 60611.

A fatigue-induced inhibitory motor reflex (Woods,J., et al., J. Neurophysiol. 58:125, 1987) has been identified as one possible mechanism underlying the decline in mean motor unit firing rate observed during fatiguing contractions in humans. Previously, we demonstrated that small diameter muscle afferents are stimulated during fatigue and may mediate this inhibitory reflex. In the present study the effect of this fatigue-induced inhibitory reflex on motoneuron rate adaptation was examined in the decerebrate cat.

MN discharge rate was recorded from medial gastrocnemius (MG) motor axons isolated from a small filament cut from the intact MG muscle nerve. Muscle force, length, EMG and MN firing rate were recorded during 10 seconds of longitudinal tendon vibration, alone, or combined with moderate levels of steady crossed-extension excitation. The pattern of MN 'late' rate adaptation, or the drop in rate over 5 seconds, beginning at 1.5 seconds following the onset of vibration, was evaluated before fatigue and during fatigue. Preliminary results from 23 MG MNs suggest that feedback from an ischemic fatigued muscle consistently modifies the pattern of MN 'late' rate adaptation, supporting the role of reflex modulation of MN rate during fatiguing contractions. Before fatigue there was a significant correlation between the initial discharge rate of a MN (measured at 1.5 seconds) and the drop in rate over 5 seconds. During fatigue alone moderate changes in the drop in rate over 5 seconds were observed and a significant increase in the drop in rate was measured in only 4 of 17 MNs recorded (one-way ANOVA, $p<0.05$). During fatigue combined with ischemia the drop in rate over 5 seconds increased in all the MNs recorded. This fatigue/ischemia-induced change was significant in 7 out of 14 MNs tested. Procaine block (0.01%) of the MG muscle nerve produced a reversal of the fatigue/ischemia-induced changes in 6 of 8 motoneurons.

367.18

OPERANT CONDITIONING OF TRICEPS SURAE H-REFLEX: THE FIRST DAYS. J.R. Wolpaw. Wadsworth Labs, NY St Dept Health & SUNY, Albany, NY 12201.

Earlier studies have shown that monkeys (Macaca nemestrina) can gradually increase or decrease the size of the spinal stretch reflex (SSR) or of its electrical analog the H-reflex (HR), and that such conditioning changes the spinal cord itself (Wolpaw & Carp, TINS 13:137-142, 1990). SSR conditioning occurs in two phases: a rapid phase I change of about 8% occurs within 6 hours and a very gradual phase II change of 1-2%/day occurs over at least 40 days. Previous data suggested similar phases in HR conditioning. The present study, comprising data from 33 HR animals (20 HRup & 13 HRdown), further evaluates change in the first days.

The HRup data showed a marked phase I increase. Initial analysis indicated that it was about twice as large as the phase I increase with SSR conditioning. In contrast, the HRdown data, while showing amplitude decline in the first days, did not appear to show an abrupt phase I decrease.

These results could indicate a fundamental difference between HRup and HRdown conditioning. However, it seems more likely that they reflect the presense of a nonspecific factor, associated with the onsets of both HRup and HRdown conditioning, that acutely increases HR size, thereby exaggerating HRup phase I change and cancelling out HRdown phase I change.
(Supported in part by NIH NS22189 & Paralyzed Veterans.)

368.1

DO MUSCLE AND MOTONEURON CHANGES ACCOUNT FOR LOCOMOTOR DEFICITS AFTER SENSORIMOTOR CORTEX LESIONS IN RATS? D.M. Basso, A.M. Gentile and D.G. Stein. Brain Research Laboratory, Rutgers-The State University, Newark, NJ 07102

This study evaluated the effects of sensorimotor (SM) cortex lesions on locomotor behavior, muscle and motoneuron (MN) morphology and metabolism. Sprague Dawley adult rats (n=24) were pre- and postoperatively trained to locomote on a narrow elevated runway. Twelve rats had bilateral SM aspirations (SM) and 12 were sham operated. After 15 days, animals were sacrificed and sections of brain, cord L_{3-5}, Soleus and Extensor Digitorum Longus were taken. Using mean run time (RT) and cinematographic measures of the hindlimb swing movement, locomotion of SM rats was found to be significantly impaired postoperatively. Although SM rats achieved preoperative RT by the 7th session, the hindlimb movement deficit persisted. There was no apparent muscle atrophy or change in metabolic activity following SM damage. Hence, the aberrant movement patterns that persist following SM removal appear unrelated to changes at the muscle level.

368.2

EFFECTS OF MOVEMENT HISTORY AND REFLEX ACTION ON MUSCLE STIFFNESS PROPERTIES OF THE DECEREBRATE CAT. R.F. Kirsch, D. Boskov, and W.Z. Rymer. Northwestern Univ. Med. School, Chicago, IL 60611.

The intrinsic stiffness properties of triceps surae muscles of the decerebrate cat, and the impact of reflex action on these properties, were examined in 10 preparations for two conditions: during the transition from the isometric state to motion produced by "step" stretches of several amplitudes and during the continuous movement imposed during various stochastic perturbations. Mean force was established by the crossed extension reflex or electrical stimulation of the muscle nerve. In 4 experiments, muscle responses were examined before and after deafferentation by dorsal rhizotomy.

Areflexive muscle responded in qualitatively different ways to the step and stochastic perturbations. Previously stationary muscle "yielded" abruptly if the muscle was stretched more than a fraction of a millimeter, with force declining while length was still increasing. After an initial transient phase, however, stochastically-perturbed muscle reached an equilibrium condition where force variations were linearly related to the length changes and no further amplitude-dependent nonlinearities were observed, even though movements larger and more rapid than those which caused yielding during "step" trials were common.

Reflex action was found to offset these intrinsic muscle properties, significantly enhancing muscle stiffness during step stretches, but having a more modest effect during stochastic perturbations. In both cases, however, the effect of reflex action appeared to be *predictive* in nature. The reflexively-mediated increase in stiffness during stochastic trials was not shifted in time by known loop delays, but simply scaled the instantaneous stiffness magnitude. Reflex responses during step stretches also preceded those expected of a negative feedback response to yielding.

It is hypothesized that reflex action in this preparation is organized in a loose, predominantly predictive manner to prevent catastrophic muscle nonlinearities rather to rigidly regulate any particular property such as muscle length or stiffness.

368.3

GOING FOR A WALK INDUCES WIDESPREAD EXPRESSION OF THE FOS PROTEIN PRODUCT OF THE C-FOS PROTO-ONCOGENE IN CENTRAL MOTOR SYSTEMS. K.R. Gogas, S.C. Ahlgren, J.D. Levine and A.I. Basbaum Depts. of Anatomy, Physiology and Medicine, UCSF, CA 94143.

Most studies that monitor the expression of c-fos to define populations of active neurons use an exogenous stimulus and/or drug. This study evaluated the pattern of Fos expression that results from a maintained behavior, namely walking on a rotarod treadmill. The rotarod was set at a slow turning speed (2rpm) so that the rats could walk uninterrupted for one hour. The rats were then anesthetized, perfused and sections of brain and spinal cord were processed for Fos-like immunoreactive (FLI) neurons. Some rats underwent unilateral dorsal rhizotomies (C5-T1) prior to testing.

The pattern of FLI in both the lumbar and cervical enlargements was comparable. Numerous cells were found in the nucleus proprius, in the medial parts of laminae V,VI and the inner substantia gelatinosa. Finally, cells were found in the ventral horn, including several densely labelled motorneurons. Labelled cells were also found in the dorsal column nuclei (DCN) and in the lateral cuneate nucleus. The cerebellum contained very extensive labelling, in the flocculus and paraflocculus and in the cerebellar hemispheres. The labeling was concentrated in dense patches of granule cells. Some folia contained labelled cells in the molecular layer. Purkinje cells were never labelled. Because of the relatively high basal level of Fos expression in the cerebral cortex, this region was not further analyzed. With the exception of the flocculus, deafferentation significantly reduced the numbers of FLI neurons in the spinal cord, medulla and cerebellum ipsilateral to the rhizotomy.

We conclude that walking results in widespread expression of the c-fos proto-oncogene; the effect of rhizotomy indicates that this is, in part, generated via input over large diameter afferents from muscle and joints. The appearance of labelled motorneurons (previously unreported even after peripheral nerve stimulation or section) and labelling in the DCN (not seen after non-noxious peripheral stimulation) indicates that the pattern of inputs may be critical to the induction of this early response gene. Supported by NS 14627, 21445, 07265 and AM32634

368.4

LOCALIZATION OF SPINAL NEURONS ACTIVATED DURING TREADMILL LOCOMOTION USING THE C-FOS IMMUNOHISTOCHEMICAL METHOD. X.Dai*, J.R.Douglas*, J.I.Nagy, B.R.Noga, and L.M.Jordan, Dept. of Physiology, University of Manitoba, Winnipeg, M.B. R3E 0W3

Immunohistochemical detection of the proto-oncogene c-fos expression has been used successfully to detect spinal cord neurons activated by sensory stimulation (Hunt *et al.*, Nature, 328:632, 1987). We have adopted this method to determine the location of spinal cord neurons which show c-fos induction during treadmill locomotion induced by stimulation of the mesencephalic locomotor region (MLR).

Cats were anaesthetized with halothane, intubated and then decerebrated at the precollicular-postmammillary level. Following a recovery period (1 hour), the MLR was electrically stimulated (50-150 μA, 0.5 msec duration pulses, 20 Hz) to induce bouts of treadmill locomotion over a 9 hour period. Control animals were subject to the same procedure but were not electrically stimulated. The animals were perfused with a mixture of 4% paraformaldehyde and 0.2% picric acid in the phosphate buffer solution ten hours after decerebration, and the spinal cord (L3-S1) was processed for c-fos immunoreactivity.

In cats subject to the locomotor task, most labelled cells were found in Rexed's laminae III, IV, VI, and VII. A bimodal distribution of labelled cells was found among Rexed's laminae, such that labelled cells in the dorsal horn were concentrated mostly in laminae III and IV. A more ventrally located group of labelled cells was concentrated in lamina VII, with some extending into lamina VI. Very few labelled cells were observed in control cats. It is suggested that cells primarily located in laminae VI and VII are involved in the production of MLR-evoked locomotion. The dorsal group of labelled cells may have been activated largely due to afferent feedback from the moving limb. The results are consistent with those obtained previously (Fortier *et al.*, Soc. Nsci. Abstr., 1988) and show that MLR stimulation activates interneurons mostly in laminae VI and VII of the lower lumber segments. Supported by the Medical Research Council of Canada.

368.5

MEDIOVENTRAL MEDULLA-LOCOMOTOR REGION SPINAL PROJECTIONS IN RAT. Y.Ishikawa*, R.D.Skinner and E.Garcia-Rill. Department of Anatomy. University of Arkansas for Medical Sciences, Little Rock, AR.

Previously we described a region in the rat medioventral medulla (MED) which, 1) can be electrically and chemically activated to produce locomotion, and 2) receives input from the mesencephalic locomotor region. The MED includes most of the NR gigantocellularis pars alpha (GiA), the rostral half of pars ventralis (GiV) and the ventralmost region of NR gigantocellularis (Gi) dorsal to GiA and GiV. True Blue (TB) was injected (0.2 µl x 6) into the cervical enlargement and 6-7 days later Nuclear Yellow (NY) was injected (0.1µl x 6) into the lumbar enlargement. After 1-2 days, the rats were anesthetized and perfused with 4% paraformaldehyde in phosphate buffer. Labeled cells were plotted on outlines of sagittal sections using a fluorescence image-analysis system (Biographics). Single TB and NY-labeled neurons were found throughout the medial medulla and caudal pons in Gi, GiA and GiV, and NR magnocellularis (MC) and pontis caudalis (PC). Single TB-labeled neurons were more numerous and their distribution extended more into MC and PC. TB-NY double-labeled neurons tended to cluster in the MED region with 58% of them being found within this small region. They represent 15% of all labeled MED cells (TB 36%, NY 49%). These results suggest that the MED-locomotor region has descending output to each of the enlargements with some projections to both. Supported by USPHS Grant NS 21981.

368.6

ENTRAINMENT OF THE LOCOMOTOR RHYTHM IN SPINAL CATS BY STRETCH OF ANKLE EXTENSOR MUSCLES. K.G.Pearson and J.M.Ramirez, Department of Physiology, University of Alberta, Edmonton, Canada.

In walking cats the transition from stance to swing in each leg is regulated by afferent input. The identity of the proprioceptors involved in this regulation has not been firmly established, although receptors associated with the hip and with the ankle extensor muscles have been implicated. To further investigate the role of proprioceptive input from ankle extensor muscles we have examined the influence of rhythmically stretching the ankle extensor muscles on the locomotor pattern generated in chronic spinal cats (3 to 10 days after spinalization). Both hindlegs in adult cats were extensively denervated leaving intact the innervation to ankle extensor and knee flexor muscles. Following decerebration and recovery from anesthetic Clonidine (50 to 100µg/Kg) was administered i.v. thus enabling sustained sequences of locomotor activity to be elicited in response to stimulation of the skin of the perineum. When the ankle extensor muscles of both legs were rhythmically stretched in antiphase the locomotor rhythm was entrained over a wide range of frequencies (0.4 to 1 Hz). At all frequencies the bursts of activity of knee flexor activity commenced immediately following the onset of the shortening phase of the stretch and following a marked decline in the force in the ipsilateral ankle extensor muscle. Currently we are attempting to establish whether the decrease in muscle length or the decline in extensor muscle force is responsible for initiating flexor burst generation. One indication for the latter is that the locomotor rhythm can be entrained by rhythmically stimulating the distal cut stump of one L7 ventral root at strengths that are below those necessary to activate γ-motoneurons. Thus our findings are consistent with the notion that a necessary condition for the initiation of the swing phase is a decline in the force in extensor muscles.

368.7

RESPONSES TO HIND PAW STIMULATION DURING BACKWARD AND FORWARD WALKING IN CATS. J. A. Buford and J. L. Smith. Neuromotor Control Laboratory, Dept. Kinesiology, UCLA, Los Angeles, CA 90024-1568.

We have previously described kinematics (Buford et al. 1988) and EMG (Buford and Smith 1989) for backward (BWD) and forward (FWD) treadmill walking in normal cats. Although details of the EMG distinguished motor patterns for BWD and FWD walking, flexors were active in swing and extensors were active in stance for both directions.

Our present studies explore cutaneous reflexes referred to as "stumbling corrective" reactions by Forssberg (1979). An object striking the paw anteriorly would be an obstacle during FWD swing, but not during BWD swing. How will the neuromuscular responses to paw stimulation differ for BWD and FWD walking? To investigate, we implanted EMG electrodes in selected hindlimb muscles of cats trained to walk BWD and FWD on a motorized treadmill (0.4-0.6 m/s). Stimuli were applied at various stages of the step cycle through subcutaneous electrodes at the anterior aspect of the paw, and EMG and ciné data were recorded.

Our prior studies demonstrated that semitendinousus (ST) was active throughout swing for BWD walking, compared to the two-burst pattern with activity at paw off and at paw contact for FWD walking. EMG data indicate that despite different patterns for BWD and FWD walking, reflex responses for ST were similar. For both directions, ST was activated at short (10-20 ms) and medium (40-55 ms) latencies for stimuli delivered in swing, and ST did not respond to stimuli in stance. The anteriorly applied stimuli tended to cause posterior motion of the paw, retarding forward limb motion during FWD swing and accelerating backward motion during BWD swing. For both directions, anterior biceps femoris (ABF), a hip extensor active in stance, was inhibited at medium (20-25 ms) latency by stimuli delivered in stance, but showed no response to stimuli delivered in swing. Kinematic responses in stance were slight.

Thus, for ST and ABF, EMG responses to dorsal paw stimuli were similar for BWD and FWD walking, suggesting similar control for both directions. Other muscles under study may show different, context-dependent responses for BWD and FWD walking. The present data support a multi-use CPG model. Supported by NIH NS19864.

368.8

GAIT-RELATED SEMITENDINOSUS EMG PATTERNS AND KINEMATICS FOR TREADMILL LOCOMOTION IN CATS. S.H. Chung, J.A. Buford, and J.L. Smith. Neuromotor Control Lab, Dept. Kinesiology, UCLA, Los Angeles, CA 90024-1586.

In a recent paper from our lab (Wisleder et al. *Exp. Brain Res.* 1990), we described gait-related changes in hip and knee joint dynamics during locomotion at a slow walk to a moderately-fast gallop. From our dynamical data, we hypothesized that biarticular posterior thigh muscles, such as the semitendinosus (ST; hip extensor and knee flexor), function differently for walking and trotting than for galloping. Here, we relate changes in ST-EMG patterns to gait-related changes in hindlimb kinematics. We recorded EMG from electrodes surgically implanted in hindlimb muscles including the proximal ST and assessed kinematics from high-speed, ciné film.

During walking and trotting, there were two ST-EMG bursts for each step, one associated with paw off (STpo) and one with paw contact (STpc). STpo onset preceded the knee E3-F reversal (ext.- flex.), occurring before paw off for walking but at paw off for trotting. Cycle period decreased markedly with increased speed, but STpo burst durations did not change. In contrast, an increase in integrated STpo burst amplitude reflected an increased range of knee flexion during swing. STpc onset preceded paw contact and coincided with the E1-E2 reversal (ext.-flex.), occurring at contact for walking but before contact for trotting. STpc burst duration and amplitude increased with speed and reflected a greater need to decelerate the hindlimb's forward motion (hip flexion and knee extension) at the end of swing. Taken together, our data suggest that the two bursts which characterized the walk and trot—STpo and STpc—are under different sensorimotor control by spinal or supraspinal circuits.

During the gallop, ST activity was markedly different. There was only one major ST burst during swing which occurred appreciably after the knee F-E1 reversal and preceded the onset of knee flexion just before contact. This burst was largely coactive with extensors at the ankle, knee and hip and may increase hindlimb stiffness for an effective stretch-shorten cycle in stance. Research supported by NIH NS 19864.

368.9

POTENTIAL SOURCES FOR DIFFERENTIAL CONTROL OF TWO SHORT-LATENCY CUTANEOUS PATHWAYS TO CAT FDL MOTONEURONS. R.E.Burke, G.N.Sholomenko and A.K.Moschovakis Lab. of Neural Control, NINDS, NIH, Bethesda, MD 20892

Previous work from this laboratory has shown that low threshold cutaneous afferents in the superficial peroneal (SP) and medial plantar (PLNT) nerves produce minimally-disynaptic excitatory postsynaptic potentials (EPSPs) in flexor digitorum longus (FDL) α-motoneurons. SP and PLNT EPSPs are differentially modulated in various step cycle phases during fictive locomotion in the decerebrate cat (Schmidt et al., *Exp. Brain Res.* 71:568, 1988; Sholomenko et al., *Soc. Neurosci. Abstr.* 15:394, 1989). This indicates that the SP→FDL and PLNT→FDL pathways are entirely separate interneuronal systems that are subject to differential control by the central pattern generator (CPG) for locomotion. We have now begun studies to determine whether there are other definable input sources that can exert differential control of the two pathways. To date we have studied the effects from two supraspinal sources, the red nucleus (RN) and Deiters' nucleus (LVN) on transmission in the SP→FDL and PLNT→FDL pathways in adult cats anesthetized with chloralose or in unanesthetized, decerebrate cats. Short trains of pulses (4 pulses, 200 Hz, 50-100μA, monopolar tungsten electrodes) delivered to RN facilitated both SP (n-25) and PLNT (n=27) short-latency EPSPs. Stimulation of LVN produced less consistent results but there was also no evidence of differential effect on SP and PLNT EPSPs in individual neurons. These descending systems thus cannot account for the differential modulation of the two pathways during fictive stepping. Testing of other input sources for differential effects is underway.

368.10

PHASIC MODULATION OF EPSPs IN EXTENSOR MOTONEURONES EVOKED BY Ib INPUT DURING FICTIVE LOCOMOTION IN THE SPINAL CAT. J.-P. Gossard, H. Hultborn*, I. Barajon*, O. Kiehn* and B. Conway*. Dept. Neurophysiology, Univ. Copenhagen, DK-2200 Copenhagen, Denmark.

Proprioceptive input from knee and ankle extensors are able to reset the fictive rhythm in spinal cats injected with nialamide and L-DOPA by apruptly terminating the flexor activity and initiating an extensor burst (Conway et al. Exp. Brain Res. 68: 643-656, 1987). This study investigates the changes in transmission in the Ib excitatory pathway to extensors that occur during fictive locomotion.

Cats underwent anaemic decerebration and spinalization (T12) under steroid anesthesia. Muscle nerves from both hindlimbs were dissected and mounted to be recorded or stimulated. Laminectomy was performed and motoneurones were recorded with K+-acetate micropipettes. Slow infusion of nialamide and L-DOPA first uncovered long-lasting FRA responses and later induced sustained fictive locomotion.

Responses to the stimulation of different nerves were recorded in ankle extensor motoneurones during the long-lasting FRA responses and later in different parts of the fictive locomotor cycle (LC). Before the L-DOPA infusion, the stimulation of plantaris (or medial gastrocnemius (G), lateral G-soleus, quadriceps; 1.6 to 2.0xT) elicited IPSPs in the impaled cells. Slow infusion of L-DOPA decreased progressively the amplitude of the IPSPs and revealed EPSPs (Ib EPSPs) of increasing amplitude (>10mV). Injection of depolarizing currents in the motoneuronal soma through the micropipette increased the amplitude of the Ib EPSPs while hyperpolarizing currents decreased it. During fictive locomotion, the amplitude of the EPSPs was profoundly modulated through the LC: minimum during the extensor phase and maximum during the flexor phase. The maximum locomotor drive potential in the recorded cells were always out-of-phase with the maximal Ib EPSP.

One possible explanation is that transmission of the Ib EPSP is occluded during the extensor phase when the "extensor center" mediating the Ib EPSP is already active.

368.11

MEDIAL GASTROCNEMIUS TENDON FORCES AND MUSCLE ACTIVITY DURING CAT SCRATCHING. P. Carlson Kuhta and J.L. Smith. Neuromotor Control Lab., Dept. Kinesiology, UCLA, Los Angeles, CA 90024-1568.

Comparison of hindlimb muscle activities between no-contact 'air scratching' in decerebrate cats and contact scratching in normally-behaving cats suggest that force feedback or other motion-related feedback may be important in prolonging the extensor burst and delaying the onset of flexor activity during paw contact (Carlson Kuhta & Smith 1989). To test this hypothesis, fine-wire electrodes were surgically implanted in tibialis anterior (TA) and medial gastrocnemius (MG) and MG-tendon forces were measured by a buckle force transducer. Scratches were elicited by ear stimulation; in some trials, EMG and force measurements were synchronized with 100 fr/s ciné film.

During each scratch cycle, the paw normally contacted the head at the base of the pinna, and during contact, peak MG tendon forces varied between 3-6 N, about 60% of the peak developed during walking (Walmsely et al. *J. Neurophysiol.* 1978). Force increases usually coincided with the onset of contact, and MG tendon forces gradually increased to a peak value during contact. The termination of contact usually coincided with peak force. MG-EMG activity preceded paw contact by 15-20 ms, and MG burst durations ranged from 48-84 ms in cycles with low forces *vs.* high-force cycles, respectively; similarly, cycle periods varied between 138-181 ms. In contrast, TA burst durations remained relatively constant, ≈ 45 ms, in all cycles, with the onset of TA-EMG coinciding with peak tendon force.

Our results suggest that ankle extensor activity varies with cycle period and peak force, suggesting that the timing and amplitude of MG-EMG is influenced by motion-related feedback, either proprioceptive or tactile *or* both. Possibly, extensor activity is first facilitated by contact or development of contractile force, but as tension increases, feedback may inhibit extensor and facilitate flexor activity, thereby preventing the development of large contact forces at the head. In contrast, extensor bursts are very brief (≈ 30 ms) in decerebrate air-scratching (Deliagina et al., *Brain Res.* 1975), a behavior in which no paw contact occurs, thereby limiting modulation by feedback on spinal centers that program scratching. Funded by NIH NS19864.

368.12

RESPONSES TO MICROSTIMULATION OF THE MEDIAL RETICULAR FORMATION DURING FICTIVE LOCOMOTION IN THE DECEREBRATE CAT. M-C. Perreault, T. Drew and S. Rossignol. Centre de Recherche en Sciences Neurologiques, Univ. de Montréal, Montréal, Québec, Canada.

Short (30ms) trains of stimuli delivered to the medial medullary reticular formation (mMRF) in the decerebrate walking cat evoke short latency phase-dependent responses in both extensor and flexor limb muscles; such trains have only weak effects on the duration and the timing of the step cycle (Drew and Rossignol, J. Neurophysiol., 55 375-410, 1984). In the present study we have examined the effects of similar mMRF stimulation during fictive walking.

Trains of stimuli (30ms train of 0.2ms pulses at 330Hz, 20-40uA) were delivered to the mMRF through glass-coated tungsten microelectrodes in three decerebrate and curarized cats. Along each electrode trajectory, the stimuli, applied at 3-5 depths, were delivered at different phases of the fictive locomotor cycle. The electroneurographic activity was monitored with monopolar cuff electrodes placed around the proximal part of sectioned motor nerves of each of the four limbs.

The stimulation evoked short latency excitatory responses (6-13ms), in both flexor and extensor motor nerves, which were often followed by a period of inhibition (lat=25-45ms). Although less common, other excitatory responses were also seen at longer latencies (25-30ms). The amplitude of these excitatory responses was modulated in relation to the phase of the cycle in which the stimulus was applied. Unlike the situation in the decerebrate walking cat, these stimuli were also capable of arresting and resetting the rhythm.

Thus, as in the decerebrate cat walking on a treadmill, stimulation of the mMRF may cause transient changes in muscle activity which are modulated as a function of the step cycle. However, the fact that these short trains may also reset the locomotor rhythm, suggests that the effect of this descending pathway on the timing of the locomotor cycle is larger in the absence of peripheral afferent feedback from the moving limbs.(Supported by the F.R.S.Q. and the M.R.C.)

368.13

SARCOMERES WORK IN THE 'ASCENDING LIMB' IN CAT MEDIAL GASTROCNEMIUS MUSCLE DURING WALKING. A.A. Caputi, J.A. Hoffer & I.E. Pose*. Dept. Clin. Neurosci., U.Calgary, Calgary, Alta. T2N 4N1 Canada.

During walking, length changes of cat medial gastrocnemius (MG) muscle fibers depend on their location in the muscle. In particular, proximal fibers move over a wider range (100-150% of "resting" length) than distal fibers (90-110%; SN Abstr 15:521, 1989). We have now investigated fiber movement *at the sarcomere level.*

End-to-end fiber length was recorded at up to four different mid-sagittal MG locations during walking, using ultrasound transit-time. Post-mortem, in the formalin-fixed muscles of four cats, fiber fascicles were teased from locations where piezoelectric crystals had been sutured, sarcomere spacings were measured optically, average sarcomere lengths were calculated and total numbers of sarcomeres per fiber were estimated. Sarcomere lengths used by each cat during walking were then estimated from the *in-vivo* fiber length recordings.

The total number of sarcomeres per fiber increased linearly from 6000 ± 500 (SD) in proximal fibers (at 20% of the MG belly length) to 11500 ± 1000 in distal fibers (at 100%). During level walking at ~.5 m/s, the amplitude of sarcomere movement decreased linearly from .70 ± .08 μm for proximal fibers to .44 ± .08 μm for distal fibers. In spite of these systematic, site-specific differences, *at mid-stance phase the average sarcomere length was 1.81± .10 μm in all measured MG muscle locations.*

Thus, although the length changes of *muscle fibers* depend systematically on fiber location, all *sarcomeres* work at the same length during the phase of walking when the MG muscle is both *active* and *loaded*. Since sarcomere lengths of 1.7-1.9 μm fall in the 'ascending limb' of the sarcomere length-tension relation for mammalian skeletal muscle, the intrinsic stiffness of MG can play a major role in the overall restorative response to small perturbations of muscle length. Whether the narrow range of sarcomere lengths used during stance is largely a property of the design of the muscle or depends on regulatory feedback awaits further investigation.

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368.14

ALPHA-MOTONEURON ACTIVITY, AFFERENT ACTIVITY AND MUSCLE FIBER MOVEMENT SIMULTANEOUSLY RECORDED FROM CAT MEDIAL GASTROCNEMIUS MUSCLE DURING POSTURE AND LOCOMOTION. J.A. Hoffer and J.L.F. Weytjens. Dept. Clinical Neurosciences, Fac. Medicine, Univ. Calgary, Calgary, Alberta T2N 4N1, Canada.

During cat locomotion, the range and time course of movement of muscle fibers depend systematically on fiber location within the medial gastrocnemius (MG) muscle[1,2]. To interpret the origin of the discharge of muscle afferents as well as to estimate the force-generating properties of motor units, it is thus necessary to take into account local muscle fiber movement, rather than muscle length. We developed a preparation to record simultaneously the activity of individual muscle afferent and efferent neurons and length and pinnation angle changes of relevant muscle fibers.

In cats trained to perform a variety of postural and locomotory tasks, the lengths[1] and pinnation angles[2] of MG muscle fibers are obtained from the transit-time of pulsed ultrasound between pairs of implanted piezoelectric crystals. Extracellular action potentials from single motor or sensory axons are recorded by floating microelectrodes chronically implanted in the MG nerve, inserted through a window cut in the wall of a silicone nerve cuff implanted around the whole sciatic nerve. Two tripolar sets of circumferential electrodes inside the cuff serve to identify the direction and velocity of axonal conduction using spike-triggered averaging[3]. Artifacts in microelectrode records caused by high-voltage excitation of the crystals are avoided by careful routing and shielding of lead wires. Stable recordings are obtained from individual axons, usually lasting one or several days.

This preparation is currently being used to study relationships between afferent and efferent firing, and between afferent firing, efferent firing and muscle fiber movement, in normal cats and in cats rendered spastic by thoracic hemicordotomy.

1: Hoffer et al., *Prog. Brain Res. 80*:75-85, 1989; 2: Caputi et al., *Soc. Neurosci. Abstr. 15*:521, 1989; 3: Hoffer et al., *J. Neurosci. Methods 4*:211-225, 1981.

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CONTROL OF POSTURE AND MOVEMENT: LEARNING AND DEVELOPMENT

369.1

SEARCHING FOR SOLUTIONS: THE ACQUISITION OF COORDINATED MOVEMENT. P. V. McDonald*, R. E. A. van Emmerik*, P. N. Kugler* and K. M. Newell. Dept. of Kinesiology, University of Illinois, Urbana, IL 61801

The process of acquiring a skill may be characterized as an exploratory search for a solution in a region defined by the confluence of constraints arising from features of the task, the learner, and the environment. The experiments reported examine the exploratory behavior individuals used in mastering redundant degrees of freedom during the acquisition of coordinated movement. Of particular interest were the search strategies used to explore the perceptual-motor workspace and the relation of these strategies to features of the workspace. Subjects were asked to minimize as quickly as possible continuous, real time error information via motion of their two elbows. The error information (z) was generated by some function, $z = f(x,y)$, where x and y were the current joint angles of the two elbows. The data reported address the significance of the gain between elbow motion and error magnitude; the effects of number of local minima; and the symmetry of the function, with respect to a performance criterion, and more importantly the form of the search trajectory through the workspace. By adopting this approach we hope to understand the construction of a perception-action loop established during learning. It is the mapping of the kinematic and kinetic field properties that constrains and organizes the perception-action cycle (Kugler & Turvey, 1987).

369.2

LOAD COMPENSATION IN HUMAN AIMED ARM MOVEMENTS. O.Bock* (SPON: European Neuroscience Association). Human Performance Lab, Inst.f.Space&Terrestrial Science, York University, Toronto, Ont., Canada.

We analysed the execution of pointing movements in humans while different loads were applied to the pointing hand. Visual feedback on arm and hand was excluded.

Hand movement paths, final positions, and normalized velocity profiles were load-independent except for the very first movement after a load change.

When weight loads (0.45-2.00 kg) were applied, movement velocity decreased and duration increased by the same factor, i.e. the velocity profiles were re-scaled in magnitude and time; rescaling depended consistently on weight size. When spring loads (35 N/m) were applied, scaling of the velocity profiles was not different from no-load trials.

To understand these findings, we propose the following hypothesis of load compensation. The controlled variable of pointing movements is a fictional external force acting on the hand; inertia-, and gravity-related components of that force are controlled separately; changes of inertia are compensated by time-scaling of the inertia-related component, while changes of gravitational (and elastic) load are compensated by magnitude-scaling of the gravity-related component. This hypothesis predicts qualitatively and quantitatively well our experimental data.

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369.3

ADAPTABILITY OF MULTI-JOINT ARM MOVEMENTS TO ALTERATIONS IN SEGMENTAL INERTIA. G.K. Kerr and R.N. Marshall*. Univ. Lab. Physiology, Parks Road, Oxford OX1 3PT, U.K. and Dept. Human Movement, The Univ. Western Australia, Nedlands, 6009, Western Australia.

Unrestrained uniplanar vertical arm movements made to a target without vision of the limb were examined using a three-dimensional motion analysis system (Vicon). The self paced movements were performed under four load conditions; unweighted, hand weighted, forearm weighted, and upper-arm weighted. Under all experimental conditions hand paths were curved and the tangential velocity profiles were unimodal. Addition of the weight resulted in hand paths that differed from unweighted limb hand paths. These differences were dependent upon the limb segment to which the weight was added. However, subjects adapted to alterations in segmental inertia towards trajectories that were typical of an unweighted limb. This adaptation was extremely rapid and occurred within one to three trials. Our data suggests that the motor control system attempts to maintain a given hand path and tangential velocity profile for a particular movement, irrespective of external load conditions.

369.4

THREE PHASES OF HUMAN MOTOR LEARNING. H.-G. Ross. Physiologisches Institut, Universität Düsseldorf, F. R. G.

Tap dancing requires intricate sequences of leg & foot movements with appropriate postural adjustments. Since these patterns interfere with established postural & locomotor routines, tap dancing is hard to learn & thus an excellent paradigm to study the acquisition of motor skills.

The training of 10 adult beginners was observed until they reached an advanced, though not perfect, stage (1 hr/week for 3 yrs). This PHASE 0 (learning the technique), which is not amenable to quantitative assessment, may be the adult analogy of a child's learning to walk.

Thereafter, 3 subjects from this group & a professional (15 yrs of experience) were asked to practice a written choreography previously unknown to them. Taps were recorded by microphones under the soles of the shoes & evaluated in terms of inter-tap interval histograms. During practice, another two learning phases could be discerned: PHASE I (professional: ca. 400; advanced: ca. 800 taps) was characterized by the absence of a detectable pattern, using the wrong foot (or wrong parts of the feet), and periods of hesitation & deliberation. After having understood WHAT to do in PHASE I, subjects switched over to PHASE II, during which the required pattern was correctly produced and facility (accuracy and speed) gradually increased.

Summary: Characteristics of PHASES I & II suggest that humans apply learning strategies like those seen in monkeys (Brooks, V.B. et al., Physiol. Behav., 31:561, 1983).

369.5

A NEW STUDY OF HUMAN MOTOR LEARNING: STRATEGY AND TACTICS FOR MOVING OBJECTS IN SIMULATED REDUCED GRAVITY.

V.B. Brooks[2], F. Hilperath*, U. Longerich*, H.-G. Ross[1], and H.-J. Freund.
Depts. of Neurology, and Physiology II[1], Univ. of Düsseldorf, F.R.G.; and Dept. of Physiology[2], Univ. of Western Ontario, London, Canada.

We show that human subjects need to adopt a behaviorally appropriate task strategy (WHAT to do) in a new manual task before they can use well-programmed movements in task context (skill tactics, HOW to do it). The two kinds of learning always follow one another in two recognizable phases.

Subjects moved a stylus on a digitizing tablet to move a cursor accurately and rapidly on a display screen from a start box to a target box. This 'step-tracking' had to be completed in 2 s and the cursor held in target for a further 1 s. Screen vision and auditory cues signalled accuracy and task completion.

The learning of a new strategy needed for a simple task, usually obscured by its rapidity in human learning, was studied by inserting a new, non-linear relation between stylus and cursor movements. The cursor was made to move with simulated increased inertia, like an object in space that requires a push to start it and another, opposite one to stop it; implemented through modulating cursor positions on the screen by velocity changes of the hand-held stylus.

Normal, naive subjects at first made unsuccessful task attempts with dysmetric movements, programmed and nonprogrammed. After 60-80 such trials, in a series of 250, each learned to adopt the correct strategy of making two successive, oppositely directed hand movements to start and then stop the simulation-induced single glide of the cursor (learning WHAT, phase 1). From then on, most hand start-movements became well-programmed (learning HOW, phase 2) with brief, clustered reaction times. Initially tremulous hand stop-movements became smoother in 100-200 trials. The two learning phases resemble those in normal animal motor learning of ordinary step-tracking tasks. (Brooks VB, Kennedy PR, Ross H-G. Physiol. Behav., 31: 561-563, 1983; Brooks VB, Watts SL. J. Motor Behav., 20: 117-132, 1988).

369.6

CHANGES IN SPATIAL PATTERNS OF MUSCLE ACTIVATION FOLLOWING PARALYSIS OF A SYNERGIST MUSCLE. M. Moniz*, J.P.A. Dewald, T.S. Buchanan, R. Beer, J. Erikson, and W.Z. Rymer. Sensory Motor Performance Program, Rehabilitation Institute of Chicago, and Departments of Rehabilitation Medicine & Biomedical Engineering, Northwestern University, Chicago, IL 60611.

In previous studies we have examined muscle activation levels associated with changes in load direction during isometric contractions and have observed orderly spatial patterns of EMG activity, traversing angular ranges up to 180 degrees, with EMG peaks located at or near the angle of optimum muscle mechanical effect [1]. In this study we have investigated the plasticity of these patterns at the wrist and elbow by paralyzing a single muscle and examining the changes in the spatial relations of EMG activity from the remaining muscles acting at the joint.

At the elbow, EMG activities in up to ten muscles were recorded with surface and intramuscular electrodes during controlled static postures. Forces were applied at the wrist and were measured in three dimensions (flexion/extension, varus/valgus, and supination/pronation) while the arm position was fixed at 90° elbow flexion, neutral pro-supination position, and 65° shoulder abduction. At the wrist, EMGs were collected from the five muscles primarily responsible for flexion/extension and radial/ulnar deviation while forces were applied at the hand. At the elbow, the biceps brachii was paralyzed by injecting carbocaine at multiple motor points. For the wrist, the ulnar nerve was blocked at the elbow, which paralyzes the flexor carpi ulnaris.

We observed that, for some muscles with moment arms similar to those of the paralyzed muscle, the peak direction of the EMG activation curves shifted orientation after neural blockade; however, the polar plots of EMG activity as a function of direction did not broaden following the block. Other muscles with moment arms further away from the blocked muscles (i.e. less synergic muscles) showed little or no change in their spatial patterns of activation. These results suggest that muscles with similar actions are able to compensate for each other within the limits dictated by the orientation of their moment arms, and that patterns of muscle co-activation can be modified quickly (within less than one hour).

[1] Buchanan et al., *J. Neurophysiol.* 62:1201-1212, 1989.

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369.7

INTERSEGMENTAL DYNAMICS OF TREADMILL STEPPING IN INFANTS AND ADULTS J. L. Jensen*, B. D. Ulrich*, E. Thelen, K. Schneider* & R. F. Zernicke.
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During their first year, infants who cannot stand or step alone will take well-coordinated, alternating steps when supported on a motorized treadmill. Here we compare the qualitative dynamics and underlying apportionment of torques during treadmill steps of 7-month-old infants and adults. Phase plane dynamics showed that infants were unstable at all three leg joints during the stance phase. In the swing, similar pendular actions were observed in the knee in both infants and adults. Qualitative differences in the hip between infants and adults pointed to differences in the underlying control strategy. Intersegmental dynamics revealed that infants maintained a strong flexor muscle torque during the entire swing phase. Adults, in contrast, exploited the inertia of the swing leg and used a flexor muscle torque only to initiate the swing. These results are consistent with a general developmental tendency for increasing exploitation of passive dynamics in the service of intentional movement.

369.8

LOAD SENSITIVE REGULATION OF PRECISION GRIP: DIFFERENCES IN YOUNG AND OLD SUBJECTS. **B.A. Hart and J.H. Abbs. Dept. Human Kinetics, University of Wisconsin-Milwaukee, WI 53201 and Speech & Motor Control Labs, Waisman Center, University of Wisconsin-Madison, WI 53706.**

Adults in their 6th and 7th decades frequently experience difficulty in manual skills requiring fine grip control. Cutaneous mechanoreceptors of glabrous skin of the hand, which mediate precise force control (Westling & Johansson, 1984, 1987), are known to undergo age related reduction in density and changes in morphology. Although decrements in somatosensory thresholds have been reported for the aged, the functional consequences of such changes, and other nervous system changes, remain to be elucidated. In this experiment we compared young (n=10, avg age= 22) and old (n=10, avg age=71) subjects' kinematic and kinetic features of precision grip and lift of a small instrumented object which was randomly varied at .5,1,2,3, or 4 Ns in vertical load. Frictional characteristics of the skin of the finger and thumb pads were assessed by determining the grip force below which the object slipped from grasp (slip force). Although slip force levels tended to be higher for the older subjects, group differences were not significant. Grip force safety margins (i.e., grip force generated for static hold in excess of slip force) were significantly greater in older subjects (m=2.5N, range=2.3 to 2.7N) than the young (m=1.45N, range=.93 to 2.4N). Further, the safety margins were uniform across loads in the old but proportional to load in the young subjects. Group differences were found for the dynamic phase of the task as well. Duration of grip force onset to initial object displacement was 30% longer for the old, who concomitantly reached a significantly higher grip force prior to displacing the object. In spite of non-significant group differences in two-point discrimination and slip force levels, the older subjects demonstrated a grip and lift pattern which was slower, overly forceful, and not well scaled to the object load. Such performance characteristics may reflect subtle changes in hand sensibility, or alternatively, age related changes in integration of sensory and motor processes.

369.9

EFFECT OF AGE AND MOVEMENT SPEED ON COORDINATION OF RISING FROM A CHAIR. M.G. Hoy. Rehabilitation Research and Development Center, VA Medical Center, Palo Alto, CA 94304-1200.

Coordination of rising from a chair depends on movement speed in young adults (Pai, Y.C. et al., Proc. Am. Soc. Biomech., 1988). Since movement speed tends to decrease with age, the purpose of this study was to determine if the elderly coordinate the task in the same way as young adults.

Seventeen active elderly (71±6 yrs) and six young (24±2 yrs) women were asked to rise from a chair at slow, normal, and fast speeds. Joint motions were quantified with a video-based motion analysis system. Reaction forces were measured using force plates mounted in the ground and the chair seat. Muscle activity data were collected for seven lower-extremity muscles.

Although some elderly women moved at the same "normal" speed as young women, they were not able to increase their movement speed as much as the young women in the "fast" trials (elderly 24%; young 36%). The vertical ground reaction force (GRF) maximum when chair contact was lost increased with speed (normal 1.2 bw (body weights); fast 1.3 bw), and the GRF minimum associated with trunk deceleration at the end of the movement decreased with speed (normal 0.8 bw; fast 0.6 bw) for both groups. The peak magnitude of muscle activity relative to the magnitude during standard voluntary contractions tended to be greater for the elderly than for the young women, especially for tibialis anterior (elderly 1.4; young 0.9) and vastus medialis (elderly 1.2; young 0.9).

Differences between young and elderly women during rising may be due to differences in movement speed and muscle strength rather than by a fundamental difference in the ability of the elderly to coordinate the movement.

This work was supported by the Department of Veterans Affairs.

369.10

WITHDRAWN

369.11

INFORMATION FEEDBACK PRODUCES INTERFERENCE IN REPRODUCING MOVEMENTS. D.E. Nicholson and R.A. Schmidt*. Motor Control Laboratory, University of California, Los Angeles, 90024.

Knowledge of results (KR), a type of information feedback, when presented frequently, degrades long-term retention of motor skills. One explanation for this finding is that frequent KR increases performance variability, and KR-induced variability is detrimental to long-term retention. UCLA undergraduates (N=80) attempted to depress a lever for 1600 msec. Subjects practiced 68 trials, with KR presented after 50% of the trials. On 16% of the 68 trials, instead of attempting to achieve the 1600-msec goal, subjects were instructed to repeat their previous movement; KR, when presented, preceded repeat instructions. After repeat instructions, performance was more consistent than when attempting a 1600-msec goal ($p<.01$). After KR, changes in performance were large, relative to when KR was withheld. Even when instructed to repeat, KR produced systematic changes in performance, relative to no-KR conditions ($p<.01$). This interference, produced during attempts to repeat movements after KR, suggests that KR causes subjects to "update" previous movement representations, leading to increased variability on subsequent trials.

369.12

A LONGITUDINAL STUDY ON THE TRANSITION TO INDEPENDENT STANCE IN CHILDREN. H. Sveistrup*, M. Woollacott, A. Shumway-Cook and G. McCollum. Univ. of Oregon, Eugene, OR 97403.

This set of experiments is part of a larger study which takes a systems approach to the study of the emergence of independent stance, determining the contributions of and interactions among sensory, motor, and musculo-skeletal factors. The development of balance control has previously been studied cross-sectionally in children but cross-sectional studies mask some of the important factors in temporal sequencing and the individuality of developmental paths. Mature postural responses to anterior platform translations consist of activation of the stretched ankle muscle (tibialis anterior (TA) at 80-100 ms), then the thigh (quadriceps (Q)) and trunk (abdominal (A)) muscles at 20-40 ms lags.

In this study longitudinal data from 4 children aged 7-14 mos were grouped into 3 developmental stages: dependent stance 1 (pulls to stand using arms), dependent stance 2 (pulls to stand using half-kneel), and independent stance. Surface EMG measured the responses from the left TA, Q, A, gastroc. (G), hamstrings (H), and trunk extensor (TE) muscles to anterior or posterior support surface perturbations. Muscle strength was also monitored across stages, by determining the % of the child's total weight that she could support.

All children were able to support their total body weight well before the emergence of independent stance. However, no organized responses were present in children for either direction of platform perturbation at the beginning of the phase 1 of dependent stance. Preliminary results showed differences in the development of "extensor" synergies (G-H-TE) vs. "flexor" synergies (TA-Q-A). The TA-Q-A synergy emerged first, with the G-H-TE synergy emerging approximately 4 weeks later. The frequency with which responses could be elicited increased gradually across phases. This was accompanied by a decrease in the absolute latency and the variability of response onset. As independent stance emerged, there was a temporary increase in response variability, and disruption in response organization followed by further refinement of response characteristics. (Supported by FCAR to H. Sveistrup)

369.13

DEVELOPMENTAL ASPECTS OF INTERSENSORIMOTOR INTEGRATION. A. J. Chicoine[2], L. Proteau[1] and M. Lassonde[2]*. [1]U.Q.T.R., Trois-Rivières, Canada, G9A 5H7, [2]Lab. de Neuropsychologie Expérimentale, Université de Montréal, Canada, H3C 3J7.

The ontogenesis of the ability to integrate different sources of afferent information in order to perform accurate aiming movement was studied in 3 groups of subjects (5-6, 11-12 and 20-30 years of age). In each group, half the subjects performed 144 acquisition trials under a condition where only the target to be reached was visually available (target only condition, TO) while the other half were allowed to see both the target and their aiming movement (full vision condition, FV). Knowledge of results (KR) was provided after each trial. Following the acquisition period, all subjects were submitted to the same transfer task performed under the TO condition, with no-KR. The root mean square error of aiming accuracy obtained under the acquisition and transfer conditions was used to compute an index of aiming deterioration. The results showed that the subjects who practiced under the FV condition suffered a large increase in error when submitted to the transfer task and this effect was more pronounced in the older groups (3.34, 2.68 and 1.50, respectively). The fact that the younger subjects showed less decrease in accuracy than older subjects when submitted to the transfer task support the notion that the improvement of aiming accuracy occurring during childhood is linked to the ability to integrate numerous sources of sensory information. These results further lead to the conclusion that the underlying principle for skilled movement acquisition is intersensorimotor integration, where the available sources of sensory feedback are integrated and used in conjunction with central planning and execution processes to form the basis of movement representation.

369.14

MOTOR LEARNING OF SINGLE-JOINT (ELBOW) MOVEMENTS WITH ASYMMETRIC VELOCITY PROFILES. J.L. McFarland, S.M. Botros*, and C.G. Atkeson. Department of Brain and Cognitive Sciences, Massachusetts Institute of Technology, Cambridge, MA 02139.

We set out to test the hypothesis that the human neuromuscular system is specialized to produce limb movements with symmetric tangential velocity profiles by asking subjects to make single-joint (elbow) arm movements with specific tangential velocity profiles. We studied the motor learning of movements with asymmetric and symmetric velocity profiles. If there were no differences in the learning or performance of these two classes of movements then we would have evidence for rejection of this hypothesis.

Subjects performed horizontal elbow movements with specific amplitudes, durations, and velocity profiles. Immediately after each trial the subject was shown a display of their actual movement (both position and velocity over time) superimposed on a template of the desired movement. Subjects typically performed more that 50 trials for each test template.

When subjects were asked to make movements with symmetric velocity profiles they learned relatively quickly. They were able to match the amplitude, duration, and peak velocity of the movements and make movements with unimodal, symmetric velocity profiles. When the same subjects were asked to make movements (of the same amplitude and duration) whose velocity profiles were asymmetric they learned more slowly. Although subjects had little trouble matching the amplitude and duration of the desired movements, they had difficulty producing the desired asymmetric velocity profiles. Furthermore, even after 50 trials subjects would intermittently make movements whose velocity profiles were distinctly bimodal.

(Supported by NIH grant AR08048 to JLM and ONR contract N000 14-88-K-0321 to CGA).

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CEREBELLUM II

370.1

SIMPLE SPIKE REPRESENTATION OF THE BODY SURFACE WITHIN CLIMBING FIBER RECEPTIVE FIELDS OF PURKINJE CELLS IN CAT CEREBELLUM. J.F. Brons, L.T. Robertson, G. Tong,. Dept. Anatomy, SD. Oregon Health Sciences Univ., Portland, OR 97201-3097

The body surface is mapped onto the cerebellar cortex by both the climbing fiber (CF) and the mossy fiber/granule cell system. The degree of congruence of body representation by both systems was studied by mapping the simple spike (SS) responses associated with receptive fields based on CF responses. Single Purkinje cells were recorded extracellularly in the intermediate zone of lobule V in decerebrate cats. CF and SS responses were elicited by mechanical stimulation of the skin of the distal extremities and the face. In almost all cases, stimulation of the receptive field representing the CF response also elicited SS responses. However, 67% of the time, excitatory or inhibitory components of the SS responses were elicited by only a part of the CF receptive field such as either the proximal or distal region. SS responses probably reflect a mapping of the body surface that only partially overlaps the somatosensory projections of the CF system. (Supported by NIH grant NS18242)

370.2

NEURONS IN POSTERIOR CEREBELLUM OF RAT RESPOND TO SOMATOSENSORY AND AUDITORY STIMULI. P.W. Archer, M.A. Meredith and B.E. Stein. Depts. of Physiology and Anatomy, Med. Coll. Va., Va. Commonwealth Univ., Richmond, VA, 23298.

The convergence patterns of various sensory modalities onto individual neurons in the posterior cerebellum were studied in Long-Evans rats anesthetized with urethane and stabilized with a skull-mounted head-holding device. The most effective natural sensory stimulus for these cerebellar neurons proved to be tactile: gentle tapping, or brushing across the hair and skin activated 75/135 neurons. The receptive fields of these tactile neurons were most frequently found on the face (41%) and were overwhelmingly ipsilateral (99%). Surprisingly, approximately one third (n=24/75) of them proved to be multisensory (bimodal). They were activated by both tactile and auditory stimuli. Since the range of stimuli delivered (e.g. only white noise; only low intensity visual stimuli) was limited, this undoubtedly represents an underestimate of the incidence of multisensory convergence here. In 42% of the bimodal neurons studied the laterality of the receptive fields matched: both were ipsilateral. In 50% of them the auditory receptive field encompassed ipsilateral and contralateral space and in 8 % the receptive fields were clearly discordant (tactile = ipsilateral, auditory = contralateral). In several cases combined tactile-auditory stimulation produced response interactions, as has been noted elsewhere in the nervous system (e.g. see Meredith & Stein, J. Neurophysiol. 56:640-662, 1986). Supported by NIH grants NS 22543 and NSO8638.

370.3

LESIONS OF THE CEREBELLAR NUCLEI, BUT NOT OF MESENCEPHALIC STRUCTURES ALTERS THE SPATIAL PATTERN OF COMPLEX SPIKE SYNCHRONICITY AS DEMONSTRATED BY MULTIPLE ELECTRODE RECORDINGS. E. J. Lang, I. Sugihara, R. Llinás Dept of Physiology & Biophysics, New York University Medical Center, 550 First Avenue, New York, NY 10016.

Multiple electrode recordings of the complex spike activity (CS) of Crus IIa Purkinje cells (PCs) were used to investigate the influence of inferior olive (IO) afferents from the cerebellar nuclei (dentate) and red nucleus in controlling the synchronicity and rhythmicity of olivary cells in female albino rats. The cerebellar nuclear projection to the IO has been shown to account for the vast majority of the GABAergic terminals found within the olivary glomeruli. If these terminals are responsible for controlling the degree of IO electrotonic coupling, then lesions of the cerebellar nuclei should cause an increase of CS synchronicity. While simultaneously recording the CS of up to 44 PCs we pressure injected a mixture of kainic acid and NMDA directly into the dentate nucleus. The injection resulted in a significant ($p<<.0005$) increase in the simultaneous (within 1 msec) firing of PCs throughout Crus IIa (the average zero-time cross-correlation between PCs increased up to 11-fold from the control value). Lesions of the mesencephalon in the region of the red nucleus were made by pressure injecting kainic acid or lidocaine. These lesions altered the frequency of the CSs, but they did not produce a change in the pattern of synchronicity, nor did they prevent increases in CS synchronicity following IP injections of picrotoxin.

The results of these experiments provides strong support for the hypothesis that the electrotonic coupling of IO neurons is modulated by GABA release within the glomeruli from afferents arising in the cerebellar nuclei and that this modulation is the basis by which the pattern of synchronicity of CS is determined. Supported by grant 13742 from NINDS.

370.4

ARE THERE SUBCLASSES OF GRANULE CELLS AND PURKINJE CELLS? C.F.Hsiao, R.Huang*, H.Mu* and C.Huang. Div. Structural & Systems Biol., Sch. Basic Life Sci., Univ. Missouri-Kansas City, Kansas City, MO 64110.

To correlate the electrophysiological and morphological properties of granule cells and Purkinje cells, we have used HRP-filled glass microelectrodes to record and stain 30 granule cells and 25 Purkinje cells from the cerebellum of the cat. Granule cells showed extracellular action potentials with broader half-widths (0.78±0.14 ms) than those of Purkinje cells (0.22±0.06 ms). Resting membrane potentials for granule cells and Purkinje cells were 40 and 65 mV respectively. The inter-spike interval of granule cells (333.3±195.4 ms) was longer than that of Purkinje cells (47.3±31.8 ms). Granule cells had small soma (5-8 μm) with 3-5 short dendrites (<30 μm). Purkinje cells had larger soma (25-40 μm) and a characteristic, two-dimensional dendritic tree structure. A tentative subclassification of granule cells into two types was attempted on the basis of the complexity of their dendritic arborization. A similar classification for Purkinje cells was made on the width of its dendritic tree structure. (Supported by PHS grant AA07643.)

370.5

THE ACTIVITY OF BLINK-RELATED PURKINJE CELLS DOES NOT AFFECT THE BLINK REFLEX J. J. Pellegrini and C. Evinger, Dept. Neurobiology & Behavior, SUNY Stony Brook, Stony Brook, NY 11794

Recordings from crus II Purkinje cells of rats reveal that corneal stimulation evokes a time locked complex spike (latency ~20ms), a pause in activity, and a subsequent burst of simple spikes, all of which correlate with the concomitant blink. The decrease in blink magnitude produced by preceding a corneal stimulus with an identical conditioning stimulus correlated with a reduction in the Purkinje cell response. In order to assess the contribution of Purkinje cell activity to the execution of reflex blinks, we recorded orbicularis oculi EMG (OOemg) responses to electrical stimulation of the cornea before, during and after inferior olive inactivation in rats anaesthetized with urethane.

Microinjections of lidocaine into the contralateral inferior olive radically altered the response of blink-related Purkinje cells to corneal stimulation. Not only was the complex spike and the pause in simple spikes which follow it absent, but the subsequent burst of activity was smaller or nonexistant. During inferior olive inactivation however, the OOemg exhibited no consistent change. Similarly, while both microinjections of lidocaine into the inferior olive and intraperitoneal injections of 3-acetylpyridine dramatically changed the Purkinje cell responses, neither altered the difference between control and conditioned OOemg responses. Also, in normal rats, complex spikes consistently occurred at lower stimulus intensities than did OOemg responses and appeared time locked to the stimulus but not the movement. These results reveal that Purkinje cell activity reflects sensory elements associated with corneal stimuli and blinking rather than modulating the real time motor commands for each blink. Supported by EY07391.

370.6

FURA-2 IMAGING OF INTRACELLULAR CALCIUM TRANSIENTS IN PURKINJE CELLS FOLLOWING GLUTAMATE IONTOPHORESIS. M. Sugimori and R. Llinás,
Dept. of Physiology and Biophysics, N.Y.U. Medical Center, 550 First Ave, N.Y., N.Y. 10016.

It has been amply confirmed that Purkinje cells generate Ca-dependent action potentials in their dendrites (Llinás and Sugimori, *J. Physiol.* 305:197, 1980). Arsenazo-3 (Ross and Werman, *J. Physiol.* 389:319, 1987) and Fura-2 (Tank et al.; *Science*, 242:773, 1988) studies indicate that such spikes invade most of the Purkinje cell dendritic tree. Using a fast Fura-2 recording system (Sugimori & Llinas, *Soc. Neurosci. Abst.*, 15:179, 1989) we determined that dendritic spike initiation starts with the generation of a plateau potential at the spiny branchlets where voltage-dependent Ca conductances reached threshold before Ca action potentials are generated in the main dendrite. In the present study, glutamic acid was iontophoresed at different sites in the dendritic tree of Fura-2 loaded Purkinje cells. The Fura signal and intrasomatic voltage were monitored simultaneously allowing a detailed analysis of the site of plateau potential activation and its relation to Ca spike generation. We found that short iontophoretic pulses of glutamate produced graded postsynaptic responses that were not accompanied by intracellular Ca^{2+} transients (ICTs). However, if the glutamic acid depolarizations reached threshold for plateau potential activation, the spiny branchlets alone demonstrate ICTs. When the threshold for dendritic activation was attained the main dendritic tree displayed ICTs following a well-defined wave which moved downwards toward the Purkinje cell soma and antidromically into the other dendrites up to the level of the spiny branchlets. These results support the view that parallel fiber activation normally regulates Purkinje cell firing via the activation of plateau potentials in the spiny branchlets. Supported by NINDS 13742.

370.7

SYNAPTICALLY INDUCED DENDRITIC CALCIUM TRANSIENTS IN GUINEA PIG CEREBELLAR PURKINJE CELLS IN VITRO. V. Lev-Ram, H. Miyakawa, N. Lasser-Ross and W.N. Ross. Dept. of Physiology, N.Y. Medical College, Valhalla, NY 10595.

We measured the spatial and temporal characteristics of synaptically activated Ca transients using high speed imaging of changes in arsenazo III absorbance and fura-2 fluorescence while recording the intrasomatic membrane potential. All or none CF responses were elicited by bipolar stimulation of the white matter. PF potentials were evoked by stimulation near the pial surface and generated graded electrical responses blocked by CNQX. CF inputs produced no detectable ligand-gated calcium entry since the Ca changes had a latency from the onset of the synaptic potential at all dendritic locations. The CF response had two components which could have different spatial distributions. One was driven by the synaptic potential and the second by the Ca spike evoked by the synaptic potential. Both components showed rapid recovery. At high rates the spike component failed at some locations but not others, suggesting localized dendritic spiking. PF synaptic potentials produced small, localized Ca transients. When the electrical response was large enough to show signs of regenerative activity larger Ca changes over a wider area were detected with the same time course as somatically evoked calcium spike transients.

Supported by the NIH, NSF, and the Whitaker Foundation.

370.8

INCREASED GLUTAMIC ACID DECARBOXYLASE (GAD) mRNA IN CEREBELLAR PURKINJE CELLS FOLLOWING CLIMBING FIBER LESION-INDUCED INCREASES IN CELL FIRING. J.Litwak*, M.Mercugliano, M.-F.Chesselet and G.A.Oltmans. Dept. of Pharm. and Mol. Biol., Chicago Med. Sch., N.Chicago, IL 60064 and Dept. of Pharm., Univ. of Pennsylvania, Philadelphia, PA 19104.

The neurotoxin 3-acetylpyridine (3-AP) selectively destroys the inferior olive-climbing fiber input to the cerebellar cortex,resulting in (1) increased Purkinje cell firing, and (2) increased GAD activity in Purkinje cell terminals. Cell firing rates are initially doubled following the lesion, but return to baseline at about 30 days post-lesion. In contrast, GAD activity increases gradually and remains elevated up to 45 days post-lesion. The increase in GAD activity may be due to an induction of enzyme synthesis in response to the increase in cell firing. If so, increases in GAD mRNA might underlie the increase in GAD activity. In the current study, in situ hybridization was used at various post-lesion intervals to determine if Purkinje cell GAD mRNA levels increase following climbing fiber lesions, and to assess factors which might explain the discrepencies between changes in Purkinje cell firing rate and GAD activity.

Adult male rats were injected with either 3-AP or saline. At 7 days post-lesion the animals were killed and the brains rapidly frozen. Tissue sections for determining GAD mRNA and GAD activity were taken throughout the extent of the deep cerebellar nuclei (DCN). At 7 days post-lesion, GAD activity at Purkinje cell projection sites in the DCN was significantly increased (+58%) above control levels. GAD mRNA levels in Purkinje cell bodies from the same animals were also significantly elevated (+42%) above control. Because the lesions increase Purkinje cell firing rates, these results suggest increased firing induces transcription of GAD mRNA, which in turn increases GAD availability in Purkinje cell terminals. Other post-lesion periods are currently being studied to determine if this relationship exists beyond the initial lesion period. (Supported in part by the Dystonia Medical Research Foundation)

370.9

CLIMBING FIBER-INDUCED DECREASES IN "SIMPLE SPIKE" DISCHARGES OF CEREBELLAR PURKINJE CELLS IN THE UVULA-NODULUS OF THE RABBIT. H. Shojaku[1] and N.H. Barmack[2], [1]Dept. of Otolaryngol., Toyama Med.& Pharm. Univ., Toyama, Japan and [2]RS Dow Neurological Sciences Inst., Good Samaritan Hosp.& Med. Ctr., Portland, OR 97209.

The cerebellar uvula-nodulus receives vestibular and visual signals conveyed by both mossy fibers and climbing fibers. Interactions between these two afferent systems may be important for determining the functional output signal mediated by Purkinje cells. In this report we describe the vestibularly- and visually-evoked climbing fiber responses (CFRs) recorded from Purkinje cells and we examine how such CFRs influence Purkinje cell simple spike responses (SSs). We have recorded from single Purkinje cells in lobules 9c, 9d, and 10 of anesthetized rabbits during vestibular and optokinetic stimulation. Most vestibularly- and visually-evoked CFRs were synergistic. For example, CFRs recorded in the left nodulus, and evoked by vestibular stimulation in the plane of the left posterior-right anterior semicircular canals were also driven by downward optokinetic stimulation of the posterior field of the left eye. CFRs evoked by vestibular sinusoidal roll stimulation discharged in phase with stimulus angular velocity or stimulus position. Without exception, if both CFRs and SSs were modulated during sinusoidal vestibular roll stimulation in a single Purkinje cell, the modulation was antiphasic. Following the occurrence of a vestibularly-evoked CFR, there was a decrease in SSs. The duration of this CF-evoked decrease was inversely proportional to the initial frequency of SSs prior to the occurrence of the CFR. Our data suggest that vestibularly-evoked CFRs play an important role in the control of SSs and that this CFR-SS interaction may determine the specificity and amplitude of postural responses.

370.10

RENAL REPRESENTATION BY THE CLIMBING FIBER SYSTEM IN THE VERMAL CORTEX OF THE CAT CEREBELLUM. G. Tong, J.F. Brons, L.T. Robertson. Dept. Anatomy, SD. Oregon Health Sciences Univ., Portland, OR 97201

The projections of renal afferent fibers by the climbing fiber system to the vermal cortex were determined for a population of the Purkinje cells in the cat cerebellum. Under alpha chloralose anesthesia, the responsiveness of the Purkinje cells by the climbing fiber system were determined by the repeated presence of the complex spikes elicited by electrical stimulation of the central cut end of the renal nerves and by mechanical stimulation of the body surface. Of 255 isolated Purkinje cells, 15% responded to electrical stimulation of the renal nerves. Almost all of these Purkinje cells also received somatosensory input, mainly from forelimb and face. The renal climbing fiber responses (CFRs) were found in lobules V, VI, and VII. The highest percentages of responses were found in sublobules Vf (62.6%) and VIIB (31.6%). The latencies of the renal CFRs were from 23 to 106 ms. The average latency of the renal CFRs in sublobule VIIB (76 ms) was significantly longer than that in lobules V (46 ms) and VI (39 ms) ($p<0.002$). The findings indicate that the renal input projects to the vermal cortex via the climbing fiber system. (Supported by NIH grant NS18242)

370.11

MODULATION OF PURKINJE CELLS BY ENKEPHALIN AND CORTICOTROPIN RELEASING FACTOR IN THE OPOSSUM'S CEREBELLUM. G.A. Bishop and J.S. King. Dept. of Anatomy and Neuroscience Program. The Ohio State University, Columbus, OH 43210

In addition to the amino acids glutamate and aspartate, several peptides have been identified within cerebellar afferent systems. Two of these peptides, enkephalin (ENK) and corticotropin releasing factor (CRF) have been co-localized within climbing and mossy fibers (Cummings and King, '90; Synapse 5:167). The iontophoretic application of these peptides reveals that they alter the firing rate of Purkinje cells. ENK suppresses spontaneous, as well as glutamate and aspartate-induced firing of isolated neurons. Preliminary studies indicate that the effect is not blocked by the opiate antagonist Naloxone (Nal). Nal applied by itself at low currents also blocks neuronal activity; moreover, when Nal and ENK are applied simultaneously the suppressive effects are greater as compared to application of either chemical alone. Iontophoresis of CRF potentiates spontaneous activity, as well as the effects of glutamate and aspartate and partially blocks the suppressive effects elicited by the iontophoretic application of ENK. The present findings are somewhat paradoxical as they indicate that ENK and CRF, which are present in afferent systems known to be excitatory, have opposite effects in regulating Purkinje cell activity. On the basis of the Nal response, the data suggest that the suppressive effects of ENK are mediated at receptive sites that are not specific for opioids. In contrast, CRF likely elicits its effects through CRF receptors which have recently been identified in the opossum's cerebellum (Cummings et al., '89; JCN 280:501). Future studies will be designed to determine the circuitry involved as well as the mechanism(s) of action of these peptides in the cerebellar cortex (Supported by NS 08798).

370.12

TEMPORAL RELATIONSHIP OF INTERACTIONS OF VISUAL AND AUDITORY STIMULI IN RAT PARAFLOCCULAR NEURONS. Brian N. Maddux, S.A. Azizi, and D.J. Woodward. *Dept of Cell Biology and Neuroscience, UT Southwestern Medical Center, Dallas, Texas 75235.*

Ongoing studies in this laboratory have been aimed at clarifying the functional role of cortical input to the cerebellum. This study posed the question: what are the temporal interactions of visual and auditory cortical signals projecting to the paraflocculus lobule? Our previous reports have demonstrated visual and auditory cortical input to the rat paraflocculus via the dorsolateral basilar pons, and interactions between concurrent stimuli have been recorded. However, it was unknown whether the interaction was confined to the time of the concurrent stimulation or whether longer lasting interactions could be observed. In this study, unit recordings from parafloccular neurons were obtained with glass micropipettes in immobilized, sedated, locally anesthetized Long-Evans rats during visual and auditory stimulation. Images (dot patterns or bars) were projected onto a screen in front of the rat, and 10 kHz sinusoidal tones were issued from a frontally placed speaker. Delivery of individual or combined stimuli was precisely controlled by a computer. The tone was sounded either coincidentally with appearance of the image or 0, 200, or 500 ms following initiation of image movement (500 ms after appearance). Concurrent visual and tone stimuli were often observed to facilitate responses over either alone. In many cases, one or both of the stimuli were below threshold to cause an observable change in firing when presented in isolation. In a subset of the units, combinations of tone and image movement resulted in augmentation of the response to subsequent image movement in the same field after delays of up to 2 seconds in studies to date. These findings argue for the existence of a process (i.e., short-term storage) which sustains an interaction between visual and auditory modalities in a time frame longer than the known synaptic processes within the cerebellar cortex. *Supported by the Biological Humanics Foundation.*

370.13

PROJECTIONS FROM RAT TRIGEMINAL NUCLEUS ORALIS TO THE CEREBELLUM. L. A. Smith and W. M. Falls, Dept. of Anatomy, Michigan State University, East Lansing, MI 48824.

Unilateral deposits of PHA-L were made into each subdivision of rat trigeminal nucleus oralis (Vo). Axons of trigeminocerebellar projection neurons traversed the ipsilateral inferior cerebellar peduncle and formed terminal arborizations of two types bilaterally in Crura I and II, and lobules 7, 8 and 9 as well as ipsilaterally in the paramedian lobule (PML). The first type, arising from neurons in dorsomedial (DM) and dorsal one-half of the border zone (BZ) subdivisions terminated in superficial and deep portions of the granule cell layer in complex mossy fiber endings. Those located superficially had beaded branches extending into the Purkinje cell layer which approximated Purkinje cell somata. The second type of DM-BZ efferent terminated between the outer portion of the granule cell layer and the Purkinje cell layer and formed a claw-shaped terminal arbor with boutons surrounding a Purkinje cell soma. Injections made in the ventrolateral (VL) subdivision labeled axons similar to the first type of DM-BZ efferents. They terminated superficially in the granule cell layer, but were principally located in PML. These findings suggest that many endings of Vo efferents in Crura I and II, lobules 7, 8 and 9 and PML are positioned to provide not only a direct synaptic influence on granule cell dendrites, but also on the output cell of the cerebellum, i.e., Purkinje cell, through synapses on its soma. (Supported by Grant DE06725)

370.14

Complex oscillations in patients with cerebellar lesions. A. Beuter, C. Labrie*, J. Milton*. Univ. of Quebec at Montreal, C.P. 8888, Succ. A, H3C 3P8 and Univ. of Chicago.

Patients with lesions in the cerebellum (CL) have deficits in preprogramming and executing voluntary movements. We compared the effect of delayed visual feedback on a simple motor task in 8 patients with uni- or pan- cerebellar lesions and 8 normal subjects, matched for age, sex and laterality. This task required the subjects to maintain a constant finger position relative to a stationary baseline displayed on an oscilloscope. The signal controlled by the finger was delayed (0-1400 ms), recorded for 80 s using a LVDT, and digitized at 102 Hz. In the absence of a delay, time series for normal subjects displayed regular low amplitude, high frequency oscillations around the baseline. Traces for patients with unilateral CL indicated a tendency for smaller tremor amplitude, on the lesioned side. We also observed sudden destabilizations of the finger producing large amplitude, simple or double oscillations lasting between 0.5 and 1.0 s. With the addition of a 1400 ms delay, time series for normal subjects displayed complex, high amplitude, low frequency oscillations around the baseline with a period between 2 and 4 times the delay. Traces for patients with CL indicated qualitatively different dynamics on the lesioned side. These results suggest that the cerebellum plays a more significant role in this task than the basal ganglia (Beuter, A. et al., Neurosci., 15:692, 1989).

370.15

POSSIBLE MECHANISMS UNDERLYING REORGANIZATION IN FRACTURED TACTILE CEREBELLAR MAPS : CORRELATIONS WITH S-1 CORTICAL PLASTICITY. C.A. Shumway, L.G. Posakony, J. Morissette, and J.M. Bower. Div. of Biology 216-76, CalTech, Pasadena, CA. 91125.

Posakony et al., this volume, demonstrated that fractured tactile cerebellar maps in rats reorganize following infraorbital nerve lesion. In this study, we explored possible mechanisms underlying crus IIa reorganization. First, we physiologically characterized the crus IIa map in adult rats immediately before and after deafferentation. On average, only 13% of the penetrations in the denervated area displayed new representations, suggesting that immediate unmasking of existing connections does not play a significant role in cerebellar reorganization. Second, we characterized maps in animals deafferented at various developmental stages, and found that the denervated region filled in predominantly with the upper incisor representation, which expanded to 5 times its normal area. This small patch was the most variable in location in normal animals, at times not even adjacent to the area innervated by the infraorbital nerve. If reorganization *were* occurring in the fractured cerebellar map itself, we would expect the deafferented patches to be invaded by afferents from neighboring adjacent patches. Third, we explored whether the observed upper incisor expansion in the fractured map might instead be correlated with changes in the *topographic cortico-cerebellar pathway*. We found that the upper incisor representation in S-1 cortex in deafferented animals was indeed much larger than that of the normal animal, increasing 10-fold. These results suggest that reorganization does *not* take place in the cerebellum but rather in the sensory afferents *projecting to* the cerebellum. Using electrical stimulation, lidocaine injections, and cortical ablations we then demonstrated that the S-1 cortex is the primary contributor to the long-latency evoked cerebellar response to tactile stimulation which is particularly resilient to peripheral lesions. Supported by NIH# 22205, BRSG# RR07003, L.P. Markey Trust, and NIMH and NIH NRSA awards to CAS and LG P.

370.16

COMPENSATORY RESPONSES TO WEIGHT AND TEXTURE CHANGES IN A PREHENSION TASK FOR CEREBELLAR PATIENTS. C. Dugas, A.M. Smith, D. Bourbonnais, L.F. Charron* and M.I. Botez*. Centre de Recherche en Sciences Neurologiques, Université de Montréal et Service de Neurologie, Hôtel-Dieu de Montréal, Québec, Canada.

Subjects were asked to grasp, lift and hold within a narrow position window an object of varying weight and texture. Three weights (200, 600 and 800 g) and two textures (polished metal and coarse grain sandpaper) were used. Three patients with an olivo-ponto-cerebellar (OPC) syndrome and two patients with advanced Friedreich's ataxia (FA) (ages 28 & 29) were compared to age-matched control subjects. The OPC patients had similar large oscillations in both the dynamic and static components of the movement which were caused by a breakdown of the normal synergy between the grip and load forces in this task. Despite this deficit the patients were able to scale the grip force appropriately according to the three loads used in this study. Moreover, these forces were always greater than for the control group. The grip forces applied to the smooth and rough surfaces for the three different weights were of similar magnitude suggesting a deficit in adapting to the surface friction. The OPC patients demonstrated a strategy that was somewhat similar to patients with sensory deficits by adopting a large security margin with greater energy expenditure. For the FA patients there were no systematic oscillations in the force traces as observed for the OPC patients. The grip and load force profiles were smooth but with a much slower ramp-like increase in the dynamic phase of the task. The force amplitude of both the static and dynamic components were always smaller than for either the control or the OPC patients. The FA patients had a much smaller security margin and a clear effect of the texture was noted in their results. These advanced FA patients did not show a clear ataxic syndrome when compared to the OPC. These results suggest that advanced FA is characterized less by asynergia than a simple loss of muscle force. Supported by MRC of Canada.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: CEREBELLUM III

371.1

FRACTURED TACTILE MAPS RETAIN FRACTURED SOMATOTOPY FOLLOWING DEAFFERENTATION AT DIFFERENT STAGES OF DEVELOPMENT. J. Morissette, L.G. Posakony, C.A. Shumway, M.G. Paulin, J.M. Bower. Division of Biology, 216-76, California Institute of Technology, Pasadena, CA, 91125.

Tactile projections to the rat cerebellar hemispheres are characterized by a series of patches representing different regions of the body surface (Shambes et al., Brain Behav. Evol. 15: 94-140, 1978). In the current experiments, we deprived the large central upper lip and upper-lip related patches found in crus IIa of their normal source of peripheral innervation by cauterizing the infraorbital branch of the trigeminal nerve. Twelve rats were deafferented between postnatal days 2 and 30, and the resulting reorganization of tactile inputs to the granule cell layer was determined using high-density physiological mapping procedures 2 to 3 months later.

Our results indicate that these cerebellar maps reorganize regardless of the postnatal day of the lesion. In each case, the map maintained an overall patchy organization, with the upper incisor representation consistently invading most of the denervated region. This result was surprising since the upper incisor crus IIa patches in normal animals are both the smallest and most variable in location. Although reorganization occurred at *all* developmental stages, we did find a developmental effect with respect to the different components of the evoked granule cell response to tactile stimulation. In normal animals, tactile evoked responses always consist of short- (5-10ms) and long- (20-50ms) latency components. In deafferented animals, some recording sites showed only the long-latency component. The number of these recording sites increased as lesions were made later in development (3% at PND2 versus 22% at PND30), suggesting developmental differences in the capacity of cerebellar projecting circuits to reorganize. In Shumway et al. this volume, the long latency response is shown to be associated with the somatosensory cortico-cerebellar pathway.

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371.2

THE VENTRAL LATERAL NUCLEUS (VL) OF THE RHESUS MONKEY MOTOR THALAMUS: LIGHT AND ELECTRON MICROSCOPIC ANALYSIS. K. Kultas-Ilinsky and I. Ilinsky. Department of Anatomy, University of Iowa College of Medicine, Iowa City, Iowa 52242

The VL, or projection zone of cerebellar afferents to the thalamus as defined earlier by Ilinsky and Kultas-Ilinsky (1987), was analyzed with respect to its neuronal composition and synaptic organization using various neuroanatomical techniques: retrograde and anterograde labeling from the cortex, anterograde labeling from the cerebellum, immunocytochemistry and quantitative morphometric analysis. Two types of neurons: thalamocortical projection neurons (PN) and GAD-positive local circuit neurons (LCN) differed in size and number. LCN:PN ratio was 1:3. Proximal parts of somadendritic membrane of PN (soma, primary and secondary dendrites) were contacted by large number of cerebellar boutons (LR type). Second major synaptic input to these sites was provided by LCN dendrites that formed symmetric synapses and frequently, but not always, were a part of a triad arrangement with cerebellar boutons. Finally, a small number of axon terminals of unknown origin were also found forming symmetric contacts at proximal locations on PN. Moreover, the relationships observed suggest a possibility of synaptic contacts on PN initial axonal segments and axon hillocks although no clear examples have been encountered as yet. The major input to distal dendritic branches of PN was from SR-type boutons that were confirmed to be of cortical origin. Synaptic input to somata of LCN was extremely sparse and was provided by small to medium size GAD-positive boutons of unidentified origin forming symmetric contacts. Glomeruli were formed at the branching sites of primary or secondary dendrites. They were encountered infrequently hence did not represent the major feature of the neuropil. Our findings provide evidence that the ultrastructure of the VL is different in principle from that of nigrothalamic afferent territory as well as of somatosensory thalamus and do not support the concept of a common blueprint of neuronal circuitry in all thalamic nuclei. Supported by NS R0124188.

371.3

DIFFERENCES IN ULTRAMORPHOLOGY AND DENDRITIC TERMINATION SITES OF SYNAPSES ASSOCIATED WITH THE ASCENDING AND PARALLEL FIBER SEGMENTS OF GRANULE CELL AXONS IN THE CEREBELLAR CORTEX OF THE ALBINO RAT. G. Gundappa-Sulur and J.M. Bower, Div. of Biology 216-76, Caltech, Pasadena, CA, 91125.

Previous electrophysiological investigations have demonstrated that focal granule cell layer activation generates an equally focal activation of the overlying Purkinje cell layer (Bower and Woolston, J. Neurophys. 49: 745-766, 1983). It was proposed that synapses associated with the ascending branch of the granule cell axon are responsible for this vertical organization of excitatory effects and that synapses of the parallel fiber segment might be responsible for more subtly modulating this ascending influence. The idea that these two segments subserve different physiological functions was supported by subsequent intracellular brain slice experiments demonstrating that the ascending branch segment generates a long duration (200-300 ms) plateau potential in Purkinje cell dendrites (Rao et al., Soc. Neurosci. Abst. 13, 602, 1987).

To further contrast the properties of the two segments of the granule cell axon, we have contrasted serial EM reconstructions of synapses associated with both. Complete reconstructions of 36 synapses demonstrate no statistically significant differences in presynaptic and postsynaptic volumes or the area of the postsynaptic density. However ascending branch synapses do contain, on average 1/3 more presynaptic vesicles than parallel fiber synapses ($p<.016$). Perhaps more interestingly, spines associated with the ascending branch have been found to arise exclusively from the smallest terminal dendrites of the Purkinje cell (average diameter .5 μm) while spines contacted by parallel fibers are exclusively associated with dendrites considerably larger in diameter (1.4-2.0 μm). This juxtaposition of parallel fiber effects between ascending branch synapses and the main dendrites of the Purkinje cell is compatible with the previously proposed modulatory role of the parallel fiber segment.

Supported by NIH (NS22205) and BRSG grant (RR07003).

371.4

POSITRON EMISSION TOMOGRAPHY IN SPINOCEREBELLAR DEGENERATION. A. Russo-Neustadt, S. Lottenberg*, A. Starr, L. Sporty* and M. Buchsbaum*, Brain Imaging Center, Departments of Psychiatry and Human Behavior and Neurology, University of California, Irvine, CA 92717.

We present two cases of spinocerebellar degeneration occurring in a family. The patients examined are brothers, ages 48 and 52, who presented with progressive muscular incoordination, decreased strength, impaired concentration, and altered social judgment. Anatomical changes, revealed by magnetic resonance imaging (MRI) are examined in conjunction with metabolic patterns evident on positron emission tomography (PET). MRI revealed degeneration of the cerebellar vermis, with normal anatomy maintained in the rest of the brain. PET showed depressed metabolic rates throughout the cerebellum, as well as the inferior frontal cortex, the posterior temporal cortex, and regions of the corpus callosum and cingulate gyrus. Neuropsychological evaluation of these patients, along with their history, revealed progressive motor deficits as well as emotional and personality changes which correspond with the metabolic deficits examined with PET. This study indicates that degenerative disorders of the cerebellum can involve subtle changes in distant areas of the brain which are involved in the efferent and afferent connections of the cerebellum and not evident grossly as structural changes.

371.5

PRETECTO-PONTINE AND INCERTO-PONTINE PROJECTIONS AND SYNAPTIC BOUTONS IN THE RAT. G.A. Mihailoff and K.W. Bourell*, Cell Biology and Neuroscience, Southwestern Medical School, Dallas, TX 75235

Previous retrograde transport studies in the rat have established that the anterior pretectal nucleus (APT) and zona incerta (ZI) contain neuronal somata that project to the basilar pontine nuclei (BPN). Further, it has been shown that each of these pontine afferents is comprised in part of GABA fibers, while the incerto-pontine system is also known to contain glutamate-positive components. The objective of the present series of experiments was to visualize the pattern of axon terminal fields formed by these two afferent systems within the BPN and, with electron microscopy, identify the synaptic boutons that arise from each axonal system. Injections of WGA-HRP were placed within the APT or the ZI and after survival periods of 24-48 hours and routine fixation and histochemical processing of the tissue, axon terminal fields were observed in the BPN ipsilateral to each injection. Only the incerto-pontine system exhibited a small contingent of labeled terminals in the contralateral ventromedial pontine region. Both the incerto- and pretecto-pontine systems established terminal fields of varying density and size in lateral, ventral and medial BPN zones. Although such projection fields occupied similar territories within the BPN, there appeared to be only minimal overlap in some areas and each system seemed to establish exclusive terminal regions, at least with respect to one another. With electron microscopy, both systems gave rise to similar types of boutons that were small to medium in size, and often formed synaptic arrangements in which a single labeled bouton contacted multiple postsynaptic profiles that included dendritic spines and shafts. These observations confirm the existence of yet additional BPN afferents, and suggest an emerging ultrastructural theme that places afferent boutons as the central profile in a glomerular-like synaptic arrangement. *Supported by USPHS grant NS12644.*

371.6

IMMUNOCYTOCHEMICAL IDENTIFICATION OF CEREBELLAR NEURONS *IN SITU* AND FOLLOWING TISSUE DISSOCIATION. L. Yousif, L. Rucker* and P.E. Hockberger. Dept. of Physiology, Northwestern University Medical School, Chicago, IL 60611.

Cell-specific immunostaining was used to identify neurons and glia in adult and developing rat cerebellum. Staining was also used to identify isolated cells following tissue dissociation (P. Hockberger et al. J. Neurosci. 9: 2258, 1989.). For the *in situ* determinations, animals were perfused with 4% paraformaldehyde, and free-floating frozen sections (adults 30μm, juvenile 60μm) were stained using either the peroxidase-antiperoxidase or the avidin-biotin technique. Isolated cells were plated onto coverslips, fixed, and stained similarly. Purkinje cells were stained using polyclonal antibodies against calbindin (1:40,000 dilution; antibody provided by Dr. Sylvia Christakos, NJ Medical School) and Peptide-19 (1:1000; Dr. James Morgan, Roche Institute), while Golgi and deep nuclei cells were stained using monoclonal antibodies against Rat-303 (1:10; Dr. Susan Hockfield, Yale). In addition Rat-302 antibodies were used to identify a novel cell type that is found only in the vermis and flocculus (1:10; S. Hockfield, Science 237: 67, 1987). Bergmann glial cells were stained using GFAP antibodies (1:8,000; DAKO Corp). Purkinje cells also stained with anti-GFAP serum in two-week old animals, but not in adult, suggesting that this antigen may be expressed in these neurons during development. This research was supported by NIH grants NS-26915 and NS-17489.

371.7

CEREBELLOTHALAMIC PROJECTIONS IN THE MOUSE. A. J. Haroian and L. M. Eisenman. Hahnemann University, Philadelphia, PA 19102 and Jefferson Medical College, Philadelphia, PA 19107.

The purpose of this study was to identify the cerebellothalamic (CBT) projection from each of the cerebellar nuclei (CN) in the normal mouse. These data will be used as the basis for future comparative studies of this projection in mutant mice with severe defects in cerebellar development. Unilateral iontophoretic injections of WGA-HRP were made into the CN and anterograde transport was detected with TMB. Cerebellar terminations were observed in the intralaminar and the ventral tier (VTN) nuclei of the thalamus. The dentate (DN) and interpositus (IN) nuclei projected to the paracentral nucleus (PC). The fastigial nucleus (FN) had a more caudal input to the central lateral and parafascicular nuclei. Each of the CN projected to the VTN. Caudally, labeling from the IN and DN was observed along the border between the ventral medial and ventral lateral (VL) nuclei. Rostrally, labeling also extended more laterally along the dorsal border of the VL adjacent to the internal medullary lamina. Terminations from the IN and parvocellular DN occupied a central zone within the rostral pole of the VL; those from the magnocellular DN were observed most laterally. Projections from the FN were modest and terminated most medially within the rostral VL. An obvious recrossing component to the ipsilateral thalamus was observed from the IN and DN. These data suggest that there is evidence of a topographic organization in CBT terminations despite an extensive overlap of terminal fields within the VTN. Grant NS16531 is gratefully acknowledged.

371.8

CEREBELLAR CHANGES FOLLOWING UNILATERAL LESION OF THE INFERIOR OLIVE IN THE ADULT RAT W.S.T. Griffin, K. Ito*, Y. Ishikawa*, M. Morrison-Bogorad+ and R.D. Skinner Depts. Anatomy and Pediatrics, UAMS, Little Rock, AR 72205; +Dept. Neurology, UTSWMC, Dallas, TX 75235

The inferior olivary nucleus (ION) is the only source of climbing fibers in the rat. The majority of these fibers innervate Purkinje cells in the contralateral cerebellum and form the largest portion of the inferior cerebellar peduncle. We hypothesized that the loss of climbing fiber innervation would result in alterations in Purkinje cell genetic expression as well as astrogliosis (numerous reactive astrocytes) in contralateral brainstem and cerebellum. Serial sections of Bouin's-fixed, paraffin-embedded cerebellum taken at different days following electrolytic lesion of the right ION were examined. Immunohistochemistry was used to determine the levels of glutamic acid decarboxylase (GAD) in Purkinje cells and to identify reactive astrocytes. At 10 days post-lesion, astrocytes in the brainstem and cerebellum contralateral to the lesion contained more GFAP and S-100 immunoreactive product in enlarged cell bodies and prominent processes, i.e., astrogliosis was found. In addition, orthograde transneuronal atrophy of Purkinje cells and decreased levels of GAD were apparent contralateral to the lesion. We conclude that atrophy and astrogliosis occur in the cerebellum by day 10 following lesion of the contralateral ION.

371.9

EVIDENCE THAT CERTAIN GABAERGIC NEURONS IN THE CEREBELLAR NUCLEI LIE OUTSIDE CLIMBING FIBER TERMNAL FIELDS. K. A. Starr and H. H. Molinari. Dept. of Anatomy, Albany Medical College, Albany, NY, 12208.

Inputs to the cerebellar nuclei (CN) include climbing fiber (CF) collaterals from the inferior olive (IO). We investigated whether GABAergic neurons in the CN may be influenced by input from these collaterals. Cells in the IO of rats were selectively destroyed using the neurotoxic drug, 3-acetylpyridine, according to the protocol of Llinas et al. (Science 190:1230, 1975). After 3, 4 or 5 days survival, rats were perfused transcardially and 30 μm-thick, transverse serial sections of the cerebelli were obtained. Every other section was processed for immunocytochemistry using a GABA antibody (Incstar Corp). These sections were used to plot the location of GABAergic cells in the CN. In adjacent sections, stained with a Fink-Heimer procedure, we determined whether these GABAergic cells were located in terminal fields of CF collaterals.

Results of this study indicate that almost half of the GABAergic cells lie outside of CF terminal fields and that many of these cells are located ventrally in the CN. It has been shown by others that neurons of the cerebello-olivary projection, particularly the GABAergic portion, are concentrated in the more ventral areas of the CN. Thus, it may be that the ventrally located GABAergic neurons that we identified as lying outside CF terminal fields may be involved in this pathway. This suggests that, at least in part, the cerebello-olivary projection may not be directly influenced by input from the IO (Supported by NSF Grant BNS-8809840).

371.10

THREE-DIMENSIONAL RECONSTRUCTION OF SPINES IN THE INFERIOR OLIVE THAT RECEIVE SOMATOSENSORY INPUT. H.H. Molinari. Department of Anatomy, Albany Medical College, Albany, NY 12208.

Spines in the inferior olive have received considerable attention because of their proposed role in electrotonic coupling. Yet their potentially large synaptic surface area suggests that they may also serve as important sites for integration of information. In the caudal half of the cat medial accessory olive (cMAO), spines constitute one of the major targets of afferents from the gracile nucleus and lumbosacral spinal cord. Both of these afferents were labeled in the present study, one by anterograde transport of wheat germ agglutinin-horseradish peroxidase and the other by degeneration. Three dimensional reconstructions of spines contacted by labeled terminals were generated from serial section electron micrographs.

Three types of spines received somatosensory input: simple stalks, club-shaped spines, and spine-crowned appendages. Virtually all of the spines received multiple inputs; most of these convergent inputs displayed the morphological characteristics classically associated with excitatory synapses. Furthermore, spines constituted the major site of convergence of afferents from the gracile nucleus and lumbosacral spinal cord. Considerably less common were gap junctions and inputs to the spines from pleomorphic-vesicle containing terminals (although two thirds of the spines participated in attachment plaques). Thus, spines in cMAO appear to be sites for integration of excitatory inputs, often from multiple somatosensory structures.

Supported by NSF grant BNS-8809840.

371.11

EFFECTS OF HARMALINE ON THE ACTIVITY OF CELLS IN THE DEEP CEREBELLAR NUCLEI OF NORMAL AND GENETICALLY DYSTONIC RATS. J. F. Lorden and J. Ervin.* Dept. Psychology, Univ. Alabama at Birmingham, Birmingham, AL 35294.

The genetically dystonic (dt) rat does not display harmaline tremor, an effect produced by rhythmic activation of the olivo-cerebellar system. Because this system appears to be intact morphologically in the mutants, functional differences have been sought between dt and normal rats. Although the spontaneous firing rate of cells in the caudal inferior olive is significantly slower than normal, cells in the dt olive respond to harmaline with rhythmic increases in firing rate indistinguishable from normal. Few vermal Purkinje cells in the dt rat, however, are activated normally after harmaline. We have now used harmaline as a probe to investigate the output of the cerebellar cortex by recording from cells in the medial and interpositus nuclei. In urethane-anesthetized rats, the spontaneous firing rate of cells was 60% higher in the dt rats than in controls. Following harmaline, however, 78% of the cells from normal rats increased their firing rate in comparison with only 20% in the mutants. Cells in normal rats showed bursts of activity following the drug that were not evident in the dt rats. The activity of cells in the cerebellar nuclei of the dt rat does not appear to be modulated normally by the cerebellar cortex. (Supported by the Dystonia Med. Res. Fdn.)

371.12

QUIPAZINE HAS DIFFERENT ELECTROPHYSIOLOGICAL AND BEHAVIORAL EFFECTS IN DEVELOPING NORMAL AND DYSTONIC RATS. V. L. Michela and J. F. Lorden. Dept. Psych., Univ. Alabama at Birmingham, Birmingham, AL 35294.

Systemic administration of the serotonin (5HT) agonist quipazine produces a high frequency tremor in the forepaws of newborn rats. In normal rats, this response declines and is no longer evident by postnatal day 16. In the dystonic rat (dt), a genetic mutant with a movement disorder that appears around postnatal days 9-10, a tremor can be elicited with quipazine throughout the postnatal period. The dose-dependent effects are blocked by prior administration of the $5HT_2$ antagonist, ketanserin. Because other studies have implicated the olivo-cerebellar system in the motor syndrome of the mutants, we compared the effects of systemic quipazine on the complex spike activity of cerebellar Purkinje cells in urethane-anesthetized normal and dt rats at 18-25 days of age. In normal rats, the frequency of complex spikes was significantly reduced from predrug rates. In the mutant rats, predrug firing rates of Purkinje cells were significantly slower than those of normal rats; however, quipazine increased the complex spike rate in the mutants. Although it is not known whether cerebellar mechanisms underly the quipazine-induced forepaw tremor, these behavioral and physiological data suggest an abnormality in 5HT systems of the dt rat. (Supported by the Dystonia Med. Res. Fdn.)

371.13

CROSSCORRELATION ANALYSIS OF NEURONAL ELEMENTS OF THE CEREBELLAR CORTEX. Iwona Zurawska and Remigiusz Tarnecki*. Dept. of Neurophysiology, Nencki Institute of Experimental Biology, 02-093 Warsaw, Poland.

Crosscorrelation is an analysis used to study the relationships between temporally disparate events. It has been previously used in studies of temporal interactions in computer simulated neuronal nets (Gerstein, G.L., 1970). In order to demonstrate the validity of crosscorrelation in real neuronal networks, we applied it to the study of the cerebellar cortex in the cat. The circuitry of this structure has been well described with microstimulation, as well as anatomical and immunocytochemical approaches. These techniques reveal both excitatory and inhibitory interactions between the neurons. We examined spontaneous activity of single cerebellar neurons, as well as the single unit potentials produced by electrical stimulation of a front limb. With crosscorrelation analysis we were able to reveal all of the previously described neuronal interactions and classify them as: direct excitatory, direct inhibitory, and those with common input from a shared source. Moreover we were able to differentiate the activity of Golgi cells from other inhibitory interneurons. Our results demonstrate the power of crosscorrelational analysis in the study of neuronal interactions.

371.14

EFFECTS OF HEMICEREBELLECTOMY ON CAT INFERIOR OLIVARY NEURONS. T.J.H.Ruigrok*,M.P.A.Schalekamp*,C.I.de Zeeuw* and J. Voogd*.(SPON: Europ. Neurosci. Assoc.) Dept. of Anat., Erasmus Univ. Rotterdam, 3000DR, the Netherlands.

After hemicerebellectomy, neurons in the cat contralateral (affected) inferior olive (IO) may either degenerate, appear unchanged or become hypertrophic.

Injections with the anterograde tracer Phaseolus vulgaris leucoagglutinin in the affected medial accessory olive (after 3 and 12 months) indicated that the axons remain within the restiform body, where they end as terminal clubs or as glomeruloid structures. Many terminal clubs expressed immunoreactivity for growth associated protein 43 (GAP-43: Benowitz and Routtenberg, TINS,10:527, 1987). Neurons in the affected IO were studied after stimulation of the mesodiencephalo-olivary pathway (MOP) and intracellularly injected with horseradish peroxidase. Injected neurons usually resemble normal olivary cells, whereas the somata of hypertrophic neurons were enlarged and studded with spiny processes. MOP stimulation resulted in monosynaptic activation followed by rebound spiking, or in long latency (120-300msec) reponses only. Dendritic Ca2+ spiking was seldomly observed in hypertrophic cells.

It is argued that cat IO neurons may survive axotomy due to a strong and continuous electrotonic coupling, caused by the destruction of the cerebellar GABAergic input. The expression of GAP-43 in terminal clubs appears not to be related to axonal outgrowth.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: OCULOMOTOR SYSTEM I

372.1

DIRECT COMPARISON OF REFLEX AND REMEMBERED SACCADES TO FLASHED TARGETS IN PARKINSON'S DISEASE. C.J. Lueck, T.J. Crawford, L. Henderson, C. Kennard. The London Hospital, Whitechapel, London E1, U.K.

It has previously been shown that remembered saccades are impaired in patients with Parkinson's disease (PD). One hypothesis is that the impairment is generated by the act of suppression of a reflex saccade during fixation before remembered saccade execution.

In order to test this hypothesis, ten patients with idiopathic PD were compared with ten age-matched controls in a task requiring the execution of four saccades to a 200 ms flashed visual target. The first (reflex) saccade was made directly to the target. Subjects then had to look back to the central fixation light which was still illuminated. Shortly thereafter, the central light was extinguished and subjects were required to make a third (remembered) saccade to the remembered location of the original flash before finally returning to centre.

Reflex and remembered saccades were compared. Latencies of reflex saccades were prolonged to over 400 ms in both groups, possibly related to the multiple-task nature of the paradigm. PD reflex saccades were significantly hypometric compared to those of controls (gain 0.82 compared to 0.97). This result differs from previous reports of reflex saccades in PD in which gains are normal, and presumably relates to the flashed nature of the target. Remembered saccades were even more significantly affected (gain 0.57 vs. 1.03). For both reflex and remembered saccades there was no significant difference in peak velocity between PD and controls.

The hypothesis is therefore disproved. The findings are discussed in relation to the theoretical requirement that a visual stimulus be present for Parkinsonian saccades to be executed normally.

372.2

CORTICOTECTAL NEURONS IN THE FRONTAL EYE FIELDS OF THE AWAKE CAT. T. Weyand, J. Malpeli, and R. LaClair*, Dept. Psych., University of Illinois, Champaign, IL 61820

We have recorded from 22 antidromically-identified corticotectal cells in the frontal eye field region of cats trained in visuomotor tasks. Head position was fixed, and gaze monitored using the scleral search coil technique. The activity of 4 cells could not be reliably influenced by any of the visual or auditory stimuli we presented, or related to task demands or eye movements. Twelve cells could be reliably driven by visual stimuli, with all but 3 possessing huge receptive fields that included most of the contralateral hemifield and the area centralis.

The most common pattern of activity was suppression whenever fixation was demanded (n=10). This included suppression of both spontaneous and visually evoked activity, beginning whenever the fixation target appeared. Spontaneous saccades not related to the task did not result in suppression. Among the other cells, three appeared to be excited by fixation and one other possessed a punctate receptive field with a large and potent suppressive surround. Activity tightly linked to oculomotor behavior was not common, but was observed among non-corticotectal cells in this region. The suppression of activity associated with task demands is consistent with the notion that the frontal eye fields are involved in target acquisition and maintenance of fixation. (Supported by NIH Grants EY06818 and EY02695)

372.3

QUANTITATIVE ANALYSIS OF THE TRAJECTORY OF ELECTRICALLY ELICITED SACCADIC EYE MOVEMENTS IN THE FRONTAL EYE FIELD AND SUPPLEMENTARY EYE FIELD OF THE MONKEY. G. S. Russo and C. J. Bruce. Section of Neuroanatomy, Yale Univ. Sch. of Med., New Haven, CT 06510.

Saccades elicited by electrical stimulation have often been classified either as being constant-vector (with roughly the same amplitude and direction regardless of the direction of gaze when stimulated) or as goal-directed (appearing to direct gaze to a specific orbital goal). We analyzed the effect of eye position on saccades electrically elicited from 30 sites in the frontal eye field (FEF) and 8 sites in the supplementary eye field (SEF). All sites had either low thresholds (<50μA) for eliciting saccades or saccade related neuronal activity, with 27 FEF and 7 SEF sites fulfilling both criteria.

Saccades were elicited while the monkey fixated a target at one of a matrix of nine locations separated by 15°. Approximately 10 saccades were elicited from each fixation target in a pseudorandom order. Electrical stimulation was through glass-coated elgiloy electrodes with exposed tips of 20-40 μm using 70 ms trains of 350 Hz biphasic pulses. At each site, the vertical and horizontal components of the elicited saccades were separately fit to the linear model: Elicited saccade = Kg * gaze position + elicited saccade from the primary position. The coefficient Kg indexes the orbital dependence of the electrically elicited saccades: Kg of 0 indicates perfectly constant-vector saccades whereas Kg of -1 indicates perfectly goal-directed saccades.

For saccades electrically elicited from FEF, Kg ranged from -0.41 to +0.01 (mean -0.17) for the horizontal component and -0.58 to +0.03 (mean -0.10) for the vertical component. For saccades electrically elicited from SEF, Kg ranged from -0.50 to 0.00 (mean -0.13) for the horizontal component and from -0.47 to 0.00 (mean = -0.15) for the vertical component.

These results seem to be at odds with previous studies associating FEF with constant-vector saccades and SEF with goal-directed saccades. Instead, the orbital dependence coefficients of FEF and SEF spanned a similar range of values (approximately -0.5 to 0) and their means did not significantly differ. We suggest that this regression analysis may be useful as an objective method for quantifying the effect of orbital position on electrically elicited saccades. Supported by PHS grant EY04740.

372.4

OCULAR TRACKING IN PATIENTS WITH UNILATERAL FRONTAL LOBE LESIONS. Mark J. Morrow and James A. Sharpe. Division of Neurology and Playfair Neuroscience Unit, The Toronto Hospital, University of Toronto, Toronto, Ontario M5T 2S8

We studied horizontal ocular tracking in 7 patients with unilateral frontal lobe tumor or infarction, using a magnetic search coil technique. We measured steady-state smooth pursuit gain to sinusoidal targets, and initial saccade and pursuit responses to unpredictable step-ramps. Four of 7 patients had asymmetrical steady-state pursuit, with lower smooth eye velocities toward the side of the lesion; lesions in these patients involved precentral cortex and underlying white matter. Three of the 4 patients with asymmetrical pursuit gain also made inaccurate saccades to step-ramp targets moving away from the lesion; these saccades underestimated target motion. Only 1 patient had asymmetrical initial pursuit responses to step-ramps.

Steady-state pursuit asymmetry is common in patients with unilateral frontal lobe lesions, but initial pursuit is usually symmetrical, implying that the frontal lobes participate in the maintenance, but not the initiation, of the pursuit response. Contralateral saccade hypometria to step-ramps signifies a deficit in directing saccades to moving targets, which may result from damage to the human frontal eye fields.

Supported by NIH Grant EY-06040 and MRC of Canada Grant MT-5404.

372.5

NEURAL ACTIVITY IN MONKEY SUPERIOR COLLICULUS DURING VISUALLY EVOKED SACCADES MODIFIED BY INTRASACCADIC ELECTRICAL STIMULATION OF SUPERIOR COLLICULUS OR FRONTAL EYE FIELDS. E. L. Keller, E. J. FitzGibbon, and M. E. Goldberg. Laboratory of Sensorimotor Research, National Eye Institute, Bethesda, MD 20892, and Smith-Kettlewell Eye Research Institute, San Francisco, CA 94115.

The amplitude and direction of a saccade evoked by electrical stimulation of the monkey frontal eye field (FEF) or superficial layers of the superior colliculus (SSC) can be modified when the stimulation is applied during an ongoing visually evoked saccade. The modified saccade has dimensions suitable for the acquisition of the target that would have been acquired by the original fixed vector electrical saccade had the intervening saccade not taken place (J. Schlag et al. *Exp. Br. Res*, 1989). Movement related neurons in the intermediate layers of the monkey SC (ISCN) discharge before saccades of a particular direction and amplitude, and their discharge may be related to dynamic motor error. We studied the activity of ISCN during saccades evoked by electrical stimulation of SSC and FEF to see if neural activity, like saccade trajectory, were modified when stimulation was applied at the beginning of a voluntary saccade. We found that ISCN activity initiated before the voluntary saccade continues through the saccade evoked by electrical stimulation of the SSC, even though the resultant eye movement was not in the movement field of the neuron. Neurons that ordinarily discharge before voluntary saccades equal to the modified electrically evoked saccade do not discharge when that saccade occurs because of prior-saccade modification. Conversely ISCN activity initiated before the voluntary saccade is terminated at the onset of the saccade evoked by electrical stimulation of the FEF. These data suggest the existence of two mechanisms for the modulation of saccade trajectory by an antecedent saccade, one above the level of the superior colliculus, a second below the colliculus.

372.6

SACCADIC EYE MOVEMENTS IN HUMAN POSTERIOR PARIETAL CORTEX LESIONS. J.-R. Duhamel, E. J. FitzGibbon, A. Sirigu, J. Grafman. Lab. Sensorimotor Research, N.E.I. and Cognitive Neuroscience Section, N.I.N.D.S., Bethesda, MD 20892.

We studied saccadic eye movements in a patient with right posterior parietal lobe damage, incomplete left homonymous hemianopia and mild neglect, using infrared and magnetic search coil techniques. We measured the latency, amplitude and velocity of saccades to stimuli appearing unpredictably at different locations within the intact portion of the patient's visual field, from different initial orbital eye positions and in "gap" and "no-gap" conditions. Saccades to contralesional visual field targets were smaller and slower than saccades to ipsilesional visual field targets. Initial orbital eye position affected saccades to contralesional targets only: a target presented at the same retinal location elicited saccades of decreasing amplitude and velocity as the initial position of the eye in the orbit shifted from the ipsilesional side to the contralesional side. In the no-gap condition (0 msec between fixation off and target on), mean saccade latency was longer for contralesional than ipsilesional targets. In the gap condition (200 msec interval between fixation off and target on), mean latencies were shorter in both directions and did not differ from one another. This was not due to a shift of the distribution toward shorter latencies but to the appearance of a second, smaller peak of express saccades (Fisher and Boch, 1983). The fact that saccadic reaction times to a contralesional stimulus can be reduced without explicitly cueing its spatial location is in accord with the notion that unilateral visual neglect results from the impaired ability of sensory input to the damaged hemisphere to release attention from its current locus (Posner et al., 1984). Posterior parietal cortex function is not, however, limited to stimulus selection, since deficits in saccade execution occur as well. The participation of posterior parietal cortex in both attentional and motor processing is consistent with current neurophysiological evidence in monkeys.

372.7

RELATIONSHIP OF MONKEY FRONTAL EYE FIELD ACTIVITY TO SACCADE DYNAMICS. M. A. Segraves, K. L. Park*, and M. E. Goldberg, Dept. of Neurobiology and Physiology, Northwestern Univ., Evanston, IL 60208, and Lab. Sensorimotor Res., National Eye Inst., Bethesda, MD 20892.

The frontal eye field (FEF) and the superior colliculus (SC) control saccadic eye movements in primates. Movement neurons in intermediate SC have discharge patterns related to the metrics of the saccade, both to velocity (Berthoz et al., 1986) and dynamic motor error (Waitzman et al., 1988). The latter have proposed that the SC lies within the feedback loop generating the saccade velocity pulse. We have now asked if activity of anatomically identified FEF neurons could also be related to the metrics of the saccade and lie within the loop.

FEF presaccadic neurons were recorded in three behaving Rhesus monkeys. We identified neurons' projections with antidromic collision: of the neurons chosen for this study, 40% projected to SC, 30% to the pons, and 30% had unidentified projections. Unit activity sampled at 1Khz was transformed to a continuous spike density function by convolving with a Gaussian function (σ = 8ms). Each neuron's spike density began to rise above baseline 100-300 ms before the start of the saccade. For 80% of the neurons examined, spike density peaked during the saccade, then began to decrease, but did not reach baseline levels until 100-200 ms after the end of the saccade. Some corticotectal neurons had their peak of spike density about 100 ms before the start of the saccade and then dropped to baseline before the end of the eye movement, but the spike density profiles did not match saccade trajectories or velocities. There was no consistent relationship between spike density and motor error. FEF neurons do not have the tight relationship to saccade metrics that certain SC neurons do. FEF activity may enable a brainstem saccade generation mechanism to proceed, and may signal target location, but does not directly influence saccadic velocity and time course.

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372.8

BIDIRECTIONAL CONTROL OF SACCADIC EYE MOVEMENTS IN THE BISECTED BRAIN. P.A. Reuter-Lorenz, H.C. Hughes, R. Fendrich & M. S. Gazzaniga. Program in Cognitive Neuroscience, Dartmouth College & Medical School, Hanover, NH.

Stimulation and recording studies reveal that cortical (FEF and parietal) and collicular mechanisms direct saccades contralaterally. In contrast, studies of hemispherectomy and callosotomy patients suggest that a single hemisphere can generate both ipsiversive and contraversive saccades. However, saccades directed into the blind field in hemispherectomy patients could be mediated by the superior colliculus (SC), as has been argued in cases of "blind-sight". In addition, previous demonstrations of ipsiversive control in callosotomy patients were taken to reflect a weak representation of the ipsilateral visual field in each hemisphere rather than bidirectional saccadic control. In this report we show that callosotomy patient JW demonstrates bidirectional control of saccades in response to a lateralized visual stimulus in the absence of interhemispheric transfer of the visual information.

Two tasks utilized the same stimulus conditions, but varied the response requirements. One task involved verbal reports of color names; in the other, JW was instructed to saccade either towards or away from the light source, or to make no response, depending on cue color. The cues were red, green or yellow LEDs unilaterally presented for 150 ms at an eccentricity of 7.0°. In the naming task, we expected that, in the absence of visual interhemispheric transfer, accuracy for LVF trials would approach chance, since JW's right hemisphere cannot control speech. J.W.'s naming accuracy for RVF cues was 100%, whereas LVF accuracy was at chance indicating that JW could not transfer the color information from the RH to the LH. In contrast, JW reliably produced both ipsiversive and contraversive saccades with normal latencies for both RVF and LVF cues (71% and 76%, respectively). Given the absence of visual transfer, these findings indicate that a single hemisphere can generate saccades in either direction, a result not readily explained by current models of oculomotor control.(supported by NIH grant PO1 NS 17778-08)

372.9

BIDIRECTIONAL PURSUIT OF LATERALIZED TARGETS IN A CALLOSOTOMY PATIENT R Fendrich P Reuter-Lorenz* H Hughes, MS Gazzaniga Dartmouth Medical School & College, Hanover NH.

While each cerebral hemisphere receives its input from the *contralateral* visual field, past investigations have suggested that each is dominant for *ipsiversive pursuit* eye motions. It follows that the leftward pursuit of targets in the left visual field, or rightward pursuit of targets in the right visual field, should require an interaction between the hemispheres. In the monkey, an anatomical pathway suited to mediate this interaction passes through the splenium of the corpus callosum. Severing this pathway in the monkey can generate a deficit in the pursuit of lateralized targets which are moving outward from the fovea (Keating, 1988).

We investigated lateralized pursuit in a human callosotomy patient. Adapting Keating's procedure, we presented the subject with "step-ramp" targets. Since the latency for pursuit is typically shorter than the latency for saccades, such stimuli can elicit a brief interval of pursuit prior to refoveation of the stimulus. Steps were 2.5° into the left or right visual field, and ramp motion was 3.3°/sec. towards or away from the fovea. In addition, we presented lateralized targets that were retinally stabilized. Such stimuli can elicit chasing smooth pursuit eye motions in normal observers.

With step-ramp stimuli, the subject's pursuit gain in the 60 ms interval just prior to his saccade to the target did not differ significantly for targets moving towards and away from the fovea (.33 vs. .29). Stabilized lateralized stimuli elicited pursuit similar to that found in normal subjects. These results suggest each hemisphere in a callosotomy patient is equally able to initiate ipsiversive and contraversive pursuit, or that pursuit can be mediated subcortically.

372.10

Electrical stimulation of the dorsomedial frontal cortex (DMFC) of the rhesus monkey. E. J. Tehovnik and K-M. Lee. Department of Brain & Cognitive Sciences, M.I.T., Cambridge MA 02139

Schlag and Schlag-Rey (1987) used electrical stimulation to show that the DMFC of monkeys is involved in the mediation of saccadic eye movements. From their study there is no suggestion of an organized motor map. We have preliminary evidence for the existence of such an organization. Electrical stimulation was used to elicit saccades from the DMFC after a monkey fixated different points in craniotopic space. Of the DMFC sites studied we found that the saccades evoked were always contraversive and that larger saccades were evoked when the eyes fixated a target in ipsiversive space and smaller saccades or no saccades were evoked when the eyes fixated a target in contraversive space. This depended on where along the rostral-caudal axis of the DMFC (about 8 mm in length) the simulation was applied: if applied rostrally, eye movements were evoked following fixation in both contralateral and ipsilateral space with the largest eye movements occurring for contralateral space. On the other hand, if stimulation was applied caudally, saccades were evoked only for gaze toward ipsilateral space, but with much shorter amplitudes than those occurring when the rostral DMFC was stimulated. Lastly, for rostral DMFC sites, when the eyes were positioned in extreme contralateral space, stimulation caused the eyes to remain fixated for the duration of stimulation; while for caudal DMFC sites, stimulation caused the eyes to remain fixated in central and contralateral space, again for the duration of stimulation. The foregoing suggests that the DMFC is intimately involved not only in saccadic generation but also in maintenance of gaze.

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372.11

EYELID MOVEMENT IN PROGRESSIVE SUPRANUCLEAR PALSY (PSP). T. C. Hain and T. T. Tsai*. Depts of Neurology and Otolaryngology. Johns Hopkins University, Baltimore, MD 21205.

PSP is a disease of humans characterized by slowing or inability to initiate vertical saccades. We measured the velocity of blinks with the magnetic search coil in 5 subjects with PSP and in 5 normal subjects.

In subjects with PSP, mean peak opening velocity was 196 ± 142 deg/sec and closing velocity was 777 ± 522 deg/sec. In normal subjects, opening velocity was 326 ± 87 deg/sec and closing velocity was 818 ± 405 deg/sec. These means are not significantly different ('t' test). The average ratio between peak opening and closing velocity, was 0.25 ± .07 in subjects with PSP vs 0.46 ± .15 in normal subjects. These means are significantly different ($p < 0.05$). Voluntary eyelid closing and opening showed a similar trend but no significant differences.

We conclude that the velocity of the opening phase of blinks is decreased in PSP, relative to the closing phase. This is consistent with the either inability to release orbicularis oculi tension, or inability to resume levator palpebrae tension.

372.12

TOPOGRAPHICAL SEGREGATION OF VESTIBULAR AND SACCADIC NEURONS IN THE POSTERIOR CEREBELLAR VERMIS OF MACAQUES. H. Sato, H. Noda, K. Ohtsuka, Y. Nagasaka* and R. Noda*. Sch. of Optometry, Indiana Univ., Bloomington, IN 47405.

Purkinje cells (P cells) bursting and pausing with saccades are confined to the oculomotor vermis (lobules VIc and VII). These cells do not modulate with head movements. When recording activity from posterior vermis, P cells responding to head rotation are often encountered above and below the folia of saccadic P cells, suggesting that vestibular and saccadic areas are juxtaposed.

Locations of P cells modulating sinusoidally during rotation of monkeys around their vertical axes were determined in reference to the recording sites of saccadic P cells. Confirming the previous results, the location of saccadic P cells was confined to vermal lobule VII and a part of folia VIc. Vestibular P cells were found in lobules VIa, b, and VIII, namely in the folia adjacent to the oculomotor vermis. P cells in the transition from saccadic to vestibular zones in lobule VI showed some changes in activity during head rotation but they were far from sinusoidal. These findings suggest that the areas of vestibular and saccadic functions occupy segregated folia in the posterior vermis. The intermediate zone may contain the P cells which receive converging eye- and head-velocity signals. (Supported by NIH grant EY 04063)

372.13

DIRECTION SPECIFIC PURKINJE-CELL ACTIVITY IN THE OCULOMOTOR VERMIS DURING TARGETING SACCADES. K. Ohtsuka, H. Noda, H. Sato, Y. Nagasaka* and R. Noda*. Sch. of Optometry, Indiana Univ. Bloomington, IN 47405.

Discharges of Purkinje cells (P cells) were recorded from lobule VIc and VII, when a macaque monkey made saccades from the central LED to one of 8 peripheral LEDs, or *vice versa*. The majority of P cells bursted during visually guided saccades in any direction. There were P cells pausing with all or some saccades in a particular direction. Even in these omnipause P cells, the degree of depression was different depending on the direction of saccade. There were also P cells bursting with saccades in one direction and pausing in the opposite direction. Saccade-related discharges were thus directionally specific in all P cells.

Typical saccadic response of the P cells consisted of a burst starting slightly after the onset of saccade during contralateral saccades. The latency of burst onset from saccade onset was shorter for smaller saccades. During ipsilateral saccades, the same P cells showed a prelude starting 40-50 msec prior to the onset, developing gradually and peaking at midsaccade. The latency of burst offset from saccade onset was also shorter in smaller saccades. Usefulness of these directionally specific P-cell activity during targeting saccades in modifying fastigial neurons will be discussed (Supported by NIH grant EY 04063).

372.14

VISUAL MOTION SIGNALS FOR PURSUIT EYE MOVEMENTS IDENTIFIED ON FLOCCULAR PURKINJE CELLS IN MONKEY. R.J. Krauzlis and S.G. Lisberger. Dept. of Physiology, Neuroscience Graduate Program, University of California, San Francisco, CA 94143

Smooth pursuit eye movements are driven by visual motion signals related to image velocity and acceleration. Since the cerebellar flocculus receives visual projections and is critical for generating pursuit, we sought to determine if its visual responses were appropriate for driving pursuit.

We recorded the activity of single floccular Purkinje cells (P-cells) in alert monkeys as they pursued a set of target motions designed to test the range of visual motion signals used by the pursuit system. The stimulus set consisted of constant velocities (5 - 30 °/s) and constant accelerations (45 - 180 °/s2) of a 0.5 degree spot. We analyzed the responses of each P-cell under the assumption that the firing rate could be modelled as the sum of four signals: eye velocity, image velocity, image acceleration, and a motion onset impulse. The eye velocity contribution to firing rate was determined from measurements of steady-state firing rate during tracking at different eye velocities. Using an iterative optimization procedure, we determined the contribution of the three visual signals required to match the actual firing rate observed during tracking of each target motion.

The population of P-cells displayed a variety of firing rate profiles during the initiation of pursuit with differences that can be attributed to variations in their sensitivities to visual motion signals and eye velocity. Most P-cells displayed responses which were combinations of all three visual signals and eye velocity. Several P-cells showed more specific responses, such as tuning for image velocity or a response dominated by a sensitivity to motion onset. Our analysis indicates that floccular P-cells receive visual motion signals appropriate to drive pursuit eye movements and that they vary in the strength of their visual and eye velocity inputs. (Supported by NSF grant BNS8616509 and a U.C. Chancellor's Fellowship to RJK)

372.15

SACCADES CONTRIBUTE TO VERGENCE IN RHESUS MONKEYS. J. S. Maxwell* and W. M. King. University of Rochester Medical School, Rochester, NY 14642.

In humans, disjunctive saccades contribute to the amplitude (up to 85%, Erkelens, et al., 1989) and speed of vergence eye movements. We show that in monkeys, saccades also interact with vergence eye movements. Rhesus monkeys were equipped with scleral search coils in both eyes and were trained to fixate near or far visual targets. Near targets required a vergence angle of 5-20 deg and far targets required no vergence. The near and the far targets were placed in the monkey's sagittal plane, aligned with one or the other eye, or offset horizontally (± 30 deg) and in depth to elicit both a saccade and a vergence movement. High velocity vergence (200-300 deg/sec) was always associated with a saccade and peak vergence velocity was correlated with saccadic velocity. During midline vergence trials monkeys often made a saccade away from and then back to the target so that no net version occurred. Midline vergence trials with saccades were faster than those without saccades (250 vs 100 deg/sec). Midline divergence movements contained a saccade more often than convergence movements (81% vs 14% of the trials). In general, divergence movements reached higher average velocities (70-100 deg/sec) than convergence movements (50-80 deg/sec) and were more stereotyped. These results show that the monkey is a suitable model for studying the neurophysiology of rapid vergence eye movements. *Supported by PHS grants RR05403, EY01319, and EY06632.*

372.16

SACCADIC EYE MOVEMENTS IN MEDICATED AND UNMEDICATED SCHIZOPHRENIC PATIENTS. T.J. Crawford, B. Haeger, L. Henderson, M. Reveley & C. Kennard. The London Hospital, Whitechapel, London E1, U.K.

Recent studies of the pathophysiology of schizophrenia have suggested abnormal functioning in the frontal lobes and basal ganglia. There have been few studies of saccadic eye movements in schizophrenia, although specific patterns of abnormalities have been found in certain neurological disorders. In this study we have used various behavioural paradigms to elicit saccades in schizophrenic patients to determine if there is a similar abnormal pattern.

The subjects studied consisted of schizophrenic patients currently taking medication (MS, n=33), schizophrenics off medication for >1 year (UMS, n=13), manic-depressive patients (MD, n=17) and normal controls (NC, n=30). The paradigms used were the reflex task (RS), the antisaccade task (AS) and the remembered target task (REM). In RS the subject made a saccade to the onset of a peripheral visual target (± 7.5, ± 15 deg), whereas in AS the subject was instructed to make a saccade to a location equally eccentric but in an opposite direction to the peripheral target. In REM the target was presented for 200 msec and the subject was instructed to make a saccade only when the central fixation was extinguished 500 msec later.

In RS there were no differences in saccadic latencies or amplitude between the four groups. In AS the patient groups showed significantly more saccadic errors (an initial saccade toward the target) than NC (MS, 63%: UMS, 47%: MD 45%). The mean saccadic amplitude remained the same for both 7.5 and 15 deg targets in the patient groups but increased in amplitude in NC. Amplitude in the patient groups was slightly reduced in the REM condition. There were no left/right asymmetries in latency or amplitude for any condition.

These results may support the concept of abnormal frontal lobe function in schizophrenia.

372.17

CHASING ILLUSIONS: SMOOTH PURSUIT OF IMAGINARY TARGETS DEFINED BY EXTRAFOVEAL CUES. J. Pola, H.J. Wyatt & B. Fortune.* SUNY College of Optometry, New York, NY.

Moving objects are often large and lack central detail, yet most studies of pursuit have used small foveal targets. A few studies have examined pursuit of extrafoveal targets, but the dependency of pursuit initiation and maintenance on target configuration is unknown.

We asked subjects to track an imaginary target (ImT), located (i) midway between two cues (small spots) 30 deg apart, or (ii) 15 deg offset from a single cue. The cues moved in a horizontal step-ramp. Responses were analyzed for latency, acceleration, peak velocity, and plateau velocity.

With an ImT between two cues, pursuit was good (slightly better for vertical and diagonal than horizontal pairs). With one cue, pursuit was degraded (most for a diagonal offset; less for horizontal; least degraded for a vertical offset). Varying cue eccentricity from 5 to 20 deg markedly degraded pursuit with one cue, but had little effect on pursuit with two cues.

We used two more cue configurations: (iii) a horizontal pair 30 deg apart, with the ImT 15 deg above the midpoint, gave good pursuit; (iv) a vertical pair, 30 deg apart, with the ImT 15 deg left or right of the midpoint, gave rather poor pursuit.

Conclusions: (i) and (ii) indicate that spatio-temporal stimulus attributes, perhaps related to perceived structure, can strongly influence pursuit guided by extrafoveal cues. (iii) and (iv) suggest that what gives such cues potency may be less a matter of wide separation than of foveal "enclosure."

372.18

INTERACTION BETWEEN EYE AND LID MOVEMENT: BLINKS ENCODED WITH VOLUNTARY GAZE SHIFTS. K.A. Manning, C. Evinger, P.A. Sibony, Depts. Neurobiology & Behavior, and Ophthalmology, SUNY Stony Brook, Stony Brook, NY 11794

A variety of observations suggest a link between blinking and eye movements in humans. First, cocontraction of the extraocular muscles accompanies voluntary blinks. Second, a blink commonly accompanies large vertical saccadic eye movements. Finally, in patients with slow saccades, a voluntary blink concomitant with a saccade increases saccade velocity to a normal value. The present study reveals that a blink also accompanies the generation of large horizontal gaze shifts.

We measured the lid closing orbicularis oculi EMG activity (OOemg) of the upper eyelid and eye and head position of both experienced and naive human subjects during voluntary saccadic eye movements or combined eye and head movements. EOGs measured eye position and a magnetic coil system showed head position. A pair of small silver plates (<2 mm diam) on the upper eyelid monitored OOemg without contamination from other muscles. At the sound of a tone, subjects made horizontal gaze shifts from the midline to targets 17 to 44° eccentric and back to the midline.

A burst of OOemg activity occurred during saccadic eye movements alone and an even more robust burst of OOemg activity accompanied combined eye and head movements. This activity in the orbicularis oculi muscle began 20 ms *before* movement of the eye. Since both extraocular and lid closing muscle activation must have been nearly simultaneous, the blink cannot be a response to corneal stimulation introduced by the eye movement. OOemg activation became apparent when maximum saccadic velocity exceeded 250-400 °/sec, increased with higher maximum saccadic velocities, but could be suppressed under certain experimental conditions. Thus, the data demonstrate that the neural program generating saccadic gaze shifts includes a blink. Supported by EY07391.

372.19

THE AMPLITUDE OF VISUALLY GUIDED SACCADES IS SPECIFIED GRADUALLY IN HUMANS. T.R. Stanford, L.H. Carney, and D.L Sparks. Dept. of Psychology, Univ. of Pennsylvania, Philadelphia, PA 19104.

The generation of an accurate visually guided saccade requires that, prior to execution, saccade metrics must be programmed to compensate for the distance and direction of the image from the fovea. Recent studies of arm movements suggest that response specification is a gradual process, dissociable from that of initiation (Hening, Vicario, and Ghez, *Exp. Brain Res.* 71:103, 1988). That the metrics of saccadic eye movements are also specified gradually might be inferred from the double-step studies of Becker and Jurgens (*Vision Res.* 19:967, 1979) and others, which demonstrated a gradual modification in the amplitude of an already programmed saccade by changing the target location slightly before movement onset. We sought to determine if this were true for saccades made to a single visual goal, and if so, to determine the time course of response specification.

Using a paradigm similar to that of Hening et al., human subjects synchronized saccade onset with the fourth of a series of equally spaced tone bursts. By presenting the target for varying amounts of time before the auditory cue, we varied the amount of time available to process information about its location from 0 - 325 ms. A rightward target LED was presented at one of three possible amplitudes with respect to a central fixation LED. Although subjects were familiar with the range of possible amplitudes, target position was randomized from trial to trial, as was the amount of time allowed for processing. Saccades begun within about 50 ms of target presentation had similar amplitudes regardless of which target was presented. This default amplitude was influenced by the range of potential targets. As the amount of time available to process visual information increased from 50 to 150 ms, saccade amplitude deviated from the default value and gradually approached that of the target.

These findings complement and extend those of double-step studies by demonstrating that the amplitude of a saccade to a single visual goal is specified gradually, and proceeds from a default value that reflects the range of potential targets. (Supported by NIH EY01189).

372.20

EFFECTS OF SACCADIC VARIABILITY ON SACCADIC PREDICTION BY NEURAL NETWORK AND CURVE FIT PREDICTORS. L A Abel and S Kolli. Ocular Motility Lab., Dept. of Biomed. Engr., Univ. of Akron, Akron, OH 44325.

Study of saccadic variability may be important when predicting saccadic endpoint from partial information. This is useful in flight simulators when, to save computation, high display resolution is used only in the vicinity of predicted eye position. We trained a small backpropagation network on 4 samples of the initial 8msec segment of 20 saccades, in the range of 1 to 30°, collected from 4 normals. Regression coefficients for actual vs predicted saccadic amplitudes were .95, .93, .88 and .81. The network training time was 5 min; for less variable subjects prediction accuracy was comparable with conventional curve fitting techniques and required much less of the saccade's duration. Next, 15 saccades of the worst and the best cases were fitted with a cumulative normal distribution function. Both the initial 8msec and entire first halves of saccades were fitted, with regression coefficients of .74 & .84 and .87 & .80, respectively. The percentage coefficient of variance was approximately the same for both peak velocity and the amplitudes of the four initial samples, for all sizes of saccades. Though this variability is within normal limits it had significant impact on both curve fitting and neural network prediction.

372.21

ADAPTIVE CHANGES IN THE AMPLITUDE OF SMALL SACCADES. Joanne E. Albano, Center for Visual Science, University of Rochester, NY 14627.

The amplitude of saccades to target displacements can be modified in response to artificially created visuo-motor errors. For large saccades (>10°), induced errors of 30% would create significant postsaccadic retinal errors, whereas, for small saccades (<3°), postsaccadic retinal errors would fall within the noise of the system. Though within the normal range of saccadic errors, it is still possible that small induced postsaccadic retinal errors are available to the mechanism controlling recalibration.

To examine this question small saccades were adapted by displacing the target by amounts equal to 30 or 40% of the size of the ongoing eye movement (Albano & King, 1989). Each experiment consisted of a set of preadaptation, adaptation, and postadaptation trials. Significant changes in the gain of saccade amplitudes were seen during the course of 100 trials. The amplitudes could be increased or decreased with positive or negative feedback. The velocity profiles of small adapted saccades were unimodal and the peak velocities were increased or decreased in the direction appropriate for the change in amplitude.

The recalibration of small saccades suggests that the mechanism responsible for the gain changes is sensitive to small dysmetrias. Somehow these unexpected induced errors are distinguished from those resulting from the normal noise of the system or alternatively an averaged error is computed and used to continuously adjust the gain. Supported by EY07344 and EY01319.

MOTOR SYSTEMS AND SENSORIMOTOR INTEGRATION: OCULOMOTOR SYSTEM II

373.1

RELATION OF THE DORSOLATERAL PONTINE NUCLEUS OF THE MONKEY TO OCULAR FOLLOWING RESPONSES. K.Kawano, M.Shidara*, and S.Yamane*. Neuroscience Sect., Electrotechnical Lab., Tsukubashi, Ibaraki 305, JAPAN

Recent studies suggest a major role of the dorsolateral pontine nucleus (DLPN) in the mediation of smooth pursuit eye movements and initial optokinetic responses (OKR). Although a close correlation between the initial OKR and ocular following responses was suggested, the role of the DLPN in the mediation of the latter was left unknown. The present experiments were concerned with the relation of the DLPN to the ocular following responses. A monkey faced a screen onto which a large field visual stimulus was projected, and its eye movements were recorded with the magnetic search coil technique. Single unit activity was recorded in the DLPN. Most of the neurons, which were activated by the movements of the visual scene, showed similar dependence on the visual properties of the stimulus to that of ocular following, e.g. preference to high speed, latency delay due to blurring and oscillation at the high temporal frequency (40Hz). Their latencies were very short. 40% of the neurons started their increase of firing rate more than 10ms before the eye movement. After the lidocaine injection in the DLPN, the gain of the ocular following reduced. On the other hand, the gain increased after the bicuculline injection. These results suggest an important role of the DLPN in the control of ocular following.

373.2

EYE MOVEMENT DURING MOTION AFTEREFFECT (MAE). S.H.Seidman*, C.W.Thomas*, W.P.Huebner, C.Billian*, and R.J.Leigh. VA Med. Center, University Hospitals and Dept. Biomed. Eng. Case Western Reserve Univ., Cleveland, OH 44106.

We measured torsional eye movements in three male Ss (ages 24-43) while viewing a circular target of 4-16 alternating light and dark sectors, subtending ~30°. The target rotated around the line of sight with angular velocities ranging from 60-180°/s for 20s, then stopped, remaining in view. Ss reported perception of motion with a potentiometer. During target rotation, Ss developed torsional nystagmus with slow phases in the direction of the stimulus (typical gain=.05). Following cessation of target rotation, Ss experienced a perceptual MAE in the direction opposite to prior target motion, with a duration of >15s. During MAE, slow phase eye movements (SEMs) were in the same direction as the perception, but did not persist as long as the MAE. Therefore, the direction of MAE was opposite to that of retinal slip. In another experiment, horizontal eye movements were recorded while Ss viewed 16 alternating vertical light and dark bars, with a stationary central fixation spot, also subtending ~30°. Targets moved horizontally at ~5 or 7.5°/s, stopping after 20s. SEMs in the direction of the stimulus occurred during target motion (typical gain=.1), despite attempts to fixate. Following cessation of motion, eye movements were suppressed but MAE in the direction opposite to prior stimulation persisted for typically 7s. In conclusion, although MAE may be accompanied by eye movements, perceived motion is not produced by these eye movements or their visual consequences. (Supp. by VAMC and NIH EY06717)

373.3

CHARACTERISTICS OF MIDBRAIN NEAR RESPONSE CELLS PROJECTING TO MEDIAL RECTUS MOTONEURONS **Yihong Zhang, Paul Gamlin, and Lawrence Mays, Department of Physiological Optics, School of Optometry, University of Alabama at Birmingham, Birmingham, AL. 35294.**

Midbrain near response cells, which are located just dorsal and lateral to the oculomotor nucleus, increase their firing rate when a nearby visual target is fixated. We have shown by antidromic activation and collision testing that a subgroup of these near response cells projects directly to the medial rectus subdivision of the oculomotor nucleus (MRS) (Zhang et al., 1989, Invest. Ophthal. & Vis. Sci. (Suppl.) 182). However, the vergence and accommodation systems are cross-coupled. Also, midbrain near response cells are reported to form a heterogeneous group, with some related to the angle of vergence, others related to lens accommodation, and still others related to both vergence and accommodation (Judge and Cumming, 1986, J. Neurophysiol. 55:915). Therefore, the present study investigated the relationship to vergence and accommodation of midbrain near response cells in general and, in particular, those cells that project directly to the MRS. Recordings from 58 near response cells in two monkeys revealed a continuous distribution of cell properties, ranging from those cells that were related predominantly to vergence to those that were related predominantly to accommodation. Seventeen of these cells could be antidromically activated from the ipsilateral MRS and, in contrast to the entire population, these cells carried a signal related mainly to vergence. We propose a model that explains the behavior of both the general population of midbrain near response cells and the subgroup of near cells projecting to the MRS. This model is based on differential strengths of accommodation and vergence input to individual neurons. (Supported by EY03463 & CORE grant P30 EY03039).

373.4

PRENATAL DEVELOPMENT OF MONKEY EXTRAOCULAR MUSCLE. John D. Porter and Robert S. Baker. Depts. of Anatomy and Neurobiology and Ophthalmology, Univ. of Kentucky Medical Center, Lexington, KY 40536-0084.

In order to better understand the basis for the unique structural-functional diversity of extraocular muscle, ontogeny of lateral rectus muscles was studied in *Macaca nemestrina* fetuses of 62-156 days gestation, using light and electron microscopy. At F62, intramuscular unmyelinated axons, enclosed in clusters (not individually) by Schwann cells, and infrequent, very primitive neuromuscular contacts were seen. Myofilaments already were organized into sarcomeres, while fiber mitochondria and internal membrane systems were nascent. By F92, presumptive singly (SIF) and multiply (MIF) innervated fiber types could be distinguished on the basis of myofibril delineation. Association of primitive with more developed myofibers was a prominent feature. Maturation of internal membrane systems was well underway; although presence of active golgi-like organelles suggested continuing elaboration of these elements. Intramuscular axons were myelinated only near point of entry into the muscle; terminal axons exhibited lengthy muscle contacts. The nmjs were primitive, with wide synaptic clefts, high vesicle content and junctional accumulations of myonuclei. No elaboration of postjunctional folds was evident. Multiple innervation of putative SIFs was apparent. By F135, axon myelination increased and synaptic cleft width was reduced, although many nmjs continued to occupy long segments of sarcolemma. Discrete clusters of vesicles appeared to mark sites that would later form definitive nmjs. Muscle oxidative capacity, evidenced by fiber mitochondrial content and microvascular network development, was clearly increased by F156, and served to define SIF subtypes. At this stage, nmjs more closely resembled those of the adult, but still lacked postjunctional folds. In all, global MIFs preceded and orbital SIFs followed all other types in attaining mature fiber characteristics.

Taken together, these observations suggest that some unique features of extraocular muscle are specified rather early in development and are not necessarily a direct consequence of the exceptionally high phasic/tonic discharge rates exhibited by oculomotor motoneurons. Supported by NEI R01 EY05464.

373.5

OPTOKINETIC NYSTAGMUS IN TURTLES: EVIDENCE OF A BEAT-TO-BEAT CYCLE GENERATOR FOR SLOW PHASE DURATIONS. C.D. Balaban and M. Ariel. *Depts. of Otolaryngology & Behavioral Neuroscience, Eye & Ear Institute, University of Pittsburgh, Pittsburgh, PA 15213.*

An analysis of optokinetic responses suggests that there is a basic cycle generator that sets the timing of nystagmus fast phases. Red-eared turtles (*Pseudemys scripta elegans*) were fitted with a search-coil contact lens for recording optokinetic nystagmus elicited by a monocular random pattern drifting at constant velocities of 0.25-42°/s. The times and eye positions at the onset and end of each fast phase were used for analysis of properties of slow phase durations (SPDs). The behavior of SPDs is consistent with a model incorporating a **basic cycle generator** (BCG), with each SPD equal to $(1 + \varepsilon)$ times the duration of the previous SPD ($\varepsilon \sim N(0, 0.1 - 0.2)$. The parameter λ, defined as the current SPD divided by the previous SPD, was used to analyze beat-to-beat operations of the BCG. The **basic cycle length** was defined as median SPD; its log was proportional to log (slow phase eye velocity). When initial eye position deviated in the slow phase (SP) direction, the SPDs reflect one cycle of the BCG. However, the SPDs 'skipped-a-beat' with probability 0.3 when initial eye position deviated in the fast phase direction; the BCG appeared to cycle a second time before producing a fast phase. The estimates of the variance of ε and p from the data were sufficient to reproduce the observed distributions of λ and SPDs across stimulus conditions. Thus, this novel model using both timing and position information can reproduce the alternation between nystagmus slow and fast phases. (Supported by NIH grants NS-00891, DC-00739, EY-05978 and MH-00815).

373.6

Gaze Pursuit of Predictable and Unpredictable Moving Visual Targets Compared to Smooth Pursuit and Vestibulo-ocular Reflex Cancellation. K. E. Cullen and R. A. McCrea Comm. on Neurobiology, Univ. of Chicago

The ability of squirrel monkeys to track both predictable and unpredictable moving targets by generating combined eye-head movements, gaze pursuit (GP), was compared to both 1) their ability to track the same target in a head fixed paradigm by generating smooth pursuit (SP) eye movements, and 2) their ability to cancel their vestibulo-ocular reflex (VORc) by fixating a target during comparable vestibular stimulation.

The accuracy (gaze velocity gain) of GP and SP was similar when the monkeys tracked predictable sinusoidally moving targets (0.25-3.5Hz, 20°/s peak velocity); while the gain of VORc was slightly higher than that of GP and SP at low frequencies of stimulation (< 1.0 Hz). The phase lag of gaze velocity during VORc with respect to stimulus velocity, was less than that of GP and SP at higher frequencies (> 1.0 Hz).

Unpredictable pseudo-random stimulation consisted of the sum of five nonharmonically related sinusoids in which the highest frequency was 1.9 Hz. The velocity of each component wave was either 5°/s or 10°/s. The gaze velocity gain during GP of unpredictable moving targets was greater than the gain of SP for all frequencies of the pseudo-random stimulus, although gaze velocity gain was greater during VORc than during either GP or SP. The phase lag of gaze velocity during VORc was less than that during GP, which in turn was usually less than that of SP. The increase in gaze velocity gain during GP compared to SP was positively correlated with the proportion of gaze velocity generated by head movement.

These results suggest that in generating combined eye-head movements during GP, monkeys make use of non-visual afferent information in addition to the visual feedback of target motion. The differential effect of this input is obscured when predictable stimuli are used, indicating that head and eye movement generation mechanisms receive input from a common predictive element.

373.7

ULTRASTRUCTURE AND CONNECTIONS OF IRIS AND CILIARY MOTONEURONS IN THE PRIMATE CILIARY GANGLION. Paul J. May. Depts. of Anatomy and Ophthalmology, U. of Mississippi Med. Ctr., Jackson, MS 39216.

The ciliary ganglion receives input from the Edinger-Westphal (EW) nucleus and contains the postganglionic motoneurons that supply the iris constrictor pupillae muscle and the ciliary muscle of the lens. There is limited data on ciliary ganglion ultrastructure in primates, despite the well developed near response present in foveate monkeys. Electron microscopic investigation of ganglia from Macaca fascicularis revealed that the motoneurons have large ovoid somata (45-70 um long) with pale nuclei and numerous stacks of rER and Golgi apparati. In stark contrast to the axosomatic calyces found in birds, the somata in macaque ciliary ganglia are nearly devoid of synaptic contacts and are instead ensheathed by glia with dark cytoplasm. Most of the synaptic contacts are found in the perisomatic neuropil, where the dominant presynaptic (presumably preganglionic) profile varies from 0.5 to 3.0 um in diameter, is filled with large (0.04 um diameter) spherical vesicles and often has scattered large (0.08 um diameter) dense-cored vesicles. Generally, the postsynaptic profiles are dendrites up to 5.0 um in diameter and their appendages (0.4 - 0.8 um). Glomerular arrangements with a central axonal element contacting several small dendritic appendages were also frequently observed. A second, very rare, profile containing only large dense-cored vesicles was also found, indicating a catecholaminergic input. To differentiate the pupillary from the lens-related motoneurons WGA-HRP was injected into the anterior chamber. The labeled pupillary ganglion cells (20%) showed no consistent ultrastructural differences from the unlabelled lens cells. In contrast to the discrete ganglionic labelling, trans-synaptic labelling was observed in most EW motoneurons, suggesting that the "lens" related EW cells may actually subserve both intrinsic components of the near response. Support: NIH Grant EY07166

373.8

IMMUNOHISTOCHEMICAL LOCALIZATION OF GLYCINE IN THE OCULOMOTOR NUCLEI OF THE GROUND SQUIRREL (Citellus tridecemlineatus).

M.A. BASSO*, S. AGARWALA*, J.K. MOORE & J.G. MAY. Depts. of Psychology and Anatomical Sciences, SUNY at Stony Brook, NY 11794.

The oculomotor nucleus (OMN) in the ground squirrel is notable in that the extraocular motorneuron pools appear to be spatially segregated to a greater degree than that seen in other mammalian species. This observation has prompted us to compare the immunohistochemical labeling patterns of selected neurotransmitters across these different motorneuron pools and across the other extraocular motor nuclei. Physiological, autoradiographic and immunohistochemical evidence from the primate and cat has implicated both GABA and glycine as the transmitters involved in the inhibitory limb of the vestibulo-ocular circuit. Recent uptake and pharmacological studies suggest that GABA may be the principal inhibitory neurotransmitter in the oculomotor and trochlear nuclei whereas glycine appears to be the principal inhibitory transmitter in the abducens nucleus (Spencer, et al. 1987, 1990). In support of this view the present study revealed heavy glycinergic reactivity throughout the abducens nucleus. Glycinergic label was also found in the oculomotor and trochlear nuclei but was meager in comparison with that seen in abducens. Though relatively sparce, this labeling was interesting in that it was seen only within restricted cellular groups along the midline and ventrolateral portions of the OMN. Supported by Sigma Xi G.I.A.(SA), NSF BNS-8896117 (JGM), NIH NS-26516 (JKM) and NIH EY-07113 (Heywood Petry).

373.9

DEFICITS IN SMOOTH-PURSUIT EYE MOVEMENTS FOLLOWING LIDOCAINE INJECTION IN MONKEY NUCLEUS RETICULARIS TEGMENTI PONTIS (NRTP). D.A. Suzuki, R.D. Yee, & K. Betelak. Dept. of Ophthalmology, Indiana Univ. Sch. of Medicine, Indianapolis, IN 46202.

A behavioral test of NRTP involvement in smooth-pursuit eye movement (SPEM) control was undertaken in light of observed inputs from the frontal eye field and the SPEM deficits resulting from frontal eye field lesions. NRTP activity was recorded in alert monkeys trained to fixate a moving 0.5 deg spot. Eye position was monitored with the scleral search coil technique. After recording pursuit-related unit activity, a 28 ga. needle replaced the μelectrode and 5-10 μl of lidocaine were injected.

SPEM gain was calculated as the ratio of eye and target velocities. Pre-injection control values were measured before and after placement of the injection needle. Following injection of lidocaine, SPEM gain decreased by 20-35%. The SPEM deficits were direction selective. Although saccadic eye movements appeared normal, the monkeys experienced difficulty in foveating a moving target. The effects of the lidocaine were diminished 20-30 minutes following injection and SPEM gains were normal at 60 minutes.

These results implicate NRTP involvement with SPEM control. Frontal eye field to NRTP projections could constitute a route that parallels the cortical visual motion (MT/MST) to dorsolateral pontine nucleus pathway. Such a parallel SPEM circuit could account for the recovery of SPEM ability following placement of lesions in the dorsolateral pontine nucleus.

373.10

The vestibulo-ocular (V-O) pathway in the chick embryo.

J.Storm-Mathisen, J.K.S.Jansen, J.C.Glover and B.O. Fischer. Depts. of Anatomy and Physiology. Oslo Univ., Oslo, Norway.

To generate appropriate eye movements during head rotation the V-O pathways must excite contralateral and inhibit ipsilateral eye motoneurons. We have preliminary information in E day 11 chick embryos on putative transmitters of certain vestibular subgroups involved in vertical V-O reflexes.

A ventral subdivision of the superior (SV) nucleus projects ispilaterally to superior oblique (SO) and rectus inferior (RI) motor nuclei. This group was retrogradely labeled from the ascending MLF. Immunhistochemically, many labeled neurons reacted with a GABA specific antiserum. Additionally, neurons of the same group were labeled autoradiographically after ^{3}H Nipecotic acid (a GABA analogue) injected into the oculomotor nucleus.

The dorsal subdivision of the SV projects to the contralateral IO and RS motoneurons. This group is labeled autoradiographically after injection of ^{3}H Asp in the oculomotor nucleus. Such injections also label a more caudal group of vestibular neurons which project to contralateral SO and RI motoneurons.

The results are consistent with observations in mammals showing that second order (V-O) neurons directly excite contralateral and inhibit ipsilateral eye motoneurone pools.

373.11

Cytoarchitectonic Parcelation of the Perihypoglossal Complex in theRat R.S. Revay and G. Aston-Jones. Dept. Mental Health Sci., Hahnemann University, Philadelphia, PA, 19102.

The perihypoglossal complex, composed of Roller's nucleus, and the nuclei intercalatus, prepositus hypoglossi (PrH), and supragenualis, is situated in the dorsomedial medulla. A subset of PrH neurons form a major afferent to the nucleus locus coeruleus (LC) as demonstrated by tract tracing studies. We examined the cytoarchitechtonics of this region to define the relation of these LC afferents to other parts of the perihypoglossal complex. Forty-micron-thick sections were cut in the three principle planes from snap-frozen, fresh brains of male Sprague-Dawley rats and Nissl stained. This method provided material with minimal tissue shrinkage. A series of transversely cut fixed brains were stained by a modified Weil method to demonstrate myelinated fibers. Ten-micron-thick sections cut from methyl/butyl methacrylate-embedded tissue provided Nissl stained sections with superior morphological detail. Careful inspection of the above material made possible a detailed description of the perihypoglossal complex. It was found that the PrH is composed of several cytoarchitonically distinct subdivisions. One of these subdivisions, located in medial suprafasicular PrH and along the dorso-lateral border of the medial longitudinal fasiculus, corresponds to the region that contains LC-projecting neurons. Thus, it appears that a cytoarchitectonically distinct region of the PrH is afferent to the locus coeruleus. Supported by USPHS grants NS24698 and DA06214.

373.12

CAT LATERAL RECTUS (LR) MOTOR UNITS: RECRUITMENT, K VALUES, AND HYSTERESIS. M.A. Snyder and S.J.Goldberg. Dept. Anatomy, Medical College of Virginia - VCU, Richmond, VA 23298.

60 antidromically identified abducens nucleus motoneurons (MNs) in 12 anesthetized cats were tested for monosynaptic recruitment by stimulation of the contralateral medial rectus subdivision of the oculomotor nucleus. These same MNs were then directly stimulated and the mechanical responses of their muscle units were recorded with the LR muscle at maximal isometric tension.

MNs, within and among cats, were not recruited based on axonal conduction velocity, contraction speed or force, fatigue index, or K value.

K values (slope of MN stimulation frequency needed to increase tetanic tension by 1 mg.) were higher for weak motor units and lower for powerful ones, (range=0.20 - 6.42; r=0.97). The highest K values were associated with fatigue resistant units and the lowest with fatigable units.

A 200 msec. continuous stimulation train of 350 pps for 10 msec., 200 pps for 15 msec., and 100 pps for 175 msec. produced a force record to be compared to that of a monotonic train of 100 pps for 200 msec. A force level hysteresis, at 100 pps, was seen in most motor units.

[Supported by BNS-8507610 and EY-07924.]

373.13

TRANSIENT RESPONSES OF PRIMATE PONTINE NEURONS FOR VISUALLY TARGETED EYE AND HEAD MOVEMENTS. L. Ling*, J.O. Phillips, and A.F. Fuchs. Regional Primate Research Center and Depts. of Psychology and Physiology and Biophysics, University of Washington, Seattle, WA, 98195.

Monkeys naturally perform gaze shifts through a combination of eye and head movements. We have developed a preparation that allows us to record extracellular single unit activity in a rhesus monkey who is free to turn his head in the horizontal plane to look at eccentric targets. Animals were trained to follow a spot of light using combined eye and head movements (with the head free to move about a vertical axis through the atlanto-axial joint) or employing eye movements alone (with the head held stationary).

We have used this preparation to record from the pontine brain stem in the region containing cells that are part of the legendary saccadic burst generator. A preliminary analysis from 13 burst neurons shows that some exhibit a sudden high frequency burst that precedes gaze movements regularly by about 70-80 msec and gives way to a gradual decrease in activity that seems to be related to parameters of the head movement. For these neurons, greater head movements are accompanied by more spikes. A similar analysis of 4 omnipause neurons shows that these cells are related to eye movement alone.

This study was supported by National Institutes of Health grants EY00745 and RR00166.

373.14

RESPONSE PROPERTIES OF MONKEY NOT NEURONS. U.J. Ilg & K.-P. Hoffmann Allgemeine Zoologie und Neurobiologie, Ruhr-Universität, Postfach 10 21 48, D-4630 Bochum, F.R.G.

Results will be presented obtained from single unit recordings in the nucleus of the optic tract (NOT) of trained monkeys working on the following paradigms.

i) Fixation of a spot while the background (random dot pattern) is moving.
ii) Pursuit of a spot across a stationary random dot or uniform background.
iii) Optokinetic nystagmus (OKN) and optokinetic after nystagmus (OKAN).

Additionally we performed experiments on anaesthetized and paralysed rhesus monkeys especially to study the response properties of antidromically identified neurons projecting from cortex to NOT.

The results can be summarized as follows: 1. NOT neurons code for direction and speed of retinal image motion (slip detectors). 2. During OKAN there is no relationship between slow phase eye velocity and NOT activity. Thus the eye velocity storage mechanism does not include the NOT, whereas NOT provides the visual input for the storage mechanism. 3. The transfer function between retinal slip and slow-phase eye velocity also describes the relationship between retinal slip and discharge rate of single NOT neurons. 4. During smooth pursuit across uniform background NOT neurons code target slip while during pursuit across a random dot pattern they code background slip suggesting that the pursuit system is not relying on NOT signals. 5. The modulation of NOT neuron activity decreases if the stimulus consists only of colour-contrast, i.e. is isoluminant. 6. The activity of NOT neurons is influenced by saccades via a visual (reafference) and a non-visual (efference copy) signal. 7. There is physiological evidence that NOT in monkey receives input from area FST of the superior temporal sulcus.

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373.15

DESCENDING CONNECTIONS OF THE MACAQUE NUCLEUS OF THE OPTIC TRACT. M.I. Mustari, A.F. Fuchs and C.R.S. Kaneko. Dept. of Physiology & Reg. Primate Res. Center, Univ. Washington, Seattle WA 98195.

Lesion studies have shown that the nucleus of the optic tract (NOT) is essential for horizontal optokinetic nystagmus. However, the neural pathways used by the NOT to support this important function have not been elucidated. We localized the NOT by single unit recording and placed small (5nl-150nl) injections of 2% WGA-HRP into the NOT of three monkeys (2 *Macaca mulatta* and 1 *M. fascicularis*). After 24-h survival, animals were sacrificed and prepared for TMB histology. The injection sites involved all of the NOT, the dorsal terminal nucleus, and other caudal pretectal nuclei, and spread in varying degrees to the rostral superficial layers of the ipsilateral superior colliculus. The principal projection of the NOT was to the ipsilateral dorsal cap of the inferior olive (dC). The NOT also had substantial reciprocal connections with the contralateral NOT and both lateral terminal nuclei of the accessory optic system. Connections with other vestibulo/oculomotor areas were disappointingly much lighter in density. At the level of the medulla, they included ipsilateral efferent connections to the nucleus prepositus hypoglossi (NPH), medial vestibular (MVN), and abducens (AB) nuclei; the inhibitory burst neuron area also received a light projection. A few retrogradely labeled neurons were present in the contralateral NPH and MVN nuclei. At the level of the pons, the NOT projected lightly to the ipsilateral nucleus reticularis tegmenti pontis (NRTP) and dorsolateral pontine nuclei (DLPN). These results show that the NOT appears to have efferent connections with structures known to be involved in oculomotor control. These include possible output pathways through the cerebellar flocculus via the dC, NRTP and the DLPN and more direct pathways through the MVN, NPH and AB.

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373.16

CYTOARCHITECTURE OF THE PRIMATE ZONA INCERTA. T. P. Ma, Y. Anavi*, X. J. Hu*, and J. A. Rafols. Laboratory of Sensorimotor Research, National Eye Institute, NIH, Bethesda, MD 20892 and Department of Anatomy and Cell Biology, Wayne State University School of Medicine, Detroit, MI 48201.

The neurons in the zona incerta (ZI) of the monkey (*Macaca mulatta*) were examined in Nissl, fiber-stained, cytochrome oxidase, and Golgi preparations sectioned in the frontal, horizontal, and sagittal planes. In the frontal plane, the ZI was oriented in the dorsolateral to ventromedial axis and could be separated into dorsal and ventral sublaminae on the basis of staining and morphological features in the Nissl, cytochrome oxidase, and Golgi preparations. In particular, the ventral sublamina was intensely stained for cytochrome oxidase. The dendrites of most neurons in both sublaminae were oriented preferentially along the long axis of the nucleus. Three neuronal types (I-III) were distinguished on the basis of features of the somata, dendrites, and axons in Golgi preparations. Type I cells had fusiform or polygonal cell bodies (long axis from 21 to 40 μm) and dendrites that radiated for up to 725 μm within the sublamina and along the axis of the nucleus. Neurons with similar dimensions were observed to be darkly stained in cytochrome oxidase preparations. Type II cells had polygonal cell bodies (18-20 μm) and thin, varicose dendrites that extended up to 300 μm. These cells were rare in our preparations. Type III cells were local interneurons that had small (12-16 μm), round cell bodies with wavy dendrites (up to 400 μm). Numerous multilobed appendages (1-3 μm) and axon-like processes originated from these dendrites and made apparent contacts with other Type III dendrites or with dendrites of Type I cells. The ZI is reported to receive projections from many centers of the brain, and has reciprocal projections with many of these centers, to the cerebral cortex, and other brainstem nuclei. Our results support the notion that complex intrinsic circuits formed by morphologically heterogeneous neuronal types exist in the primate zona incerta to integrate these inputs.

374.1

LOCOMOTOR ACTIVITY AND ACTIVE AVOIDANCE IN RATS: EFFECTS OF INTRAHIPPOCAMPAL INJECTIONS OF NEUROTRANSMITTER ANTAGONISTS. L.S.O.Brito* and G.N.O.Brito. Setor de Neurociencias, UFF, Brasil.

Previously, we showed that lesions in the septo-hippocampal system increase general activity, but do not alter the performance of a one-way active avoidance task. Seventy five male Wistar rats underwent surgery for implantation of bilateral 22 ga. cannulae aimed at the dorsal hippocampus. Rats were tested for general activity and one-way active avoidance after 1ul injections of one of the following drugs: Krebs (N=8); scopolamine (9 ug/ul, N=8; 18 ug/ul, N=8); sulpiride (5 ug/ul, N=9; 10 ug/ul, N=9); propranolol (5 ug/ul, N=9; 10 ug/ul, N=8); cimetidine (0.75 ug/ul, N=8; 1.5 ug/ul, N=8). The results showed that only intrahippocampal injections of scopolamine increase activity, and that there were no drug effects on total number of avoidances in the one-way active avoidance task. We conclude that disruption of cholinergic mechanisms in the hippocampus mimic some of the behavioral effects of lesions in the septo-hippocampal system.

Research supported by FUNPPENE.

374.2

PROTECTIVE EFFECT OF A CHOLINOMIMETIC DRUG, α-GLYCERYLPHOSPHORYLCHOLINE (α-GFC) ON SCOPOLAMINE-RELATED DEFICIT OF MEMORY AND BRAIN ACETYLCHOLINE (ACH) LEVELS IN THE RAT. C. Lopez*, A. Gagnoni*, F. Battaini+, S. Govoni*^, and M. Trabucchi+, Inst. Pharmacol. Sci., Univ. Milan, + Dept. Exp. Med. Biochem. Sci., Univ. Rome, ^ Dept. Pharmacobiol., Univ. Bari, Italy.

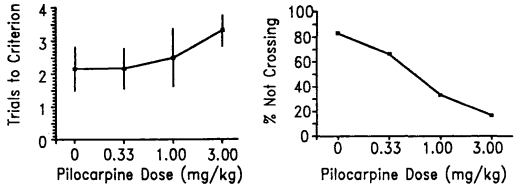
The cholinergic system has been implicated since many years in the process of learning and memory in animals and man.The cholinolytic drug, scopolamine (a well known amnestic drug) produces impairment in the acquisition of the passive avoidance task. The studies reported here investigated the effects of α-GFC on the performance of passive avoidance (step-down). Male Wistar rats (150-200 g) were given α-GFC (0.1, 0.3, 0.6, 1.0 g/kg; 1, 5, 20, 48 hours before the training session). Scopolamine was injected 30 minutes before the training session (0.75 mg/kg s.c.). The levels of ACH were determined using HPLC with electrochemical detection (Damsma, G. et al., J. Neurochem., 45:5, 1985). The results indicate that rats receiving 0.3 and 0.6 g/kg i.g. α-GFC, 5 hours before training, perform significantly better in comparison with those treated with scopolamine (mean latency times : 155~, 293~, 25 sec. respectively. ~p<0.01). The peak effect was obtained with 0.6 g/kg i.g. α-GFC, while rats receiving 0.1 and 1 g/kg did not differ from the scopolamine treated group. α-GFC prevented partially the decrease of ACH levels induced by scopolamine in cortex but not in striatum. However, the time course of the behavioral response and that of ACH levels were not superimposable. These results suggest that changes in ACH levels mediate the antiamnestic effect of α-GFC, though this neurotransmitter may not be the only correlate of the behavioral response.

374.3

DOSE-DEPENDENT EFFECTS OF PILOCARPINE ON ACQUISITION AND RECALL OF STEP-THROUGH PASSIVE AVOIDANCE IN THE RAT. W. Jeffrey Wilson[1] & Jennifer A. Cook[2], [1]Dept. of Psychological Sciences, Indiana-Purdue University, Fort Wayne, IN 46805; [2]Dept. of Psychology, Emory University, Atlanta, GA 30322.

Sprague-Dawley rats received one of four doses of pilocarpine (0, .33, 1.00, & 3.00 mg/kg, n=6/group) prior to both the training and test session of a step-through passive avoidance procedure. In training, the 1.0 mA shock was escapable, and the session continued until the rat remained on the safe side for 60 s. Test session continued until the rat stepped through or for 600 s. Pilocarpine produced a dose-dependent increase in the number of trials required to reach criterion, and a dose-dependent decrease in the number of rats in each group that performed perfectly in the test. Dose-dependent increases in latency to cross, and to escape shock, occurred in training. These motor and memory effects interacted to produce a biphasic effect of drug dose on latency to cross in the test.

We have previously shown that a high dose of pilocarpine impairs both learning and performance of this task, as does scopolamine. The present results demonstrate that low doses of a muscarinic agonist do not produce effects opposite to those produced by scopolamine, and suggest that both increases and decreases in central cholinergic activity can disrupt memory.



374.4

DIFFERENTIAL IMPAIRMENT OF DISCRIMINATION LEARNING IN RATS DUE TO NUCLEUS BASALIS MAGNOCELLULARIS LESIONS OR SCOPOLAMINE TREATMENT. A. E. Butt, J. E. Dencoff, B. G. Cooper*, K. Nopp-Dvorak*, and G. K. Hodge. Department of Psychology, University of New Mexico, Albuquerque, NM 87131.

Changes in learning and memory were related to disruptions of cholinergic function in a neurotoxic and pharmacologic animal model of Alzheimer's disease. The effects of bilateral ibotenic acid (IBO) lesions of the nucleus basalis magnocellularis (nbm) and the subsequent response to scopolamine administration were evaluated in multiple tests of discrimination learning in rats. IBO (10 μg/μl) infused bilaterally into nbm impaired performance as determined by the failure of IBO-treated rats to decrease nonrewarded (SD−) responding relative to rewarded (SD+) responding. The IBO group also had more difficulty than controls in adjusting to subsequent transitions in reinforcement contingency parameters. Lesion-induced deficits diminished within 6 weeks post-surgery. Scopolamine (0.06 mg/kg, i.p.) was then administered on alternate test days, which retarded response rate and decreased total responding for both groups; although performance was slower, accuracy relative to animals' spontaneous performance was unimpaired. Results suggest that the nbm is involved in the acquisition of adaptive behaviors relying on accurate responding to environmental cues, but that scopolamine-induced deficits may partly reflect other components in addition to any disruption of memory. The qualitative differences shown in response to nbm lesions and scopolamine treatment suggest that injury of basal forebrain systems may model Alzheimer's disease better than the global cholinergic inhibition caused by scopolamine.

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374.5

SCOPOLAMINE SUPPRESSES BOTH LOCOMOTION AND OBJECT CONTACT IN A FREE EXPLORATION SITUATION.

M. J. Renner, D. L. Dodson, & P. A. LeDuc. Department of Psychology, Memphis State University, Memphis, TN 38152

The anticholinergic drug scopolamine is widely reported to have amnestic properties. Buhot, et al. (*Psychobiology*, 1989, *17*, 409-417), recently reported that pre-session cholinergic disruption with scopolamine decreases time spent in proximity to novel objects while increasing locomotor behavior. In the experiment reported here, male Long-Evans hooded rats (*Rattus norvegicus*, 80 days old) were given access to an arena containing objects on three consecutive nights. On Day 1 subjects were familiarized with the apparatus and procedure: Each was injected with saline (SAL), and 12 minutes later given access to the arena under low light. Subjects were randomly divided into four groups, with half given SAL and half given scopolamine (SCO, 1 mg/kg) on Day 2 and Day 3, resulting in SAL-SAL, SAL-SCO, SCO-SAL, and SCO-SCO groups. Videotapes of these sessions were scored according to a standardized protocol (Renner, *J. Comparative Psychology*, 1987, *100*, 94-100), which allows separate quantification of locomotor, general, and object interaction behaviors. Scopolamine suppressed object investigation (both gross contact measures and indices of interaction character) whenever present: Day 2 drug experience had significant effects on Day 3 behavior in the arena, but this appeared to be mediated through suppression of Day 2 investigatory behavior. Scopolamine's arousal-modulating effects must also be considered: In contrast to Buhot, et al. (who used a forced-exploration situation) in this free-exploration context SCO suppressed locomotor behavior. This study replicates and extends Buhot, et al.'s conclusion that anticholinergics impair information gathering rather than affecting memory directly, which calls into question memory-related explanations of cholinergic treatments unless controls for behavior during the purported "learning" event are included.

374.6

THE DURATION OF A SHORT-TERM MEMORY IMPAIRMENT FOLLOWING CHRONIC SCOPOLAMINE EXPOSURE DEPENDS ON THE AGE WHEN THE TREATMENT IS ADMINISTERED. R. Paylor, Psychology Dept., University of Colorado, Boulder, CO 80309

Long-term scopolamine treatment is known to result in a transient (less than 10 days) increase in the number of hippocampal muscarinic receptors in both neonatal and adult rats (J. Ben-Barak & Y. Dudai, Brain Res., 193, 309-313, 1980). Previously, we demonstrated that rats exposed to a long-term scopolamine treatment during the early postnatal (PN) period (PN day 1 to 19) produced a selective impairment in the short-term memory processes underlying performance on a conditional spatial discrimination task (CSD task). The memory imairment, however, lasted at least 41 days which is much longer than receptor numbers are known to be above normal following chronic scopolamine treatment.

The present experiments were designed to determine if the enduring memory impairment following scopolamine treatment is restricted to subjects treated when the CNS is immature. Rats were, therefore, administered the 19 day scopolamine treatment when they were 70 days old. Two, 11 or 41 days following the last injection subjects were tested on the CSD task. Results showed that subjects given either a 2 or 11 day recovery period were capable of learning the CSD task, but were impaired when their short-term memory based performance was further challenged. Subjects given a 41 day recovery period, however, appeared to be unimpaired.

The present results, together with the results from subjects treated during the early postnatal period demonstrates that (a) chronic exposure to scopolamine produces a specific behavioral impairment which can not be easily explained by a receptor number hypothesis and (b) the duration of the impairment depends upon the maturation of the CNS at the time of treatment..

374.7

AGE-DEPENDENT EFFECTS OF HIPPOCAMPAL MUSCARINIC RECEPTOR BLOCKADE ON MEMORY-BASED LEARNING IN THE RAT E.C. Pollock, M.W. Lilliquist, N.J. Lobaugh & A. Amsel. Dept. of Psychology & Inst. for Neuroscience, University of Texas at Austin, 78712

Hippocampal cholinergic blockade has been shown to produce a deficit in response suppression in passive avoidance learning (Blozovski & Hennocq, Psychopharm., 76:351, 1982), in which external cues, rather than memory of the previous trial, guide behavior. The present study examined the effects of intra-hippocampal atropine (ATR) on a discrimination task, patterned alternation (PA), that requires intact short-term memory of the previous trial. In 16-17-day-old pups, ATR treatment disrupted acquisition by interfering with approach to the goal, but did not eliminate PA at 8-s intertrial interval (ITI) if acquired under saline. We also tested rats at two stages of hippocampal development (16-17 and 28-32 days of age), using a modified training procedure, to examine the effects of ATR on suppression in PA learning at 15-s ITI. Relative to controls, no deficit was observed in the younger age group; however, the older atropine subjects demonstrated a suppression deficit on nonrewarded trials. In contrast, the suppression deficits seen by Blozovski et al. were observable in pups as young as 13 days of age, perhaps because of the aversive rather than appetitive nature of passive avoidance. This evidence suggests that there is increased involvement of the hippocampus in the acquisition of this memory-based learning task as the rat pup matures. Supported by NSF grant BNS 8609877 and NIAAA grant AA67052.

374.8

THE EFFECTS OF RO 23-4749, A FURO[2,3-C]PYRIDINE DERIVATIVE, ON LEARNING AND MEMORY IN MICE. G.P. Vincent, E. Chiang,* G. Olson* and J. Sepinwall. Neurobiology and Obesity Research and Medicinal Chemistry II, Hoffmann-La Roche Inc., Nutley, NJ 07110.

Ro 23-4749, a furo[2,3-c]pyridine derivative, was evaluated in mice in various tests to assess its activity as a potential cognition enhancing agent. In CF1 mice, Ro 23-4749 protected over a wide range of doses (1 to 300 mg/kg, PO) against electrobrain shock (EBS) disruption in retrieval of an active avoidance response. A peak recovery of function of 68 percent was obtained at a dose of 300 mg/kg. In a time course evaluation using this peak active dose, the compound was active only after a 60 minute pretreatment. When administered to "poor learning" mice (doses of 100 and 300 mg/kg, PO) immediately after training, Ro 23-4749 significantly increased the number of avoidance responses made in the subsequent testing session 24 hours later. In C57BL/10 mice, Ro 23-4749 significantly decreased the latency to find the hidden platform in the Morris water maze test. Active doses were 1 to 300 mg/kg, PO, with the exception of 3 mg/kg. These results demonstrate that Ro 23-4749 enhances several stages of memory processing, including acquisition, storage and retrieval.

374.9

BEHAVIORAL CONDITIONING INCREASES MUSCARINIC$_2$ RECEPTOR BINDING IN LIMBIC THALAMUS AND CORTEX. B.A. Vogt, M. Gabriel, L.J. Vogt*, A. Cox, E.J. Jensen*, Y. Kubota and E. Kang. Dept. of Pharmacology, Boston Univ. Sch. of Med., Boston, MA & Dept. of Psycology, Beckman Inst., Univ. of Illinois, Urbana, IL

Little is known of the molecular mechanisms which subserve learning and memory in the mammalian brain. This study analyzes M_2 receptor regulation which occurs in tandem with training-induced neuronal plasticities during discriminative avoidance learning. Rabbits were trained to one of 5 stages of performance wherein hopping in a wheel to a CS^+ prevents negative reinforcement. Upon reaching a particular stage the brains were sectioned, incubated in ^{3}H-oxotremorine-M and autoradiographed. In the parvocellular part of the antero-ventral thalamic nucleus there was a progressive increase in OXO-M binding which peaked when rabbits met the criterion stage of learning. Binding in the magnocellular part increased only during the criterion stage. Increases in binding also occurred in mid levels of cingulate cortex and were more pronounced at the stage when the rabbits first performed a significant discrimination. Binding in AVp and cortex were highly correlated.

The parallel increases in M_2 receptor binding and discriminative neuronal activity throughout training provides a new approach for analyzing the mechanisms of behaviorally-relevant neuronal plasticities. Furthermore, neuronal plasticities in some limbic structures may depend on up-regulation of muscarinic receptors. (Air Force Office of Scientific Res.)

374.10

PHYSOSTIGMINE DECREASES A NEOPHOBIC RESPONSE WITHOUT AFFECTING SELECTIVE ATTENTION. M.A. Pelleymounter and M.J. Cullen,* Dept. of Neuroscience, Oberlin College, Oberlin, Ohio 44074.

It has been suggested that the amnestic effects of scopolamine are the result of a deficit in maintenance of attention, rather than the result of a deficit in storage, or "memory". The present study was designed to further test this idea by observing the effects of physostigmine, a cholinesterase inhibitor which has been widely used to **reverse** memory deficits, on two forms of attention; neophobia and selective attention. Selective attention was tested using the latent inhibition paradigm. Long-Evans male rats were either pre-exposed or not pre-exposed to three 72 dB, 30 sec tones presented in Coulbourn test cages (Day 1). Licks per sec were measured throughout all training and test sessions. On Day 2, all rats were exposed to the 30 sec tones followed by a 0.4 mA, 1 sec footshock. On the test day, rats were presented with the 30 sec tones and lick suppresion ratios were calculated. Physostigmine (0.06mg/kg) or saline was injected either 30 min prior to the pre-exposure session (Day1) or 30 min prior to the tone-shock pairing session (Day 2). Pre-exposed rats that received saline on both Day 1 and Day 2 showed significantly less suppression than their counterparts that were not pre-exposed, indicating that these rats had attended to the tone presentation on Day 1 and had failed to associate it with shock. Physostigmine treatment on either day did not alter this pre-exposure effect, indicating that it did not affect selective attention. These rats were then tested in a neophobia paradigm, where they were given either dilute grape juice or water to drink for 15 min sessions, followed by a 15 min presentation of water. Rats were injected with physostigmine or saline 30 min prior to the presentation of grape juice or water. All rats showed significant suppression to the novel flavor, but physostigmine-injected rats showed significantly less suppression ($p<.05$). Further, physostigmine-injected rats did not show significantly greater habituation to the novel flavor on the second day of exposure to grape juice than did saline controls. These results could suggest that while physostigmine does not affect selective attention, it may actually disrupt a more general aspect of attention that involves detection of a novel stimulus, as in the neophobic reaction to a novel flavor.

374.11

A COMPARISON OF THE EFFECTS OF CHOLINESTERASE INHIBITORS AND MUSCARINIC AGONISTS ON ACQUISITION OF A SPATIAL WATER-MAZE TASK BY C57BL/10j MICE. W. Lipinski, C.J. Moore, C. Bay, M. Smith, J. Kinsora, M. Dickerson and R.E. Davis. Parke-Davis Div. of Warner-Lambert Co., Ann Arbor, MI 48105.

C57BL/10J mice have difficulty acquiring a spatial water-maze task. This deficit can be ameliorated by administration of cholinesterase inhibitors. It is thought that cholinesterase inhibitors improve performance through their ability to elevate central cholinergic function. Muscarinic agonists also enhance central cholinergic function but through a different mechanism than cholinesterase inhibitors. An investigation was conducted to compare the relative ability of these two classes of cholinomimetics to improve acquisition of a spatial water-maze task in B10 mice.

Acetylcholinesterase inhibitors (tacrine, HP029, physostigmine, RA 10) showed greater efficacy than the muscarinic agonists (CI-969, CI-979, arecoline, oxotremorine, RS 86) in reducing the behavioral impairment of the B10 mice. This difference was most noticeable on the first day of testing. These results suggest that cholinesterase inhibitors are more effective than muscarinic agonists in improving performance deficits of B10 in the water maze. This may reflect differences in the mechanism by which these classes of cholinomimetics enhance central cholinergic activity. Alternatively, cholinesterase inhibitors may affect noncholinergic systems and changes in these systems may mediate their effect on mouse water-maze performance.

374.12

MUSCARINIC SUBTYPE INVOLVEMENT IN HABITUATION OF LOCOMOTOR ACTIVITY. M. J. Buckley and M. W. Decker. Neuroscience Research, Abbott Labs, Abbott Park, IL 60064.

The muscarinic cholinergic receptor, particularly the M1 receptor subtype, has been targeted in the development of cognition enhancers for the treatment of Alzheimer's disease. Toward further characterizing the behaviors mediated by M1 and M2 cholinergic receptor subtypes, we compared the effects of scopolamine (SCOP) and methylscopolamine (MSCOP), two non-selective muscarinic antagonists, with those of pirenzepine (PZ), an M1-selective antagonist, on mouse locomotor activity.

On each of 2 consecutive days, activity was measured for 1 min time bins over the course of 3 min. in male, CD1 mice. Systemically-administered SCOP (1.0 mg/kg) effected an increase in locomotor activity on day 1 and a failure to habituate (defined as a time-dependent decrease in activity) both within the day 1 session and between the 2 sessions. Systemically-administered MSCOP, which does not readily cross the blood-brain barrier, had no such effect. Centrally-administered PZ (1.0 ug, i.c.v.) had an effect similar to that of systemically-administered SCOP, although the between trial habituation effect did not quite reach statistical significance ($p<.07$). Much higher doses of PZ (100 ug, i.c.v.) were required to attenuate the hypothermic effects of the muscarinic agonist oxotremorine (0.5 mg/kg, i.p.). In contrast, central administration of MSCOP at doses that significantly attenuated oxotremorine-induced hypothermia (0.3, 1.0 & 3.0 ug) produced less marked increases in locomotor activity and only a borderline trend toward reduced habituation of activity on day 1 (dose by time interaction, $p<.06$).

Results suggest a dissociation between M1-mediated cholinergic effects on locomotion and habituation and M2-mediated cholinergic effects on temperature regulation.